OnabotulinumtoxinA Treatment for Neurogenic Detrusor Overactivity and the Prevention of Autonomic Dysreflexia Following Spinal Cord Injury

by

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Abstract

Individuals with high-level (>T₆) spinal cord injury (SCI) are prone to the development of a dangerous episodic hypertensive condition called autonomic dysreflexia (AD). The urinary bladder is the number one trigger of AD and is attributed to a condition called neurogenic detrusor overactivity (NDO). Intravesical injections of OnabotulinumtoxinA (Botox) into the detrusor muscle of the bladder in a dose of 200 Units (U) provides effective treatment for NDO. Following Botox, a few studies observed a reduction in AD during urodynamic studies (UDS). In this dissertation, I quantitatively assessed the efficacy of 200 U of intravesical injected Botox into 20 sites of the detrusor muscle on reducing AD severity, frequency and impact on AD-related quality of life (QoL) and bladder-related QoL. A total of 14 individuals (11 male; 3 female), mean age 45 ± 11 years, injury duration of 21 ± 12 years with a traumatic, chronic (> 1 year) SCI at ≥T₆ level underwent arterial blood pressure (BP) and heart rate (HR) monitoring according to an AD cut-off criteria of an increase in systolic BP (SBP) by ≥20 mm Hg above baseline SBP during UDS and 24-hr ambulatory BP monitoring (ABPM). Visit #1 consisted of a UDS pre-screening assessment with BP and HR monitoring. Participants who met the AD cut-off criteria were enrolled and completed 24-hr ABPM, the AD questionnaire, and bladder questionnaire. During Visit #2 (one week later), participants received the Botox injections by the urologist. During Visit #3 (one month later), participants repeated all components of Visit #1. During post-Botox UDS #2, there was a significant reduction in AD severity as per average SBP change (Δ) (P = <0.001) and maximum SBP (P = <0.001). There was a significant reduction in bladder-related AD severity SBPΔ (P = 0.001) and frequency (P< 0.001) as well as overall AD severity (P = 0.005) and frequency (P = 0.001) during post-Botox 24-hr ABPM. Significant improvements were found in AD-related QoL (P = 0.0015) and bladder-related QoL (P = 0.0005). AD was abolished in 8/14 (57%). Botox may prove a viable treatment option to reduce AD severity and frequency due to NDO.
Preface

This dissertation is original, unpublished, independent work by the author, R.J. Fougere. All of the work presented henceforth was conducted in the Autonomic Research Laboratory at the International Collaboration on Repair Discoveries located in the Blusson Spinal Cord Centre in affiliation with the University of British Columbia, Vancouver campus. R.J. Fougere was responsible for writing the ethics proposal and receiving ethics approval, implementation and coordination of the study through participant recruitment, screening, coordination of study protocol visitations, conduction of all pre and post-Botox cardiovascular assessments during urodynamic studies and 24-hr ambulatory blood pressure monitoring, questionnaire administration, and all aspects involved in the data collection, data entry, performance of statistical analyses, writing, and editing of this thesis document.

Dr. Andrei Krassioukov provided expertise, advice, guidance, and research supervision throughout the conduction of this study and was responsible for writing the grant proposal and study design. Dr. Krassioukov conducted all neurological examinations, provided confirmation of autonomic dysreflexia, in addition to providing assistance for cardiovascular interpretations during urodynamic studies and 24-hr ambulatory blood pressure monitoring. Dr. Krassioukov also provided editorial assistance for this thesis document.

Dr. Mark Nigro confirmed diagnosis of all participants with NDO as failures of anticholinergic therapy; followed by providing a prescription for 200 U of Botox for NDO with the required prophylactic antibiotic. Dr. Mark Nigro performed thirteen of the fourteen Botox injections at the Blusson Spinal Cord Centre and oversaw the urodynamic studies protocol for eleven participants.

Dr. Daniel Rapoport performed one of the fourteen Botox injections in addition to over-seeing the urodynamic studies protocol for three participants.
Registered Nurse Teresa Lim and Licensed Practical Nurse Colleen McLean conducted all pre and post-Botox urodynamic studies in the Blusson Spinal Cord Centre.

A version of Chapter III will be submitted for peer review. R.J Fougere was responsible for writing the manuscript, conduction of data collection and analysis, and implementation and coordination of the study protocol. Dr. Krassioukov was responsible for study concept formation and design. All co-authors made significant contributions toward editing the manuscript.

Components from the Chapter II literature review describing previously conducted randomized controlled trials assessing the efficacy, safety and impact on quality of life following Botox treatment for neurogenic detrusor overactivity, and detrusor sphincter dysynergia in persons with SCI will be submitted for peer review in a systematic review titled: “Botulinum neurotoxin for the management of dysautonomias: An evidence-based systematic review on randomized controlled trials”. R.J. Fougere was responsible for writing the manuscript. R.J. Fougere conducted the literature review and selection of studies to be included in addition to the first round of analyses and scoring of studies. Dr. Katharine Currie assisted with the second round of the systematic review and editing of the manuscript in addition to providing editorial assistance with this thesis document. Dr. Krassioukov oversaw all aspects of the systematic review ensuring that the review procedures adhered to the highest standards and editing of the manuscript.

The Rick Hansen Institute provided financial support for this thesis investigation and all Botox was provided by in-kind donation from Allergan Inc. The data from this research investigation was obtained with approval by the UBC Clinical Research Ethics Board and Vancouver Coastal Health Research Institute under Certificate # H12-02215.
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List of Abbreviations

Δ change
>T₆ high thoracic
ABPM ambulatory blood pressure monitoring
AD autonomic dysreflexia
AD HR-QoL autonomic dysreflexia health-related quality of life
ADFSCI Autonomic Dysreflexia Following Spinal Cord Injury
AIS American Spinal Injury Association Impairment Scale
ANS autonomic nervous system
BP blood pressure
BoNT botulinum neurotoxin
Botox onabotulinumtoxinA
CIC clean intermittent catheterization
CVD cardiovascular disease
DBP diastolic blood pressure
DSD detrusor sphincter dysynergia
EMG electromyography
HR heart rate
I-QoL incontinence quality of life
LUT lower urinary tract
MAP mean arterial pressure
NDO neurogenic detrusor over activity
Pves intravesical pressure
SCI spinal cord injury
SBP systolic blood pressure
UDS urodynamic studies
UI urinary incontinence
UUT upper urinary tract
QoL quality of life
Acknowledgements

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“...to know even one life has breathed easier because you have lived. This is to have succeeded.”

– Ralph Waldo Emerson (1803-1882)
To my family
Chapter 1: Introduction

1.1. Background and Rationale

A spinal cord injury (SCI) is one of the most debilitating and catastrophically life-altering accidents a person can experience. In Canada, an estimated 1,237 new traumatic injuries occur each year, resulting in approximately 85,556 Canadians living with a SCI\(^1\). Unbeknownst to many, SCIs exert among the most expensive expenditures of the entire Canadian healthcare system. Close to $3 billion dollars is spent per year on treatments for acute and chronic SCI health-related complications\(^2\)–\(^4\). Significant advances in the delivery of medical care services during the acute phase have led to a considerable increase in survival rates following a traumatic SCI\(^3\). Individuals with SCI are now living longer and healthier lives than ever before. Despite this however, the incidence of morbidity and mortality due to more chronic-related cardiovascular disease (CVD) complications continues to impact the SCI population at greater rates compared to able-bodied counterparts\(^5\)–\(^8\). As a result, CVD is now recognized as the leading cause of death in those with SCI\(^3\),\(^4\),\(^9\). One of the most prominent autonomic dysfunctions to occur in up to 90% of individuals with a high-level (≥T\(_6\) level) SCI\(^10\)–\(^15\), is an episodic hypertensive condition called autonomic dysreflexia (AD)\(^16\),\(^17\). AD is defined by episodic bouts of high blood pressure (BP) in response to noxious or non-noxious stimuli below the level of injury\(^15\),\(^18\); and results from a loss of a drive to sympathetic spinal preganglionic neuronal control over the heart and blood vessels\(^17\),\(^19\),\(^20\). Evidence available from animal studies has revealed a worsening of AD with time\(^18\),\(^21\).

In the majority of episodes of AD (up to 85%), irritation of the urinary bladder due to a urinary tract infection (UTI) or bladder stones\(^22\) are the leading cause. Furthermore, spontaneous
uninhibited contractions of the detrusor muscle of the bladder due to a condition called *neurogenic detrusor overactivity* (NDO) are considered the leading triggers for AD following SCI. OnabotulinumtoxinA (Botox), has been found to provide safe and highly effective treatment for NDO in those with SCI. There is preliminary evidence that Botox might also have beneficial effects on AD. In the one previously conducted study assessing the efficacy of Botox on AD, no arterial blood pressure (BP) or heart rate (HR) measures were taken, and no specified definition of AD was provided. Self-report was the sole outcome measure used to assess the efficacy of Botox on AD. Although self-report may provide valuable information, it is highly advised against as a primary outcome measure due to high levels of response bias. To date, no quantitative assessments of bladder-related AD frequency and severity have been performed on humans. Accordingly, the primary objective for this thesis investigation is to directly and quantitatively assess the efficacy of Botox treatment on reducing AD frequency and severity in individuals with SCI and NDO.

### 1.2 Overview of Document

Chapter II- titled: *Review of the Literature* has been partitioned into three sub-sections and will provide an introduction to key concepts surrounding the main objectives of this thesis. Part I will discuss cardiovascular and autonomic dysfunction. Part II will discuss bladder function following SCI. Part III will discuss Botox and QoL. The thesis investigation is provided in Chapters III-V with the overall methodology described in Chapter III, the results in Chapter IV, and the discussions, limitations, future directions and conclusions in Chapter V.
Chapter 2: Review of the Literature
Part I: Introduction to the Cardiovascular System after Spinal Cord Injury

2.1 Overview of the Autonomic Nervous System

The autonomic nervous system (ANS) provides fundamental regulation and operation of the human body. Considered the guardian of *le milieu intérieur* (the environment within), the ANS offers protective stability to vital bodily tissues and organs. Responsible for automatic regulation of operating systems below the level of consciousness, the ANS is comprised of a central and peripheral division. The central division is enclosed in bone and a blood-brain barrier, and is comprised of the brain (cortex, hypothalamus, and brainstem) and spinal cord. The central division is the main processing centre, responsible for sending, receiving and interpreting information from all areas within the body. Neuronal properties of the central division are comprised of preganglionic neurons which travel via preganglionic fibres and synapse on post ganglionic neurons within the autonomic ganglia or peripheral nervous system. The peripheral division contains no outer protection making it highly vulnerable to toxins and mechanical injury. Comprised of the autonomic ganglia and receptors located outside of the brain and spinal cord (postganglionic neurons), the peripheral division serves as a communication relay centre from the limbs and target organs to the brain and spinal cord and vice versa.

The ANS is then further divided into two main sub-divisions. The first sub-division called the *sympathetic nervous system*, controls activation of “fight or flight” responses. Sympathetic nervous system outflow originates from the sympathetic spinal preganglionic neurons, the majority of which are located in the lateral horn of the spinal gray matter between
T1-L2 spinal segments. Following their departure from the spinal segments, the axons of these neurons then synapse onto the sympathetic paravertebral ganglia (ganglionic sympathetic neurons) and finally onto postganglionic fibres of these neurons providing innervation to peripheral target organs17. Sympathetic innervation to the upper extremities, respiratory system, and cardiovascular system are provided from the sympathetic circuits of the upper thoracic cord segments between T1-T5. Sympathetic innervation to major vasculature beds in the gut (splanchnic vascular region), lower extremities and pelvic organs are received from the between the T6-L2 spinal segments. For the lower urinary tract (LUT) including: detrusor, bladder neck, and internal urethral sphincter, sympathetic innervation is provided from between the T10-L2 spinal segments which allows for relaxation of the bladder and urine storage45.

The second sub-division of the ANS called the parasympathetic nervous system, aids in the slowing down of or “rest and digest” component. The more rostral parasympathetic nervous system outflow occurs from preganglionic neurons located in the nuclei of four cranial nerves (III, VII, IX, and X). The cranial component of the parasympathetic nervous system contain neurons that are localized within the midbrain, pons, and medulla oblongata; responsible mostly for HR control, lacrimation, salivation, and digestion. The more caudal component of the parasympathetic nervous system resides in neurons located within the lateral gray horn located between the S2-S4 spinal segments, and are responsible for bladder contraction and sphincter relaxation to facilitate voiding, defecation of the bowels and sexual arousal46,47. The parasympathetic division of the ANS innervates mostly visceral structures through the cranial nerves or within the abdominal-pelvic region and so, of these two sub-divisions, the sympathetic
division exerts the greatest influence over symptomatic autonomic dysfunctions as it has widespread effects on both visceral and somatic bodily structures\textsuperscript{47,48}.

Historically, following a traumatic SCI, the severity of damage to the spinal cord was assessed according to the International Standards for Neurological Classification of Spinal Cord Injury including the American Spinal Injury Association Impairment Scale (AIS)\textsuperscript{49,50}. These standards allow for documentation of the degree of motor and sensory function loss according to a muscle function grading scale of zero (total paralysis) to five (normal movement) and a sensory impairment scale using light touch and pin prick with a scale ranging from zero (absent sensation) to two (normal sensation)\textsuperscript{51}. More recently, the importance of documenting ANS dysfunctions (i.e., bladder, bowel, sexual and general autonomic function) were recognized and the International Standards to document remaining Autonomic Function after Spinal Cord Injury were created to be utilized as an adjunct to the previously established standards\textsuperscript{52,45}.

\textbf{2.2 Cardiovascular Function and Autonomic Control}

Two clinically important measures used to assess cardiovascular health include HR and BP. HR provides a measure of the number of cardiac cycles per minute, is expressed in beats per minute (bpm), and has a clinically therapeutic target range of 60 to 80 bpm in adults\textsuperscript{47}. HR is typically controlled intrinsically by the cells of the sino-atrial node; however, the ANS may exert extrinsic control as both the sympathetic and parasympathetic divisions innervate the sino-atrial node and atrioventricular node\textsuperscript{47}. Sympathetic innervation to the heart stems from between T\textsubscript{1}-T\textsubscript{5} thoracic spinal segments, while parasympathetic innervation to the heart is provided extra spinally via the vagus nerve (cranial nerve X). Sympathetic postganglionic neurons release
adrenergic agonists (i.e., norepinephrine), while parasympathetic postganglionic neurons release cholinergic agonists (i.e., acetylcholine). Adrenergic agonists activate adrenergic receptors (i.e., alpha_1, alpha_2, beta_1, beta_2, and dopamine) leading to an increase in the rate and force of contraction, while cholinergic agonists activate M2 muscarinic receptors leading to the reduction in rate and force of contraction. Cholinergic agonists act as vasodilators within the central nervous system providing an inhibitory effect to reduce HR contractility.

The second clinically relevant measure is arterial BP, which is the force exerted against the vascular walls when blood is ejected from the left ventricle during a cardiac contraction. Arterial BP varies between a maximum systolic BP (SBP) and minimum diastolic BP (DBP) measured in millimetres of mercury (mm Hg) and has a clinically therapeutic target range of 120/80 mm Hg in adults. Mean arterial pressure (MAP) is then the average pressure within the arteries over one cardiac cycle and is used to demonstrate perfusion to vital organs and is calculated as: \[ \text{MAP} = \frac{\text{SBP} + 2 \times \text{DBP}}{3} \] with a therapeutic target range of 70-110 mm Hg in adults. Changes in BP are mostly mediated by a core network of neurons located within vasomotor centers of the hypothalamus, rostral ventrolateral medulla, and spinal cord, which detect changes in arterial blood concentrations leading to either arteriolar vasoconstriction or vasodilation. It is important to note that the majority of arteries within the body only receive sympathetic innervations through adrenergic agonists.

Following changes in arterial BP, two autonomic negative feedback reflex systems are activated via the vagus nerve in an attempt to maintain cardiovascular homeostasis. Both reflex systems receive innervation by the glossopharyngeal nerve (cranial nerve IX). The first reflex is called the baroreceptor reflex and the second is the chemoreceptor reflex. Baroreceptor reflexes...
(located in the heart and major blood vessels) adjust cardiac output and peripheral resistance to help maintain normal bodily arterial pressures\textsuperscript{47}. Activation of the baroreceptor reflex causes one of two outcomes in the sympathetic nervous system; inhibition via the release of acetylcholine or stimulation via the release of norepinephrine. Chemoreceptor reflexes (located primarily in carotid bodies) on the other hand, respond to changes in arterial blood gases (carbon dioxide, oxygen) and pH levels by elevating arterial pressure in order to maintain adequate blood oxygenation and peripheral blood flow to vital tissues and organs\textsuperscript{47}. Visceral sensory fibres also run with the autonomic nerves, carrying sensory impulses from the heart, greater splanchnic blood vessels, respiratory and gastrointestinal system which assist in BP regulation by responding to changes in arterial gases\textsuperscript{58}.

The final key component involved in autonomic control of the sympathetic nervous system relevant to cardiovascular function are the visceral splanchnic blood vessels (including the greater, lesser, least and lumbar splanchnic nerves). Axons for the preganglionic neurons for the splanchnic blood vessels pass through the sympathetic chain ganglia and then synapse on postganglionic neurons in the collateral ganglia providing direct innervation to visceral structures in the abdominal and pelvic region\textsuperscript{47}. Of highest cardiovascular significance are the greater splanchnic vessels that exit via the T\textsubscript{5}-T\textsubscript{9} spinal segments. At the T\textsubscript{6} level in particular, resides the visceral splanchnic sympathetic outflow (splanchnic vascular bed) which provides innervation to major vasculature beds in the gut and contains the largest amount (29\%) of total circulating blood volume of the entire body at rest\textsuperscript{5}. Due to its location, the splanchnic vascular bed plays an imperative role in systemic BP regulation.
2.3 Sympathetic Nervous System Dysfunction after Spinal Cord Injury (SCI)

Following a traumatic SCI, individuals with high-level (≥T₆ thoracic segment) injuries experience an even greater disruption in the sympathetic nervous system compared to those with low-level (<T₆ thoracic segment) injuries¹⁹,²⁰,⁵⁹. As previously mentioned, sympathetic afferent sensory nerve fibres arriving to the main cardiac control centre (medulla) assist in responding to changes in BP with the help of baroreceptors and chemoreceptors. Individuals with low-level SCIs will generally have preserved sympathetic innervation to the vasculature (splanchnic vascular bed in particular) with normal baroreceptor and chemoreceptor mediated reflexes to maintain cardiovascular homeostasis²⁰. However, in those with high-level SCIs, severe dysfunction over regulatory BP control are experienced due to a decreased drive to sympathetic spinal preganglionic neuronal control over vasomotor tone, HR, and the splanchnic blood vessels¹⁷,¹⁹,²⁰,⁶⁰,⁶¹,²².

