The role of urodynamics detecting neuropathic bladder dysfunction in non-urological diseases

Ágnes Rita Martonosi M.D.

Ph.D. Thesis

Szeged

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I. PUBLICATIONS

1.1. Publications related to the subject of the thesis

I. **Martonosi ÁR,** Pázmány P, Kiss S, Dembrovszky F, Oštarijaš E, Szabó L.
Urodynamics in Early Diagnosis of Diabetic Bladder Dysfunction in Women: A Systematic Review and Meta-Analysis.
MED SCI MONIT. 2022 Jul 9;28:e937166.
doi: 10.12659/MSM.937166. PMID: 35808810; PMCID: PMC9278270.
Q2, IF: 3.386

II. **Martonosi ÁR,** Pázmány P, Kiss S, Földi M, Zsákai A, Szabó L.
Urine flow acceleration in healthy children: A retrospective cohort study.
NEUROUROL URODYN. 2023 Feb;42(2):463-471.
doi: 10.1002/nau.25123. Epub 2022 Dec 27. PMID: 36573908.
D1, IF: 2.367

III. **Martonosi ÁR**, Pázmány P, Kiss S, Zsákai A, Szabó L.
INvesTigating the Abnormality of detrusor ConTractility by uroflowmetry in diabetic children (INTACT Trial): protocol of a prospective, observational study.
BMJ OPEN. 2022 Nov 14;12(11):e062198.
doi: 10.1136/bmjopen-2022-062198. PMID: 36375985; PMCID: PMC9664277.
Q1, IF: 3.007

1.2. Publications not closely related to the subject of the thesis

I. **Ágnes Rita Martonosi,** Piroska Pázmány, Márió Mikóczi, Diana Molnár, Zsuzsanna Zsófia Szalai, László Szabó. Necrotizing fasciitis and toxic shock syndrome due to Streptococcus pyogenes in a female adolescent – A case report. **J Pediatr Surg Case Rep.** 2023 March;90:102582 doi:10.1016/j.epsc.2023.102582
Q3, IF:-

II. **Martonosi ÁR**, Pázmány P, Fukász Á, Rudolf J, Kovács É, Szakács Z, Szabó L. Differential Diagnostic Challenges in the COVID-19 Pandemic: Renal Abscess After SARS-CoV-2 Infection in a Young Adolescent. **Am J Case Rep.** 2022 Mar 13;23:e935190. doi: 10.12659/AJCR.935190. PMID: 35279666; PMCID: PMC8928230.
Q3, IF:-

III. **Martonosi ÁR**, Soós A, Rumbus Z, Hegyi P, Izsák V, Pázmány P, Imrei M, Váncsa S, Szakács Z, Párniczky A. Non-invasive Diagnostic Tests in Cystic Fibrosis-Related Liver Disease: A Diagnostic Test Accuracy Network Meta-Analysis. **Front Med (Lausanne).** 2021 Jul 27;8:598382. doi: 10.3389/fmed.2021.598382. PMID: 34386504; PMCID: PMC8353091.
Q1, IF: 5.058

IV. Veres Klára, Gál Andrea Izabella, Szabó András, Szentirmai Réka, Zsigmond Borbála, **Martonosi Ágnes Rita**, Szalai Zsuzsanna Zsófia. A Heim Pál Országos Gyermekgyógyászati Intézet Bőrgyógyászatán észlelt SARS-CoV-2 infekcióval kapcsolatos esetek ismertetése és irodalmi áttekintés. **Bőrgyógyászati és Venerológiai Szemle.** 2021 97. évf. 1. 36-44. doi:10.7188/bvsz.2021.97.1.5

V. Izsák VD, Soós A, Szakács Z, Hegyi P, Juhász MF, Varannai O, **Martonosi ÁR**, Földi M, Kozma A, Vajda Z, Shaw JA, Párniczky A. Screening Methods for Diagnosing Cystic Fibrosis-Related Diabetes: A Network Meta-Analysis of Diagnostic Accuracy Studies. **Biomolecules.** 2021 Mar 31;11(4):520. doi: 10.3390/biom11040520. PMID: 33807165; PMCID: PMC8065857.

Q2, IF: 6.064

VI. Juhász MF, Varannai O, Németh D, Szakács Z, Kiss S, Izsák VD, **Martonosi ÁR**, Hegyi P, Párniczky A. Vitamin D supplementation in patients with cystic fibrosis: A systematic review and meta-analysis. **J Cyst Fibros.** 2020 Dec 18:S1569-1993(20)30940-1. doi: 10.1016/j.jcf.2020.12.008. Epub ahead of print. PMID: 33349585.
D1, IF: 5.527

VII. **Martonosi Ágnes Rita**, Scheuring Noémi, Karoliny Anna, Lőrincz Margit: Szondatáplálásra szoruló öt hónapos csecsemő táplálási zavarának kezelése. **Gyermekgyógyászat.** 2018; 69. évfolyam, 3. szám, 181-185. oldal

1.3. Scientific metrics

Number of publications **related to the subject of the thesis**: 3 (3 first author)
Cumulative impact factor of publications related to the thesis: 8.76
D1: 1, Q1: 1, Q2: 1, Q3: 0, Q4: 0

Number of **total accepted/published articles**: 10 (7 first author)
Cumulative impact factor of the published articles: 25.409
D1: 2, Q1: 2, Q2: 2, Q3: 2, Q4: 0

Number of total citations by **MTM2**: 6
6 independent

Hirsch Index: 1
MTMT ID: 10040200
https://m2.mtmt.hu/api/author/10040200/summary

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II. LIST OF ABBREVIATIONS

ADA	American Diabetes Association
ÁRM	Ágnes Rita Martonosi
BMI	body mass index
BOO	bladder outlet obstruction
BPH	benign prostate hyperplasia
CAD	cardiovascular autonomic dysfunction
CAN	cardiovascular autonomic neuropathy
CENTRAL	Cochrane Central Register of Controlled Trials
CFRD	cystic fibrosis – related diabetes
CI	confidence interval
DC	diabetic cystopathy
DM	diabetes mellitus
ECG	electrocardiography
GCP	good clinical practice
GFR	glomerular filtration rate
HgA1c	haemoglobin A1c
HIV	human immunodeficiency virus
HOGYI	Heim Pál National Pediatric Institute
ICMJE	international committee of medical journal editors
INTACT Trial	INvesTigating the Abnormality of detrusor ConTractility Trial
IEC	independent expert committee
LUT	lower urinary tract
LUTS	lower urinary tract symptoms
MCC	maximum cystometic capacity
mL	millilitre
$P_{det}Q_{max}$	maximal detrusor pressure at maximal flow rate
PI	principal investigator
PP	Piroska Pázmány
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
Q_{acc}	urine flow acceleration (Q_{max}/TQ_{max})

Q_{ave}	average urine flow rate
Q_{max}	maximum urine flow rate
QTc	corrected QT interval
QUIPS	QUality In Prognostic Studies
REML	restricted maximum-likelihood
SC	steering committee
SD	standard deviation
SDNN	standard deviation of the normal-to-normal interval
SE	sensitivity
SEC	seconds
SLE	systemic lupus erythematosus
SP	specificity
SPIRIT	Standard Protocol Items: Recommendation for Interventional Trials
STROBE	sTrengthening the Reporting of OBservational studies in Epidemiology
SUPPL	supplementary
TNF	tumour necrosis factor
TQ_{max}	time to maximum flow rate
κ	Cohen's kappa

III. INTRODUCTION AND THE AIM OF THE PHD THESIS

3.1. URODYNAMICS

Urodynamic studies are functional tests that assess pressure-flow relationship of the upper and lower urinary tract [1-10]. The main purpose of urodynamics is to evaluate the mechanisms of lower urinary tract function and dysfunction. Types of urodynamic testing are reported in *Supplementary (Suppl.) Table 1.*

Uroflowmetry is an essential, non-invasive, easy-to-use, widely accessible, and quick urodynamic diagnostic tool in the evaluation of voiding function [7, 11-13]. It measures the amount of voided urine [in millilitres (mL)] per unit of time (in sec). Urine flow rate measurements are generally used to determine lower urinary tract (LUT) function and dysfunction. The elements of uroflowmetry examinations are (1) voided volume (measured in mL), (2) voiding time (measured in sec), (3) maximum or peak urine flow rate (Q_{max}, measured in mL/sec), (4) average urine flow rate (Q_{ave}, measured in mL/sec), and (5) time to maximum flow rate (TQ_{max}, measured in sec.) Acceleration of the detrusor muscle contraction (Q_{acc}) is a calculated uroflowmetry value, which is the ratio of Q_{max} and TQ_{max}, measured in mL/sec^2; and refers to the increased flow rate in a period of time from the beginning of urination to the peak value.

Although Q_{acc} characterizes the bladder function, only a few studies have addressed it [14-19]. Q_{acc} may be increased in children with urgent and frequent micturition, as well as with urge incontinence. Possible damage in the detrusor muscle function may also impair Q_{acc}, therefore a change or a decrease in the acceleration of the detrusor muscle contraction can be an early sign of the damage in the muscle and/or of the innervation. Q_{acc} may be changed in outflow obstruction, such as in benign prostate hyperplasia (BPH). Q_{acc} has been found to be superior to Q_{max} in the diagnosis of bladder outlet obstruction (BOO) in adult men with BPH [14]. Furthermore, Q_{acc} may be reduced if a patient has autonomic neuropathy [e. g. diabetic cystopathy (DC)][15]. The relationship between Q_{max} and Q_{acc} may characterize the urinary tract disfunction. In urinary tract obstruction, Q_{max} decreases while Q_{acc} remains normal. In bladder dysfunction caused by the impair of detrusor muscle contraction (e.g. in autonomic neuropathy), both Q_{max} and Q_{acc} decrease. Therefore, Q_{acc} (in combination with Q_{max}) might replace invasive pressure-flow studies.

3.2. NEUROPATHIES AND THEIR EFFECT ON BLADDER FUNCTION

3.2.1. Types of peripheral neuropathies

Neuropathy is one of the most bothersome and diverse neurological conditions which is caused by damage to the nerves of peripheral nervous system. The damage can impair sensation, movement, gland, or organ function [20]. Neuropathy can disrupt several physiological processes impairing the quality of life. It is mainly a part or a complication of different diseases, not an independent condition itself. There are hundreds of diseases or conditions that can lead to nerve damage, which include chronic alcoholic and non-alcoholic liver diseases, chronic kidney or metabolic disorders [diabetes mellitus (DM), hyperlipidaemia etc.], haematological or neurological conditions, infections [human immunodeficiency virus (HIV), syphilis], toxins (chemotherapy, drug-induced, alcohol), nutritional deficiencies (vitamin B12, vitamin E, copper), immune-mediated diseases [systemic sclerosis, sarcoidosis, systemic lupus erythematosus (SLE), Sjögren syndrome, celiac disease], polyneuropathies arising from allergic reactions, and hereditary diseases [21-31]. Diabetic neuropathy is highlighted the most by its clinical and prognostic significance which affects one third of the patients with neuropathy, although its pathomechanism still remains poorly understood [32-34].

Neuropathies can be categorized according to the types of the affected nerve fibre (sensory, motor, or autonomic neuropathy), the number and distribution of nerves (mononeuropathy, mononeuritis multiplex, or polyneuropathy), or the process affecting the nerves (compression, inflammation, or toxic). They can be acute or chronic, and may be reversible or permanent [35].

3.2.2. Sensory and motor neuropathies

Sensory neuropathy can result in loss of sensation throughout the body, but mainly on the hands or feet. It is classified by the size of the nerves (small and large fibre neuropathy). The aetiology of sensory neuropathy includes immune-mediated, metabolic, nutritional deficiencies, toxic, infectious, and hereditary diseases [24, 36].

Motor nerve neuropathy is most commonly associated with muscle weakness, muscle cramps, muscle shrinking, and fasciculations.

Sensory and motor peripheral neuropathies are one of the most bothersome complications of type 2 DM, with a global prevalence of 35.78% (in Europe 48.14%) among adult patients, and

2.6–11% among children [37-42]. The most common form is symmetric generalized polyneuropathy which is a well-known microvascular complication of type 2 DM. The diagnosis of peripheral neuropathy is based on nerve conduction tests and electromyography.

3.2.3. Autonomic neuropathy

3.2.3.1. Autonomic neuropathy

Autonomic neuropathy is caused by the damage of autonomic nerves, which can manifest in cardiovascular (disturbances of heart rate, orthostatic hypotension), genitourinary (impair of the detrusor muscle function), gastrointestinal (dysphagia, vomiting, abdominal pain, malabsorption), thermoregulatory, pupillomotor and sudomotor symptoms [43-45]. DM is a major cause of autonomic neuropathy with an increased risk of morbidity and mortality [46-48]. Autoimmune diseases (rheumatoid arthritis, SLE, Sjögren), toxins, infections (HIV, Lyme, botulism), neurodegenerative diseases (Parkinson's) are the most common cause of autonomic neuropathy. The diagnosis is based on laboratory, electrophysiological testing, and most importantly on the sensitive and reproducible measures of autonomic function [49, 50].

3.2.3.2. Cardiovascular autonomic neuropathy

Cardiovascular autonomic neuropathy (CAN), which occurs in 2.5–90% of diabetic patients [51], is associated with abnormalities of vascular dynamics and heart rate control [52-56]. The clinical symptoms might vary from tachycardia, orthostasis to myocardial infarction [51, 57]. Diagnosis is based on sympathetic and vagal autonomic function examinations [58, 59]. The gold standard examinations are cardiac autonomic reflex tests, including heart rate, blood pressure and sudomotor responses [60-64]. CAN may be assessed by measuring heart-rate variability [65-67], and by the five reproducible and standardised cardiovascular reflex tests described by Ewing et al in 1980 [60, 68-70]. Three of the five tests assess parasympathetic function: heart rate response to deep breathing, to standing, and the Valsalva manoeuvre. Two tests evaluate sympathetic function, which are blood pressure responses from lying to standing and at sustained handgrip. The characteristics of cardiovascular autonomic dysfunction tests are demonstrated in *Suppl. Table 2.*

3.2.3.3. Diabetic cystopathy

Diabetes and urological problems are common and have a prominent effect on the quality of life. DC is a well-recognized urological complication of diabetic autonomic neuropathy [71, 72] which occurs in 25–90% of patients [73]. It can impair the detrusor muscle function, leading to LUT problems [74, 75]. The pathogenesis of diabetes induced bladder dysfunction is multifactorial: (1) alterations in detrusor muscle physiology, (2) neuronal impairment, and (3) urothelial dysfunctions are the main participating factors [76]. The classic triad of DC is decreased bladder sensation, impaired bladder emptying with postvoid residual volume, and increased bladder capacity [77-79]. The earliest manifestation is impaired sensation that increases the threshold for initiating the micturition reflex, which is followed by a decreased detrusor activity. It causes incomplete bladder emptying, consequently increased postvoid residual volume, lower Q_{max}, bladder overdistension, and ultimately urinary retention. Therefore, patients usually have overactive bladder or overflow incontinence, including urinary frequency, urgency, incontinence, and nocturia [77, 78, 80, 81]; which are listed among the lower urinary tract symptoms (LUTS) [81, 82]. DC gradually progresses over time from an initial compensated (typically manifests as overactive bladder) to a later decompensated (typically manifests as underactive bladder) phase that cause storage or voiding LUTS [83, 84]. Assessment of DC includes urodynamic measurements [85] and LUT symptoms questionnaires [86].