Following high-level SCI, a reduced ability of the ANS to detect and respond to acute changes in arterial BP have been found to result from damage to the baroreceptor reflexes⁶². While the ability of the baroreceptors to detect changes in BP remains intact, the ability in function to regulate arterial BP change is lost. Following SCI, an inability to regulate BP leads to significant alterations in cardiovascular homeostasis causing dramatic swings in SBP between excessively low hypotensive episodes called orthostatic hypotension to uncontrollably high hypertensive episodes called autonomic dysreflexia (AD). These dramatic swings can wreak
havoc on health related-quality of life (HR-QoL) and may potentially exacerbate the progression of CVD in those with SCI over the long-term.

### 2.4 Autonomic Dysreflexia

As mentioned previously, one particular condition affected in those with high-level SCIs due to an inability to regulate arterial BP is AD. First described as an occurrence in the spinal-injured population by J. Hilton in 1860; AD is considered a medical emergency resulting in an episodic hypertensive crisis due to noxious or non-noxious stimuli below the level of injury, resulting in a massive uncontrollable activation of the sympathetic outflow centre (visceral splanchnic vascular bed). AD is defined clinically as an elevation in arterial SBP ≥20 mm Hg or DBP by ≥10 mm Hg from average seated or supine baseline parameters.

Common sympathetic nervous system signs and symptoms which accompany the increase in SBP or DBP include: flushing of the skin, hyperhidrosis, goosebumps, muscle spasms, in addition to complaints of a pounding headache, nasal congestion, blurred vision, chills, tingling sensations, dizziness, and feelings of anxiety and fatigue. Additionally, a paradoxical bradycardia will typically occur due to increased vagal activity in an effort to reduce cardiac output and help stabilize rising arterial SBP. AD is reported to occur frequently and oftentimes asymptotically in up to 90% of individuals with a high-level SCI. If AD is poorly managed and SBP elevates to dangerously high levels of up to 300 mm Hg, myocardial infarction, stroke, intracranial haemorrhage, retinal detachment, seizure, and even death, may result. A total of 32 cases of death and life threatening complications resulting from AD have been previously described.
The development of AD is typically seen within four to six weeks following the dissipation of spinal shock\textsuperscript{83,84}. However, AD has been noted to occur as early as four days following cervical SCI\textsuperscript{71,85}. With time, the severity of AD is shown to progress due to plastic changes taking place within the spinal cord and the progressive establishment of inappropriate connections within the central and peripheral nervous system\textsuperscript{18,86,87}. At the present time, pathophysiological mechanisms underlying the direct cause of sympathetic nervous system dysfunction leading to AD have still not been completely elucidated\textsuperscript{88}. However, several mechanisms related to the autonomic circuitry have been discovered leading to greater understanding of contributing mechanisms. At the present time, the most widely accepted explanation results from the loss of supra-spinal inhibitory input to sympathetic spinal circuits secondary to the destruction of descending vasomotor pathways\textsuperscript{46,54,20}.

Other pathophysiological mechanisms have been attributed toward morphological changes occurring in sympathetic preganglionic neurons\textsuperscript{86,89}, sprouting of dorsal root afferents causing the formation of new inappropriate afferent fibre inputs to spinal preganglionic neurons\textsuperscript{21,90,91}, the development of peripheral alpha-adrenoceptors and peripheral micro-vascular adrenoceptors causing a hyper-responsiveness due to an up-regulation below the injury level\textsuperscript{19,92,93}, and an overexpression of intra-spinal sprouting of nerve growth factor causing hyperactivity\textsuperscript{94}. Over time, the sustained hyper-responsiveness seen in more chronic SCI is thought to manifest as AD\textsuperscript{10}.

Severity of injury damage (i.e., complete or incomplete injury) has also been found to play a significant role in the severity of sympathetic disruption experienced. Individuals with
cervical AIS A (motor and sensory complete) SCIs have reported greater severity of AD episodes compared to those with incomplete AIS B injuries\textsuperscript{95,96}. In one study by Curt and colleagues only 27\% of incomplete (AIS B, C, or D) quadriplegics presented with signs of AD compared to 91\% of complete quadriplegics\textsuperscript{97}.

In 85\% of AD cases, the urinary bladder is the leading trigger resulting from bladder distension due to insufficient frequency of clean intermittent catheterization (CIC), NDO, a blocked catheter due to the build-up of sediments, kinked catheter, urinary tract infection (UTI), or the development of bladder stones\textsuperscript{13,15,98}. Other non-bladder related causes of AD may include bowel impaction, haemorrhoids, lower leg spasticity, pressure sores, skin infection, or an ingrown toenail, to name a few\textsuperscript{22,99}. Iatrogenic manipulations during required assessments including UDS and cystoscopy have also been associated with activation of urinary bladder afferents leading to the triggering of AD\textsuperscript{100,101}. Other common procedures also known to elicit AD include electrical muscle stimulation and vibrostimulation for ejaculation\textsuperscript{102,5}.

In the case for NDO as a stimulus for AD, bladder filling acts as a noxious stimulus causing significant changes in intravesical pressure (Pves) within the bladder causing involuntary uninhibited detrusor contractions resulting in a spastic and hyper-reflexive bladder which may cause urinary incontinence (UI) and retention, leading to the elicitation of AD\textsuperscript{25,36,103}. During a typical AD episode resulting from an over distended bladder or due to NDO, stretch receptors located within the bladder wall pick up the irritating sensory impulses and attempt to transmit the afferent sensory information through the dorsal horn of the spinal cord up to the primary somatosensory cortex of the brain. As the message travels up the dorsal columns and spinothalamic tract, collateral connections become activated increasing sympathetic efferent
(motor) neuronal activity until it reaches the lesion level where the SCI occurred\textsuperscript{75}. However, once the sensory signal reaches the lesion level it cannot continue on any further as it is blocked. This subsequently activates an automatic exaggerated hyper-reflex like response (known as AD) below the level of the injury, causing widespread peripheral vasoconstriction from the visceral splanchnic outflow centre\textsuperscript{22,104}. Unless the noxious or non-noxious stimulus is identified and promptly removed, the sympathetic nervous system will remain in a state of prolonged activation, which can lead to detrimental outcomes.

First-line treatment to stabilize arterial SBP during an acute episodic bout of AD includes: 1) transferring the individual into a seated upright position in an attempt to decrease arterial BP using the orthostatic reflex 2) loosening constrictive clothing and 3) quickly assessing, identifying and promptly removing any potential triggering stimulus. In the event that all attempted non-pharmacological measures failed and SBP remains at an elevation of >150 mm Hg, pharmacological intervention with an anti-hypertensive agent such as a calcium channel blocker is suggested (i.e., Nifedipine, Captopril or Nitro Paste)\textsuperscript{12,105}. The administration of a fast-acting antihypertensive agent should however, be given with the utmost of caution. Individuals with cervical complete SCIs typically have an already lower than average resting arterial SBP (ranging from 90-100 mm Hg). Therefore, cerebrovascular deterioration or syncope could potentially occur at greater incidences in these individuals due to a rebound effect by going from a state of hypertension to a sudden and severe state of hypotension\textsuperscript{106,5,107}.
2.4.1 Silent Autonomic Dysreflexia

Another dangerous and disconcerting cardiovascular condition that typically occurs in those with cervical complete SCIs, again due to a lower than average resting arterial BP, is a type of AD called silent AD. Silent AD is defined the exact same clinically as regular occurring AD, except that it occurs in the complete absence of any sympathetic nervous system signs or symptoms\textsuperscript{108}. In one study conducted by Linsenmeyer and colleagues silent AD was found to occur in 40\% of SCI participants during BP monitoring while undergoing UDS bladder assessment\textsuperscript{100}. In a follow-up study by Giannantoni and colleagues, silent AD was again found to occur in seven of the 20 participants who experienced AD during UDS\textsuperscript{102}. Silent AD is estimated to occur in approximately 35\% to 43\% of all individuals with SCI\textsuperscript{100,97,102}.

In cervical complete injured individuals, silent AD has been reported to occur frequently and chronically in up to 60-70\% of individuals\textsuperscript{109}. An increase in SBP by as little as 20\% or 20 mm Hg from a baseline resting SBP of 90 mm Hg to 110 mm Hg may go unnoticed due to the lack of symptoms despite the sustained vascular damages occurring\textsuperscript{109}. Spinal injured individuals with a chronic (>2 years post injury) motor complete SCI were also found to have more severe silent AD as discovered by Liu and colleagues during UDS assessment\textsuperscript{110}. Over the long-term, shear stress imposed upon blood vessel walls due to fluctuations in BP may cause significant changes in plasma fibrinogen levels leading to platelet dysfunction, endothelial vasculature, progression of atherosclerosis, and ultimately an increased predisposition toward the development of CVD\textsuperscript{111,112}. 
2.4.2 Twenty-four hour Ambulatory Blood Pressure Monitoring (24-hr ABPM) for AD

It is known that individuals with high-level SCI experience dramatic fluctuations in arterial BP and HR multiple times a day in response to bladder filling, CIC (approximately 4-6 times/day), bowel routine, lower-leg spasticity, transfers, wheeling, and simply at rest\(^{113}\). With time, cardiovascular irregularities may occur so often that individuals accept BP dysregulation as a part of everyday life following a SCI. In an effort to capture the severity and frequency of arterial BP and HR fluctuations more precisely and accurately, a non-invasive clinical tool called 24-hr ABPM has been utilized and proven highly effective at providing a more comprehensive assessment of true BP and HR\(^{114}\). Automatically pre-set recordings are configured through computerized program software prior to initiation and are typically programmed to begin recording every 15-30 minutes during the daytime period (typically from 07:00 AM to 23:00 AM) and then every one-hour during the nighttime period (typically from 23:00 AM to 07:00 AM) over a 24-hour time frame. Individuals undergoing ABPM are encouraged to document specific events or activities in an activity log throughout the 24-hours to aid in the identification of causal cardiovascular irregularities.

In the able-bodied population, ABPM is widely used as a prognostic tool to assess cardiovascular morbidity and mortality risk\(^{115-118}\). At the present time, ABPM is accepted as superior to routine one-time clinic BP measurements\(^{119,120}\). This is due to the issue of *white coat hypertension* where individuals persistently experience elevated BP during clinic visits but then resume normal BP when outside the clinic during ABPM\(^{121}\). In a study conducted by Krum and colleagues, individuals with tetraplegia SCI and the presence of AD, were found to experience
significantly greater BP and HR variability compared to individuals with SCI without the presence AD and able-bodied persons\textsuperscript{122}. The frequency of specific bouts of AD can also be captured through the use of ABPM as shown in a previous study by Curt and colleagues where high peaks in SBP accompanied by decreased HR and documentation of sympathetic nervous system responses of headache, sweating, and goosebumps were found to occur in 70\% of individual’s with a complete tetraplegia SCI\textsuperscript{67}. When used in conjunction with the AD component of the Autonomic Dysreflexia Following Spinal Cord Injury (ADFSCI) questionnaire\textsuperscript{123,124}, ABPM provides a strong clinical basis for the assessment of AD. To date, there have been no reports to support a placebo effect when worn short-term (one day) or long-term (one week)\textsuperscript{125–127}, demonstrating ABPM a reliable clinical tool.

The benefits of ABPM to clinicians and researchers are abundant including the ability to take a more in-depth assessment of the frequency and severity of cardiovascular irregularities during activities of daily living (i.e., before and after CIC, bowel routine, transfers, wheeling, while in seated or supine position, during sleep, early morning, while at work etc.). ABPM also allows for greater insight into the severity of autonomic dysfunction according to the absence of signs or symptoms during cardiovascular fluctuations (i.e., silent AD) as well as nighttime circadian BP patterns, HR fluctuations, and the absence or presence of nocturnal dip which can be used as a significant predictor of CVD risk when absent\textsuperscript{128,67}. ABPM is also useful for assessing the efficacy of certain pharmacological treatment interventions for cardiovascular health\textsuperscript{115,119,129}. Overall, ABPM continues to gain popularity within the clinical setting due to its ease of use, discreet concealment and lack of invasiveness.
2.4.3 Health-Related Quality of Life (HR-QoL) and AD

Cardiovascular dysregulation resulting from repetitive daily bouts of hypotension and hypertension cause significant disturbances in an individual's perceived HR-QoL\textsuperscript{63,64}. HR-QoL has been described as a multidimensional construct used to examine the ways in which disability, disease, or different treatment strategies come to affect a person's general functioning with respect to emotional, psychological, and social well-being\textsuperscript{130}. Individuals with SCI not only experience a significant impairment in physical functioning but also in psychological, emotional and social functioning\textsuperscript{131}.

Uncontrolled swings in arterial BP can be both physically and mentally draining leading to a cascade of negative events due to feelings of fatigue\textsuperscript{132}, which may then lead to delays in rehabilitation due to a lack of energy to perform exercises, thereby negatively impacting QoL and the ability to function. In one study, individuals with autonomic dysfunction were found to experience significantly elevated levels of anxiety and sadness compared to those without autonomic dysfunction\textsuperscript{133}. At the present time, validated and reliable questionnaires used to assess the impact of BP variability on AD-related QoL are limited. Previous questionnaires include the Autonomic Symptom Profile which is a 169 item questionnaire used to measure autonomic symptoms and was not designed specifically for individuals with SCI\textsuperscript{134}. To date, only one questionnaire, the ADFSCI questionnaire has been used to measure self-reported frequency and severity of symptoms during hypotension and hypertensive episodes during 24-hr ABPM. The ADFSCI questionnaire has been found to provide a reliable assessment of self-reported AD symptoms when used in conjunction with 24-hr ABPM\textsuperscript{123}.
Impairments in QoL resulting from AD can occur for numerous reasons. However, most often, the cause is the urinary bladder. A conceptual model was used to demonstrate the far-reaching effects a single bout of AD can have on QoL.\(^6^3\) For example, if an individual is unable to decipher the underlying cause of an AD episode, overwhelming feelings of anxiety, pain, and panic-stricken fear ensue which then lead to feelings of significant psychological and emotional distress, especially if the individual is alone at the time of the AD event or has limited hand function for which to remove the noxious stimulus causing the AD event (i.e., kinked catheter, ability to drain the bladder)\(^6^3\). Strategies aimed at improving bladder management through regular CIC or through the use of pharmacological treatments have assisted in improving QoL however, the effects on the bladder and its role on AD prevention are still not entirely known.

**Part 2: Introduction to Bladder Dysfunction after Spinal Cord Injury**

**2.5 Urinary Function**

The LUT is comprised of the urinary bladder, bladder neck, urethra, and striated muscles of the external urethral sphincter\(^1^3^5,^1^3^6\). There are two main functions of the LUT which is to allow for urine storage and periodic urine expulsion\(^5^2,^1^3^6–^1^3^8\). In order for the LUT to function appropriately however, a harmonious coordination between numerous complex neuronal circuits within the pontine region of the brainstem, lumbosacral spinal cord and peripheral ganglia must exist. Three sets of peripheral nerves aid in the regulation of the LUT which include: the sacral pelvic splanchnic nerves, thoracolumbar (hypo-gastric) sympathetic nerves, and the somatic (pudendal) nerves\(^1^3^9,^1^3^5\). The sacral pelvic splanchnic nerves carry parasympathetic innervation from the S\(_2\)-S\(_4\) spinal segment through cholinergic muscarinic receptors and help regulate
emptying of the bladder by controlling the internal urethral sphincter. The pelvic splanchnic nerves also influence motility for defecation and sexual functions\textsuperscript{47}. The hypo-gastric nerves (superior and inferior) contain mostly nociceptive afferents and provide sympathetic innervation to the bladder from T\textsubscript{10}-L\textsubscript{2} spinal segments\textsuperscript{45}. The hypo-gastric nerves are responsible for urinary storage and continence as mediated by sympathetic excitatory alpha-adrenergic receptors in the trigone, bladder neck and internal urethra; while urine expulsion is mediated by inhibition of the beta-adrenergic receptors located within the smooth muscle cells of the detrusor (bladder) which allow for contraction\textsuperscript{45}. The mostly nociceptive afferent containing pudendal nerves provide innervation to striated muscles of the external urethral sphincter which is under voluntary control\textsuperscript{139}. During bladder filling, the sympathetic nervous system predominates and during bladder emptying the parasympathetic nervous system predominates.

In the normal functioning bladder, afferent sensory information is picked up by mechanosensitive myelinated (A-delta) or unmyelinated (C-fibre) axons in the LUT and carried to second-order neurons within the spinal cord\textsuperscript{140}. Once in the spinal cord, the fibres synapse on interneurons which then project to Lissauer’s tract at the apex of the dorsal horn sending out collaterals medially and laterally which extend into the deep layers of laminae V-VII and X at the dorsal horn base\textsuperscript{141, 140}. As medial and lateral connections are made, sympathetic circuits initiating spinal reflexes are activated which then mediate detrusor muscle relaxation and bladder neck contraction, allowing for urine storage\textsuperscript{45}. During bladder emptying, stimulation of the hypo-gastric and pudendal nerves which allows for relaxation of the internal and external sphincters is blocked by supraspinal centres through the removal of sympathetic inhibition over parasympathetic receptors allowing for contraction of the detrusor muscle\textsuperscript{141, 139}. 
Normal functioning of the micturition reflex is also required in a normal functioning bladder. The micturition reflex is mediated by a spinobulbospinal pathway passing through the pontine micturition coordination center within the rostral brainstem\textsuperscript{142}. Under voluntary control by higher order centres within the cerebrum, the micturition reflex assists with maintaining appropriate coordination of the bladder neck and detrusor muscle by relaxing the detrusor muscle to allow for the storage of urine under low $P_{ves}$ and then contraction of the detrusor and expulsion of the urine through the bladder neck\textsuperscript{142}.