3.3. BLADDER DYSFUNCTION IN NON-UROLOGICAL DISEASES

Micturition is a well-coordinated process that starts with sensation of bladder filling, data is then transmitted to the central nervous system, processed by the centre and efferent pathways, resulting in a voiding response. The bladder has dual function: it stores urine at a low pressure, and empties it at a specific time at a high pressure. During the normal storage phase, the bladder is continuously filled with urine, and at a given moment sensation of bladder fullness appears, followed by the urge of urination [72].

The detrusor muscle is a smooth muscle that is located within the walls of the bladder, and allows the bladder to contract and excrete urine, as well as to relax or hold urine. The detrusor muscle is continuous with the internal urethral sphincter. The combination of detrusor muscle contraction and urethral sphincter relaxation leads to urination. The detrusor muscle is

controlled by the autonomic nervous system. The parasympathetic nervous system stimulates M3 muscarinic stretch receptors in the bladder walls through the pelvic nerve fibres, which leads to the contraction of the detrusor muscle for urination. The sympathetic nervous system stimulates beta-3 receptors in the bladder which causes relaxation of the detrusor muscle. Sensory fibres detect pain from overdistention [87].

Detrusor muscle pathology can lead to urinary retention, incontinence, or a combination of them. Damage to the detrusor muscle from chronic overdistention can lead to fibrosis of the muscle with weakness in the contraction. It is well-known that loss of sensation leads to bladder distension with an increased bladder capacity. DM and neurological conditions (stroke, multiple sclerosis etc.) are the leading causes of detrusor nerve control degeneration. In long-standing DM, bladder emptying is incomplete because of impaired detrusor activity and failure of the internal sphincter to open adequately, and urine flow is reduced. Immunomodulatory drugs e.g. cytotoxic agents (doxorubicin, cyclophosphamide, platinum-based antineoplastic agents, vinca alkaloids, epothilones, taxanes, proteasome inhibitors etc.) [21, 88, 89] can also damage the detrusor muscle function. Untreated abnormalities of the detrusor muscle can lead to deterioration of the upper urinary tracts as well.

3.4. AIM OF THE RESEARCH AND PHD THESIS

Our hypotheses are that (1) urodynamics can provide a fine and early diagnosis of bladder dysfunction not only in urological, but also in non-urological diseases; (2) urodynamics are underused in the diagnosis of non-urological diseases (emphasising the lack of use in diabetes care in Hungary); (3) Q_{acc} has not been widely used in the diagnosis of urological diseases and not known in the diagnosis of non-urological diseases; and (4) DC is understudied compared to other diabetic complications.

Therefore, our main aim is to assess the use of urodynamics in non-urological diseases. Under the research, we were focusing on autonomic neuropathy caused by different non-urological diseases (especially DM) since recent studies suggest that uroflowmetry might determine autonomic neuropathy earlier than CAN symptoms occur [15, 90, 91]. Until now, Q_{acc} was a less frequently adapted uroflowmetry parameter, but as previous studies suggest that it might indicate the deviation of detrusor muscle function earlier than other uroflowmetry parameters (e.g. Q_{max}, Q_{ave}), therefore it seems to be a better indicator of diabetic autonomic neuropathy [15] than cardiovascular dysfunction tests (Ewing tests) [60]. Since DC can reduce the quality

of life, it is urgent to be addressed before resulting in complications. Only a few urodynamic studies have been performed in non-urological diseases so far (in particular to investigate DC [71, 80, 92-95]); and furthermore, traditional uroflowmetry parameters (Q_{max}, Q_{ave}) are not sufficient enough to confirm autonomic neuropathy, and Q_{acc} is not widely used. Therefore, in order to prove our hypothesis, firstly we wanted to compare the urodynamic parameters of diabetic women and healthy controls, as the literature on diabetic women is scant.

To do so, a meta-analysis was performed which is a well-known statistical analysis to systematically synthetize the findings of single studies [96-98].

Normative reference values of bladder function in healthy adult women [99, 100], and in healthy paediatric population [101, 102] have been widely studied. Since there is no consensus on the cut-off values of Q_{acc} which limits the use of it, our second aim was to establish normal ranges of urine flow acceleration in both genders by a wide range of voided volumes, in order to assess the difference of Q_{acc} values between diabetic and healthy population.

And finally, we designed a clinical trial with diabetic and healthy children to evaluate the diagnostic accuracy [sensitivity (Se), specificity (Sp), positive and negative predictive value] of uroflowmetry in the detection of autonomic neuropathy.

We believe that the results of urodynamic studies in DC and autonomic neuropathy will support the early diagnosis of autonomic neuropathy in other non-urological diseases as well.

IV. METHDOS

4.1. CHAPTER I: META-ANALYSIS

4.1.1. Search strategy

The meta-analysis was reported as per the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 Statement [97], and the study was in line with the protocol registered on 23 May 2021 in PROSPERO (CRD42021256275).

Our primary aim was to compare the urodynamic parameters of diabetic women to those of healthy women, but the eligible studies according to our inclusion criteria did not provide sufficient raw data on healthy female population. Since a direct comparison could not be implemented in diabetic and non-diabetic patients, we conducted a single-arm meta-analysis and positive event rates were pooled for statistical analysis.

Furthermore, our intent was to detect changes in voiding in diabetic women with cystopathy compared to diabetic women without peripheral neuropathy by uroflowmetry, but the eligible studies did not contain sufficient raw data to conduct the analysis. Otherwise, we fully adhered to the study protocol.

Two review authors [Ágnes Rita Martonosi (ÁRM) and Piroska Pázmány (PP)] independently carried out the systematic literature search in Embase, MEDLINE (via PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL), and the Web of Science. The authors applied the following keywords: (urodynamic or uroflow* or uroflowmetry) and (diabetes or diab*) on 04 November 2021. In each database, they applied “All text” or “All fields” in the search bar avoiding any filters or restrictions regarding publication year, language and place of origin. They reviewed the included studies to find any articles previously missed in the original search.

4.1.2. Selection and eligibility

The authors included case-control and cohort studies, as well as full-text articles and conference abstracts in the synthesis of quantitative and qualitative data to reduce selection bias. Case reports featuring single patients were excluded. In case of potentially overlapping study populations (based on authors, sites, patients and urodynamic parameters), those with better quality of data were included.

Eligible studies had to provide data on diabetic women, with or without voiding disorder, and urodynamic parameters [13], which include uroflowmetry and cystometry parameters [103, 104]. Regardless of the method of measurement (with ultrasonography or via catheter), postvoid residual volume (in mL) was also included in the analysis. Studies with both sexes were included if they performed subgroup analysis for diabetic women.

Articles examining patients after kidney transplantation or surgery affecting the genitourinary tract, patients with neurogenic bladder dysfunction and other neurological disease (e.g. progressive neurological conditions such as Parkinson's disease, dementia, multiple sclerosis etc.) except peripheral neuropathy; pregnant women and women within first 6 months of postpartum, end-stage kidney disease or kidney transplants were excluded, since they can influence urodynamic parameters per se, causing indistinguishable confounding factor.

The records were selected via a standard three-phased process including titles, abstracts and full-texts independently by the 2 review authors (ÁRM and PP) with EndNote X9.1.1 software (2020 Clarivate™ Analytics, Philadelphia, PA, USA). They resolved any disagreements occurred in any phase through consensus, and calculated the Cohen's kappa (κ) as well in each phase to test interrater reliability.

4.1.3. Data extraction

The 2 independent review authors (ÁRM and PP) extracted the data into a purpose-designed data collection table, with any disagreements resolved by consensus. The following data were extracted from each study: (1) study information (first author, year of publication, recruitment period, country of origin); (2) study design and methodology (retrospective versus prospective, inclusion and exclusion criteria, single versus multicentre study); (3) patient information (number of patients, mean age, definition of diabetes, type of diabetes, diabetes duration, body mass index (BMI), haemoglobin A1c (HgA1c) values, diabetes treatment, diabetic complication); (4) LUTS assessment and symptoms, DC and peripheral neuropathy existence; and (5) urodynamic parameters of diabetic patients [voided volume, postvoid residual, Q_{max}, maximal detrusor pressure at maximal flow rate ($P_{det}Q_{max}$,) volume at first sensation, and maximal cystometric capacity (MCC)].

4.1.4. Statistical analysis

κ was calculated to test interrater reliability. It ranges from -1 to +1, where values ≤ 0 indicates no agreement (which is unlikely in practice), 0 represents the amount of agreement that can be expected from random chance, and 1 represents perfect agreement between the review authors. κ results should be interpreted as the followings: values ≤ 0 as indicating "no agreement", 0.01–0.20 as "none to slight", 0.21–0.40 as "fair", 0.41– 0.60 as "moderate," 0.61–0.80 as "substantial", and 0.81–1.00 as "almost perfect" agreement [105].

For data synthesis, the random-effects model with restricted maximum-likelihood (REML) estimation was used in all cases; means and 95% confidence intervals (CIs) were calculated. The calculated effect sizes were visualized in forest plots. Heterogeneity was tested using Cochrane's Q and the I^2 statistics. I^2 statistic represents the percentage of the total variability across studies: 30% to 60%, 50% to 90%, and 75% to 100% corresponded to "moderate", "substantial" and "considerable" degrees of heterogeneity, respectively, based on the Cochrane's handbook for Systematic Reviews of Interventions [106]. Q test was considered significant if $p<0.1$. Statistical analyses were carried out using R statistical software (version 4.0.5) and package *meta (*version 4.18-1). A single-arm meta-analysis was created based on urodynamic parameters, and the results were graphically presented on forest plots.

4.1.5. Risk of bias assessment

The risk of bias of the studies were evaluated by 2 independent review authors (ÁRM and PP) using the Quality In Prognostic Studies (QUIPS) tool [107]. The result of the assessment was graphically demonstrated; any disagreements were resolved by consensus among the review authors.

To judge overall risk, the review authors (ÁRM and PP) described studies with a low risk of bias as those in which at least 5 of the 6 important bias domains [(1) study participants, (2) study attrition, (3) prognostic factor measurement, (4) outcome measurement, (5) study confounding, and (6) statistical analysis] were rated as having low risk of bias. If there was at least 1 domain rated as high risk, or more than 3 domains rated as moderate risk of bias, the overall risk of bias was deemed high. All other variations were determined as moderate risk of bias.

When the study design could not be identified, study attrition was not evaluated and was thus labelled as 'grey'. Grey means not applicable or not reported.

There were not enough studies in the analyses to evaluate publication bias by funnel plots and statistical tests.

4.2. CHAPTER II: URINE FLOW ACCELERATION NOMOGRAMS

4.2.1. Study design and eligibility

A single-centre, retrospective cohort study was conducted with 270 healthy children and adolescents. The study protocol was based on the previously designed study [101]. Age (years), gender (boys/girls), weight (kg), and height (cm) were collected. Each child urinated multiple times (spontaneously in calm conditions, at first desire to void after 15 mL/kg liquid consumption, and at maximal sensation of bladder fullness). Reliability of the tests was not examined as the children's first urination occurred upon arrival at the Urodynamics Laboratory, and the second urination occurred at the first urge to urinate after 15 mL/kg liquid consumption. Thus, almost everyone voided a different volume during the second urination. There were no children with Q_{acc} values below 10^{th} percentile or above 90^{th} percentile.

Eligibility criteria were healthy children aged between 6 and 18 years, without any acute or chronic (neurological, gastrointestinal, pulmonological, cardiac, urological, nephrological etc.) disease or medicine consumption. Exclusion criteria therefore were the following conditions: (1) acute febrile condition (≥38 °C core temperature) in the past seven days; (2) acute or chronic urinary tract or kidney disease: renal insufficiency (glomerular filtration rate (GFR) ≤ 60 mL/min per 1.73 m^2), urinary tract infection; (3) urological disease: bladder cancer, urolithiasis, urethral stricture, posterior urethral valve, meatal stenosis, previous genitourinary surgery, conditions causing urinary outflow problems (phimosis, hypospadias, vesicoureteral reflux); (4) neurological disorders (multiple sclerosis, transient ischaemic attack, transverse myelitis, myelocele, meningomyelocele, previous spinal cord operation, or operation which might injure the sacral nerve plexus etc.); (5) medicines taken which can cause neuropathy: cytostatic agents, immunosuppressive agents [tumour necrosis factor (TNF) -alfa inhibitors], cardiovascular medicines (statins, digoxin, amiodaron), antimicrobial agents (nitrofurantoin, linezolid, voriconazole, itraconazole, antituberculotics, metronidazole, fluoroquinolone), anti-ulcerative agents (cimetidin), neuropsychological agents (levodopa, phenytoin); (6) psychiatric disorders

that prevent participation/collaboration in the study; (7) constipation; (8) patients who are pregnant or gave birth in the last 12 months; (9) voided volume less than 20 mL; and (10) postvoid residual more than 15% of voided volume [102, 108]. Children who had LUTS were not enrolled as well.

During spontaneous urination, children voided smaller volumes (20-50 mL) with lower Q_{ave} and Q_{max} values. In 7.9% of the total micturition, either Q_{max} or Q_{ave} was less than 5 mL/sec. According to the normal nomograms [101], the values of Q_{ave} 2.5 mL/sec and Q_{max} 5 mL/sec are above 5% (between 5-10 percentile values), which means they are within the normal range. The first step of the study's protocol, children were asked to void spontaneously regardless the need of urination. After that, children voided when they felt the first sensation of bladder filling (after drinking 15 mL/kg liquid), which were clearly larger volumes with higher Q_{max} and Q_{ave} values. So they did not void small amounts all the time, which means they did not have a small bladder capacity. Therefore, we think these small voided volumes with lower Q_{max} and Q_{ave} values are acceptable. Given there were only a few numbers of voiding, which does not change the normal values of Q_{acc}, we did not exclude these urinations from the analysis to reduce selection bias. These data confirm our hypothesis that at spontaneous voiding, healthy younger children void smaller amounts of volumes with lower Q_{max} and Q_{ave} values.

Since Q_{max} and Q_{ave} values have already been presented (Miskolc Nomograms [101]), and our current study was focused on Q_{acc} values, we did not wish to publish Q_{max} and Q_{ave} values again. In our previous study [101] Q_{ave} and Q_{max} values were divided into three body surfaces (<0.92 m^2, between 0.92 and 1.42 m^2, and > 1.42 m^2) and not into age (basically younger children have lower body surfaces). As we did not want to deviate from the original study protocol, we planned to have a subgroup analysis based on body surfaces. Unfortunately, due to the low number of children in each subgroup – according to the sample size calculation – data were not enough to make valid analyses.

The study was reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) 2004 Statement [109]. The study was approved by the Heim Pál National Paediatric Institute's Local Ethics Committee (registration number: KUT-37/2021, Date: 23 July, 2021). The data were obtained from a previous uroflow study [101] carried out between 1990–1992 at Child Health Centre, Borsod Abaúj-Zemplén County Teaching Hospital, Miskolc, Hungary. Some part of the data have been published previously [101], but acceleration

values have not been reported so far. The research and methodology used for data collection are explained in detail in the previous study [101].