### 2.6 Urinary Dysfunctions after Spinal Cord Injury (SCI)

In the spinal injured bladder, disruption in the neuronal circuitry between the bladder and the brain significantly jeopardizes voluntary control over the functioning of the LUT and micturition reflex\textsuperscript{137}. Destruction to central inhibitory pathways means the cerebrum can no longer receive afferent sensory stimuli signals from stretch receptors within the bladder wall indicating the need to void. Immediately following a SCI, a temporary condition called \textit{spinal shock} sets in due to the absence of reflexes caudal to the injury\textsuperscript{83}. Spinal shock is experienced by all individuals regardless of lesion level and persists for a few days to weeks depending the severity of injury and time taken for the return of reflexes\textsuperscript{143,144}. In the interim, the urinary bladder becomes areflexic and flaccid resulting in complete urinary retention\textsuperscript{17}. As time progresses from four days to approximately one month, most deep tendon reflexes begin to re-emerge\textsuperscript{144}. Upon return of the deep tendon reflexes, the bladder experiences one of two outcomes; one outcome occurs in persons injured $<$T\textsubscript{12} spinal cord level or at the peripheral nerves where damage imposed upon the spinal micturition reflex in the cauda equina region (T\textsubscript{12}-
L₁) resulting in a flaccid (areflexic) bladder that fails to contract. The second and most common outcome in the majority of SCIs is damage >T₁₂ spinal cord level or at the peripheral nerves, resulting in a bladder dysfunction called NDO, where the bladder becomes spastic and hyper-reflexive leading to involuntary detrusor contractions.\(^\text{142}\)

NDO results from a disconnection in the communication pathways between the pontine micturition centre and sacral segments in control of the muscles of the bladder.\(^\text{145}\) In this condition, simultaneous and uncoordinated activation of parasympathetic neurons which innervate the smooth muscle cells of the detrusor, and somatic neurons which innervate the urethral sphincter contract involuntarily due to changes in Pves within the bladder under low filling volumes\(^\text{24, 146, 142}\). The involuntary uninhibited detrusor contractions act as a noxious stimulus causing subsequent activation of the sympathetic nervous system triggering urinary leakage and the elicitation of AD.\(^\text{36, 24}\) NDO is confirmed through UDS where spontaneous involuntary detrusor contractions are seen during bladder filling.\(^\text{147, 148}\)

Another condition that frequently occurs alongside NDO is detrusor sphincter dysynergia (DSD). In this condition, a lack of coordination between the detrusor muscle of the bladder and external urethral sphincter muscle cause involuntary bladder contractions of the detrusor and subsequent reflex contractions of the urinary sphincter.\(^\text{149}\) DSD is characterized by uninhibited detrusor contractions with simultaneous contraction of the pelvic floor muscles resulting in an increase in electromyography (EMG) activity, (measured in millivolts) causing urinary retention and dangerously elevated Pves. A lack of coordination causes very high elevations in Pves and may predispose individuals toward vesicoureteral reflux of urine into the upper urinary tract (UUT).
2.7 Urodynamic Studies (UDS)

UDS are considered the “gold standard” of bladder function assessments and provide a valid, reliable, and objective diagnostic procedure to guide bladder management through the evaluation of the UUT and LUT\textsuperscript{150-153}. The ultimate goal of UDS is to protect the kidneys\textsuperscript{150}. In one study conducted by Ho and colleagues, the reproducibility of UDS for NDO in persons with SCI was assessed at two different time points. Overall findings revealed a significant agreement between corresponding parameters during the filling and voiding phase, leading to the conclusion that UDS is a valid and reliable test for intra-subject assessments\textsuperscript{154}.

During UDS, one catheter attached to a transducer is passed into the bladder through the urethra while a second catheter is passed into the rectum. The transducers allow for the measurement of Pves and abdominal pressures. The bladder is then filled with warm sterile water (37°C) at a controlled rate of 30 mL/min to mimic the most natural filling\textsuperscript{155}. UDS allows clinicians to evaluate bladder and sphincter function parameters which include: maximum cystometric capacity, the amount of volume in the bladder before the person indicates voiding can no longer be delayed (range in adults is 300-600 mls); maximum detrusor pressure, the highest detrusor pressure recorded; maximum urethral pressure; leak point pressure, the measurement of intravesical pressure at the instant of urine leakage; post-void residuals, the amount of urine left in the bladder after voiding; first uninhibited detrusor contraction; Pves, the pressure within the bladder which varies with independent contractions of the detrusor muscle and is assessed using a double lumen catheter for filling and measuring pressure; abdominal pressure, the pressure outside the bladder measured vaginally or rectally; detrusor pressure, a
component of Pves obtained by subtracting (Pves - abdominal pressure = detrusor pressure); detrusor contraction, confirmed when there is no change in abdominal pressure during a rise in Pves; and EMG to record bioelectrical activity of striated pelvic floor muscles and verify DSD.

During UDS, three main areas of bladder function are observed for including: sensation, detrusor activity, and urinary sphincter function\textsuperscript{52,156}. When documenting sensation, three time points are observed: at first sensation during filling when the individual becomes aware of the bladder (typically occurs at 90 to 150 mls); at first desire to void or perform CIC in those with SCI (typically occurs at 300 to 600 mls); and at leak point pressure (if any)\textsuperscript{150}. The second main bladder function observation is detrusor activity and documentation includes observations of a detrusor that is normal, overactive, underactive or acontractile\textsuperscript{52}. Detrusor activity can be assessed based on changes in Pves and is measured in cm H\textsubscript{2}O, or is measured at the first uninhibited detrusor contraction with EMG. The third main area of observation is urinary sphincter function which is documented as normal urethral closure, incompetent closure (occurrence of leakage in the absence of detrusor contraction), DSD where detrusor contraction occurs alongside involuntary contractions of the urethral and/or peri-urethral striated muscle during voiding, or a non-relaxing sphincter causing obstruction of the urethra and reduced urine outflow\textsuperscript{156}.

In a retrospective review involving 42 individuals with SCI undergoing routine UDS over a four-year time period, 64% were found to have developed UUT complications resulting in low bladder compliance due to increased Pves\textsuperscript{157}. In another study by Linsenmeyer and colleagues, annual UDS were used to assess 96 individuals with SCI, discovering that 82.6% required some form of urological intervention (mostly medication), 13% required a non-urological intervention, and 4.3% required both\textsuperscript{158}. While physical examination is useful for bladder and sphincter
function assessment, alone it cannot assess specific bladder parameters affecting the UUT\textsuperscript{159}. Therefore, UDS should be conducted annually in order to monitor and prevent the progression of UUT and LUT complications.

2.8 Autonomic Dysreflexia (AD) during Urodynamic Studies (UDS)

Dating back to 1947, Guttmann and Whitteridge were the first to report a relationship between bladder distension and the development of AD upon assessment of 30 individuals with SCI and NDO during UDS\textsuperscript{160}. Not until many years later, in 1979 was the concept of AD resulting from bladder distension in spinal injured individuals with NDO and concomitant DSD revisited. At that time, Perkash and colleagues discovered that AD was a spinal reflex mediated by afferents arising from the bladder wall due to the release of excessive stimulus impulses when stretched during bladder filling\textsuperscript{161}. Earlier UDS conducted in high-level SCI animal models and humans have revealed intrinsic reflex contractions that occur during bladder filling and fail to occur in persons with intact spinal cords\textsuperscript{162–164}. In 1996, a pivotal study conducted by Linsenmeyer and colleagues confirmed these previous findings following the assessment of 45 individuals with high-thoracic SCIs during UDS with simultaneous BP and HR monitoring. Utilizing an AD cut-off criteria of an increase in SBP $\geq 160$ mm Hg and DBP $\geq 90$ mm Hg, a total of 35/45 (78\%) of their participants experienced AD and 15/35 (43\%) were found to experience silent AD during uninhibited detrusor contractions\textsuperscript{100}. It was concluded that bladder distension alone was not the direct cause of AD, but also the result of uninhibited detrusor contractions resulting from NDO\textsuperscript{100}.
In another pivotal study conducted by Giannantoni and colleagues two years following, again utilizing BP and HR monitoring during UDS. A total of 48 individuals with high-thoracic SCI were assessed according to an AD cut-off criteria of an increase in SBP ≥150 mm Hg and DBP ≥100 mm Hg. Following UDS, all individuals experienced an increase in SBP with 20/48 (42%) exceeding the AD criteria. Of those 42%, seven experienced silent AD. Of the 20 individuals who met the AD criteria, three individuals experienced AD at the moment of the uninhibited detrusor contraction, 11/20 (55%) at the peak of uninhibited detrusor contraction, and six at maximum volume infusion/maximum bladder capacity. In the same study, all participants were taking anticholinergic medications for which study results concluded that treatment with anticholinergic medication was insufficient at preventing bladder related AD.

In another study by Huang and colleagues, BP and HR monitoring were used to assess bladder function during UDS in 120 individuals with high-level SCI using an AD cut-off criteria of an increase in SBP ≥20 mm Hg from baseline. AD incidence was found to occur in 36.7% overall and in 42.6% with high-level SCI and even in those injured <T6 (at the T7 level) in 15.4%, no significant changes in HR were found in 75% of individuals and 22.7% experienced a decrease in HR by ten beats per minute during AD.

In 2013, Huang and colleagues conducted another observational study to investigate factors associated with silent AD in 42 individuals (21 with symptomatic AD and 21 with silent AD) with SCI and NDO during UDS with BP and HR recording and an AD cut-off criteria of an increase SBP ≥20 mm Hg from baseline. It was discovered that individuals with symptomatic AD experienced greater elevations in DBP and then more rapid SBP/DBP increments and was found to be negatively correlated with age, and speculated to be due to decreased baroreceptor
sensitivity that develops with time\textsuperscript{166}. In 2013, Linsenmeyer and colleagues conducted a prospective cross-sectional study on 96 chronic (>2 years post injury) individuals with SCI (70 >T\textsubscript{6} and 25 <T\textsubscript{6}) during UDS with BP and HR monitoring according to an AD cut-off criteria of SBP >140 mm Hg from baseline at one year and again the following year. Findings from this study revealed a new onset of AD in 10.9\% at year two due to decreased capacity, increased fibrosis, reduced compliance, and an increased risk for vesicoureteral damage\textsuperscript{158}. Additionally, silent AD occurred in 40\% in individuals who reported to history or awareness of any AD symptoms, and the development of involuntary detrusor contractions following the first year of UDS was found to be a leading contributor toward the development of AD at year two.

Given the significant findings from these previously conducted studies utilizing BP and HR monitoring during UDS assessment is becomes quite evident that UDS are both an essential and highly reliable screening tool to assess bladder function and risk of development of AD in a safe and controlled clinical setting. As urinary bladder stimuli are attributed as the leading cause of AD due to involuntary uninhibited detrusor contractions\textsuperscript{5,100,102,167}, concomitant arterial BP and HR monitoring is recommended throughout UDS given the detrimental cardiovascular risks associated with undetected AD\textsuperscript{168}.
In Figure 1, a sample arterial BP report from a study participant during UDS is provided to demonstrate both the severity and frequency of AD bouts. In Figures 2 and 3, sample-tracing reports from a study participant during pre-Botox UDS then post-Botox UDS is provided to demonstrate how Botox affects the bladder and arterial BP.

Figure 1. A sample of the data generated during urodynamics studies (UDS) to demonstrate multiple systolic blood pressure (SBP) fluctuations indicative of autonomic dysreflexia (AD) in response to bladder filling. During the first ten minutes of UDS, average resting arterial SBP remains stable at approximately 100 mm Hg. After 12 minutes, the first spike in SBP reaches 170 mmHg during an uninhibited detrusor contraction accompanied by an increase in Pves detrusor pressure to 35 cm H₂O. After 15-20 minutes of infusion, SBP peaks at a maximum of 202 mm Hg resulting in a 100 mm Hg increase from average baseline SBP. At this point a leak occurred and the individual reported SNS responses of headache, sweating, and goose bumps. Bladder filling was stopped and the bladder was emptied. In the 25-30 minute time period SBP began to stabilize and return to baseline following voiding. SNS signs and symptoms of AD dissipated.
Figure 2. A sample tracing report from a study participant during pre-Botox urodynamic studies (UDS) assessment. First sensation was documented after ten minutes of bladder filling; the participant experienced their first involuntary uninhibited detrusor contraction at a volume of 334 mls. Intravesical pressure (Pves) increased from 12 cm H₂O (baseline) to 53 cm H₂O. Detrusor pressure (Pdet) also increased from 4 cm H₂O (baseline) to 41 cm H₂O. Electromyography (EMG) activity also increased from 48 (baseline) to 77 demonstrating detrusor sphincter dysynergia alongside neurogenic detrusor overactivity. Systolic blood pressure (SBP) increased from 107 mm Hg (baseline) to 155 mm Hg, resulting in an average SBP Δ of 48 mm Hg. The participant experienced lower leg spasms, facial flushing, Goosebumps, and reported a “mild headache” indicative of autonomic dysreflexia (AD). At a volume of 373 mls the participant leaked and filling was stopped.
Figure 3. A sample tracing report from a study participant during post-Botox urodynamic studies (UDS) assessment. First sensation was documented after ten minutes of bladder filling at a volume of 150 mls due to a reported bladder pressure sensation rather than an involuntary uninhibited detrusor contraction. Intravesical pressure (Pves) remained in a clinically health range of 13 cm H2O at a bladder volume of 385mls. Detrusor pressure (Pdet) also remained low at 11 cm H2O. Electromyography (EMG) activity remained stable. Systolic blood pressure (SBP) increased from 94 mm Hg (baseline) to 104 mm Hg, resulting in an average SBP Δ of +10 mm Hg at maximum infusion. There was no bladder leak. No signs or symptoms of AD were experienced.
2.9 Bladder Management after Spinal Cord Injury

Current non-surgical bladder management strategies for individuals with SCI include: placement of an indwelling catheter, CIC, condom drainage, and bladder training techniques\textsuperscript{169}. Surgical options include: sphincterotomy, bladder augmentation, urinary diversion or neuro-stimulation devices\textsuperscript{169}. Management of the bladder through pharmacological intervention is with anticholinergic agents, and Botox treatment\textsuperscript{170}.

At the present time, CIC is the preferred long-term bladder management strategy and proven the most practical, effective, and safest way to achieve a catheter free state in SCI persons\textsuperscript{170,171}. First introduced by Dr. Jack Lapides in the early 1970’s, CIC has been used as an effective bladder management strategy for over 40 years now\textsuperscript{171}. Frequency of CIC is recommended four to six times per day with maximum bladder volumes of 500 mls. The benefits of CIC include: improved blood circulation in the bladder wall making the bladder mucous membrane more resistant to bacteria\textsuperscript{172,171}, reduced incidence of UTI\textsuperscript{173}, preserved renal function\textsuperscript{174,175}, improved bladder emptying and incidence of UI through a reduction in Pves pressure, increased self-esteem due to a reduced dependence on healthcare professionals or family, less sexual restrictions allowing for the enjoyment of a physical relationship, all of which significantly improve HR-QoL and bladder-related QoL\textsuperscript{156}.

In addition to CIC, pharmacological treatment for NDO in those with SCI is also a common bladder management strategy. First-line treatment for NDO is with anticholinergic or antimuscarinics medications (i.e., oxybutynin (Ditropan®), tolterodine (Detrusitol®), or solfenacin (Vesicare®))\textsuperscript{170}. Both types of medications prevent the binding of the neurotransmitter
acetylcholine to its receptor site in nerve cells causing inhibition of efferent parasympathetic nerve impulses responsible for involuntary movement of smooth muscle cells (detrusor muscle). When effective, benefits of anticholinergics include: a reduction in involuntary bladder contractions, enhanced bladder compliance, decreased urinary frequency, urgency and UI\textsuperscript{52,24}.

Despite the many benefits, anticholinergics are not without bothersome side effects. The severity of side effects experienced by individuals is the most limiting factor of these medications and the leading cause for non-compliance or discontinuation. The most common reported unwanted side effects include dry mouth, constipation, drowsiness, blurred vision, fatigue and more recently reports of cognitive impairments\textsuperscript{170,24,176}. In order to effectively manage UI, higher dosages are gradually needed as tolerance builds resulting in even more pronounced side effects\textsuperscript{177}. In some individuals, the efficacy of anticholinergic medications decreases with time or individuals develop resistance\textsuperscript{177}. An inability to effectively control symptoms of NDO through pharmacological management with anticholinergic medications had been found to correlate significantly with reports of decreased I-QoL and overall QoL\textsuperscript{42,178}.

In these cases, second-line treatment with Botulinum Neurotoxin (BoNT) (i.e., Botox) has been found a highly efficacious and safe alternative for treating NDO. BoNT works in a similar manner to anticholinergic and antimuscarinics in that acetylcholine is preventing from leaving the presynaptic membrane, resulting in temporary paralysis of the detrusor muscle for up to nine months\textsuperscript{29,179}. 
Part 3: Introduction to Botulinum Neurotoxin

2.10 A Historical Overview

Historical records of BoNT date back to the 17th century when Justinus Kerner (1786-1862), a poet and German physician, first documented the effects of BoNT as a deathly food-borne illness following the consumption of uncooked German blood sausages\textsuperscript{180,181}. Kerner hypothesized that the toxin was produced under anaerobic conditions and exerted its action on the autonomic and motor nervous system\textsuperscript{180}. Nearly 100 years later in 1870, another German physician by the name of John Muller coined the term “botulism” originating from the Latin root word “Botulus” to mean sausage\textsuperscript{182}. Twenty-five years following in 1895, Van Ermengem isolated the very first anaerobic Clostridium botulinum bacterium responsible for the production of this neurotoxin\textsuperscript{181}. Dr. Edward Schantz and Carl Lamanna then went on to successfully isolate the specific neurotoxin itself in 1944\textsuperscript{182}. The first successful treatment with BoNT took place in 1980, when ophthalmologist physician Dr. Alan Scott effectively treated strabismus in humans using a BoNT Type A serotype\textsuperscript{182,183}. Soon after in 1984, The United States Food and Drug Administration (FDA) approved the first commercially manufactured BoNT A serotype called OnabotulinumtoxinA under the trade name Botox® (Allergan, Irvine, CA, USA Inc.) for the treatment of blepharospasm\textsuperscript{183,182}.