4.2.2. Measurements and outcomes

Urinary bladder function was assessed by uroflowmetry, and postvoid residual volume was detected by ultrasonography. All examinations were performed by László Szabó (nephrologist and urodynamic specialist). Data were collected using a targeted questionnaire, each measurement was documented by László Szabó. Uroflowmetry was performed using a Uroflow-cystometer (X0002, Metripond, Hungary) which determined Q_{max}, Q_{ave} and TQ_{max}. Voided volume (mL), voiding time (sec), Q_{max}, Q_{ave} (mL/sec), and TQ_{max} (sec) were measured; Q_{acc} (mL/sec^2) was calculated. Q_{max} and Q_{ave} were defined according to the International Children's Continence Society [8]. Voided volume was measured manually using a graduated cylinder; boys voided in a standing, girls in a sitting position. Ultrasound was accomplished before and after micturition. Scans were obtained with a real time ultrasound scanner (Hitachi EUB 40.5 MHz transducer) using a direct scanning technique. Postvoid bladder diameter (mm) was measured by ultrasonography and converted to bladder residual volume (mL). Manual data collection was turned into a digital form which was carried out by ÁRM, data were tabulated and encoded. Data validation was conducted by PP, Mária Földi and Szabolcs Kiss. All patients' data are stored securely.

Different uroflow parameters can only be compared if voided volumes are the same, since uroflow parameters highly depend on voided volumes. Therefore, children were allocated to one of the two groups by gender, and then Q_{acc} was determined by voided volumes.

4.2.3. Statistical analysis

All descriptive statistic calculations were carried out with MS Excel (version 16.52, Microsoft Corporation, 2019). Quantile method was used to establish the 3–97th percentile levels with SPSS statistical software package (version 25.0, Armonk, NY: IBM Corporation, US). For presentation, the nomograms were expressed in centile forms, prepared for girls and boys separately. The centile curves of acceleration by voided volume were estimated by using lmsChartMaker Pro 2.3 software (Medical Research Council, UK 1997–2006; Cole and Green 1994; Cole and Pan 2004) based on the LMS method [110]. Normal nomograms of Q_{acc} in paediatric population were visualized in percentile forms and graphically as well. When data

show normal distribution mean ± standard deviation (SD), in other cases, median [range] was used. Data imputation was not applied. The sample size (n) was estimated by the following equations [111]:

$$n = \frac{z^2 \times p(1-p)}{e^2};\ n' = \frac{n}{1+\frac{z^2 \times p(1-p)}{e^2 \times N}}$$

, where z: z score (z = 1.96 for 95% confidence level), e: margin of error, N: population size of healthy children aged between 6 and 18 years, p: population proportion by using with confidence level = 95%, margin error = 5%, population proportion = 85% (healthy children were selected for the nomogram construction, proportion value was set by considering all the illnesses, diseases, medications and health status conditions that can influence uroflowmetry parameters in children) and population size = 1 827 520 (Hungarian Central Statistical Office, data for healthy children aged between 6 and 18 years in Hungary: https://www.ksh.hu/docs/hun/xstadat/xstadat_eves/i_zoi002a.html). The estimation revealed that a sample size of at least 196 healthy children (aged between 6 and 18 years) would be necessary for the study.

4.3. CHAPTER III: INTACT STUDY

4.3.1. Study design and patient enrolment

It is a prospective, observational, single-centre clinical trial. The study protocol is constructed in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) 2013 Statement [112].

The inclusion criteria are children aged 5–18 years (boys, girls) with type 1, type 2 and monogenic DM who are treated at the Endocrinology Department and Outpatient Clinic of Heim Pál National Pediatric Institute (HOGYI, Budapest, Hungary) will be enrolled. The definition of diabetes is based on the American Diabetes Association (ADA) criteria [113].

Healthy volunteer children aged 5–18 years (boys and girls) without any acute or chronic disease will be enrolled in the control group, and the same tests will be performed on them as on diabetic children. Control subjects will be recruited by the HOGYI's Volunteer Recruiting Program in kindergartens and schools.

Diabetic children with the following conditions will be excluded from the study: (1) acute febrile condition (≥38 °C core temperature) in the past seven days; (2) acute or chronic urinary

tract or kidney disease: renal insufficiency (GFR ≤ 60 mL/min per 1.73 m^2 [114]), urinary tract infection; (3) urological disease: bladder cancer, urolithiasis, urethral stricture, posterior urethral valve, meatal stenosis, previous genitourinary surgery, conditions causing urinary outflow problems (phimosis, hypospadias, vesicoureteral reflux); (4) cystic fibrosis-related diabetes (CFRD); (5) neurological disorders (multiple sclerosis, transient ischaemic attack, transverse myelitis, myelocele, meningomyelocele, previous spinal cord operation, or operation which might injure the sacral nerve plexus); (6) medicines taken which can cause neuropathy [115]: immunomodulatory drugs such as Immunosuppressive agents (TNF-alfa inhibitors (adalimumab, infliximab, etanercept), interferon), Cytostatic agents (cyclophosphamide, platinum-based antineoplastic agents, vinca alkaloids, epothilones, taxanes, proteasome inhibitors [21]), Cardiovascular medicines (statins, digoxin, amiodaron), Antimicrobial agents (nitrofurantoin, linezolid, voriconazole, itraconazole, antituberculotics, metronidazole, fluoroquinolone), Anti-ulcerative agent (cimetidin), and Neuropsychological agents (levodopa, phenytoin); (7) psychiatric disorders that prevent participation/collaboration in the study; (8) constipation (defined according to the Rome IV criteria [116]); (9) voided volume <20 mL; (10) patients who are pregnant or gave birth in the last 12 months; (11) lack of consent of the patient or legal representative; or the patient or legal representative withdraws his or her voluntary consent during the study.

Children with voided volume <20 mL, and postvoid residual volume >15% of the voided volume will be excluded in the healthy control group.

All patients who meet the inclusion criteria will be informed of the possibility of taking part in the INvesTigating the Abnormality of detrusor ConTractility Trial (INTACT Trial). Informed consent will be signed by the patient, or in the event of incapacitated status, by the patient's legal guardian; for details, see *Suppl. Table 3.* The consent will be obtained by the Steering Committee (SC) members. Those enrolled in the study will be monitored yearly regardless they reach the age limit of 18 years during the recruitment period. The flowchart of the participants according to the SPIRIT 2013 statement is demonstrated in *Figure 1.*

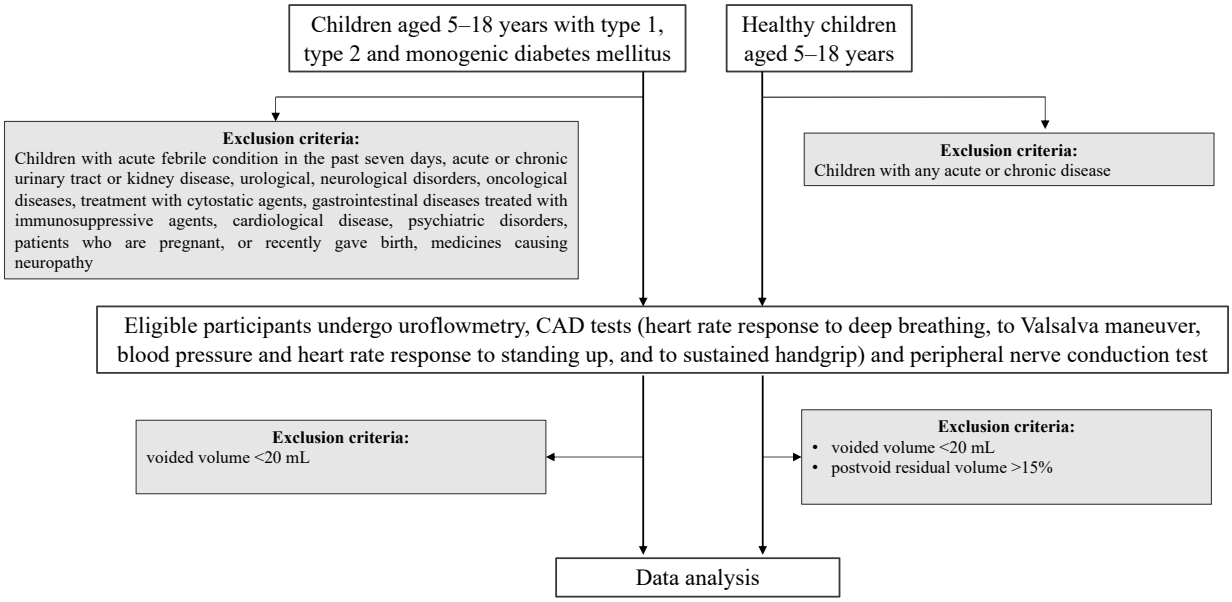


Figure 1. Flow chart of the participants according to the SPIRIT 2013 statement.
CAD, cardiovascular autonomic dysfunction; SPIRIT, Standard Protocol Items: Recommendation for Interventional Trials.

4.3.2. Patient and Public Involvement

Patients will be involved in the design and conduct of this research. During the feasibility stage, the priority of the research question, choice of outcome measures, methods of recruitment and the trial will be informed by a discussion with patients and the patient's legal guardian through a focus group session and a structured interview. Once the trial is published, participants will be informed of the results through a consultation.

4.3.3. Data collection

The questionnaire used in the study is shown in *Suppl. Table 4.*

4.3.3.1. Baseline characteristics

(1) Date of birth, age (years), gender (boys/girls), race (white/black/Indian/Asian/other); (2) weight (kg), height (cm), body surface calculated by Mosteller formula [117], body mass index (BMI) and BMI percentiles; (3) diet, alcohol consumption, and smoking habits; (4) regular medicine consumption (drug active ingredient); (5) physical status, vital parameters [axillary temperature (°C), respiratory rate (respirations/min), oxygen saturation measured by pulse

oximetry (%), heart rate (beats/min), non-invasive blood pressure (mmHg), and capillary refill time (sec)] will be recorded.

4.3.3.2. Clinical symptoms

Urge to urinate (urgency), daytime urine incontinence, nocturnal urination, nocturnal enuresis, frequency of bowel movement, and consistency of stool will be recorded.

4.3.3.3. Diabetes anamnesis

Type of diabetes, time of diagnosis, treatment (oral antidiabetics, diet, insulin), method of insulin administration (subcutaneous injection, pump), use of sensor–pump, the total number of diabetic ketoacidosis, HgA1c value (%), fasting glucose value (mmol/L), and postprandial glucose value (mmol/L) will be recorded.

4.3.3.4. Fluid balance in the past 48 hours

48-hour consumed liquid and outflow fluid flow will be documented.

4.3.3.5. Laboratory parameters

Urine rapid test (*Medi-Test Combi 9+leuko*®), C reactive protein (mg/L), white blood cell count (G/L), absolute neutrophil count, absolute lymphocyte count, red blood cell count (T/L), haemoglobin (g/L and conversion: mmol/L), haematocrit (%), platelet count (G/L), glucose (mmol/L and conversion: mg/dL) blood urea nitrogen (mmol/L and conversion: mg/dL) , creatinine (µmol/L and conversion: mg/dL, carbamide (mg/dL and conversion: mmol/L), estimated glomerular filtration rate (mL/min), aspartate aminotransferase/glutamic- oxaloacetic transaminase (U/L), alanine transaminase/ glutamic pyruvic transaminase (U/L), gamma-glutamyl transferase (U/L), lactate dehydrogenase (U/L), alkaline phosphatase (U/L), natrium (mmol/L), potassium (mmol/L), chloride (mmol/L), calcium (mmol/L), albumin (g/L), serum total protein concentration (g/dL), and C-peptide (ng/mL) will be collected. Cut-off values will be determined by the Department for Laboratory Medicine of HOGYI, Budapest, Hungary.

4.3.3.6. Body composition analysis

Body composition analysis (*InBody*®) – which determines the impedance by age, gender, body type or ethnicity – will be executed. Total body water, lean body mass, dry lean mass, skeletal

muscle mass, and body fat mass will be measured; basal metabolic rate and body fat percentage will be calculated. The device will be calibrated according to the prescribed instructions for use by a skilled technician.

4.3.3.7. Uroflowmetry parameters

Uroflowmetry parameters will be recorded at spontaneous voiding and at the first sensation of bladder filling after 15 mL/kg liquid consumption. Urinary bladder function will be assessed by uroflowmetry, and postvoid residual volume will be detected by ultrasonography (*SonoSite M Turbo®*). Uroflowmetry will be performed using a uroflow-cystometer (*UroDoc Frytech*), which determines Q_{max}, Q_{ave} and TQ_{max}. Voided volume (mL), voiding time (sec), Q_{ave}, Q_{max} (mL/sec), and TQ_{max} (sec) will be measured; Q_{acc} (mL/sec^2) will be calculated. Q_{max} and Q_{ave} are defined according to the International Children's Continence Society [8]. The voided volume will be measured by the uroflow-cystometer device; boys void in a standing, girls in a sitting position. Postvoid bladder diameter (mm) will be measured by ultrasonography and converted to bladder residual volume (mL). The device will be calibrated according to the prescribed instructions for use by a skilled technician. The examinations will take approximately 10 minutes.

4.3.3.8. Cardiovascular autonomic dysfunction tests [51, 60, 68, 69, 118]

Cardiovascular autonomic dysfunction will be assessed by the Ewing tests. The characteristics of cardiovascular autonomic dysfunction tests are demonstrated in *Suppl. Table 2.*
During the examination, electrocardiogram (ECG) and blood pressure values will be recorded continuously with the reflex tests, as well as a 1 min rhythm strip to calculate the standard deviation of the normal-to-normal interval (SDNN). ECG recording will be executed using 1, 6, and 12 leads. The captured ECG will be taken from a recording it creates a moment and from the amount of data stored in 1 min, averaged default value calculation is implemented as the followings: mean heart rate, heart rate distribution, P wave duration, PR and RR interval, QRS duration, ST segment, QT duration, corrected QT interval (QTc) according to the Bazett's formula. Normative values will be reported on the ECG record, as well as the heart rate of the patient.

4.3.3.9. Peripheral neuropathy examination

Peripheral neuropathy will be evaluated by a nerve conduction test. The device measures motor conduction in the lower extremities. It operates at two dedicated frequencies in order to perform a thick myelin sheath cordless fibre (5Hz) and thin myelinated nerve fibre (2000Hz) examination. The device will be calibrated according to the prescribed instructions for use by a skilled technician.

The duration of the cardiovascular autonomic dysfunction examinations is about 15-20 minutes, and the peripheral neuropathy examination (based on the patient's attention) takes 5-10 minutes per limb.

4.3.4. Recruitment period

Recruitment period of the entire study: the planned starting date of the study is March 2022, and the planned completion date is March 2028. During the first year period, all the previously diagnosed and treated diabetic patients, as well as the newly diagnosed ones, will be examined. The metabolic status will be evaluated in parallel with autonomic and peripheral neuropathy. After this period, patients will be followed-up for 5 years (regardless they reach the age of 18 years), and the same tests will be performed on the same individuals who were previously enrolled in the study, and all data (listed in *4.3.3. Data collection* section) will be collected from them. During the 5-year follow-up period, data will be regularly collected every year. The follow-up will be continued even if we find abnormalities either in the peripheral or autonomic neuropathy examinations.