BoNT is a purified protein available in seven distinctly different serotypes (A-G)\textsuperscript{184}. Presently, there are five Food and Drug Administration approved BoNT products available on the market, four of which are derivatives of the BoNT A serotype and a fifth which contains a BoNT B serotype\textsuperscript{184,179}. Studies have suggested BoNT serotype A as having the longest half-life,
followed by types C, B, F and E. BoNT serotype A preparations are most commonly used for the majority of intramuscular injections, while serotype B preparations are reserved for individuals resistive to serotype A. At the present time, Botox in particular is used to treat over 26 various medical conditions. A few of the more commonly indicated therapeutic uses include: urological dysfunctions, muscular dystonia, upper and lower limb spasticity, hyperhidrosis, achalasia, Sialorrhea, glabella facial lines, migraine headaches and depression.

2.11 Mechanism of Action

BoNT is produced by the Clostridium botulinum bacterium and in its most natural form is considered the most poisonous substance known to humankind with an average lethal dose (LD₅₀) ranging from 0.1 to 1 ng/kg. The core neurotoxin is produced as a single inactive polypeptide chain of 150 Kilo-Daltons which is cleaved by tissue proteinases into an active di-chain molecule consisting of a heavy chain weighing 100 Kilo-Daltons and a light chain weighing 50 Kilo-Daltons bridged together by a disulfide bond. The mechanism behind the action of BoNT is through the inhibition of acetylcholine release at the somatic and autonomic presynaptic nerve terminals. Initially, the heavy chain of the toxin binds to the neuronal membrane, followed by internalization of the toxin through receptor-mediated endocytosis. The light chain then inhibits neurotransmitter release by blocking protein activity of the SNAP-25 protein at presynaptic nerve terminals. The end result is temporary paralysis of muscles and glands innervated to that particular region. BoNT exerts its main effect on alpha-motor neurons through the inhibition of parasympathetic acetylcholine release. Figure 4 provides an illustration of how Botox works in the bladder to prevent AD from occurring.
Figure 4. Diagram illustrating how Botox (X) prevents autonomic dysreflexia (AD). Botox modulates afferent sensory and motor stimuli from entering the spinal cord by preventing the release of acetylcholine from the presynaptic membrane and into the neuromuscular junction. With a relaxed detrusor, activation of uninhibited detrusor contractions are prevented from occurring which results in a reduction in Pves and subsequent reduction of the activation of AD.
2.12 Treatment of Neurogenic Detrusor Overactivity (NDO) and Detrusor Sphincter Dysynergia (DSD) with Botulinum Neurotoxin (BoNT)

Urological uses of BoNT were first demonstrated by Dykstra and colleagues when Botox was injected into the external urinary sphincter for the treatment of NDO and concomitant DSD in persons with SCI. Schurch and colleagues later confirmed the efficacy of Botox for the treatment of NDO in persons with SCI. This was followed by two pivotal phase III randomized, double-blind placebo-controlled trials, confirming the safety and efficacy of 200 U of Botox for NDO in persons with SCI and Multiple Sclerosis with an efficacy of nine to ten months. In 2011, the Food and Drug Administration approved Botox injections for the treatment of UI resulting from NDO in persons with SCI and Multiple Sclerosis. In Canada, approval of Botox injections for NDO in those with SCI was received in June 2012 by the Common Drug Review of the Canadian Agency on Drugs and Technologies in Health. The Ministry of Health Services under Special Authority provides coverage for Botox treatment for individuals with SCI and NDO every nine months.

Botox injections for NDO exert robust therapeutic benefits on all bladder function parameters during UDS including: maximum bladder capacity, detrusor pressure, Pves, involuntary detrusor contractions, maximum urethral pressure, post-void residuals and results in a significant reduction in urinary urgency, UI, UTI development and of greatest importance, improvements in QoL. Following Botox treatment, the reduction in Pves also leads to a reduced risk of vesicoureteral reflux and deterioration of the UUT over the long-term.
Traditionally the effect of Botox on treating NDO was attributed only toward its ability to block the presynaptic release of acetylcholine from parasympathetic efferent nerve terminals resulting in an inhibition of neural bladder contractions. More recent studies have however, demonstrated that SNARE proteins also inhibit the release of neurotransmitters glutamate and substance P which are known to cause vasodilation and the release of pro-inflammatory mediators (i.e., bradykinin, prostaglandins, histamine and serotonin) which can lead to endothelial dysfunction and the progression of CVD\textsuperscript{184,201}. As a result, Botox has since been suggested to also exert positive benefits in reducing nociceptive pain transmission and assists in the inhibition of inflammation by acting as a temporary blockade. In another study conducted on rats, Botox was found to exert a significant influence over the inhibition of sensory receptor expression in suburothelial fibres as well; as evidenced following endoscopic sub-mucosal biopsy where Botox was found to reduce the expression of inflammatory markers: TRPV1, P2X\textsubscript{3}, and neuropeptides substance P, calcitonin gene-related peptides and glutamate following injection into the bladder lumen resulting in a reduction of afferent nerve stimulation due to bladder irritants\textsuperscript{202,203}.

For the treatment of NDO with Botox, according to the American Academy of Neurological Levels of Evidence\textsuperscript{204}, there are three randomized controlled trials with Class I Level A Evidence (effective)\textsuperscript{26,28,205}, four prospective randomized controlled trials; three with Class I Level A Evidence\textsuperscript{26,31,33} and a fifth with Class II Level B Evidence\textsuperscript{34} (probably effective). As a result of these studies, it was found that 200 U of Botox exerts the greatest therapeutic effects on providing safe and highly effective treatment for NDO by reducing UI, improving QoL and urinary bladder parameters for up to 12 months\textsuperscript{26,29,33,204,206–208}. In a recent review by
Seth and colleagues, data from a total of 691 SCI persons with NDO were pooled and findings indicated that following Botox treatment for NDO, 65% of individuals no longer experienced involuntary detrusor contractions as evidenced by UDS assessment six weeks following treatment. For treatment of DSD in SCI persons, four RCTs exist, one with Class I Level B Evidence and three with Class II Level B Evidence. For treatment of DSD, BoNT has been found to provide safe and probably effective treatment for those with SCI. The need for more randomized clinical trials with larger sample sizes will help confirm efficacy for treatment of DSD in SCI.

2.13 Efficacy of Botulinum Neurotoxin (BoNT)

The efficacy of BoNT injections into smooth muscle autonomic neurons (i.e., detrusor muscle) compared to striated skeletal muscle is known to be physiologically different. Re-establishment of neuromuscular connections due to the regrowth of new motor endplate units is not typically seen until approximately six to nine months in smooth muscle vs. striated skeletal muscle which is typically seen within three to four months. In a study conducted by Haferkamp and colleagues, comparison between the efficacy of Botox injections into striated skeletal muscle and detrusor smooth muscle of 24 SCI participants was assessed. It was found that injection into the detrusor muscle resulted in minimal axonal sprouting following endoscopic biopsy compared to striated muscle, concluding that Botox lasts comparatively longer when injected into the detrusor muscle (average six months) versus striated skeletal muscle (three to four months). The onset of effect usually appears by two weeks but in some before, reaching maximum peak within six weeks. In another study by Schurch and colleagues, Botox
injected into the detrusor muscle of the bladder was found present in 11 participants at nine months\textsuperscript{25} and in a few other studies was noted to exert benefits in some up to 12 months\textsuperscript{26,29,33,204,206–208}.

The recovery of neuronal activity has been attributed to two discoveries: first, is that active axonal sprouting occurs in response to the secretion of nerve growth factor from denervated muscles producing temporary re-innervation in the early phase of recovery; and secondly that during the later phase of recovery, vesicular neurotransmitter release has been found to return to the original nerve terminal\textsuperscript{179,216}. Other studies have suggested that central-nervous system-neurite growth inhibitors may impede neuronal sprouting pathways in peripheral motor neurons in persons with NDO\textsuperscript{213,217,218}. Data on optimal interval time between reinjection due to axonal sprouting for NDO is still limited\textsuperscript{212}. At the present time, reinjection is typically no sooner than 12 weeks but depends on resource availability, efficacy and individual reported-symptoms\textsuperscript{219}. More longitudinal studies are required in order to assess whether BoNT injections for NDO are required life-long or if permanency is achieved at a specific point\textsuperscript{212}.

### 2.14 Bladder Histology following Botulinum Neurotoxin (BoNT)

Inquiries concerning the long-term impact Botox may have on bladder atrophy, submucosal/urothelium changes, dysplasia, or fibrotic changes have been raised and addressed in several studies\textsuperscript{213,219–221}. In one study by Apostolidis and colleagues, mild fibrosis was found in only 2.2\% of biopsies equally before and after repeated Botox treatment at four and 16 weeks, and no dysplasia or significant inflammatory changes were found on the bladder urothelium/suburothelial following assessment of bladder biopsies after Botox treatment for
NDO\textsuperscript{222}. In another study, Botox was found to have no influence on the presence of muscle cell junctions and did not induce motor neuron death or degeneration\textsuperscript{213,223}. One study has indicated that Botox may induce atrophy of the paralyzed muscle leading to structural changes within the bladder wall over the long term\textsuperscript{224}. Additionally, axonal sprouting, development of extra junction acetylcholine receptors, and re-innervation of the muscle may also occur thereby slowly reversing the muscle denervation effects produced by Botox\textsuperscript{224}. At the present time, further longitudinal studies examining the pathology of the neurogenic bladder in those with SCI following long-term treatment is needed\textsuperscript{225}. In another study by Comperat and colleagues, histopathology comparisons of the bladder were made between individuals who received Botox before and those who did not. In those who did not respond to Botox, fibrosis was found to be present in the bladder wall, indicating that Botox may not be an ideal treatment for individuals with pre-existing fibrosis\textsuperscript{220}. In the short-term, histopathology findings have indicated that repeated Botox injections do not cause muscle cell damage or excessive connective tissue deposition in the bladders of individuals with NDO\textsuperscript{213}.

2.15 Safety and Adverse Events

When administered in its recommended dosage of 200 U in the bladder, Botox provides safe and highly effective treatment for UI resulting from NDO\textsuperscript{226}. Botox has been used as a treatment option for more than 25 years now with no long-term side effects reported\textsuperscript{227}. Botox is comprised of proteins foreign to the body and therefore, neutralizing antibodies may be formed against it\textsuperscript{188}. In some individuals, the development of neutralizing antibodies can render the Botox ineffective. In order to counteract the potential for antibody formation, it is recommended
to inject the lowest effective dose at the longest possible interval\textsuperscript{225}. At the present time, the development of neutralizing antibodies has not been conclusively linked to treatment failure\textsuperscript{225,228}. For NDO, no studies to date have found any issues with the development of neutralizing antibodies\textsuperscript{24}.

A total of eight RCTs have assessed the impact of BoNT for NDO using Botox in all but one study which used A/Abo (Dysport)\textsuperscript{31}. In all studies, Botox was found to provide effective treatment for NDO with an excellent safety profile\textsuperscript{211}. The most common side effects following treatment were the development of UTI in approximately ¼ of individuals who received treatment\textsuperscript{25,29}. Important to note as well, is that numerous studies have found the likelihood of developing a UTI following Botox injections the same for individuals conducting CIC regularly or undergoing UDS assessment\textsuperscript{29,30,206,229–231}. Other common side effects include: increased post-void residuals and some mild hematuria\textsuperscript{28,25}, urinary retention requiring CIC\textsuperscript{28,29,231}, muscle weakness\textsuperscript{30,34} and mild transient pain at the injection site\textsuperscript{25}. For treatment of DSD and NDO, one randomized controlled trial and two prospective randomized controlled trials have reported mild generalized weakness\textsuperscript{41,29} (following a 300 U Botox injection dose) and an initial exacerbation of UI in one individual\textsuperscript{39}. With the recommended 200 U dose of Botox, no individuals have reported any life-threatening adverse events resulting from systemic distal effects due to toxin spreading. The development of AD has been reported to occur during the injection of Botox, as the procedure itself is an iatrogenic trigger. In individuals prone to the development of AD, a lidocaine anaesthetic can be applied prior to the injections to reduce the incidence of AD.
2.16 Sparing of the Trigone during Botulinum Neurotoxin Injections

The trigone is a highly sensitive triangular region within the internal urinary bladder that is formed by two ureteral orifices and one internal urethral orifice\textsuperscript{232}. The orifices function as valves to allow the entrance of urine into the bladder and prevention of urine backflow into the ureters\textsuperscript{47}. The majority of studies conducted where Botox was injected into the detrusor have suggested avoidance of the trigone in order to prevent vesicoureteral reflux into the kidneys\textsuperscript{233}. It is also been suggested that by injecting the trigone, complications may arise due to disruption of the cholinergic blockade which innervates sensory, adrenergic and non-cholinergic pathways from the trigone\textsuperscript{234}. A few studies have assessed the impact of Botox injections into the trigone for overactive bladder and idiopathic detrusor overactivity concluding no complications\textsuperscript{202,235–237}. Despite these findings, no studies have yet injected the trigone of individuals with NDO and further investigation is still needed to determine whether trigone injection is associated with improved UDS outcomes or may be more appropriately used in persons with overactive bladder vs. NDO.

2.17 Quality of Life (QoL) Following Botulinum Neurotoxin Treatment

Individuals with SCI and NDO have reported significant improvements in both overall HR-QoL bladder-related QoL following Botox treatment for NDO\textsuperscript{42,178,206,219,231,238–243}. In a total of four studies assessing the impact of Botox for NDO in SCI persons following the completion of the 7-item Incontinence Quality of Life Questionnaire, significant improvements in all studies were reported\textsuperscript{33,219,42,244}. In another study by Sussman and colleagues, HR-QoL data from two
pivotal QoL studies\textsuperscript{26,33} on Botox treatment for NDO were pooled together. Results demonstrated that at weeks six and 12, there were significant improvements in both Botox injected groups versus the placebo. At week six, a greater proportion of Botox-treated individuals in both groups versus the placebo group reported being somewhat or very satisfied and significant progress toward or complete achievement of UI was a leading factor. Significant improvements in mood have also been reported including a reduction in feelings of depression, frustration or anxiety about being embarrassed due to UI following Botox treatment\textsuperscript{32}. The impact of having to initiate CIC in some participants following Botox due to increased post-void residuals was assessed in two studies concluding that initiation of CIC actually lead to more significant self-reported improvements in QoL as reports of greater self-control were found\textsuperscript{245,246}.

\textbf{2.18 \ The Effect of Botox on Autonomic Dysreflexia (AD)}

To date there is one animal study which examined 20 U of Botox in 44 female rats following a T\textsubscript{4} spinal cord transection. At three weeks post injury, arterial BP and HR during UDS which would typically result in AD, was blocked by the Botox treatment in the Botox treated group compared to the sham group (non-Botox treated group). Botox was found to reduce maximum voiding pressures and therefore the number of uninhibited detrusor contractions and lowering of nerve growth factor concentrations in the bladder and the T\textsubscript{4} dorsal root ganglia (following extraction and quantification by ELISA). Collectively, their observations suggest Botox has the potential therapeutic benefit of controlling AD resulting from NDO\textsuperscript{36}.

In several previously conducted studies involving the use of Botox for individuals with SCI and NDO or NDO and DSD, signs and symptoms of AD were observed as reduced\textsuperscript{24–}
The first ever previously conducted study to report observations of a reduction in AD symptoms following Botox treatment in individuals with SCI during UDS assessment was from a study conducted by Dykstra and colleagues. A total of 11 men with SCI and NDO with DSD, received low doses of Botox treatment each week for three weeks followed by UDS assessment with a primary objective to assess the efficacy of Botox on bladder function for NDO. Interestingly at the end of week three, 5/11 (45%) of the men self-reported reduced signs and symptoms of AD while the efficacy for NDO lasted an average of 50 days. 

AD has been self-reported as reduced following Botox injections into the external urethral sphincter where four individuals reported AD before Botox following which only one of the four underwent Botox treatment experiencing complete abolishment, while the remaining three underwent treatment with placebo and AD was self-reported to persist. In another study where Botox was injected transperineally into the external urethral sphincter, 7/18 (39%) of SCI persons reported AD before treatment, followed by an improvement in 6/7 (86%) in self-reported symptoms of AD following treatment at the one-month follow-up, indicating that there may be multiple options for injection sites. Additional benefits following external urethral sphincter injections included a reduction in vesicoureteral reflux, and incidence of UTI. Several studies have examined the effect of Botox injections into multiple sites of the detrusor between 20-30 sites, and 40 sites. Schurch and colleagues observed an abolishment of AD in three SCI persons with tetraplegia at the six week follow-up period, and an average efficacy of up to 9-months, while Kuo and colleagues reported “less AD” by 5/7 (71%) who had initially self-reported having AD.
The most recent investigation by Chen and colleagues\textsuperscript{35} involved 34 SCI participants with thoracic and cervical injuries, diagnosed with NDO and DSD. The efficacy of repeated intravesical injections of 200 U Botox into 40 sites of the detrusor muscle at baseline and six months, on reducing AD severity during UDS assessment and net change in UI and using the Urogenital Distress Inventory and the Incontinence Impact Questionnaire were assessed during UDS at intervals of three months until one year. Results from their study concluded that the repeated dose of Botox injections provided at six months was ineffective at reducing AD at the final 12-month follow-up UDS assessment as 31/34 (92%) self-reported still having AD. However, at their six-month follow-up UDS assessment, complete abolition of AD was self-reported by three individuals and significant reductions of AD were self-reported by 18/34 (53%) of participants. Another three participants reported no effect whilst 10 reported an exacerbation of AD. The authors concluded that AD might resolve, persist or become exacerbated following Botox treatment for individuals with SCI and NDO with concomitant DSD. Despite an improvement in 21/34 (62%) (21/34) in self-reported AD at the six-month follow-up, a number of considerations regarding study design, reliability and validity were found.