4.3.5. Withdrawal of a subject from the study

Patients will not be included in the per-protocol analysis if: (1) during the trial any exclusion criteria meet; (2) data required for the primary endpoints are missing.

4.3.6. Sample size calculation and power analyses

The trial will start with a pilot period when the first 50 diabetic and 50 healthy children will be assessed. This will be followed by a short evaluation period, during which the principal investigator (PI) and the study team could make adjustments to the study protocol to ensure feasibility. Based on the preliminary data of the pilot period, the investigators plan to carry out

a sample size calculation in order to decide the timing of the interim analyses. Based on the estimated number of items, auditing trial conduct is planned after the first year and every year, on the basis which the SC will suggest changes if necessary.

4.3.7. Data analysis plan and outcomes

The primary endpoint is the diagnostic accuracy (Se, Sp, negative and positive predictive values) of the uroflowmetry test compared to the cardiovascular autonomic dysfunction (CAD) tests in the detection of autonomic neuropathy. The secondary endpoints are (1) the existence of peripheral and autonomic neuropathy in diabetic children in parallel with the metabolic status (prevalence and incidence of peripheral and autonomic neuropathy), (2) differences in metabolic status (weight, height, body surface, BMI, laboratory parameters, body composition), (3) fluid turnover, and (4) clinical symptoms of diabetic patients comparing to healthy children.

All descriptive statistic calculations and analyses will be carried out with MS Excel (version 16.52, Microsoft Corporation (2019) and SPSS (version 24, IBM Corporation 2016). Results will be characterised as either false positive, true positive, false negative or true negative. Using these data, sensitivities, specificities and odds ratios will be calculated. Basic statistical parameters, that is, mean, standard deviation, standard error of the mean, median, minimal and maximum values of the variables for the groups of diabetic children and members of the control groups will be estimated. The centile distribution of the variables will be estimated by using lmsChartMaker Pro 2.3 software (Medical Research Council, UK 1997–2006) based on the LMS method [110, 119]. First, in the statistical analysis, the values of the above listed and detailed examinations will be measured (*see 4.3.3. Data collection* section) and evaluated whether there is a significant difference between diabetic patients and healthy controls: in the case of continuous variables Student's t-test and ANOVA analysis for normally distributed variables, while Mann-Whitney and Kolmogorov-Smirnov tests in the case of not normally distributes variables; furthermore χ^2 analysis will be used for testing distributions' homogeneity in the case of variables having discrete probability distribution. The statistical relationship and association between the variables will be tested by correlation, regression and contingency table analyses and Kendall's tau test. Univariate and multivariate analyses will be performed to assess the prognostic variables that affect the urinary bladder functions of diabetic children. As a second phase of the analysis, after executing basic and comparative statistical analyses of the

studied variables, the best cut-off values will be determined to differentiate early neuropathy from healthy controls (by estimating Se, Sp, positive and negative predictive value, likelihood ratio positive, likelihood ratio negative and characteristic of receiver operative curves). In the third phase, we will assess the diagnostic accuracy of each diagnostic test to differentiate early neuropathy (by estimating Se, Sp, predictive values, likelihood ratios, area under the receiver operating characteristic curve, overall accuracy and diagnostic odds ratio). Significance will be set at p=0.05 level in the statistical analyses. We are not planning any statistical methods to handle missing data; data imputation will not be carried out. For each endpoint, a data quality table will be created in order to assess the extent of the potential bias provided by the missing data.

For dividing children into nutritional status subgroups, age-dependent cut-off values will be used. The following subgroups will be made during statistical analyses: (1) ages (5–9, 10–14, >14 years); (2) BMI (BMI percentiles, and <18.5, 18.5–25, 25–30, 30–35, >35 kg/m^2, respectively at the age 18 years); (3) type of diabetes (type 1, type 2, monogenic DM); (4) time of diagnosis (<5, 6–9, >10 years); (5) treatment (oral antidiabetics, diet, insulin); (6) method of insulin administration (subcutaneous injection, pump); (7) number of total diabetic ketoacidosis (0–1, 2–5, 6–9, >10x); (8) HgA1c value (<5.7, 5.7–6.4, >6.4%).

4.3.8. Ethics and dissemination

The study will be conducted following the Declaration of Helsinki. It will be managed in compliance with the study protocol, good clinical practice (GCP), designated standard operating procedures, and Hungarian laws and regulations. This protocol, in its current version, was approved by the Local Scientific and Research Ethics Committee of the HOGYI Medical Research Council (ethical approval number KUT-37/2021). The study was registered in ClinicalTrials.gov Protocol Registration and Results System under the registration number NCT05247840 on 18 February 2022. The corresponding centre and designer of the trial is the HOGYI, Budapest, Hungary. The study was designed by the SC and Independent Expert Committee (IEC). The SC will be led by László Szabó (PI, paediatrician and specialist in nephrology and urodynamics). SC members will be: ÁRM (paediatrician), PP (physician), and Szabolcs Kiss (physician). Primary supervision of the study will be provided by the SC; it will make decisions concerning all important questions (e.g. premature termination of the study, drop-outs during the study). All recommendations will be filed. SC serves as a data monitoring

committee as well. If the study expands to multicentric, so does the number of SC members. The IEC will include two paediatric endocrinologists (Zsolt Vajda and Zsuzsanna Almássy), a paediatric neurologist (Beáta Rosdy), a paediatric oncologist (György Péter), and a paediatric gastroenterologist (Anna Karoliny). The task of the IEC is to monitor the trial regularly, to improve adherence to the protocol, and to provide recommendations to the SC. An early quality assessment check will be performed on the first 50 patients. The IEC will perform an independent assessment of the trial-related documents and activities, with the aim of ensuring the respect of subjects' rights, safety and well-being and to guaranteeing the plausibility of the clinical data. The IEC will report to the SC. The SC will discuss all the information and – if differences would be expected to have any bearing on the interventions and outcomes of the study – the study needs to be reassessed, and the IEC will make recommendations regarding either re-evaluation of the extension of the recruitment period or the number of study centres, power calculation, or termination of the trial. Furthermore, the study centre will include a patient representative. To comply with current ethical regulations, the study will have an independent physician and a safety manager as well. Since there are no unknown drugs or therapy used in the study, no adverse or serious adverse events are expected. In the trial, IEC will examine safety variables after every 25 patients have completed. Investigators will report adverse or serious adverse events on a separate form which will be sent to the IEC. The SC will discuss and, if the adverse effect is confirmed, it will be reported to the relevant institutional and national ethical committee.

4.3.9. Centres

The study will start as a single-centre trial, but additional centres are planned to be involved in the future.

V. RESULTS

5.1. CHAPTER I: META-ANALYSIS

5.1.1. Study selection

Out of 1750 records (MEDLINE, n = 454; Embase, n = 773; CENTRAL, n = 63; and Web of Science, n = 460), a total of 140 articles were assessed for eligibility by full text, of which 10 studies [120-129] were used in the quantitative synthesis. κ of the title selection was 0.99 (99.7% agreement), 0.98 (99.3% agreement) of the abstract selection, and 0.99 (99.8% agreement) of the full-text selection. The flowchart and reasons for exclusions on full-text assessment is illustrated in *Figure 2*.

5.1.2. Study characteristics

The baseline characteristics of the included studies are reported in *Table 1*. Studies took place in 9 different countries, and were published between 2002 and 2020. Six studies were prospective [120, 121, 125, 126, 128, 129], 2 were retrospective cohorts [123, 127], and 2 studies did not provide sufficient information about study design [122, 124].

5.1.3. General characteristics of diabetic women

We included 10 studies to the quantitative synthesis that reported on a total of 2342 diabetic patients, including 2055 patients (87.7%) with LUTS. The majority of the patients had type 2 diabetes. In 7 studies, the type of diabetes was reported [120-124, 128, 129], while in 3 studies [125-127] it was not, although they had a small number of patients. The mean age of the study populations ranged between 52.75 ± 9.2 and 64.7 ± 11.1 years, the mean duration of diabetes ranged between 8.04 ± 0.69 and 12.42 ± 7.3 years, the mean BMI ranged between 22.8 ± 2.4 and 33.2 ± 7.8 kg/m^2, and the mean HgA1c ranged between 6.05 ± 2.38 and 9.1 ± 2.6 %. The demographic characteristics of diabetic patients are presented in *Table 2*.

5.1.4. Urodynamic parameters of adult diabetic women

The urodynamic parameters of the diabetic females were assessed qualitatively. These data are represented in *Table 3*.

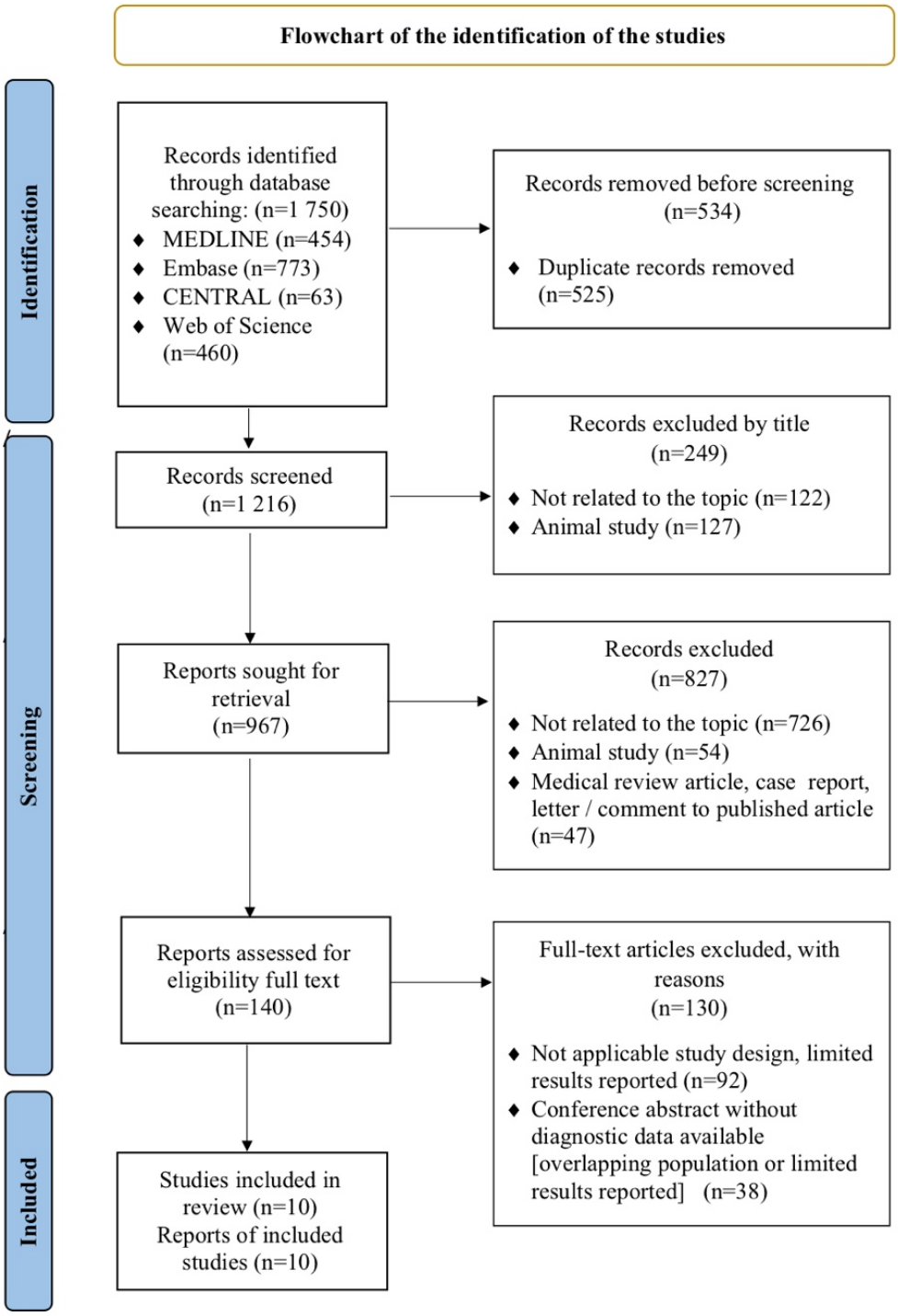


Figure 2. PRISMA Flow Diagram of the selection of the studies.

The algorithm of the study selection; out of the 1,750 records, 10 full-text articles were used in the final analysis.

First Author	Country (centre) and recruit-ment period	Study design	Inclusion criteria	LUTS					No. of Pts. with DC (%)	No. of Pts. with peri-pheral neuro-pathy (%)
				LUTS assess-ment	No. of Pts. with LUTS (%)	No. of Pts. with urge inconti-nence (%)	No. of Pts. with stress inconti-nence (%)	No. of Pts. with OAB (%)		
Al Timimi et al. 2020[120]	Iraq (single) 2018-2019	Prospec-tive cross-sectio-nal	patients with T2DM at least 5 years with LUTS	NA	71 (100%)	3 (4.2%)	3 (4.2%)	13 (18.3%)	31 (43.7%)	NA
Chang-xiao et al. 2014[121]	China (multi) 2010–2013	Prospec-tive cross-sectio-nal	women aged ≥18 years with DM	IUA/ ICS	1525 (93%)	NA	NA	918 (55.9%)	1558 (95%)	NA
GalÍ et al. 2015[122]	Italy (single) 2008–2010	NA	patients with T2DM at least 5 years with moderate/ severe LUTS	IPSS, QoL, OAB-q, ICI-SF	19 (100%)	14 (73.7%)	4 (21%)	15 (79%)	NA	14 (73.7%)
Golabek al. 2012[123]]	Rep. of Ireland (single) 2004–2008	Retro-spec-tive cohort	diabetic female with OAB, defined as an involuntary rise in detrusor pressure of greater than 5 cm H_2O during filling	NA	29 (100%)	15 (51%)	0	29 (100%)	29 (100%)	NA
Lee et al. 2007[124]	Taiwan (single) 2002–2003	NA	women with T2DM with no concurrent neurologic disorder or	AUA-SI	47 (100%)	NA	NA	0	47 (100%)[#]	18 (38.3%)

			medical conditions that could interfere with voiding function, without bladder dysfunction							
Løwenstein et al. 2021[125]	Denmark (multi) 2016-2020	Prospective randomized	adult women with symptoms of urinary incontinence, urgency and nocturia	ICIQ-UI SF, ICIQ-OAB	31 (100%)	NA	4 12.9%	4 (12.9%)	NA	4 (12.9%)
Malik et al. 2020[126]	USA (multi) 2010-2014	Prospective	female patients with urology-based voiding dysfunction and no neurologic disease	NA	96 (100%)	10 (11%)	44 (45%)	21 (24%)	NA	NA
Shin et al. 2016[127]	South-Korea (single) 2008–2015	Retrospective cohort	women without BOO who were diagnosed with SUI	NA	92 (100%)	0	92 (100%)	0	NA	NA
Tai et al. 2009[128]	Taiwan (single) 2005-2007	Prospective	women with T2DM, age 50-75 years	AUA-SI IUSS	100 36.7%	49 (18%)	30 (11%)	NA	NA	52 (19.1%)
Yenilmez et al. 2008[129]	Turkey (single) 2004–2007	Prospective cross-sectional	patients with T2DM and LUTS	NA	45 (100%)	NA	NA	NA	0*	17 (37.7%)

Table 1. Baseline characteristics of the included studies.