Most notably, no definition or specified SBP cut-off criterion for what constituted AD was provided. There were no baseline hemodynamic measurements taken prior to UDS or throughout for which to confirm AD during the UDS assessment. The outcome measure for AD relied solely on self-report using two three-point classification scales; one for AD severity (1-mild, 2-moderate, or 3-severe) and another for AD frequency (1-occasionally, 2-often, 3-frequently), but only at the six-month and 12-month follow-up. Additionally, within the same study more than half of their total sample size had cervical injuries (85%) making the reliability
of self-reported AD as an outcome measure all the more difficult considering the high rates of silent AD experienced in this population. Given this, it becomes quite evident that the need for a more objective and quantifiable assessment of the efficacy of Botox on reducing the severity and frequency of AD is needed. Therefore, the purpose of this thesis investigation was to examine the effect of Botox on the severity and frequency of AD during UDS and bladder-related events using objective and quantifiable assessments.

2.19 Summary of Objectives

Using 200 U of intravesical Botox injections into 20 sites of the detrusor muscle in males and females with chronic (>1 year post-injury), traumatic, high-level SCI and NDO, the objectives of the study were:

1) To quantitatively assess the efficacy of intravesical Botox injections in individuals with SCI and NDO in reducing the severity of AD (SBPΔ, max SBP) one month post-Botox treatment during urodynamic studies (UDS).

2) To quantitatively assess the efficacy of intravesical Botox injections in individuals with SCI and NDO in reducing the severity (SBPΔ, max SBP) and frequency (# of events) of bladder-related AD one-month post-Botox treatment using 24-hour ambulatory blood pressure monitoring (ABPM).

3) To assess the impact of intravesical Botox injections in individuals with SCI and NDO on improving signs and symptoms of AD, AD-related quality of life (QoL) score, and incontinence-related QoL score one-month post-Botox treatment.
2.20 Hypotheses

1) There will be a significant reduction in AD severity (SBPΔ, max SBP) in individuals with SCI and NDO one-month post-Botox treatment during UDS.

2) There will be a significant reduction in the severity (SBPΔ, max SBP) and frequency (# of events) of bladder-related AD events in individuals with SCI and NDO one-month post-Botox treatment during 24-hr ABPM.

3) There will be a significant improvement in AD signs and symptoms, AD-related QoL score, and incontinence-related QoL score in individuals with SCI and NDO one-month post-Botox treatment.

3.1 Methods

3.1.1 Participants

Inclusion criteria included males and females, 18-65 years of age with a chronic (>1 year post injury) traumatic SCI at or above the sixth thoracic spinal segment, documented presence of AD and NDO during UDS, hand function to perform CIC, and a lack of successful treatment with anticholinergic therapy for NDO. Additionally, all participants had to have a stable neurological condition, good command of English, and ability to provide informed consent. Exclusion criteria included documented traumatic brain injury, previous genitourinary disease or operation, multiple injury levels, pregnancy, current UTI, or an unwillingness to perform CIC’s.

A total of 53 individuals were screened for eligibility following physician referral or poster advertisement. Of the 53 screened, four declined participation as their AD was stated as beneficial to everyday life. Of the remaining 49 individuals, 25 met the inclusion/exclusion criteria and underwent baseline pre-screening UDS to confirm AD according to our criterion of an increase in SBP $\geq 20$ mm Hg. Following baseline UDS, three of the 25 were disqualified for not meeting the BP criteria, leaving a total of 22 eligible participants. Of the 22 individuals, 15 completed study requirements to entirety; however, one participant was removed from the study at the end due to the discovery of a traumatic brain injury. Two participants dropped out of the study (deciding against trying Botox), and two were lost at follow-up (one relocated to another
country and the other to another province). Participant characteristics are presented in Table 1. Fourteen individuals (11 male, 3 female) were included in this study. All participants were > 2 years post injury. Four had thoracic injuries (T3-T5) of which three were AIS A and one AIS B. The remaining ten had cervical injuries (C4-C8) of which four were AIS A, three AIS B, and three AIS C.

### 3.1.2 Protocol

This study employed a prospective, non-randomized open-label study design with pre and post comparisons. All testing took place at the Blusson Spinal Cord Centre, in Vancouver, British Columbia, Canada from April 2013 to June 2014. All protocols were approved by the University of British Columbia’s Clinical Research Ethics Board and the Vancouver Coastal Health Research Institute Ethics Board. A written informed consent was provided by all individuals prior to participation. All participants were instructed to avoid the performance of exercise 24-hrs prior to testing, and abstain from the consumption of prescribed anticholinergic medications and caffeine and alcohol on the day of UDS testing. No participants were self-reported smokers, however, one participant was utilizing medical cannabis for pain relief. Testing took place over a total of six weeks per participant with UDS testing occurring in the morning between 0800-1200 hrs in a temperature-controlled room (21°C). A summary of the study procedures is provided in Figure 5. During visit one, the UDS pre-screening assessment was performed and the inclusion criterion of documented AD during UDS was confirmed, following which eligible participants completed the questionnaires and 24-hr ABPM. All individuals were prescribed prophylactic antibiotics (ciprofloxacin 500 mg twice daily) to take five days before the Botox injections. One
to two weeks later, participants returned to receive the Botox treatment. One month following the Botox treatment, participants returned to repeat all assessments from visit one.

**Figure 5. Flow diagram summary of study procedures.**

### 3.2 Measurements

#### 3.2.1 Neurological Evaluation

Neurological evaluation of level and severity of damage to motor and sensory pathways was conducted in accordance with the International Standards for Neurological Classification of SCI\(^6\). In brief, this examination included the assessment of motor function of key muscles in the upper and lower extremities using an established scale (muscle power graded as 0-5; for a total of 20 muscles in the four limbs) and sensory evaluation to light touch and pin prick in 28 dermatomes of the body. Sensory scores for each dermatome were assigned as 0 = absent, 1 = abnormal, and 2 = normal.
3.2.2 Urodynamic Studies (UDS) Assessment

The UDS were conducted in accordance with the principles set forth by the International Continence Society\textsuperscript{248,249}. Bladder function parameters were based on the International Spinal Cord Injury Urodynamic Basic Data Set\textsuperscript{250} and included: maximum capacity, maximum detrusor pressure, maximum detrusor pressure at first involuntary uninhibited detrusor contraction, post void residual, maximum urethral pressure, abdominal pressure, detrusor pressure. Primary outcome measures for hemodynamic variables included arterial BP, HR, and MAP utilizing the automated Dinamap BP machine during bladder infusion. Secondary outcome measures for bladder function parameters included: volume (mls) at first uninhibited detrusor contraction, compliance (cm H$_2$O$^{-1}$), maximum detrusor pressure (cm H$_2$O), and the number of contractions before urinary leakage, and volume at urinary leakage or maximum volume achieved (mls). All secondary bladder function parameters were documented at the following time points during UDS assessment: at initiation of infusion, at first bladder sensation, at first urge to conduct CIC, or at the point of urinary leakage, at maximum SBP and finally at maximum volume infusion. These two specific UDS parameters are presented in Table 6.

The UDS assessment consisted of multichannel pressure recording technology using cystometry with warm sterile water (37 °C) filled at a fixed rate of 30 mls per minute through a pump to a double lumen catheter (6Fr, Laborie, Canada) while participants were in a supine position. Abdominal pressure was measured with an intrarectal balloon catheter (10Fr, Laborie, Canada). Pelvic floor EMG (Aquarius TT, Laborie Model 94-R03-BT, Montreal, Quebec, Canada) was recorded using a bipolar wire electrode in the urethral sphincter to document
involuntary detrusor contractions with simultaneous contraction of the pelvic floor muscles which helps in the identification of DSD alongside NDO. Filling was stopped when the participant experienced urine leakage, bladder filling reached 500 mls, SBP reached ≥180 mm Hg or upon participant request. UDS have undergone psychometric testing by previous investigators and has been found to provide a valid, reliable, and objective diagnostic procedure to guide bladder management through the evaluation of the UUT and LUT\textsuperscript{150–153,251}. The reproducibility of UDS for NDO in persons with SCI was assessed at two different time points, in a study by Ho and colleagues demonstrating significant agreement between corresponding parameters concluding UDS as a valid and reliable test for intra-subject assessment\textsuperscript{154}.

### 3.2.3 Blood Pressure (BP) Protocol during Urodynamic Studies (UDS)

The BP protocol utilized during the UDS is illustrated in Figure 6. During UDS, discrete measurements of arterial BP and HR (DinamapV100, GE Medical Systems CareScape\textsuperscript{TM}, Fairfield, CT, USA) were conducted using an automatic sphygmomanometer with an inflatable cuff wrapped around the non-dominant upper arm. Following five minutes of rest on the UDS bed, three supine baseline measurements were recorded to establish baseline parameters. The average supine SBP was used as the value from which the presence or absence of AD was determined (i.e. if SBP was ≥20 mm Hg above average supine SBP). The UDS assessment was then initiated, and BP and HR were recorded every minute for the rest of the procedure at pre-set intervals of one minute with approximately 30-40s required for each inflation. During the UDS assessment, Hemodynamic measurements were documented in correspondence to initiation of infusion, first sensation, and first urge to perform CIC or at urinary leak, maximum SBP and
SBP at maximum volume infused. Signs and symptoms of AD (i.e., headache, sweating, goose bumps, chills/tingling sensation) were also documented.

3.2.4 Autonomic Dysreflexia Health Related-Quality of Life (AD HR-QoL) Questionnaire

The AD HR-QoL Questionnaire is derived from the original version of the AD subsection of the ADFSCI questionnaire which was much more comprehensive in order to assess AD frequency and severity on a daily basis and specifically when the bladder is full. The ADFSCI questionnaire was created for use in clinical practice and research to assess BP instability and was designed using the Delphi technique by an expert panel experienced in SCI treatment. The ADFSCI questionnaire is a 24-item self-reported questionnaire. The questionnaire

Figure 6. Illustration of summarized blood pressure protocol utilized during Urodynamic Studies.
consists of demographics, medications, frequency/severity of symptoms during AD and hypotensive events. In a study by Hubli and colleagues, the ADFSCI questionnaire was utilized for test-retest reliability assessment when combined with 24-hr ABPM on two separate occasions. In their study, the number of AD events over the 24-hour period and the BP variability were significantly correlated given the consistency and reproducibility, of the patients' self-reported total AD score and daily AD frequency to the quantitative measurements recorded\(^{123}\). The questionnaire was found to provide a reliable assessment of self-reported AD symptoms over a 24-hr time frame when used in conjunction with 24-hr ABPM\(^{123}\).

The AD component of the ADFSCI questionnaire includes 10 items using a 5-point scale (1-never, 2-rarely, 3-sometimes, 4-often, and 5-very often) to score the frequency and severity of AD symptoms. Only the AD-component of the ADFSCI questionnaire has been found reliable for assessing self-reported symptoms of AD in individuals with SCI when used in conjunction with 24-hr ABPM\(^{123,252}\). The AD HR-QoL questionnaire employed a 4-point scale (1-never, 2-rarely, 3-sometimes, 4-frequently) to assess the frequency and severity of AD experienced on a daily basis and when the bladder is full. The impacts of AD symptoms on performance in daily life were also assessed by ‘Yes’ or ‘No’ questions followed by a severity ranking scale from 0-9 points (0- never interferes with performance in daily life to 9- interferes with performance in daily life most). Scoring was based on a total of 0-204 points with higher scores indicating greater severity and frequency of AD episodes and therefore a more negative impact on perceived AD-related QoL. A copy of the AD HR-QoL questionnaire is included in Appendix A.
3.2.5 Incontinence Quality of Life (I-QoL) Questionnaire

The I-QoL Questionnaire is a self-administered previously validated and reliable disease-specific questionnaire that measures bladder-related QoL in individuals with neurogenic bladder\textsuperscript{253,241}. The questionnaire is formatted as a 22-items divided into 3 sub-scales: 1) avoidance and limiting behaviour (8 items); 2) psycho-social impact (9 items); and 3) social embarrassment (5 items). Scoring is based on a five-point response scale with values ranging from one (extremely) to five (not at all). Scores are then tallied and transformed into a scale score ranging from 0-100 points with higher scores indicating a greater QoL. A copy of the I-QoL Questionnaire is included in Appendix B.

3.2.6 Twenty-four Hour Ambulatory Blood Pressure Monitoring (24-hr ABPM) Protocol

Following UDS, 24-hr ABPM was performed using the Meditech Card (X) plorel (Meditech Ltd., Budapest, Hungary) as previously described\textsuperscript{254}. At the present time, 24-hr ABPM is accepted as superior to routine one-time clinic BP measurements\textsuperscript{119,120}. Compared to a single one-time clinic visit, 24-hr ABPM has been found to provide a highly effective and comprehensive assessment of true BP and HR\textsuperscript{114}. No reports to support a placebo effect when worn short -term (one day) or long-term (one week)\textsuperscript{125–127} have been found demonstrating ABPM a reliable clinical tool. A standard adult-sized cuff for an arm circumference of 24-32 cm was fixed on the non-dominant arm (left arm). A mercury sphygmomanometer comes attached to the monitor and recordings were taken every 15 minutes between 07:00 AM and 23:00 AM which represented the daytime period, and then every one-hour from 23:00 AM and 07:00 AM,
which represented the nighttime period. Daytime and nighttime BP and HR values were determined from the average of all measurements during each time period. All participants completed an activity log to indicate the time before and after each CIC, transfers, time they transferred into supine position to sleep then seated when they woke-up, or any other times they felt AD occurring. Additionally, all participants were instructed to manually record a BP measurement during each event by pressing the button ‘1’ followed by the button ‘2’ located on the front of the ABPM device.

Participants were instructed to maintain their routine activities while avoiding exercise and excessive physical exertion. Participants were also requested to avoid scheduling their regimented bowel routine on the same day of wearing the ABPM. Prior to starring 24-hr ABPM, three consecutive baseline measurements of BP (separated by one minute) were recorded (DinamapV100, GE Medical Systems CareScape™, Fairfield, CT, USA) with the participant seated in their own wheelchair, following five minutes of rest. The average of the three seated BP and HR values were used as the baseline from which the presence or absence of AD bouts during the daytime 24-hr ABPM period were determined. Any measurement that had a SBP ≥20 mm Hg above the average seated baseline value was documented as a bout of AD, and the corresponding cause was obtained from the activity log. AD severity was calculated by subtracting the highest recorded SBP from the respective baseline. Calculation of AD during the night required another baseline BP due to change in position from seated to supine and due to a physiological nocturnal dip that is found absent in those with AIS A tetraplegia. A nocturnal dip is described as a physiological circadian BP pattern where SBP and DBP are reduced by 10% during sleep113,122.
3.2.7 Botox Injections

Botox injections were conducted according to previously established clinical protocol by a trained urologist at the Vancouver General Hospital. One cycle of 200 U of OnabotulinumtoxinA (Botox®; Allergan Inc., CA, USA) was diluted in 15 mls normal saline and injected into the detrusor muscle at 20 sites (10U per site) using a flexible scope with a 6 French injection needle (Olympus Flexible Visera Cysto-Nephro Videoscope CYF Type V2). Previous comparative studies have found a dose of 200 U to provide the most therapeutic effects for the treatment of NDO and DSD in those with SCI\textsuperscript{40,42}. The trigone was spared to avoid inducing vesicoureteral reflux into the UUT. In participants with AIS ‘A’ (motor and sensory complete injuries), bladder sensation was absent and so no anesthesia was required. In all other participants, a local anesthesia was utilized with instillation of 50 mls of 2\% lidocaine into the bladder mucosa prior to the procedure to avoid the provocation of AD. BP was monitored continuously throughout the procedure, which required no more than 30 minutes. All spontaneously reported adverse effects were recorded including cause, type, incidence, and severity.

3.3 Statistical Analysis

Statistical analyses were performed using SPSS, version 19.0 (SPSS, Chicago, IL, USA). Normal distribution of the data was assessed using the Shapiro-Wilk test of normality. Differences in continuous variables for primary outcome measures: SBP, DBP, MAP, and HR, pre and post-Botox treatment during UDS and 24-hr ABPM were compared using one-tailed paired-sample Student’s \textit{t}-tests for normally distributed data, and Wilcoxon signed-rank test for
non-normally distributed data. Differences for secondary outcome measures for bladder function parameters including: volume (mls) at first uninhibited detrusor contraction, compliance (cm H$_2$O$^{-1}$), maximum detrusor pressure (cm H$_2$O), and the number of contractions before urinary leakage, and volume at urinary leakage or maximum volume achieved (mls), were assessed using the same method as for continuous primary outcome measures. Questionnaire data for the I-QoL Questionnaire and the AD-HR-QoL Questionnaire was also assessed using Wilcoxon signed-rank tests. A one-tailed test was chosen due to the small sample size and strong hypothesis concerning the directionality of the effect of Botox on reducing AD severity and frequency. A $P$-value less than 0.05 were considered significant. Data are reported as mean ± standard deviation.
Chapter 4: Results

Participant characteristics are described in Table 1. Common side effects of the Botox injections included: UTI in 5/14 (36%), headache lasting one week post treatment 3/14 (21%) and pain at the injection site that lasted two days following treatment 2/14 (14%). There were no reports of systemic side effects in our study.