Ten full-text articles were included with a total of 2 342 diabetic female patients from nine countries. The majority of the patients (2 055 – 87.7%) complained LUTS. 1 620 diabetic patients had urodynamic measurements, therefore included in the meta-analysis.

[#]Diabetic cystopathy was defined postvoid residual volume greater than 100 mL. [*]Diabetic cystopathy was defined as an increase in bladder capacity (more than 500 mL), impaired bladder sensation and decrease bladder contractility.

Abbreviations: AUA-SI: American Urological Association Symptom Index; DC – diabetic cystopathy; DM – diabetes mellitus; ICIQ-OAB – International Consultation of Incontinence Questionnaire – Overactive bladder questionnaire; ICIQ-UI – International Consultation of Incontinence Questionnaire Urinary Incontinence Short Form; ICI-SF – International Consultation on Incontinence – Short form; IPSS – International Prostate Symptom Score; IUA/ICS: International Urogynecological Association/International Continence Society Standardization of Terminology Reports; IUSS – Indevus Urgency Severity Scale; LUTS – lower urinary tract symptoms; NA – not available data (not reported); OAB – overactive bladder (detrusor overactivity); OAB-q – Overactive Bladder Questionnaire; Pts– patients; QoL – Quality of life Questionnaire ; SUI – stress urinary incontinence; T2DM- type 2 DM.

Study	Definition of diabetes	Number of patients (Percentage of T2DM in %)	Mean age (years) ± SD	Mean diabetes duration (years) ± SD	Mean BMI (kg/m²) ± SD	HgA1c (%) ± SD	Diabetes treatment Number of patients with treatment (Percentage in %)		
							Oral antidiabetic agent	Insulin	Diet and exercise
Al Timimi et al. 2020[120]	WHO (Alberti and Zimmet 1998[#]) criteria	71 (100%)	62 ± 13	12.2 ± 4.1	28.9 ± 4.57	NA	NA	NA	NA
Changxiao et al. 2014[121]	ADA	1640 (95)[*]	52.75 ± 9.2	8.04 ± 0.69	NA	6.8 ± 1.87	1107 (67.5)	384 (23.4%)	103 (6.2%)

Galĺ et al. 2015[122]	NA	19 (100)	63.1 ± 10.0	11.9 ± 5.2	28.8 ± 2.3	9.1 ± 2.6	11 (57.9%)	8 (42.1%)	0
Golabek et al. 2012[123]	NA	29 (89)	53.84 ± 16.0	NA	NA	6.05 ± 2.38	NA	NA	NA
Lee et al. 2007[124]	NA	47 (100)	63.6 ± 9.3	12.42 ± 7.3	25.3 ± 2.7	7.7 ± 1.4	NA	NA	NA
Løwenstein et al. 2021[125]	NA	31 (not reported)	64.7 ± 11.1	11.1 ± 10.1	31.8 ± 5.5	6.7 (6.4-7.2) ‡	15 (47%)	3 (9.7%)	NA
Malik et al. 2020[126]	NA	96 (not reported)	57.6 ±12.2	10.3 ± 8.5	33.2 ± 7.8	7.5 ± 2.1	NA	13 (13%)	NA
Shin et al. 2016[127]	ADA	92 (not reported)	58.34 ± 8.25	9.24 ±7.63	NA	7.27 ± 1.43	NA	NA	NA
Tai et al. 2009[128]	NA	272 (100)	63.1 ± 9.8	11.6 ± 8.5	22.8 ± 2.4	7.3 ± 1.2	234 (86%)	16 (5.9%)	4 (1.5%)
Yenilmez et al. 2008[129]	NA	45 (100)	60.1 ± 1.4§	11.4 ± 1.0†§	28.8 ± 0.8†§	7.3 ± 0.3†§	NA	13 (35%)†	NA

Table 2. Demographic characteristics of women with diabetes of the included studies.

diabetes was defined according to Alberti et al 1998 (Alberti, K. G. M. M., Zimmet, P.Z. 1998 Definition, diagnosis and classification of diabetes mellitus and its complications Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabetic Medicine* 15 (7): 539-553); * from the 1640 patients 918 had uroflow measurement therefore were included in the our meta-analysis; † data of 37 diabetic women after uroflowmetry without major complication after the procedure are presented; ‡ data in median, 25th percentile (first figure in the brackets) and 75th percentile (second figure in the brackets); § data presented in mean and standard error **Abbreviations:** ADA – American Diabetes Association; BMI – Body mass index ; NA – not available data (not reported); SD – standard deviation; T2DM – type 2 diabetes mellitus

First Author	Mean voided volume (mL) ± SD	Mean postvoid residual volume (mL) ± SD	Mean Q_{max} (mL/sec) ± SD	Mean $P_{det}Q_{max}$ (cmH_2O) ± SD	Mean first sensation of bladder filling (mL) ± SD	Mean cystometric capacity (mL) ± SD
Al Timimi et al. 2020[120]	NA	127 ± 15	14 ± 1.3	NA	NA	426 ± 414
Changxiao et al. 2014[121]	NA	323 ± 79.7	9.6 ± 7.1	32.4 ± 13.2	238.1 ± 58.3	624 ± 117.4
GalÍ et al. 2015[122]	NA	12.1 ± 14	19.8 ± 3	NA	165.5 ± 55.3	380 ± 78
Golabek et al. 2012[123]	414.59 ±154.87	5 (0-35)†	22.331 ± 9.99	40.69 ± 22	NA	447 ± 118.95
Lee et al. 2007[124]	239.4 ± 173.6	104.9 ± 59.1	15.2 ± 1.2	NA	NA	NA
Løwenstein et al. 2021[125]	327 (293-348)*	NA	27.6 ± 11.1	22.5 ± 10.8	139 ± 119	NA
Malik et al. 2020[126]	NA	99 ± 46	19 ± 15	27 ± 18	174 ± 179	493 ± 284
Shin et al. 2016[127]	274.73 ±131.92	33.24 ± 55.63	23.55 ± 10.26	26.78 ± 15.4	173.4 ± 75.84	NA
Tai et al. 2009[128]	199.5 ± 85.2	74.3 ± 30.5	13.9 ± 7.2	NA	NA	NA
Yenilmez et al. 2008[129]	NA	55.4 ± 11#	24.8 ± 1.3#	34.1 ± 1.5#	166 ± 10#	495 ± 23#

Table 3. Baseline characteristics of urodynamic parameters of diabetic women in the included studies.

From the 2 342 patients, 1620 had urodynamic measurements, therefore included in the meta-analysis. * data presented in median, and range in the brackets; # data presented in mean and standard error; † data in median, 25th percentile (first figure in the brackets) and 75th percentile (second figure in the brackets). **Abbreviations:** NA – not available data (not reported); $P_{det}Q_{max}$ – maximal detrusor pressure at maximal flow rate ; Q_{max} – maximum flow rate; SD – standard deviation.

5.1.4.1. Voided volume

The pooled event rates show that mean voided volume in diabetic women (n = 471) is 288.21 mL [95% CI: 217.35–359.06] with a considerable level of heterogeneity (I^2 = 98%) (*Figure 3 Panel A)*.

5.1.4.2. Postvoid residual volume

The pooled event rates represent that mean postvoid residual volume in diabetic women (n = 1589) is 93.67 mL [95% CI: 31.35–155.99] with a considerable level of heterogeneity (I^2 = 100%) (*Figure 3 Panel B*).

5.1.4.3. Q_{max}

The mean Q_{max} in diabetic women (n = 1620) is 18.80 mL/sec [95% CI: 15.27–22.33] with a considerable level of heterogeneity (I^2 = 99%) (*Figure 3 Panel C*).

5.1.4.4. $P_{det}Q_{max}$

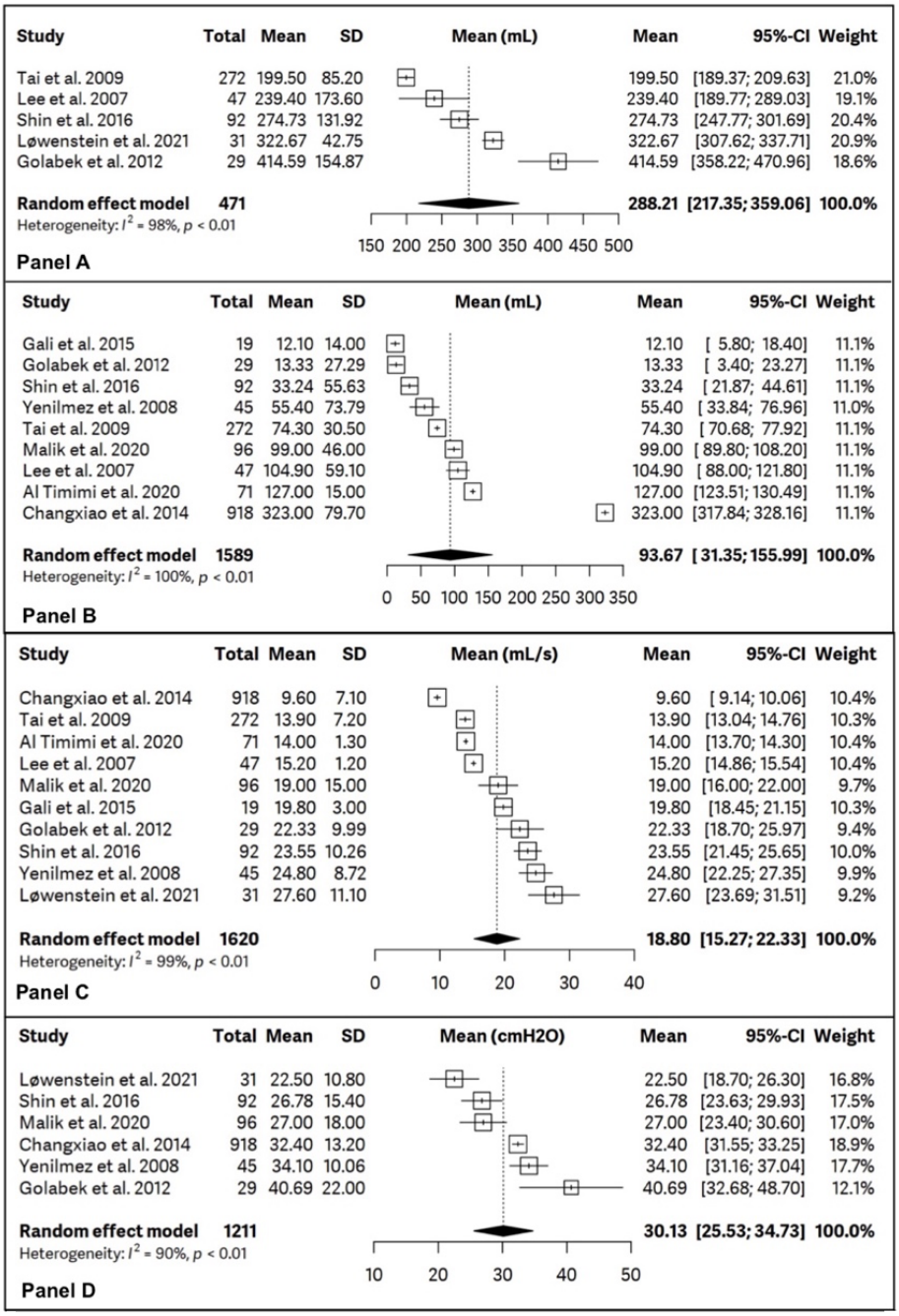
The mean $P_{det}Q_{max}$ in diabetic female population (n = 1211) is 30.13 cmH_2O [95% CI: 25.53–34.73] with a considerable level of heterogeneity (I^2 = 90%) (*Figure 3 Panel D*).

5.1.4.5. First sensation of bladder filling

The mean first sensation of bladder filling in diabetics (n = 1201) is 178.66 mL [95% CI:150.59–206.72] with a considerable level of heterogeneity (I^2 = 97%) (*Figure 3 Panel E)*.

5.1.4.6. Cystometric capacity

The mean MCC in diabetic women (n = 1178) is 480.41 mL [95% CI:409.32–551.50] with a considerable level of heterogeneity (I^2 = 98%) (*Figure 3 Panel F).*



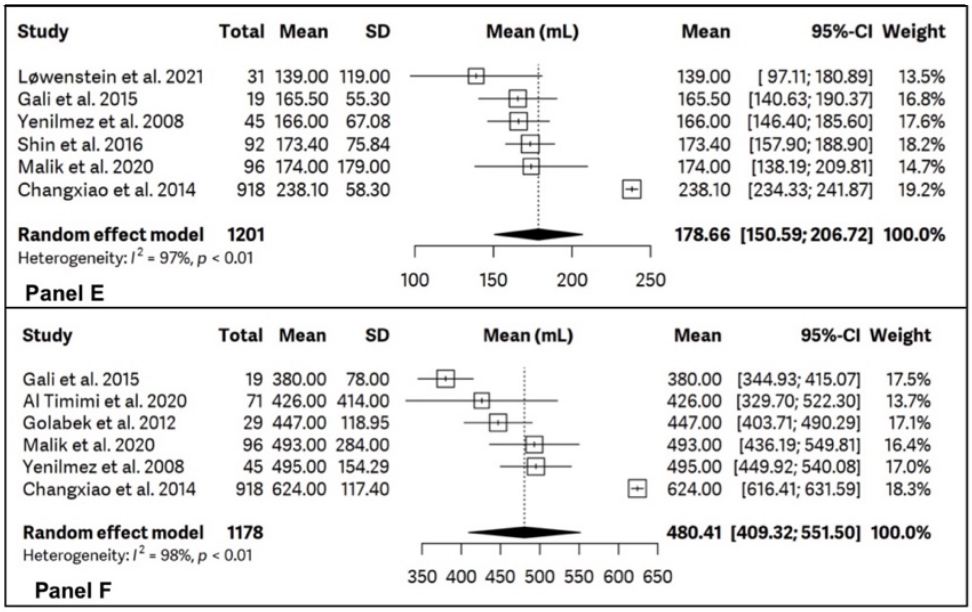


Figure 3. Forest plots of the urodynamic parameters of adult diabetic women.
Panel A represents the pooled mean voided volume of diabetic women (n = 471). *Panel B* shows the pooled mean postvoid residual volume of diabetic women (n = 1589). *Panel C* demonstrates the pooled mean Q_{max} values in diabetic women (n = 1620). *Panel D* presents the pooled mean $P_{det}Q_{max}$ values in diabetic female population (n =1211). *Panel E* reveals the pooled mean first sensation of bladder filling in diabetics (n = 1201). *Panel F* unveils the pooled mean maximum cystometric capacity (MCC) in diabetic women (n = 1178).

5.1.5. Risk of bias assessment

A summary of the risk of bias assessment is visually presented in *Suppl. Figure 1.*
In the analysis of female diabetic patients' urodynamic parameters, the majority of the studies had a high overall risk of bias [120, 122-127, 129]. The main reasons include confounding factors, such as unreported (1) diabetes definition, (2) diabetes duration, (3) HgA1c, (4) diabetes treatment, (5) BMI, (6) LUTS assessment, (7) device and methodology of the uroflowmetry measurement, (8) measuring method of postvoid residual volume, and (9) statistical analysis. Existence of pyuria was a confounding factor as well. One study had moderate overall risk of bias because not all patients were included in the urodynamic analysis, and an assessed confounding factor was that BMI was not reported [121]. One study was reported as having low overall risk of bias [128].

5.2. CHAPTER II: URINE FLOW ACCELERATION NOMOGRAMS

5.2.1. Study selection and baseline characteristics

Out of 270 healthy children, 208 were enrolled in the analysis who performed 404 micturition. 62 children were excluded due to the following reasons: 33 children were excluded due to voided volume less than 20 mL, and 29 children were excluded due to a postvoid residual volume of more than 15% of voided volume. Data quality tables are presented in *Suppl. Table5.* The baseline characteristics of girls/boys and all patients and uroflowmetry parameters are presented in *Table 4.*

Out of 208 children, 94 are female and 114 are male. The mean age of the total population is 9.68 ± 3.09 years, the median weight is 32 [14–78] kg, the mean height is 138.76 ± 19.21 cm, and the mean body surface is 1.19 ± 0.28 m^2. The median voided volume is 130 [20–460] mL, the median voiding time is 10 [3–56] sec, the median TQ_{max} is 3 [1–14] sec, the median Q_{ave} is 11.7 [2.5–36.6] mL/sec, the median Q_{max} is 20.5 [5–50] mL/sec, the median Q_{acc} is 6 [0.81–25] mL/sec^2, and the median postvoid residual volume is 1.83 [0–38.62] mL.

	Median	**Standard error**	**Minimum value**	**Maximum value**
Age (years) n= 94/114/208	10/9.9/10	0.3/0.3/ 0.21	3/3/3	16/17/17
Weight (kg) n= 93/113/206	32/32/32	1.22/1.28/ 0.89	14/15/14	71/78/78
Height (cm) n= 79/98/177	140/141.5/ 141	2.0/2.05/ 1.44	92/89/89	170/179/ 179
Body surface (m^2) n=80/99/179	1.18/1.18/ 1.18	0.03/0.02/ 0.02	0.6/0.6/ 0.6	1.8/1.8/ 1.80
Voided volume (mL) n= 169/ 235/404	130/140/ 130	6.86/6.09/ 4.55	20/20/20	375/460/ 460
Voiding time (sec) n= 169/ 235/404	10/10/10	0.52/0.43	3/3/3	48/56/56
Time to Q_{max} (sec) n=169/ 235/404	3/4/3	0.15/0.14/ 0.10	1/1/1	12/14/14

Q_{ave} (mL/sec) n=169/ 235/404	12.5/11.1/ 11.7	0.47/0.35/ 0.28	2.5/2.5/2.5	34/36.
Q_{max} (mL/sec) n=169/ 235/404	23/19/20.5	0.62/0.50/ 0.39	5/5/5	50/50/50
Q_{acc} (mL/sec^2) n=169/ 235/404	7.25/5.4/6	0.31/0.25/ 0.20	1.12/0.81/ 0.81	19.5/25/25
Postvoid residual (mL) n=169/ 235/404	1.40/1.90/1.83	0.48/0.40/0.30	0/0/0	38.62/29.1/ 38.62

Table 4. Baseline characteristics and uroflowmetry parameters of girls/boys/all children included in the study.

Out of the 208 children, 114 are male, 94 are female. Altogether, 404 (235, 169) micturition was performed.

Out of the 94 girls with 169 urinations, the mean age is 9.71 ± 2.95 years, the median weight is 32 [14–71] kg, the mean height is 138.13 ± 17.84 cm, and the mean body surface is 1.18 ± 0.27 m^2. The median voided volume is 130 [20–375] mL, the median voiding time is 10 [3–48] sec, the median TQ_{max} is 3 [1–12] sec, the median Q_{ave} is 12.5 [2.5–34] mL/sec, the median Q_{max} is 23 [5–50] mL/sec, the median Q_{acc} is 7.25 [1.12–19.5] mL/sec^2, and the median postvoid residual volume is 1.40 [0–38.62] mL.

Out of the 114 boys with 235 urinations, the mean age is 9.65 ± 3.21 years, the median weight is 32 [15–78] kg, the mean height is 139.27 ± 20.32 cm, and the mean body surface is 1.20 ± 0.29 m^2. The median voided volume is 140 [20–460] mL, the median voiding time is 10 [3–56] sec, the median TQ_{max} is 4 [1–14] sec, the median Q_{ave} is 11.11 [2.5–36.6] mL/sec, the median Q_{max} is 19 [5–50] mL/sec, the median Q_{acc} is 5.4 [0.81–25] mL/sec^2, and the median postvoid residual volume is 1.9 [0–29.10] mL.

5.2.2. Normative urine flow acceleration nomograms

Figure 4 and *Figure 5* demonstrate the nomograms of Q_{acc} by voided volumes in girls and boys, respectively.

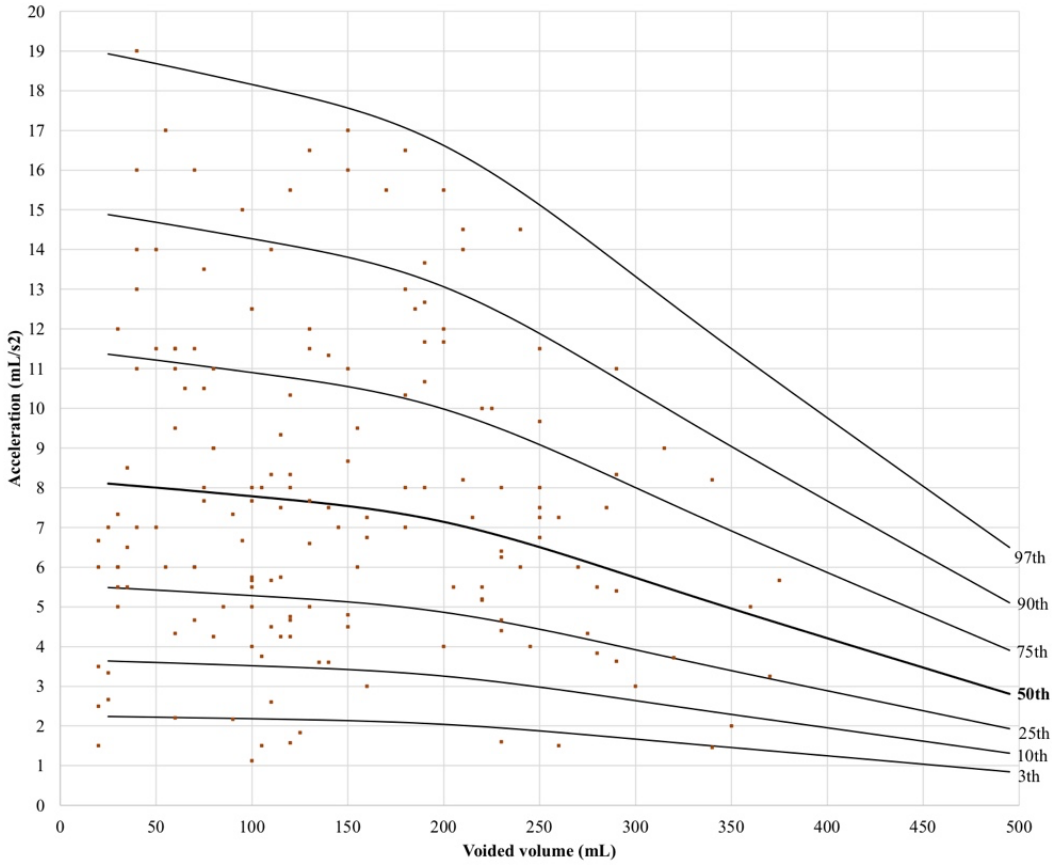


Figure 4. Urine flow acceleration (Q_{acc}) nomogram for girls

Q_{acc} is demonstrated by voided volumes in female population ≤18 years of age.

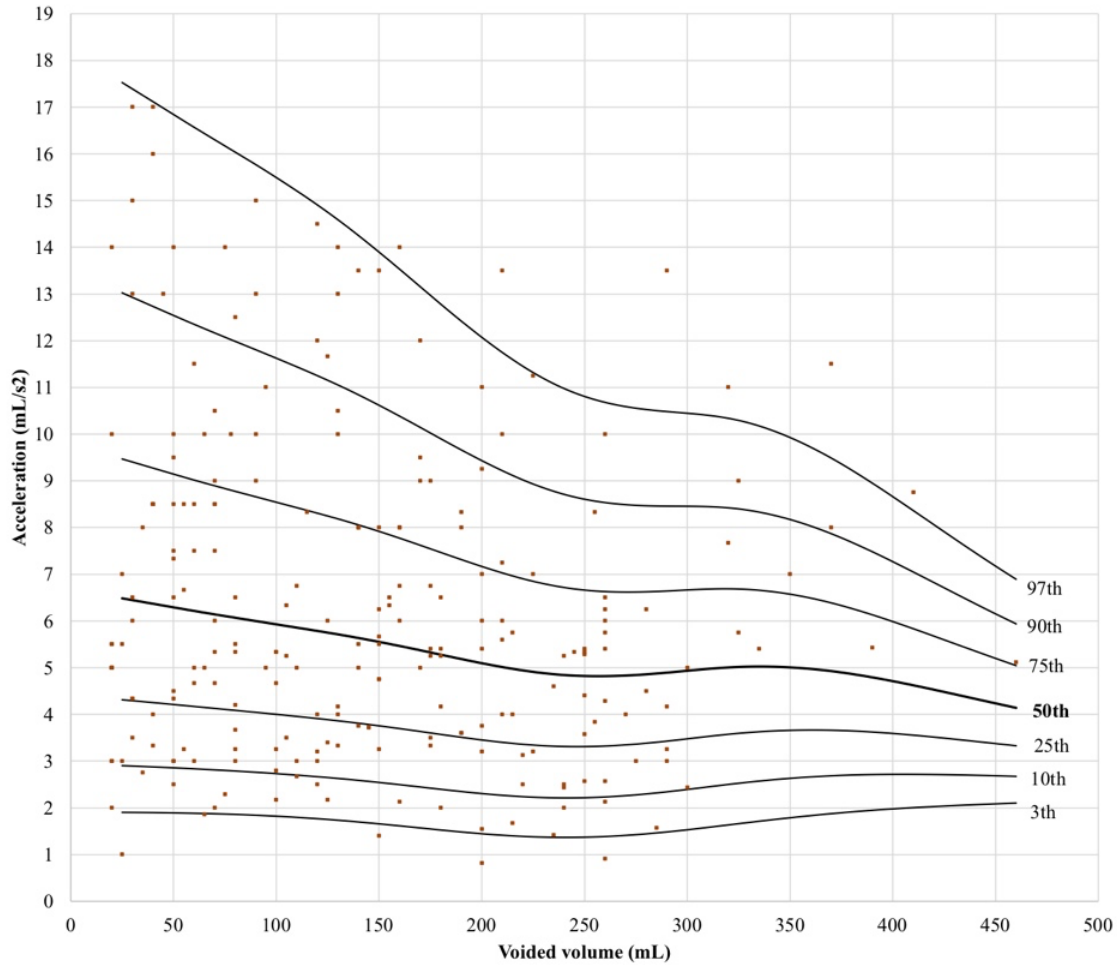


Figure 5. Urine flow acceleration (Q_{acc}) nomogram for boys

Q_{acc} is demonstrated by voided volumes in male population ≤18 years of age.

5.3. CHAPTER III: INTACT STUDY

5.3.1. Data management

The SC members will perform the examinations listed in the section *4.3.3. Data collection*. Data will be handled by the SC. Manual data collection will be turned into a digital form by a study data manager. Insufficient data will be filtered when digitized from the paper. At the end of each examination, before releasing the patient, a data quality and validity check will be performed. Once the data have been digitized, another data check will be executed. The PI will ensure that the digitalized data are accurate, complete and legible. Any missing, implausible or inconsistent recordings will be referred back to the investigator who performed

the examinations. Patients with missing information will be asked via a phone call to fulfil the missing data. All changes will be documented. All data will be retained for 10 years. Manually collected data will be stored safely in the corresponding centre (HOGYI) and only be accessible to the SC members. Digitization will only take place under the supervision of the PI. When digitised, data are separated, tabulated and encrypted, and patient data are stored securely. Digitized data will be anonymized as well (the patent's personal data will be coded and stored separately for identification). There are no other contractual agreements that limit such access for SC members. Other participants (study manager, IEC members) do not have access to the final trial dataset. The study protocol is free for public access. Any changes or deviations in the study protocol will be updated on clinicalTrials.gov. The changes will be reported to the research ethic committee as well. Authorships will be implemented according to the International Committee of Medical Journal Editors (ICMJE) recommendations [130]. Results will be submitted for publication in a peer-reviewed journal.

VI. DISCUSSION

6.1. CHAPTER I: META-ANALYSIS

Lower mean voided volume, Q_{max} and $P_{det}Q_{max}$; as well as higher mean postvoid residual volume, first sensation of bladder filling, and cystometric capacity in the diabetic group was detected compared to healthy women [99, 100].

6.1.1 Diabetic women

6.1.1.1. Voided volume

Lower voided volume in diabetes is a surprising finding, because higher voided volumes are generally expected in diabetic patients with higher fluid turnover; and even much higher volumes in autonomic neuropathy [71, 78, 131]. However, in diabetic patients, according to the literature [132, 133], the residual urine is larger as the autonomic neuropathic bladder cannot completely empty the bladder, and there will be residue in it. On the other hand, if we add the emptied amount and the residual volume, there is a clearly higher bladder capacity in diabetic patients [132]. This phenomenon could be explained by the selection of different stages and duration of diabetes. This clinical heterogeneity is also indicated by the statistical heterogeneity.

6.1.1.2. Q_{max} and $P_{det}Q_{max}$

Q_{max} is always lower in patients with an autonomic neuropathic bladder [11] due to impaired detrusor muscle function, which agrees with our findings. A smaller voided volume always has a lower Q_{max} [101, 134], but in this case the bladder also contains residual volume; and this should be taken into account during contraction, so the value is even worse. At maximum flow, the detrusor pressure ($P_{det}Q_{max}$) is lower in DM due to the dysfunction and abnormal innervation. Lee at al. [124] reports lower Q_{max} in the diabetic group without bladder dysfunction (n = 135, Q_{max}:19.9± 0.7 mL/sec), which is even more profound with bladder dysfunction (n = 47, Q_{max}:15.2 ± 1.2 mL/sec) compared to healthy women (n = 197, Q_{max}: 25.8 ± 8.4 mL/sec).