Table 1. Participant characteristics

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Stature (cm)</th>
<th>Mass (kg)</th>
<th>Lesion Level</th>
<th>AIS</th>
<th>Time post injury (yrs)</th>
<th>Injury Cause</th>
<th>Anticholinergic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51</td>
<td>F</td>
<td>167</td>
<td>55</td>
<td>C₄</td>
<td>A</td>
<td>8</td>
<td>MVA</td>
<td>Ditropan 5mg BID</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>F</td>
<td>165</td>
<td>64</td>
<td>C₄</td>
<td>A</td>
<td>14</td>
<td>MVA</td>
<td>Ditropan 5mg TID</td>
</tr>
<tr>
<td>3</td>
<td>62</td>
<td>M</td>
<td>180</td>
<td>98</td>
<td>C₅</td>
<td>C</td>
<td>18</td>
<td>Fall</td>
<td>Ditropan 2.5mg BID</td>
</tr>
<tr>
<td>4</td>
<td>43</td>
<td>M</td>
<td>175</td>
<td>84</td>
<td>C₅</td>
<td>C</td>
<td>27</td>
<td>MVA</td>
<td>Ditropan 5mg BID</td>
</tr>
<tr>
<td>5</td>
<td>44</td>
<td>M</td>
<td>183</td>
<td>63</td>
<td>C₆</td>
<td>A</td>
<td>19</td>
<td>Skiing</td>
<td>Detrol LA 12mg OD</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>M</td>
<td>178</td>
<td>70</td>
<td>C₆</td>
<td>B</td>
<td>34</td>
<td>MVA</td>
<td>Ditropan 5mg BID</td>
</tr>
<tr>
<td>7</td>
<td>43</td>
<td>M</td>
<td>183</td>
<td>81</td>
<td>C₆</td>
<td>C</td>
<td>24</td>
<td>Diving</td>
<td>Toviaz 4mg OD</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>F</td>
<td>178</td>
<td>53</td>
<td>C₇</td>
<td>A</td>
<td>18</td>
<td>MVA</td>
<td>Ditropan 5mg BID</td>
</tr>
<tr>
<td>9</td>
<td>28</td>
<td>M</td>
<td>178</td>
<td>68</td>
<td>C₇</td>
<td>B</td>
<td>8</td>
<td>MVA</td>
<td>Ditropan 5 mg TID</td>
</tr>
<tr>
<td>10</td>
<td>46</td>
<td>M</td>
<td>165</td>
<td>45</td>
<td>C₈</td>
<td>B</td>
<td>40</td>
<td>MVA</td>
<td>Ditropan 2.5mg BID</td>
</tr>
<tr>
<td>11</td>
<td>38</td>
<td>M</td>
<td>182</td>
<td>95</td>
<td>T₃</td>
<td>B</td>
<td>21</td>
<td>MVA</td>
<td>Ditropan 10mg BID</td>
</tr>
<tr>
<td>12</td>
<td>44</td>
<td>M</td>
<td>157</td>
<td>70</td>
<td>T₄</td>
<td>A</td>
<td>18</td>
<td>Fall</td>
<td>Toviaz 8mg OD</td>
</tr>
<tr>
<td>13</td>
<td>31</td>
<td>M</td>
<td>178</td>
<td>60</td>
<td>T₅</td>
<td>A</td>
<td>10</td>
<td>Biking</td>
<td>Ditropan 5mg BID</td>
</tr>
<tr>
<td>14</td>
<td>62</td>
<td>M</td>
<td>183</td>
<td>91</td>
<td>T₅</td>
<td>A</td>
<td>42</td>
<td>MVA</td>
<td>Detrol LA 4 mg OD</td>
</tr>
<tr>
<td>Mean</td>
<td>45 ± 11</td>
<td>168 ± 26</td>
<td>71 ± 16</td>
<td></td>
<td></td>
<td></td>
<td>21 ± 12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AIS, American Spinal Cord Injury Association Impairment Scale; BID, twice daily; C, cervical; mg, milligram(s); MVA, motor vehicle accident; T, thoracic; TID, three times daily; OD, once daily.
4.1 Hemodynamic Outcome Measures during Urodynamics

The severity of AD during UDS is presented in Figure 7. Maximum SBP during the UDS was decreased following Botox treatment (Figure 5A, \( P<0.001 \)), in 13/14 participants by an average \( \pm\) SD of 161 mm Hg \( \pm 27 \) to 138 \( \pm 14 \) mm Hg resulting in an overall average reduction in maximum SBP by 23 mm Hg. One participant experienced an increase in maximum SBP (pre-Botox from 145 mm Hg to 164 mm Hg post-Botox). This individual in particular had the highest lesion level (C₄) with an AIS A SCI and NDO with DSD (Participant No. 2, Table 1). While another two participant’s maximum SBP remained unchanged (No. 9, Table 1) at 138 mm Hg pre and post Botox treatment, and 137 mm Hg (pre) to 138 mm Hg (post) (No. 13, Table 1). SBP change (\( \Delta \)) (maximum SBP increase minus average resting supine baseline SBP) was also decreased following Botox treatment (Figure 5 B, \( P<0.001 \)) from an average of 45 \( \pm 25 \) mm Hg to 22 \( \pm 11 \) mm Hg. Before Botox treatment, the maximum SBP\( \Delta \) was 100 mm Hg and minimum was 20 mm Hg. After Botox treatment, the maximum SBP\( \Delta \) was 61 mm Hg and minimum was 13 mm Hg. Before Botox treatment, all 14 participants experienced AD based on the criteria of SBP\( \Delta \geq 20 \) mm Hg, and after Botox treatment complete abolition of AD was experienced by over half of the participants 8/14 (57%). Of the remaining, six individuals who still experienced AD during UDS, all experienced a reduction in average SBP\( \Delta \) post-Botox by 37% overall. The average reduction in amplitude of SBP\( \Delta \) was 43% overall.
Figure 7. Systolic blood pressure (SBP) responses during urodynamic studies (UDS) assessment pre and post-Botox treatment. **Panel A:** maximum SBP achieved during UDS assessment. **Panel B:** change (Δ) in SBP during UDS assessment. Circles represent pre-Botox measurements. Squares represent post-Botox measurements.

Analyses of all hemodynamic variables assessed during UDS are presented in Table 2. Baseline (supine) BP and HR were unchanged following Botox treatment (all $P>0.050$). One month following Botox, SBP, DBP and MAP were lower at all measurement time points including first sensation (all $P<0.001$), first urge to perform CIC (all $P<0.001$), maximum SBP (all $P<0.050$), and maximum infusion (all $P<0.050$). HR at first sensation was significantly higher post-Botox ($P<0.030$), while HR at all other time points was unchanged.
Table 2. Hemodynamic changes during urodynamic assessment pre and post-Botox treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Botox</th>
<th>Post-Botox</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>114 ± 17</td>
<td>116 ± 14</td>
<td>0.340</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>63 ± 10</td>
<td>64 ± 10</td>
<td>0.401</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>81 ± 11</td>
<td>80 ± 13</td>
<td>0.321</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>73 ± 16</td>
<td>73 ± 12</td>
<td>0.492</td>
</tr>
<tr>
<td><strong>First sensation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>147 ± 27</td>
<td>116 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84 ± 16</td>
<td>64 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>108 ± 19</td>
<td>94 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>59 ± 14</td>
<td>67 ± 8</td>
<td>0.030</td>
</tr>
<tr>
<td><strong>First urge to perform CIC</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>147 ± 27</td>
<td>128 ± 13</td>
<td>0.001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>84 ± 16</td>
<td>71 ± 9</td>
<td>0.001</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>108 ± 19</td>
<td>94 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>59 ± 14</td>
<td>62 ± 9</td>
<td>0.201</td>
</tr>
<tr>
<td><strong>Maximum arterial SBP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>180 ± 28</td>
<td>144 ± 19</td>
<td>0.003</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>88 ± 13</td>
<td>77 ± 10</td>
<td>0.012</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>116 ± 17</td>
<td>102 ± 11</td>
<td>0.011</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>56 ± 14</td>
<td>62 ± 13</td>
<td>0.062</td>
</tr>
<tr>
<td><strong>Maximum infusion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>154 ± 27</td>
<td>139 ± 20</td>
<td>0.022</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85 ± 17</td>
<td>76 ± 10</td>
<td>0.031</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>111 ± 19</td>
<td>99 ± 12</td>
<td>0.012</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>56 ± 14</td>
<td>56 ± 13</td>
<td>0.241</td>
</tr>
</tbody>
</table>

Abbreviations: CIC, clean intermittent catheterization; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial blood pressure; SBP, systolic blood pressure. Data are expressed as mean ± SD.

Subjective symptoms (i.e., headache, light-headedness, tingling/chills, sweating) and objective signs (i.e., Goosebumps, facial flushing, sweating) of AD are presented in Table 3.

Before Botox injections, 12/14 (86%) of participants experienced signs or symptoms, with the most common responses being sweating. As previously described, six participants had AD during the post-Botox UDS according to not meeting the AD-cut-off criteria (No. 3, 4, 6, 9, 10, and 11, Table 1). Of those six, three reported a “warm/tingling” sensation (No. 9, 10, and 11).
The fourth reported a “tingling” sensation in addition to goosebumps and sweating (No. 4, Table 1), while the fourth and fifth participants experienced silent AD (SBPΔ ≥20 mm Hg in the absence of signs or symptoms). Of the two participants who experienced silent AD (No. 6 and 3, Table 1), one of the two experienced silent AD both pre and post-Botox treatment (No. 3, Table 1). This individual experienced among the lowest SBPΔ both pre and post-Botox treatment from baseline but still met the AD cut-off criteria. For the other individuals who experienced silent AD, the one individual in pre-Botox was also the individual to experience an increase in maximum SBP post-Botox treatment (No. 2, Table 1), and still experienced AD following treatment, but became symptomatic. The other individual in post-Botox, still also experienced AD as well, but became asymptomatic (No. 6, Table 1). Of the eight who did not experience AD during the post-Botox UDS, three still reported feeling “slightly sweaty” or a “pressure sensation” at maximum bladder capacity (Participant No. 1, No. 9, and No. 13).

Table 3. Self-reported signs and symptoms of autonomic dysreflexia during urodynamic assessments

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Botox (%)</th>
<th>Post-Botox (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweating</td>
<td>7/14 (50%)</td>
<td>1/14 (7%)</td>
</tr>
<tr>
<td>Light-headedness</td>
<td>3/14 (21%)</td>
<td>0/14 (0%)</td>
</tr>
<tr>
<td>Tingling/chills</td>
<td>6/14 (43%)</td>
<td>3/14 (21%)</td>
</tr>
<tr>
<td>Goosebumps</td>
<td>6/14 (43%)</td>
<td>1/14 (7%)</td>
</tr>
<tr>
<td>Headache</td>
<td>4/14 (29%)</td>
<td>0/14 (0%)</td>
</tr>
<tr>
<td>Facial Flushing</td>
<td>1/14 (7%)</td>
<td>0/14 (0%)</td>
</tr>
<tr>
<td>Asymptomatic AD</td>
<td>2/14 (14%)</td>
<td>2/14 (14%)</td>
</tr>
</tbody>
</table>
4.2 Twenty-Four Hour Ambulatory Blood Pressure Monitoring (24-hr ABPM) Outcome Measures

A sample 24-hour ABPM report is presented in Figure 6.

Figure 8. Sample 24-hour ambulatory blood pressure monitoring data report demonstrating a reduction in autonomic dysreflexia (AD) bouts following Botox treatment. In Panel A: (pre-Botox) maximum systolic blood pressure (SBP) was 172 mm Hg and a total of 17 bouts of AD documented. In Panel B: (post-Botox) maximum SBP was 153 mm Hg, resulting in a reduction by 19 mm Hg and a >50% reduction in AD frequency resulting in only nine bouts of AD. White shading represents the daytime period. The gray shading represents the nighttime period. Vertical black lines indicate a BP measurement where the top of the line represents SBP and the bottom of the line represents diastolic BP. The horizontal black line represents heart rate. Black circles represent AD events per the AD-cut off criteria.
Following Botox treatment there was a significant reduction in the frequency of bladder-related AD events (Figure 9A, \( P < 0.001 \)) from an average of 8 ± 6 (maximum: 26 events; minimum: 4 events) pre-Botox to only 1 ± 2 (maximum: 4 events; minimum 0 events) AD bladder-related events post-Botox. There was also a significant reduction in severity of AD as per a reduction in the amplitude of average SBP \( \Delta \) (Figure 9B, \( P = 0.001 \)). Average SBP\( \Delta \) pre-Botox was 58 ± 27 with a maximum SBP\( \Delta \) of 103 mm Hg and minimum SBP\( \Delta \) of 16 mm Hg. After Botox treatment this was reduced to an average SBP\( \Delta \) of 35 ± 22 with a maximum SBP\( \Delta \) of only 68 mm Hg and minimum SBP\( \Delta \) of 11 mm Hg. This resulted in an overall reduction in average SBP\( \Delta \) by 23 mm Hg following Botox treatment.

Figure 9. Frequency (A) and severity (B) of bladder related autonomic dysreflexia (AD) events during 24-hour ambulatory blood pressure monitoring. Panel A: total number of bladder related AD events. Panel B: change (\( \Delta \)) in systolic blood pressure during bladder related AD events. Circles represent pre-Botox measurements. Squares represent post-Botox measurements.
Overall AD (inclusive of both bladder-related AD and non-bladder related AD bouts) frequency and severity were reduced post-Botox (Figure 10). Non-bladder AD (exclusive of bladder-related AD) frequency (# AD events: 4 ± 4 vs. 3 ± 3, \( P = 0.001 \) for pre vs. post-Botox) and severity (SBP\( \Delta \): 58 ± 28 mm Hg vs. 35 ± 22 mm Hg, \( P = 0.005 \) for pre vs. post-Botox) were also reduced following Botox treatment. In two individuals, average SBP\( \Delta \) for overall AD severity was to have increased following Botox treatment.

![Figure 10](image.png)

**Figure 10.** Frequency (A) and severity (B) of overall autonomic dysreflexia (AD) events during 24-hour ambulatory blood pressure monitoring. **Panel A:** total number of overall AD events. **Panel B:** change (\( \Delta \)) in systolic blood pressure (SBP) during overall AD events. Circles represent pre-Botox measurements. Squares represent post-Botox measurements.
Hemodynamic measures from the 24-hr ABPM are presented in Table 4. Nighttime BPs and HR were unchanged post-Botox, while daytime SBP and MAP increased by 5 mmHg \( (P<0.050) \). In terms of 24-hr outcomes, daytime maximum SBP was reduced \( (P = 0.010) \) by an average of 17 mm Hg post-Botox, while maximum SBP prior to CIC \( (P = 0.001) \) was reduced by 24 mm Hg. This resulted in an average reduction 30 mm Hg of SBP\( \Delta \) prior to CIC \( (P = 0.001) \).

Table 4. Hemodynamic measures during 24-hr ABPM pre and post-Botox treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Botox</th>
<th>Post-Botox</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Daytime Values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>111 ± 14</td>
<td>116 ± 13</td>
<td>0.021</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>64 ± 7</td>
<td>67 ± 10</td>
<td>0.092</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>80 ± 12</td>
<td>85 ± 9</td>
<td>0.031</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>79 ± 13</td>
<td>75 ± 10</td>
<td>0.112</td>
</tr>
<tr>
<td><strong>Nighttime Values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>101± 10</td>
<td>103 ± 8</td>
<td>0.342</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>59 ± 10</td>
<td>58 ± 7</td>
<td>0.381</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>72 ± 9</td>
<td>73 ± 7</td>
<td>0.151</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>61 ± 16</td>
<td>62 ± 14</td>
<td>0.372</td>
</tr>
<tr>
<td><strong>24 hr Outcomes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Max SBP Day (mm Hg)</td>
<td>169 ± 24</td>
<td>152 ± 17</td>
<td>0.010</td>
</tr>
<tr>
<td>Max SBP Night (mm Hg)</td>
<td>128 ± 22</td>
<td>123 ± 11</td>
<td>0.201</td>
</tr>
<tr>
<td>Max SBP before CIC (mm Hg)</td>
<td>160 ± 18</td>
<td>136 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>Average SBP ( \Delta ) before CIC (mm Hg)</td>
<td>49 ± 22</td>
<td>19 ± 16</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: CIC, clean intermittent catheterization; DBP, Diastolic blood pressure; HR, heart rate; MAP, mean arterial blood pressure; SBP, systolic blood pressure. Data are mean ± SD.
4.3 Questionnaire Data

There was a significant improvement in AD HR-QoL (Figure 11A, \( P=0.0015 \)) questionnaire scores following Botox treatment, where a reduction in total score is indicative of an overall improvement in AD HR-QoL. Before Botox treatment, the average total AD HR-QoL score was 118 ± 26, with a maximum score of 171 points and a minimum score of 87 points reported. Following Botox treatment, the average total AD HR-QoL score was reduced to an average total score of 82 ± 23 with a maximum score of 119 points and a minimum score of 57 points, resulting in an average reduction in total average score by 36 points and a reduction in maximum score by 52 points. In total, 12/14 (86%) of participants self-reported an improvement in AD HR-QoL following Botox treatment.

Two participants (14%) (No. 1 and 3, Table 1) reported no noticeable improvement in their AD signs and symptoms following Botox treatment. Participant No. 1 had an increase in score from 88-106 points, while participant No. 3 had an increase in score from 116-119 points. Participant No.1 was below the AD-cut-off criteria following Botox treatment, but was one who still reported feeling “tingling/chills sensation” following treatment. This participant thought that the AD bouts had worsened in terms of severity and overall daily bouts and bladder-related bouts of AD. The increase in score for Participant No. 3 was the one individual who also experienced silent AD during UDS pre and post Botox. This participant self-reported a worsening of AD severity, overall AD on a daily basis and bladder-related. Another participant (No. 9, Table 1), although reported experiencing a slight improvement in score (88-85 points), was below the AD-cut-off criteria in post-Botox UDS, but had the same maximum SBP during UDS (138 mm Hg
pre and 138 mm Hg post). Individual subsections for each component of the AD HR-QoL Questionnaire is displayed in Table 5.