6.1.1.3. Postvoid residual volume, first sensation of bladder filling, and cystometric capacity

Autonomic neuropathy reduces the sensation of the bladder [90, 135, 136], so it is well understood that the onset of the first urge to urinate in diabetic patients occurs at higher bladder volumes. Bladder capacity is always measured higher during cystometry and is markedly higher in diabetic patients [135, 137]. Lee at al. [124] reported that 1.6% of diabetic women had bladder capacity >500 mL and 0% in the healthy control group; 25.8% of patients had voiding dysfunction in the diabetic group and 3.5% in the healthy population; and 14.8% of diabetic patients and 1.5% of healthy people have postvoid residual >100 mL.

6.1.2. Non-diabetic women

Haylen et al. [134] found that Q_{max} and Q_{ave} are strongly related to voided volume. Afraa et al. [138] found Q_{max} values ranging between 23–32 mL. Wyman et al. [99] included 3090 healthy women ranging in age from 19 to 91 years from 24 studies to their meta-analysis of normative reference values for bladder function parameters. They found 334 mL [95% CI: 299–350] for mean voided volume, 12 mL [95% CI: 4–20] for mean postvoid residual volume, and 28 mL/sec [95% CI: 27–30] for mean Q_{max}. Sorel et al. [100] included 1416 adult patients to their systematic review. They found 338 mL [SD:161] for mean voided volume, 23.5 mL [SD:10] for mean Qmax, 15.5 mL [SD:25] for mean postvoid residual volume. Mahfouz et al. [139] found 175 mL for first sensation of bladder filling, and normal MCC between 300–500 mL. These findings agree with our results.

6.1.3. Diabetic adults with and without LUTS

As diabetes progresses, LUTS also appears [135, 140] which suggests more pronounced bladder damage, so it is understandable that in DM with LUTS, higher voided volumes suggest greater bladder capacity compared to the diabetics without LUTS. The same more pronounced damage is indicated by increased detrusor muscle weakness in diabetic patients with LUTS, compared to those without voiding symptoms. Higher detrusor pressure at maximum flow only fits into this pattern if it indicates a LUTS outflow disturbance. The rate of residual urine is higher in diabetics who also have LUTS.

6.1.4. Strength of the study and limitations

To the best of our knowledge, this is the first meta-analysis that synthetizes quantitative data about urodynamic measurements of female patients with diabetes. Nonetheless, the strength of our meta-analysis is the use of a comprehensive and precise search strategy and data extraction. Uroflowmetry is a non-invasive, widely accessible, quick and easy-to-use urodynamic diagnostic tool to evaluate voiding function [11] and to determine LUT dysfunction, and it might detect subtle voiding modifications in neuropathic patients before LUT symptoms manifest [90], therefore it might be a useful tool in the early diagnosis of dysfunction of the detrusor muscle. The main limitation is that we could not directly compare the parameters of diabetic and non-diabetic women, as there were insufficient studies that directly compared these two groups of patients. The lack of definition of DC is also a limitation; only 2 studies reported it [124, 129].

6.2. CHAPTER II: URINE FLOW ACCELERATION NOMOGRAMS

We have previously established normal reference values for maximum and average urine flow in children [101], which have been adopted by the International Continence Society and recommended in the 2nd edition of the Book of Incontinence [141] for those who study urine flow in children. The uroflow patterns of children with LUT symptoms [142-145] are still under evaluation. In our previous study [101], we did not establish normal reference values for Q_{acc}, although a difference was observed in children with DM [15]. Therefore, we have established nomograms for normative reference values of Q_{acc} in paediatric population (girls and boys separately) by voided volumes in centile forms, and found an inversely proportional correlation between voided volumes and Q_{acc} parameters.

The diversity of Q_{acc} by percentiles is huge. We recommend that values between the 25^{th} and 75^{th} percentiles should be accepted as normal, which in boys is between 3.9 and 8 mL/sec^2 for a voided volume of 150 mL. If we get values below 25% or above 75%, we recommend further examinations. Theoretically, a Q_{acc} value above 75% could be due to overactive bladder dysfunction.

Up to 350 mL of voided volume, higher Q_{acc} values were observed in girls than in boys. This phenomenon might be explained by the shorter and straighter urethra in the female population.

Above 350 mL of voided volume, Q_{acc} values below the 50th percentile were minimally lower in girls than in boys. A relatively small number of cases may play a role in the latter.

None of the children had BOO, as can be suggested based on the shape of the uroflow curve. According to the Miskolc Nomograms [101], none of the children had plateau-shaped flow curves. Furthermore, reduced flow parameters can be observed in BOO (Q_{max} and Q_{ave} values are below 5%). Both Q_{max} and Q_{ave} values in the Miskolc Nomograms were above the 10th percentile. These findings can be confirmed with pressure/flow urodynamic tests (and video urodynamics), but due to their invasive nature, they are not performed routinely. We have not examined Q_{acc} in BOO so far, but according to Wen et al. [14], Q_{acc} might be a better indicator than Q_{max} in the examination of BOO.

Micturition represents complex neuromuscular mechanisms involving structures of the LUT and nervous system. Voiding symptoms refer to a disease involving several factors of dysregulation; although the absence of voiding symptoms is not a guarantee of normal micturition.

Hubeaux et al. [146] found that almost one-third of women without voiding complaint has been actually shown to have abnormal uroflowmetry.

Mattiasson and Teleman [147] investigated the urethral motor function in incontinence women. Q_{acc} has been significantly increased in patients with incontinence: Q_{acc} was 13 ± 17.8 (2.2); 20 ± 18.9 (2.8); and 32 ± 24.9 (4.9) degrees [mean ± SD; (Standard Error)] for incontinence, naive incontinence and no incontinence, respectively.

Cucchi [18] concluded that all of the obstructed patients have lower values of acceleration than the controls, but patients with detrusor instability (today, the correct name is overactive bladder) tend to show higher Q_{acc} than those with stable bladders. He also found that the measurement of Q_{acc} is a simple and reliable test to aid in the diagnosis of detrusor instability in stress incontinent women [18].

Q_{acc} might have a role in the detection of detrusor muscle dysfunction, as well. Karasu et al. [16] found higher values of Q_{acc} in intrinsic sphincter deficiency stress incontinent women than stress incontinent ones alone, and they hypothesized that Q_{acc} might be a more reliable parameter for urethral resistance and tonus. Wen et al. [14] reported the Q_{acc} to be superior to Q_{max} in diagnosing bladder outlet obstruction in men with BPH.

Acceleration of urine flow might provide a finer diagnosis of the relationship between abnormalities of the LUT and DM [15] as well.

We believe that Q_{acc} might be used primarily to detect damage to the detrusor muscle (especially to detect reduced detrusor contraction caused by DM). We consider that Q_{acc} is a better indicator of diabetic autonomic neuropathy than CAD tests (Ewing tests), because in our previous study [15] Q_{acc} levels were significantly decreased not only in diabetic children with CAD, but also in diabetic patients without CAD.

Theoretically, it can be assumed that Q_{acc} values do not decrease in the case of a reduced Q_{max} – if there is a urethral stricture in the background. While in the event of hypocontractility they might be a decrease. But as the disease progresses, the detrusor muscle transforms, and its contractility deteriorates, therefore reduced Q_{acc} values appear. Thus, Q_{acc} examinations might be suitable even as a replacement for invasive urethral pressure profile, but we have not performed such tests so far.

By the evaluation of Q_{acc}, there will be more biological indicators to assess the aetiology of the urinary problems and the effect of different diseases and treatments for voiding function to get the appropriate and precise treatment for patients.

Since there are only a few studies evaluating Q_{acc} values in adults and paediatric population with different diseases; furthermore, normative reference values of Q_{acc} are lacking, we found it important to establish normal ranges of Q_{acc} in both genders by voided volumes in children.

The strength of this study is the novelty of the evaluation of normal reference values of Q_{acc}, which includes a relatively large number of children with Q_{acc} calculation.

The main limitation of this study is the possible selection bias due to the retrospective design.

6.3. CHAPTER III: INTACT STUDY

The main objective of the INTACT trial is to evaluate the diagnostic accuracy (Se, Sp, positive and negative predictive value) of uroflowmetry. To do so, we compare uroflowmetry to the gold standard neuropathy tests in diabetic children and healthy controls. To the best of our knowledge, this is the first prospective clinical trial evaluating early signs of neuropathy by simultaneously uroflowmetry, cardiovascular autonomic dysfunction tests, and peripheral nerve conduction test in paediatric patients with diabetes and healthy controls.

CAN is one of the most studied forms of autonomic neuropathy, which is a frequent and early complication of diabetes, and DC is a frequent urological complication of diabetic autonomic

neuropathy. Although progression of DC is believed be related to the duration of diabetes, and poor metabolic status; animal studies raised the question whether changes in the bladder function begin to occur soon after its onset [148, 149]. Previous studies have suggested that DC is not the prime urodynamic finding in diabetics. Kaplan et al. [79] found that detrusor overactivity was the most common finding. Kebapci et al. [84] came to the conclusion that classic DC occurs in only 44% of women with type 2 DM, followed for a mean of 13.85 years; more common findings are detrusor overactivity, stress and urge incontinence.

Translational Research takes scientific discoveries made in the laboratory and transforms them into new treatments [150]. Therefore, the sooner the early signs of DC are discovered, the earlier the therapeutic modifications can be initiated (tight glycaemic control), which can improve the quality of life. Uroflowmetry can highlight the progressive nature of diabetes – starting with storage changes, then developing voiding dysfunction due to detrusor overdistension, to the decompensated phase. As early alterations in voiding patterns can be seen during the urodynamic examinations before bothersome urinary symptoms are recognized by patients, urodynamics, mostly uroflowmetry, can contribute to the early diagnosis of DC. Therefore, the inclusion of routine uroflow measurements in the current guidelines of diabetes management is crucial.

VII. CONCLUSIONS

Urodynamic tests were primarily invented for the investigation of diseases of the lower urinary tract, but there is a rising number of data that they can be used in non-urological diseases as well. They can detect and monitor the progression of the diseases, and can even be used as screening tests. Uroflowmetry is a non-invasive, widely accessible, quick and easy-to-use urodynamic diagnostic tool to evaluate voiding function [11] and to determine LUT dysfunction. Diabetes is an important independent risk factor for LUTS. Urodynamics can detect early alterations in voiding function, which might help to apply interventions to delay or prevent the onset of diabetes to limit difficulties in voiding. Uroflow might detect subtle voiding modifications in neuropathic patients before LUT symptoms manifest [90], and it might be a useful tool in the early diagnosis of dysfunction of the detrusor muscle. Therefore, the use of uroflowmetry may be considered in current diabetes guidelines, and regular uroflow measurements can contribute to the early recognition of DC.

Normal reference values for urinary flow acceleration were established in percentile forms in children. As acceleration of urine flow can provide a finer diagnosis of abnormalities of the LUT and various chronic diseases (DM etc.), our results could form a basis on studies about the diagnostic significance of uroflow parameters in different non-urological diseases in children. As soon as studies – comparing the Q_{acc} values of healthy children and patients with different diseases – identify diagnostic cut-off values, the use of the normal reference values of Q_{acc} can be easily translated to everyday clinical practice. According to the currently available literature, Q_{acc} is an important tool to aid in the diagnosis of LUT symptoms. By establishing normative reference values, interpreting the different Q_{acc} parameters might help clinicians evaluate different diseases. Since we evaluated the Q_{acc} patters of healthy asymptomatic paediatric population, we formed the basis of future prospective studies. Further prospective studies comparing healthy children and paediatric population of different diseases with or without LUT symptoms will be needed to establish cut-off values to differentiate normal and abnormal uroflow patterns (voided volume, voiding time, Q_{ave}, $Q_{max,}$ TQ_{max}) as well.

In the INTACT Trial, by simultaneously assessing uroflowmetry, cardiovascular autonomic dysfunction tests, and peripheral nerve conduction test in paediatric patients with diabetes and healthy controls, diagnostic accuracy of uroflowmetry can be evaluated in the detection of neuropathy.

VIII. AUTHOR'S OWN CONTRIBUTION

8.1. Martonosi at al. Medical Science Monitor, 2022

The author performed the database search and read the articles for eligibility, collected the data from the articles to the study database, performed the bias analysis and quality assessment, completed the PRISMA checklist. The author drafted the majority of the manuscript and edited the tables and figures.

8.2. Martonosi et al. Neurourology and Urodynamics, 2023

The author designed a part of the study, performed the majority of the acquisition of data, and part of the statistical analysis. The author drafted the majority of the manuscript and edited the tables and figures.

8.3. Martonosi et al. BMJ Open, 2022

The author studied the available literature, played a key role in the study design, wrote the majority of the manuscript and edited the study figures and tables.

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XI. SUPPLEMENTARY MATERIAL

Supplementary Table 1. Types of urodynamic studies and measurements.

Simple, non-invasive examinations	1. Documentation of 24-hour fluid intake and voiding 2. Volume at first desire to void 3. Maximal bladder capacity measurement 4. Documentation of incontinence 5. Bladder wall thickness measurement by ultrasound 6. Uroflowmetry 7. Postvoid residual bladder volume measurement by ultrasound 8. Surface electromyography to indirectly measure pelvic floor and sphincter muscle contractility
Complex, invasive examinations	9. Cystometry: intravesical, urethral, abdominal pressures during bladder filling and voiding 10. Video-cystometry 11. Urethral pressure profile test 12. Neurophysiological examinations
Complex examinations evaluating the upper urinary tract	13.Whitaker test
Isotope examinations	14. Dynamic renal imaging 15. Isotope uroflowmetry

Supplementary Table 2. The characteristics of cardiovascular autonomic dysfunction tests.

Name of the cardiovascular test	Characteristics of the test	Autonomic nerve system
Heart rate response to deep breathing	The patient breathes deeply in a sitting position. The maximum and minimum heart rates during each breathing cycle are measured, and the mean of the differences during three successive breathing cycles are taken to give the maximum-minimum heart rate.	parasympathetic function
HR response to standing up	The patient lies on a couch and then stands up unassisted. An immediate increase in heart rate happens immediately, maximal amount is at about the 15th beat after starting to stand, followed by a relative bradycardia, maximal around the 30th beat. This will be calculated as the 30:15 ratio, which means the ratio of the longest R-R interval around the 30th beat to the shortest R-R interval around the 15th beat.	parasympathetic function
Valsalva manoeuvre	The patient blows into a mouthpiece at a pressure of 40 mmHg for 15s. Normally the heart rate increases, followed by a rebound bradycardia. During the manoeuvre, the ratio of the longest and shortest R-R interval will be measured. The Valsalva ratio will be calculated as the mean ratio from three consecutive Valsalva manoeuvres.	parasympathetic function
Blood pressure response to standing up (Orthostatic hypotension)	The blood pressure (in mmHg) is measured in a lying and standing position using a blood pressure meter. The difference in systolic blood pressure is considered to be the extent of the change in postural blood pressure. Systolic pressure is considered to be abnormal when a fall >20 mmHg after standing up is observed.	sympathetic function

Blood pressure response to sustained handgrip	Handgrip is maintained at 30% of the maximum voluntary contraction using a handgrip dynamometer up to a maximum of 5 min, and the blood pressure is measured each minute. The difference between the diastolic blood pressure just before the release of the handgrip, and before starting, is taken as the measure of response.	sympathetic function

Each of these five tests is assigned a score of 0 for normal, 0.5 for borderline, and 1 for abnormal results. The sum of these 5 scores – which is the Ewing score – is used to assess the severity of cardiovascular autonomic dysfunction. Patients with an Ewing score ≥ 2 form the cardiovascular autonomic dysfunction + (positive) group, and those with less than 2 form the cardiovascular autonomic dysfunction – (negative) group.