Significant improvements in bladder-related health following completion of the I-QoL questionnaire was also demonstrated following Botox treatment (Figure 11B, $P = 0.0005$). An increase in total score indicates an improvement in bladder-related QoL. Before Botox treatment, the average total I-QoL score was 78 ± 17, with a maximum score of 95 points and minimum of 41 points. Following Botox treatment, the average total I-QoL score was significantly increased to an average total score of 96 ± 11 with a maximum score of 108 points and minimum of 77 points, resulting in an average increase in total score by 18 points. Following Botox treatment, 100% (14/14) of participants self-reported improvements in bladder-related health. Individual subsections for each component of the I-QoL Questionnaire is displayed in Table 5.

![Figure 11. Total scores for the (A) autonomic dysreflexia health related quality of life (AD HRQoL), and (B) incontinence quality of life (I-QoL) questionnaire pre and post-Botox treatment. Circles represent pre-Botox measurements. Squares represent post-Botox measurements.](image-url)
Table 5. Questionnaire subsections

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Botox</th>
<th>Post-Botox</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AD HR-QoL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily Basis AD</td>
<td>20 ± 5</td>
<td>14 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Bladder-Related AD</td>
<td>18 ± 4</td>
<td>13 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Daily Basis AD Severity</td>
<td>14 ± 3</td>
<td>11 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>Bladder-Related AD Severity</td>
<td>16 ± 3</td>
<td>12 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>AD Interference in Daily Life</td>
<td>13 ± 2</td>
<td>10 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>Severity of Interference in Daily Life</td>
<td>23 ± 10</td>
<td>14 ± 6</td>
<td>0.001</td>
</tr>
<tr>
<td>AD Severity in Past 2 Weeks</td>
<td>12 ± 4</td>
<td>8 ± 6</td>
<td>0.021</td>
</tr>
<tr>
<td>AD Frequency in Past 2 Weeks</td>
<td>3 ± 1</td>
<td>1 ± 1</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>I-QoL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Avoidance Limiting Behaviour</td>
<td>28 ± 5</td>
<td>35 ± 3</td>
<td>0.001</td>
</tr>
<tr>
<td>Psychosocial Impact</td>
<td>35 ± 3</td>
<td>41 ± 4</td>
<td>0.001</td>
</tr>
<tr>
<td>Social Embarrassment</td>
<td>16 ± 6</td>
<td>20 ± 5</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Abbreviations: AD, autonomic dysreflexia. Data are mean ± SD

4.4 Bladder Function and Systolic Blood Pressure Parameter Outcome Measures during Urodynamic Studies

Bladder function and SBP parameters are presented in Table 6. Following Botox treatment, the volume at first uninhibited detrusor contraction was significantly increased by an average of 158 mls \((P<0.009)\) while SBP was significantly reduced by an average of 16 mm Hg \((P<0.036)\). Significant improvements in bladder compliance were found resulting in an average increase of 26 cm \(H_2O\) \(^{-1}\) following Botox treatment \((P<0.001)\). Maximum detrusor pressure was significantly reduced by an average of 21 cm \(H_2O\) following Botox treatment \((P<0.001)\), in addition to SBP by an average of 19 mm Hg at the maximum detrusor pressure \((P<0.002)\). The number of detrusor contractions before a participant experienced a urinary leak was significantly reduced by an average of 2.93 contractions following Botox treatment \((P<0.018)\). The volume within the bladder before a urinary leak or at maximum bladder volume was significantly
increased by an average of 188 mls following Botox treatment ($P<0.003$). While SBP remained significantly decreased by an average of 22 mm Hg following Botox treatment ($P<0.008$). All participants were taking anticholinergic medications unsuccessfully pre-Botox (Table 1), and all participants discontinued taking anticholinergic medications by week two following the Botox treatment. Additionally, 8/14 (57%) experienced a urinary leak during the pre-Botox UDS assessment, while continence was restored in 12/14 (86%) of those participants with initial urinary leakage ($P = 0.01$) during post-Botox UDS assessment. Also notable, is that at baseline UDS 7/14 participants presented with NDO only and the remaining 7/14 presented with NDO and DSD as evidenced by EMG.

Table 6. Bladder function and SBP parameters during UDS pre and post-Botox treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Botox</th>
<th>Post-Botox</th>
<th>P-value (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume at First Contraction (mls)</td>
<td>257 ± 38</td>
<td>415 ± 67</td>
<td>0.009</td>
</tr>
<tr>
<td>SBP at First Contraction (mm Hg)</td>
<td>132 ± 6</td>
<td>116 ± 4</td>
<td>0.036</td>
</tr>
<tr>
<td>Compliance ($cm H_2O\cdot^{-1}$)</td>
<td>15 ± 5</td>
<td>41 ± 12</td>
<td>0.001</td>
</tr>
<tr>
<td>Max Detrusor Pressure (cm H_2O)</td>
<td>38 ± 4</td>
<td>17 ± 2</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP at Max Detrusor Pressure (mm Hg)</td>
<td>147 ± 7</td>
<td>128 ± 4</td>
<td>0.002</td>
</tr>
<tr>
<td>Constructions (#) before Leak</td>
<td>3 ± 1</td>
<td>.07 ± 1</td>
<td>0.018</td>
</tr>
<tr>
<td>Volume before Leak/Max Volume (mls)</td>
<td>366 ± 61</td>
<td>554 ±47</td>
<td>0.003</td>
</tr>
<tr>
<td>SBP at Max Volume (mm Hg)</td>
<td>161 ± 7</td>
<td>139 ±4</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Abbreviations: CIC, clean intermittent catheterization; Pves, intravesical pressure; SBP, systolic blood pressure. Data are mean ± SD.
Chapter 5: Discussion, Future Directions, and Conclusions

5.1 General Discussion Statement

To our knowledge our study is the first human-conducted study to quantitatively show the efficacy of Botox treatment for management of NDO on reducing the severity and frequency of AD events in individuals with SCI. Furthermore to our knowledge, this is the first study to utilize the AD component of the ADFSCI Questionnaire and provide assessment of how modifications in the severity and frequency of AD bouts impact AD-related QoL. Collectively, the results from our investigation demonstrate that intravesical injections of Botox in a dose of 200 U in 20 sites of the detrusor muscle (trigone sparing) in individuals with traumatic, chronic (>1 year post SCI), high-level SCI, is effective at reducing the severity and frequency of bladder related and overall AD bouts, as well as improving QoL one-month following treatment.

5.2 Effect of Botox on hemodynamic parameters during Urodynamic Studies

AD is a medical emergency triggered by afferent stimuli originating from the urinary bladder 85% of the time. Activation of AD leads to a massive sympathetically mediated vasoconstriction of the splanchnic vascular bed. At the present time, no previously conducted studies in humans utilizing arterial BP/HR monitoring following Botox injections for NDO in persons with SCI have been done. In the one animal study utilizing arterial BP/HR monitoring following Botox injections for NDO in female rats following UDS assessment; AD was reported
as eliminated at the three week follow-up in addition to a significant reduction in Pves and number of uninhibited detrusor contractions\textsuperscript{36}.

In our first hypothesis we proposed there would be a significant reduction in AD severity one-month post-Botox treatment in individuals with SCI and NDO during UDS. Indeed Botox was effective at reducing AD severity one-month following treatment according to a reduction in average maximum SBP during UDS and amplitude of AD as per a reduction in average SBPΔ. Improvements in the reduction of AD in our study were found greater to those reported in the study by Chen\textsuperscript{35}. In our study, 8/14 (57\%) of participants experienced an attenuation of AD at the four-week follow-up. In the study by Chen and colleagues, AD was only reported as abolished in three individuals, and only 15 individuals reported a greater than 50\% improvement in the reduction of self-reported AD signs and symptoms at the six month follow-up. It is important however, to keep in mind the differences that exist in methodology between the Chen study and our study. Core differences lie in the follow-up time frame, the use of an AD-cut-off criteria and the use of quantitative measurements during UDS assessment. For Chen and colleagues, the follow-up was set to six-months, and in our study it was four-weeks. As Botox, is known to exert greatest effects at four to six weeks, our follow-up time period was a critical point when the efficacy of Botox on ameliorating AD would most likely be seen. Given the duration of Botox in the detrusor of approximately six to nine months\textsuperscript{25,30} it would be expected that the efficacy of Botox might start to wane at six-months. Additionally, since the only other study that has quantitatively assessed arterial BP and HR during UDS following Botox treatment was conducted in animals, we again are not provided with the best means of comparison since a human model was utilized in our study.
Our baseline UDS screening process was established according to recommended clinical guidelines to confirm the presence of AD according to an increase in SBP $\geq 20$ mm Hg from baseline which allowed for a more rigorous, objective, quantitative and therefore more reliable method to assess the efficacy of Botox on attenuating AD. Great difficulties were found when attempting to compare the results found in the Chen study with our results due to the differences in protocol. In our BP protocol for UDS, a one-minute interval of automated arterial BP inflations was utilized in order to allow for accurate capturing of frequently occurring fluctuations in arterial BP and HR. In some of the earliest conducted studies to utilize BP monitoring during UDS to screen for the development of AD, Linsenmeyer and colleagues performed BP inflations manually every 45 seconds during bladder filling demonstrating an average increase in SBP to 169 mm Hg in the AD group at first uninhibited detrusor contraction, which was slightly higher compared to our participants who experienced an average increase in SBP of $147 \pm 27$ mm Hg at “first sensation” which is correlated with the first uninhibited detrusor contraction using one-minute intervals.

Giannantoni and colleagues used intervals of every two-minutes\textsuperscript{102} and found SBP to be greatest at first uninhibited detrusor contraction, whereas in our study, we found SBP to be greatest as the bladder reached its maximum capacity demonstrating that more frequent recordings might yield better accuracy. In more recent hemodynamic evaluations assessing the incidence of AD during UDS, Huang and colleagues used a three-minute interval in their 2011 study and then decided it was best to go with a two-minute interval in their 2013 study, yielding
hemodynamic outcome measures similar to those of our pre-Botox assessments with a maximum SBP of 168 ± 33 mm Hg and average SBPΔ of 52 ± 24 mm Hg in their symptomatic AD group during UDS\textsuperscript{165,255}, which was comparable to ours with a maximum SBP of 161 mm Hg ± 27 and SBPΔ of 45 ± 25 mm Hg.

The chosen AD cut-off criteria is also another area of interest when objectively quantifying AD. In previous literature reports Linsenmeyer and colleagues used an AD cut-off criteria of an arterial SBP/DBP \( \geq 160/90 \) mm Hg and reported AD to occur in 35/45 (78\%) of their high thoracic population\textsuperscript{100}. In the study by Giannantoni and colleagues, the AD cut-off criteria was set at a BP of \( \geq 150/100 \) mm Hg resulting in a total of 20/48 (42\%) of participants to demonstrate AD. In 11/20 (55\%) of their participants, AD was experienced at the peak of uninhibited detrusor contraction and in 6/20 (30\%) at maximum volume infusion. In our study we saw similar findings in our pre-Botox UDS BP data where maximum SBP occurred at the peak of an uninhibited detrusor contractions just before reaching maximum bladder capacity. In the two studies by Huang and colleagues, an AD cut-off criteria according to the guidelines in the Consortium for Spinal Cord Medicine\textsuperscript{105} of an increase in SBP by \( \geq 20-40 \) mm Hg from baseline were used to confirmed an AD diagnosis\textsuperscript{165,255}. In the 2011 study, reports of AD were observed to occur in 36.7\% overall and in 42.6\% with high-level SCI. In our study, we employed a similar AD-cut off criteria of an increase in SBP by \( \geq 20 \) mm Hg as per the Consortium for SCI\textsuperscript{105} and the International Standards on documentation of remaining Autonomic Function\textsuperscript{45}. By utilizing a \( \geq 20 \) mm Hg in SBP based on individualized baseline resting measures, AD can be more accurately assessed, especially as cervical injured individuals typically have an already low
resting arterial BP. By not utilizing established criteria, there is a chance that AD could be overestimated or underestimated as in the study by Chen and colleagues.

Results from our study revealed significant reductions in AD frequency and severity using only 20 injections sites in the detrusor compared to the study by Chen that used 40 detrusor injections sites. Our study therefore demonstrated that effective attenuation of AD signs and symptoms and improvements in bladder function may potentially be achieved best with less bladder injections. However, a randomized controlled trial comparing injection frequency between 20 and 40 sites would be required to confirm this possibility. Additionally, Before Botox treatment, SBP increases were typically seen at the first involuntary uninhibited detrusor contraction followed by a period of stabilization and then numerous continued gradual spikes in SBP as pressures changed eliciting further detrusor contractions. In our participants, maximum SBP was generally found to peak closer to maximum bladder capacity or infusion, demonstrating that both detrusor contractions and distension of the stretch receptors may initiate activation of AD. Similar findings were found by Giannantoni et al., Linsenmeyer et al., and Tsai et al., 27,100,102.

In our study, one individual experienced an increase in maximum SBP following Botox treatment (145 mm Hg pre-Botox to 164 mm Hg post-Botox). Potential explanations for this increase might have been due to the fact that the participant was our highest lesion level (level C4) and had an AIS A motor and sensory complete SCI, in addition to NDO with DSD, that was not found to be resolved following Botox treatment as per an increase in EMG activity from 4 to 22 millivolts right before experiencing a urinary leak. Additionally, 24-hr ABPM on this individual revealed an absence of nocturnal dip which has been found to correlate strongly with
increased CVD risks in persons with cervical complete SCIs. Before Botox, average resting supine BP was extremely hypotensive at 74/48 mm Hg and upon bladder filling and the participant failed to experience a gradual rise in BP as volume increased, reporting “first sensation” after only 43 mls, reaching a maximum SBP of 145 mm Hg, with a Pves of 32 cm H₂O, demonstrating a non-compliant highly pressurized bladder. Following Botox treatment, UDS parameters were significantly improved. “First sensation” was reported at a Pves of 16 and bladder volumes increased to 211 mls. Interestingly enough however, is that EMG activity, which is correlated with DSD, was still highly active following Botox treatment, reaching an activity level of 23 at the same time AD was activated.

Among the most common triggers within the bladder capable of initiating the cascade of afferent stimulation to the spinal cord are uninhibited detrusor contractions due to increases in Pves which pose as a noxious stimulus leading to the development of AD (Figure 2). When Botox is injected into the detrusor muscle, there is an inhibition of the vesicular release of acetylcholine from the presynaptic nerve terminal through the neuromuscular junction where it would then go on to bind to the cholinergic receptor site in the muscle. Typically, the effect of Botox will be evident within two weeks following which the smooth muscle of the detrusor will lose its ability to contract and will be temporarily paralysed for approximately six to nine months. Parasympathetic nervous transmission originating from painful or non-painful bladder afferents is essentially blocked preventing the subsequent occurrence of uncontrolled contractions of the detrusor muscle which would trigger AD.

The efficacy of Botox has been linked to its inhibitory effects on sensory neuron action as well as sensory neuropeptides from the urothelium. As Botox reduces Pves leading to an
increase in bladder capacity/compliance it allows the bladder to function as it normally should, holding volumes equivalent to that of an able-bodied bladder. It has also been proposed that noxious stimuli (i.e., bacterial UTI infection which causes inflammation of the bladder and subsequent release of nerve growth factor) might inadvertently elicit the activation of AD and so injections of Botox into the suburothelial/urothelium space may also exert effects on reducing “non-noxious” stimuli by acting as a protective barrier for the bladder\textsuperscript{257}. In a few studies, greatest improvements in cardiovascular outcomes following Botox treatment during UDS were reported during a follow-up of six weeks\textsuperscript{25,222,40}. It is plausible then that perhaps had our follow-up been six-weeks vs. four-weeks, Botox may have had more time to exert maximal effect and perhaps even greater hemodynamic responses might have been achieved in those who both met and did not meet the AD-cut off criteria.

Self-reported signs and symptoms of AD were documented throughout the UDS assessment and found to be significantly reduced following Botox treatment which had similar findings to the study by Tsai and colleagues where improvements in self-reported AD were experienced by 6/7 (86%) following Botox treatment for NDO and DSD at week 4 during UDS\textsuperscript{27}. The Tsai study however, only used 100 U Botox and was injected transperineally. In the study by Kuo “less AD” was reported by five participants following Botox treatment\textsuperscript{42}. Our study findings were found to contrast the Chen study where self-reported common signs and symptoms of AD were not found to be significantly reduced at the six-month follow-up\textsuperscript{35}.

Of the 57% (n = 8) who met the AD-criteria, 38% (n = 3) (Participant No. 1, 9, and 13) reported feeling mild AD despite remaining below the clinically recommended AD guidelines for SBP. These participants were also the same participants to have the lowest self-reported
improvements in AD- HR-QoL scores. Additionally, the maximum SBP during UDS Participant No. 9 and 13 remained unchanged demonstrating that perhaps Botox may not have had enough time to exert the maximum effect in these two participants at the four-week follow-up. Of the six participants to not meet the AD cut-off criteria, silent AD occurred in two individuals with cervical AIS A injuries demonstrating a potential weakening effect of sympathetic nervous system responses which may be linked to potential failure. Additionally, in our study, EMG recordings revealed that half of the participants 7/14 had NDO only while the other half 7/14 had both NDO and DSD. Changes in Pves in those with NDO and DSD are known to cause more severe uninhibited detrusor contractions and therefore the combination of concomitant NDO and DSD may have played a role. The results from this component of the study support the notion that AD results from Pves changes due to bladder distention which cause uninhibited detrusor contractions, acting as a noxious stimulus initiating the chain of events leading to the activation of AD.

5.2.1 Limitations

The main limitation of this study was the small sample size. Had a larger sample-size been achieved, a more accurate and representative reflection of the efficacy of Botox treatment on the attenuation of AD in our selected population could have been drawn. In our protocol, we advised participants to refrain from taking their anticholinergic medication only on the day of testing. In other studies, a minimum of three days\textsuperscript{206,258}, and even as long as five days\textsuperscript{31} in order to try to assess true baseline bladder function parameters in the absence of any influence of anticholinergics. A few participants took their anticholinergic medication the morning of the
baseline assessments. Given that all participants had discontinued their use of anticholinergics at post-Botox assessments, this discrepancy in medication state may have influenced their measurements. One of the participant’s had previously received Botox injections for the bladder two years prior; however, the likelihood of this exerting any influence would likely be non-existent as Botox has only been found effective in the detrusor muscle for up to nine months\textsuperscript{205,211}. Additionally, the duration of bladder filling during UDS can vary and therefore may affect peak SBP and should be standardized in the future.

5.3 Effect of Botox on AD during 24-hr Ambulatory Blood Pressure Monitoring

The influence of the descending spinal sympathetic tract on cardiovascular function can be assessed utilizing 24-hr ABPM\textsuperscript{259}. Previous studies have been conducted to assess the severity and frequency of AD during daily living in those with SCI\textsuperscript{67,124,260–262}. The efficacy of medical antihypertensive drugs on improving cardiovascular outcomes have also previously assessed utilizing 24-hr ABPM\textsuperscript{263–266}. To our knowledge, this was the first study ever conducted to quantitatively investigate the efficacy of intravesical injected Botox for NDO on reducing the severity and frequency of AD utilizing 24-hr ABPM.

Botox was found to exert significant effects on reducing the maximum SBP just prior to conducting CIC, which was significantly reduced from 160 mm Hg (pre-Botox) to only 136 mm Hg (post-Botox). Additionally, the average amplitude of SBPΔ before conducting CIC was also significantly reduced from an average SBPΔ of 49 mm Hg (pre-Botox) to only 19 mm Hg (post-Botox). Results from this study demonstrated that over a 24-hr time period, upon key activities
that would otherwise elicit AD, (i.e., bladder filling and just prior to CIC) BP was demonstrated as contained within a healthy therapeutic clinical range demonstrating the efficacy Botox exerts over the bladder for reductions in AD severity and frequency. The changes in bladder function parameters also resulted in improved bladder management in our patients: in some individuals, CICs were being performed up to ten times per day, due to small volume of the urinary bladder and frequent episodes of AD triggered by bladder contraction due to even small volumes of urine in the bladder. Frequent and repeated CICs act as a noxious stimulus to the urinary tract afferents bladder, and therefore results in exacerbation of episodes of AD. Therefore, reduction in frequency of CICs may have also attributed to the overall reduction in bladder-related AD

Following Botox treatment, we also observed another interesting phenomenon including a reduction not only bladder-related but also overall AD events. The explanation for this effect could be due in part to AD episodes that prior the Botox treatments were occurring during the day with moments, leg spasm, transfers, or another non bladder related activities (Figure 1). Prior to Botox treatment, the bladder was highly sensitive and so perhaps addition over-exertions and sudden changes in movement may have also caused stimulation to the bladder causing AD. However, after Botox, as the bladder is paralysed, every-day movements (i.e., transfers, wheeling, etc.) that would typically disturb the bladder unintentionally may be prevented from doing so. All participants demonstrated an increase in bladder capacity and compliance and a decreased sensation/urge to perform CIC unnecessarily. In two individuals, the number of AD events increased following Botox treatment which could be linked to the fact that one individual reported lower leg spasticity which was uncontrolled with Baclofen and the other individual
reported the presence of a skin infection (cellulitis) on the lower leg at that time. Both of which may have been acting as noxious stimuli eliciting the activation of non-bladder related AD.

As the urinary bladder is known to be the leading contributor of dangerously elevated SBP levels in 85% of all AD episodes, the effects of Botox on reducing SBP and subsequent activation of AD are tremendous. Figure 8 also provides a good example of the effects Botox has on reducing maximum SBP leading to a significant reduction in bladder-related and overall AD bouts. The increase in SBP we saw following Botox at baseline is likely attributed to regular day-to-day BP variability. Additionally, average nighttime hemodynamic variables were not found to change significantly following Botox treatment. In a previously conducted study using 24-hr ABPM, nighttime hemodynamic variables were also found unchanged in 91% (10/11) individuals with complete tetraplegia SCI due to an absence of physiological rhythmicity of circadian BP. In our study, 5/14 (36%) were AIS A cervical injured and also experienced an absence of physiological nocturnal dipping. In the remaining 5/14 (36%) of cervical incomplete injured, two did not experience nocturnal dipping which could be due to the ABPM impairing deep sleep and three did experience nocturnal dipping which was also experienced in the 4/14 (29%) of thoracic injured participants. Results from this study demonstrated that over a 24-hr time period, upon key activities that would otherwise elicit AD, (i.e., bladder filling and just prior to CIC) BP was demonstrated as contained within a healthy therapeutic clinical range.

5.3.1 Limitations

One of the major limitations of the ABPM is its reliance on participant compliance, including accurately completing the activity log, taking self-measurements whenever there is an event (i.e.,
transfers, CIC, change in position from seated to supine, etc.), and being in an optimal position during measurements (i.e. not using one’s arms). Another limitation is missing the recording of silent AD, which may occur unnoticed and therefore is not recorded manually by the participant.

5.4 Effect of Botox on Quality of Life (Questionnaire Data)

At one-month following Botox treatment self-reported AD-related QoL was significantly improved. In terms of improvements in AD HR-QoL outcomes, participant’s self-reported significant reductions in AD frequency due to reduced bladder sensations, which would otherwise cause AD and the urge to perform CIC. There were self-reports of a reduction in “daily headaches”, feelings of “body tingles and chills”, “sweating” “less shirt changing”, and reports of improved sleep quality. Episodes of AD have previously been found to be associated with increased feelings of anxiety. In one study, a direct relationship was observed between individuals with affective distress due to chronic pain and AD. Following Botox treatment, feelings of less worry and anxiety were reported suggesting that over the long-term, as Botox reduces the number of bladder-related events, improvements in mood, anxiety, pain, and emotional factors involved in the triggering of AD may also be improved.

Following Botox treatment self-reported bladder-related QoL was improved after one-month where an increase in overall score demonstrated a significant improvement in I-QoL scores. Improvements in bladder-related health following Botox treatment were similar to previous studies. In the Chen study, the Urogenital Distress Inventory and Incontinence Impact Questionnaire was used following Botox treatment for NDO in SCI participants but only found improvements in the Urogenital Distress Inventory. In another
study by Kuo and colleagues, an overall 78% rate of satisfaction for QoL using the Urogenital Distress Inventory and Incontinence Impact Questionnaire was reported following Botox injections for individuals with SCI and NDO with DSD\textsuperscript{42}. In our study participants self-reported no longer needing to wear a condom catheter during the night or briefs due to UI, greater control and consistency over bladder and CICs, discontinuation of “annoying” anticholinergic medications which were reported to cause “constipation, dry mouth, dry eyes, fatigue, and nausea”. One participant exclaimed: “I got to wear my underpants to bed for the first time in almost 10 years”. Other reports included the ability to “finally get a good night sleep” and “sleep through the night”, due to not having to wake up in the middle of the night to perform a CIC.

Following Botox treatment complete continence was reported by 86% which was similar to reports in the study by Schurch where continence was reported in 89% at the 6-week UDS follow-up following 200 U of Botox treatment for NDO in SCI persons\textsuperscript{40}. In the same study, UI was attributed as the leading reason for improvements in bladder-related QoL. Following Botox treatment, three individuals self-reported a reduction in the number of CICs. This suggests that improvements in consistency due to a reduction in unnecessary CIC may decrease the risk of hazards imposed upon the urethra and bladder and also reduce the incidence of bacteria being introduced to the bladder to cause a UTI.

Participant satisfaction is an indicator of whether participants believe a treatment to be worthwhile and if they would chose the same treatment again\textsuperscript{267}. A significant number of participants involved in this study reported a high level of satisfaction in both bladder and cardiovascular function following Botox treatment. In fact, a total of 36% of participants stated that Botox was “life changing” and one participant is quoted as saying “I am the happiest I have
ever been over the past 20 years now that my AD and bladder is fixed”. After receiving Botox treatment, many of the study participants stated that they wished they had known about Botox sooner due to the positive and dramatic impact on their QoL. Results from this study indicate that Botox has a significant influence over health-related and bladder-related QoL.

5.4.1 Limitations

With any questionnaire, there is the potential for self-report bias where participants are aware of the expected outcomes following Botox treatment, and therefore may be more apt to provide responses in favour of the desired outcomes.

5.5 Bladder function parameters

Following Botox treatment, for all three time points during UDS assessment, there was a reduction in sensation of urgency which is supported by evidence demonstrating the influence Botox exerts over the denervation of afferent sensory impulses and efferent motor impulses\(^ {33}\). It is recommended as a standard of clinical practice that Pves should be maintained < 40 cm H\(_2\)O in order to prevent vesicoureteral reflux and to preserve the UUT and prevent damage to the kidneys\(^ {167,268}\). Prior to the Botox treatment, Pves at first urge to perform CIC, at maximum SBP and at maximum infusion were all above the clinically recommended standards with one maximum Pves reaching a dangerously high level of 82 cmH\(_2\)O. Following Botox treatment all Pves were significantly reduced and maintained well below the suggested clinically safe range for protection of the UUT, even at maximum infusion volume, only reaching an average of 34 cm H\(_2\)O or maximum of 45 cm H\(_2\)O.
The results seen in our study with respect to reduction in Pves following Botox treatment are similar to previous literature\textsuperscript{27,39–42,269,270}. However, our study results were found to be in contrast to the UDS results from the study by Chen and colleagues which reported no changes among participants whose AD improved or did not improve\textsuperscript{35}. This can be attributed to their six-month follow-up period versus our one-month follow-up period. In our study, we propose the improvements in Pves to be directly correlated with the reduction in the severity and frequency of AD experienced following Botox treatment as indicated previously with an improvement of 57% of AD cases and a decrease in amplitude by 37% in the remaining participants. As Botox reduces Pves leading to an increase in bladder capacity and compliance it allows the bladder to function as it normally should, holding volumes equivalent to that of an able-bodied bladder.

Bladder volume was also an important variable to consider following Botox treatment, as bladder distention is a known contributor of AD. There was a significant increase in bladder volume at first urge to conduct CIC, maximum SBP, and at maximum infusion which were similar findings to previously conducted UDS\textsuperscript{27,39–42,269,270}. These increases demonstrate the powerful effects Botox has on improving bladder capacity and compliance but more importantly the powerful benefits Botox exerts over blocking AD from occurring. Before Botox treatment, bladder distension was a defining contributor to the development of AD. However, following Botox treatment, as a result of the reduction in involuntary activation of uninhibited detrusor contractions and subsequent decrease in Pves leading to increased bladder compliance, AD was not elicited and if it was, the amplitude and severity was significantly reduced.
5.6 Future Research

Future research in this field should be aimed at conducting a large randomized, double blind, placebo controlled clinical trial with a control arm. As it would be unethical to have a placebo group not receiving any pharmacological intervention for the treatment of NDO. Individuals would then need to be allocated to one of two groups: Group a) Botox treatment with placebo anticholinergic “sugar” pill, or Group b) non-Botox treatment group treated with a Botox substitute such as lidocaine whilst still taking an anticholinergic medication specifically for NDO. As our study was only able to assess individuals one month following the Botox treatment, future research should be aimed at including more long term evaluation studies of the efficacy Botox exerts on reducing AD severity and frequency during UDS assessments and 24-hr ABPM. From clinical practice it is known that intravesical Botox has an approximated efficacy of an average of nine months. However, we do not know how long the Botox will exert effects on the reduction of sign and symptom severity and frequency of AD following intravesical injections of Botox. It would then be suggested to participants undergo UDS assessment at baseline, six-weeks, and then every three months thereafter up until 1 year post-Botox treatment or until the participant report decreased efficacy and the request for reinjection. Another area that can be further investigated is the development of clinical guidelines on cardiovascular monitoring and education of healthcare providers with respect to the importance of following a BP monitoring protocol during UDS assessment as a key safety measure while conducting UDS.
5.7 Conclusions

In conclusion, the first hypothesis was accepted, as there was a significant reduction in AD severity one month following Botox treatment during UDS. Botox treatment provides a simple and highly effective therapeutic use for the amelioration of AD during UDS in individuals with chronic SCI and NDO. The second hypothesis was also accepted, as there was a significant reduction in severity and frequency of bladder-related AD events as evidenced by 24-hr ABPM. This study demonstrated 24-hr ABPM is a highly effective tool to assess hemodynamic response to a specific intervention and is useful to assess the severity and frequency of AD episodes in individuals with SCI during activities of daily living. The third hypothesis was also accepted as Botox had a positive and meaningful impact on improving the QoL of individuals with SCI. Finally, following Botox we also demonstrated improvements in bladder function parameters including Pves and volume at imperative times where AD would normally occur.

Episodes of AD are associated with life-threatening events among individuals with SCI. Our study demonstrated that Botox treatment for the management of NDO may provide a viable alternative for the successful alleviation of episodes of AD and possibly amelioration of CVD health risks after SCI. Given the significant risks associated with the development of AD, any therapeutic strategy aimed at reducing acute and chronic bouts of AD from occurring in this very special group of individuals with abnormal autonomic control, is not only a high priority for health care providers but can also mean the difference between life or death.
References


Appendices

Appendix A: Autonomic Dysreflexia Health Related-Quality of Life Questionnaire

Please tick check (“√”) the appropriate box.

1. Do you have episodes of autonomic dysreflexia (a condition where your blood pressure rises very fast, usually because of a painful stimulus below the level of your lesion, resulting in symptoms such as severe headache, sweating, excessive spasms, hot/cold flashes, nasal congestion, etc.)?
   □ Yes    □ No    □ Unsure

2. On a Daily basis, how often do you experience the following symptoms?

   a. Excessive sweating above the level of injury:
      □ Frequently □ Sometimes □ Rarely □ Never

   b. Excessive headaches:
      □ Frequently □ Sometimes □ Rarely □ Never

   c. Goosebumps:
      □ Frequently □ Sometimes □ Rarely □ Never

   d. Dizziness:
      □ Frequently □ Sometimes □ Rarely □ Never

   e. Nausea:
      □ Frequently □ Sometimes □ Rarely □ Never

   f. Fatigue:
      □ Frequently □ Sometimes □ Rarely □ Never

   g. Passing Out:
      □ Frequently □ Sometimes □ Rarely □ Never

   h. Light headed:
      □ Frequently □ Sometimes □ Rarely □ Never

   i. Blurred Vision:
      □ Frequently □ Sometimes □ Rarely □ Never
3. When your bladder is full, how often do you ever experience the following symptoms?

a. Excessive sweating above the level of injury:
   - Frequently
   - Sometimes
   - Rarely
   - Never

b. Excessive headaches:
   - Frequently
   - Sometimes
   - Rarely
   - Never

c. Goosebumps:
   - Frequently
   - Sometimes
   - Rarely
   - Never

d. Dizziness:
   - Frequently
   - Sometimes
   - Rarely
   - Never

e. Nausea:
   - Frequently
   - Sometimes
   - Rarely
   - Never

f. Fatigue:
   - Frequently
   - Sometimes
   - Rarely
   - Never

g. Passing Out:
   - Frequently
   - Sometimes
   - Rarely
   - Never

h. Light headed:
   - Frequently
   - Sometimes
   - Rarely
   - Never

i. Blurred Vision:
   - Frequently
   - Sometimes
   - Rarely
   - Never

4. In the following table, please indicate how these experiences affect you:

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Daily Basis</th>
<th>Full Bladder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive sweating above the</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td>level of injury</td>
<td>Moderately</td>
<td>Moderately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Mildly</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>Never</td>
</tr>
<tr>
<td>Goosebumps</td>
<td>Severe</td>
<td>Severe</td>
</tr>
<tr>
<td></td>
<td>Moderately</td>
<td>Moderately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Mildly</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>Never</td>
</tr>
<tr>
<td>Symptoms</td>
<td>Daily Basis</td>
<td>Full Bladder</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Dizziness</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Modately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Never</td>
</tr>
<tr>
<td>Nausea</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Modately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Never</td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Modately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Never</td>
</tr>
<tr>
<td>Passing out</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Modately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Never</td>
</tr>
<tr>
<td>Light headed</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Modately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Never</td>
</tr>
<tr>
<td>Blurred vision</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe</td>
<td>Modately</td>
</tr>
<tr>
<td></td>
<td>Mildly</td>
<td>Never</td>
</tr>
</tbody>
</table>
4. Do these symptoms interfere with your performance in your daily life? If yes, indicate the order of significance by circling the number (i.e., if you feel dizziness affects your performance the most, circle 9; if you feel that nausea never interferes with you performance, circle 0):

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Interfere with performance?</th>
<th>Ranking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excessive sweating</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Excessive headache</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Goosebumps</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Dizziness</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nausea</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Passing out</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Light headedness</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

5. In a whole, how severe was your most recent episode of AD in the past 2 weeks?

(Not significant) 0 1 2 3 4 5 6 7 8 9 (Most Severe)

6. In a whole, how unpleasant was your episode of AD in the past 2 weeks?

(No unpleasantness) 0 1 2 3 4 5 6 7 8 9 (Most Severe Unpleasantness)

7. In a whole, how frequent did AD happened in the last 2 weeks

- Three or more times per day
- Once a day
- Once a week
- Once every 2 weeks
- Once a month
- Others, please specify
Appendix B: Incontinence-Quality of Life Questionnaire

Avoidance and limiting behavior domain

I worry about not being able to get to the toilet on time
I worry about coughing/sneezing because of my incontinence
I have to be careful standing up from sitting
I worry about where toilets are in new places
It’s important for me to make frequent trips to the toilet
It’s important to plan every detail in advance because of my incontinence
I have difficulty getting a good night’s sleep because of my incontinence
I have to watch how much I drink because of my incontinence

Psychosocial impacts domain

I feel depressed because of my incontinence
I don’t feel free to leave home for long periods because of my incontinence
I feel frustrated because my incontinence prevents me doing what I want
My incontinence is always on my mind
My incontinence makes me feel unhealthy
My incontinence makes me feel helpless
I get less enjoyment out of life because of my incontinence
My incontinence limits my choice of clothing
I worry about having sex because of my incontinence

Social embarrassment domain

I worry about others smelling urine on me
I worry about my incontinence getting worse as I get older
I worry about being embarrassed or humiliated by my incontinence
I worry about wetting myself
I feel I have no control over my bladder