Supplementary Table 3. Informed consent and parental information about the process of the INTACT Trial.

Heim Pál National Pediatric Institute

Heim Pál National Pediatric Institute INTACT Trial
Statement of consent

Statement of consent to the INTACT Trial of Heim Pál National Pediatric Institute

Name of the patient: ..... Patient's insurance ID: ☐☐☐ ☐☐☐

Date of birth: ..... The name of the patient's mother:.....

Name of the patient's legal guardian: .....

Address: .....

The child's chronical illness: .....

The intended examination procedure

1. Name of the procedures: uroflowmetry, blood sample collection, body composition analysis (Inbody), body height, body weight, non-invasive blood pressure, and heart rate measurement, measurement of O_2 saturation, electrocardiography, measurement of peripheral nerve conduction in the lower limb
2. Preparation and process: detailed in the document titled as 'Parental Information on the process of the INTACT examination'
3. Risks and complications: According to the current state of modern medicine, there are no known complications of uroflowmetry examination, the examination does not carry any risk. The main risk of blood sampling is discomfort, pain and possible bruising at the needle penetration site. These complications are usually mild and go away shortly after the tests are done. Rare side effects may include oedema, thrombosis, nerve injury, allergies, and iatrogenic anaemia [1]. There is no risk either during the body composition analysis (InBody). There is no known risk of measuring height, body weight, O_2 saturation. A rare risk of measuring blood pressure is pain, or possible nerve injury [2]. When measuring nerve conduction, an infection may develop at the site of insertion of the needle, and the test may cause discomfort [3] as well.
4. There are no alternative methods of the examinations: The tests are voluntary.
5. Aims and expected Outcome: Since the diagnosis of autonomic and peripheral neuropathy is complex and might be inconvenient for children, our aim is to detect early abnormalities in bladder muscle function in diabetic children in an easy and painless way before the manifestation of autonomic or peripheral neuropathy.
6. Scheduled date: Since patients have annual control examination due to their chronical disease, we plan to carry out all the examinations at the time of the annual follow-up.

PARENTAL INFORMATION about the process of the INTACT Trial

Dear Parent and Relative!

Depending on the metabolic state of your child, minor but detectable abnormalities in the nervous system that affect both the autonomic and sensory nerve pathways may add up over time. Assessing these has been a time consuming process so far.

In this trial, we would like to test a simpler, easy-to-perform, painless method that infers the existence of nervous system abnormalities from the dynamics of urination.

The first part of the study consists of urinating to a special toilet (uroflowmetry), which is not stressful and does not require preparation. The test is performed at a pre-arranged time (in parallel with the annual control examinations), and approx. it's duration is 10 minutes.
In the second part of the study, the annual control blood sampling, pulse and blood pressure measurements will be performed in the supine and standing positions. In addition, a body composition (InBody) analysis will be performed to derive body composition through impedance measurements.

In the third part of the study, we use a special instrument to measure possible nerve damage with sensors placed on different areas of the lower extremities. This part of the study takes approx. 20-30 minutes. The results obtained will be compared with the uroflowmetry values.

The aim of the study is to explore whether more time-consuming tests for neuropathy can be triggered by a faster, simpler (non-invasive) urination test.

Test procedure:
Upon arrival, spontaneous urination begins in the special toilet, followed by data collection, annual blood collection, body composition (InBody) analysis.
The child then consumes 15 ml of fluid per kilogram of body weight and urinates again in the special toilet at the first urge of urination. At the bottom of the device the measuring sensor is connected to a computer. Pulse and blood pressure measurements and sensory function tests are performed during the waiting period.
Bring the child to the test in the morning with a full bladder, and bring any fluid you want.

Thank you for supporting the success of the study.

Supplementary Table 4. Questionnaire used in the INTACT study.

Heim Pál National Pediatric Institute

INTACT Trial

Patient Questionnaire

Please fill or underline the correct answer.

1. Patient personal details

Name: ……..

Insurance number: ……

Date of birth: ……..

Gender: F / M

Address: ……..

Phone number: ……...

E-mail address: ……

Who has signed the statement of consent?

patient / parent / guardian / other relative

Country: Hungary

City: Budapest

Institute: Heim Pál National Paediatric Institute

Name of the expert physician:

Name of the physician who executes the examination:

Date of the examination:

2. Contact person, availability of the contact person

Contact person: patient / parent / guardian / other relative / nursing home

Name: ……

Address: ……

Phone number: ……

E-mail address: ……....

3. Patient's history

Diet: yes / no

If yes: name of the diet:

vegetarian / lacto-ovo vegetarian / vegan / raw vegan / paleo / gluten free / lactose free / dairy free / ketogenic / PKU diet / other (name of the diet): ……

Alcohol consumption: yes / no

If yes: Frequency: occasionally / monthly / weekly / daily

Amount (g/occasion):.....

For how many years? .....

Total alcohol consumption in the last 2 weeks: .....

If not: Did you drink alcohol earlier? yes / no

If yes: Frequency: occasionally / monthly / weekly / daily

Amount (g/occasion):.....

For how many years?.....

How long ago did you stop drinking alcohol?.....

Guide for estimation of the amount:

1 dl beer (4.5 vol. %) = ~3.5 g alcohol

1 dl wine (12.5 vol. %) = ~10 g alcohol

1 dl hard drink (50 vol. %) = ~40 g alcohol

Smoking: yes / no

If yes: Amount (cigarettes/day):.....

How many years ago have you started? .....

Pack year (a physician will calculate it) .....

If not: Did you smoke earlier? yes / no

If yes: Amount (cigarettes/day):.....

For how many years?.....

Pack year: (a physician will calculate it) .....

How long ago did you stop smoking? .....

48-hour total fluid intake and voided volume (fluid balance)

(This document will be filled by the patient before the examination)

Total fluid intake in 24 hours:

1. day: ……
2. day: ……

Total voided volume in 24 hours:

1. day: ……
2. day: ……

Time	Consumed liquid (mL)	Voided liquid (mL)
07:00		
08:00		
09:00		
10:00		
11:00		
12:00		
13:00		
14:00		
15:00		
16:00		
17:00		
18:00		
19:00		
20:00		
21:00		
22:00		
23:00		
00:00		
01:00		
02:00		
03:00		
04:00		
05:00		
06:00		
07:00		
08:00		
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00:00		
01:00		
02:00		
03:00		
04:00		
05:00		
06:00		
07:00		
08:00		
09:00		
10:00		
11:00		

QUESTIONNAIRE FILLED BY PHYSICIAN

Name of the patient: ……

Insurance number: ……..

Date of birth: ……...

Race: White / Black / Indian / Asian / Other: **…….**

1. Medications

Medications taken regularly: yes / no

If yes:

name of the medication:.....

active substance:.....

dose (without unit, number only)..... unit (gram, milligram, etc.).....

if fluid, concentration (e.g. 10%, 1g/2mL, etc.).....

how many times per day (e.g. 3) .....

method of administration: intravenous / oral / enteral / subcutaneous /other, if other: please describe:.....

2. Physical status of the patient before the examinations

Body length (cm):.....

Body weight (kg):.....

Body surface (m^2):.....

BMI (kg/m^2):.....

Respiratory rate (/min):.....

Oxygen saturation measured by pulse oximetry (%):.....

Heart rate (HR) (/min):.....

Capillary refill time (sec):.....

Blood pressure (NIBP) (mmHg): :.....

Body temperature (°C): :.....

Area of the measurement:.....

3. Clinical symptoms

Urgent urination: yes / no

Daily urine incontinence: yes / no

Urination during night time: yes / no

Nocturia: yes / no

Frequency of bowel movement: daily / every two days / less frequently

if yes: frequency:.....

Consistency of the stool: normal / hard / loose / liquid

4. Diabetes anamnesis

Type of diabetes: Type 1 / Type 2 / MODY

Time of diagnosis: .....(year)

Treatment of diabetes:

- Oral antidiabetics: yes / no

  if yes: active agent: .....

amount / day: ..... mg / daily

- Diet: yes / no

  if yes: amount of carbohydrate per day:..... g CH / day

- Insulin: yes / no

  if yes: amount: ..... unit / day

Way of insulin administration:

- Subcutaneous injection (syringe or pen): yes / no
- Pump: yes / no

Use of sensor–pump: yes / no

Diabetic ketoacidosis:

Total number of ketoacidosis since the diagnosis of the disease:..... occasion

Number of ketoacidosis last year:..... occasion

Ketoacidosis requiring Intensive Care Unit treatment: yes / no

if yes: total number of ICU treatment: ..... occasion

HgA1c value in the last 3 months: .....%

Fasting glucose concentration at the time of the last measurement: .....mmol/l

Date of the measurement: .....

Postprandial glucose concentration at the time of the last measurement:.....mmol/l

Date of the measurement: .....

Diabetic complication: yes / no

If yes:

Neuropathy: yes / no

Retinopathy: yes / no

Nephropathy: yes / no

5. Laboratory parameters

CRP (C–reactive protein) (mg/L)	
Erythrocyte sedimentation rate (ESR) (mm/h)	
White blood cell (WBC) count (G/L)	
Absolute neutrophil count:	
Absolute lymphocyte count:	
Red blood cell (RBC) count (T/L)	
Haemoglobin (g/L conversion to mmol/L)	
Haematocrit (%)	
Thrombocyte count (G/L)	
Glucose (mmol/L conversion to mg/dL)	
HbA1c value (% conversion to mmol/mol)	
C-peptide (mg/mL)	
Triglyceride (mmol/L)	
Cholesterol (mmol/L)	
Uric acid (mmol/L conversion to mg/dL)	
Creatinine (μmol/L conversion to mg/dL)	
Carbamide (mg/dL conversion to mmol/L)	
AST/GOT (U/L)	
ALT/ GPT (U/L)	

GGT (U/L)	
Lactate dehydrogenase LDH (U/L)	
Alkaline phosphatase (U/L)	
Natrium (mmol/L)	
Potassium (mmol/L)	
Calcium (mmol/L)	
Amylase (U/L)	
Lipase (U/L)	
Albumin (g/L)	
Serum total protein concentration (g/dL)	
Result of urine rapid test	

Cardiovascular autonomic dysfunction tests

Schellong test

NIBP in a supine position (mmHg): ……

HR in a supine position (/min): ……...

NIBP after line up (mmHg): ……

HR after line up (/min): ……...

NIBP after 1 minutes in a standing position (mmHg): ……

HR after 1 minutes in a standing position (/min): ……...

NIBP after 5 minutes in a standing position (mmHg): ……

HR after 5 minutes in a standing position (/min): ……...

1)HR response to deep breathing: ……..

2)HR response to deep breathing: ……..

3)HR response to deep breathing: ……..

NIBP before sustained handgrip examination starts: ……..

NIBP to sustained handgrip in the 1st minute: ……...

NIBP to sustained handgrip in the 2nd minute: ……..

NIBP to sustained handgrip in the 3rd minute: ……...

NIBP to sustained handgrip in the 4th minute: ……...

NIBP to sustained handgrip in the 5th minute: ……...

NIBP just before release of handgrip: …….

Valsalva maneuver:

HR during the maneuver (/min): …….

HR after the maneuver (/min): ……

Uroflowmetry

Spontaneous voiding:

Voided volume (mL): ……

Voiding time (sec):…….

Time to Qmax (s):……...

Q_{ave} (mL/s):…….

Q_{max} (mL/s):……..……..

Q_{acc} (ml/sec^2):…….

Ultrasonographic examination

Before voiding:

bladder wall thickness (mm): …….

bladder diameter (mm): ……..

After voiding:

bladder wall thickness (mm): …….

bladder diameter (mm): ……..

residuum (mL): ……...

Voiding at first sensation:

Voided volume (mL): …….

Time to first sensation (min):……..

Voiding time (sec):…….

Time to Qmax (s):……...

Q_{ave} (mL/s):…….

Q_{max} (mL/s):……..……..

Q_{acc} (ml/sec^2):…….

Ultrasonographic examination

Before voiding:

bladder wall thickness (mm): …….

bladder diameter (mm): ……..

After voiding:

bladder wall thickness (mm): …….

bladder diameter (mm): ……..

residuum (mL): ……...

InBody examination

Body composition analysis:

Weight (kg):……

Height (cm):……

BMI(kg/m^2):……

Total body water (litres):.....

Protein (kg):.....

Minerals (kg):.....

Body fat mass (kg):.....

Muscle-fat analysis:…….

Skeletal muscle mass (kg): .....

Lean body mass (kg): .....

Dry lean mass (kg):…….

Basal metabolic rate(kcal):……..

Percent body fat (%):.....

Supplementary Table 5. Data quality of all patients.

EPIDEMIOLOGY	**OVERALL**	**UPLOADED DATA**	**%**
Age	94/114/208	94/114/208	100/100/100%
Gender	94/114/208	94/114/208	100/100/100%
Weight	94/114/208	93/113/206	98,9/99,1/99%
Height	94/114/208	79/98/177	84/85.9/85%
Body surface	94/114/208	80/98/179	85.1/86.8/86%
Total	***470/570/1040***	***440/538/978***	***93.6/94.3/94%***
UROFLOWMETRY PARAMETERS	**OVERALL**	**UPLOADED DATA**	**%**
Voided volume	169/235/404	169/235/404	100%
Voiding time	169/235/404	169/235/404	100%
Time to Q_{max}	169/235/404	169/235/404	100%
Q_{ave}	169/235/404	169/235/404	100%
Q_{max}	169/235/404	169/235/404	100%
Qa_{cc}	169/235/404	169/235/404	100%
Postvoid residual	169/235/404	169/235/404	100%
Total	***1183/1645/2828***	***1183/1645/2828***	***100%***

208 children 94 girls, 114 boys performed 404 (169/235) micturition.

Supplementary Figure 1. A summary of the risk of bias assessment.

QUIPS tool-

Risk of bias assessment

QUIPS assess risk of bias in studies of prognostic factors.

Concerns about risk of bias and applicability were rated as “low”, “moderate” or “high” in six domains 1)study parti-

cipation, 2)study attrition, 3)prognostic factor measurement, 4)outcome measurement, 5)study confounding, as well as 6) statistical analysis and reporting.

To judge overall risk, the review authors (ÁRM and PP) described studies with a low risk of bias as those in which at least five of the six important bias domains were rated as having low risk of bias. If there was at least one domain rated as high risk, or more than three domains rated as moderate risk of bias, the overall risk of bias was deemed high. All other variations were determined as moderate risk of bias.

Abbreviations: QUIPS: quality in prognostic studies

Supplementary Figure 1 Panel A: Risk of bias assessment on study level [A] and across studies [B] assessing mean voided volume (mL) in diabetic female population.

A

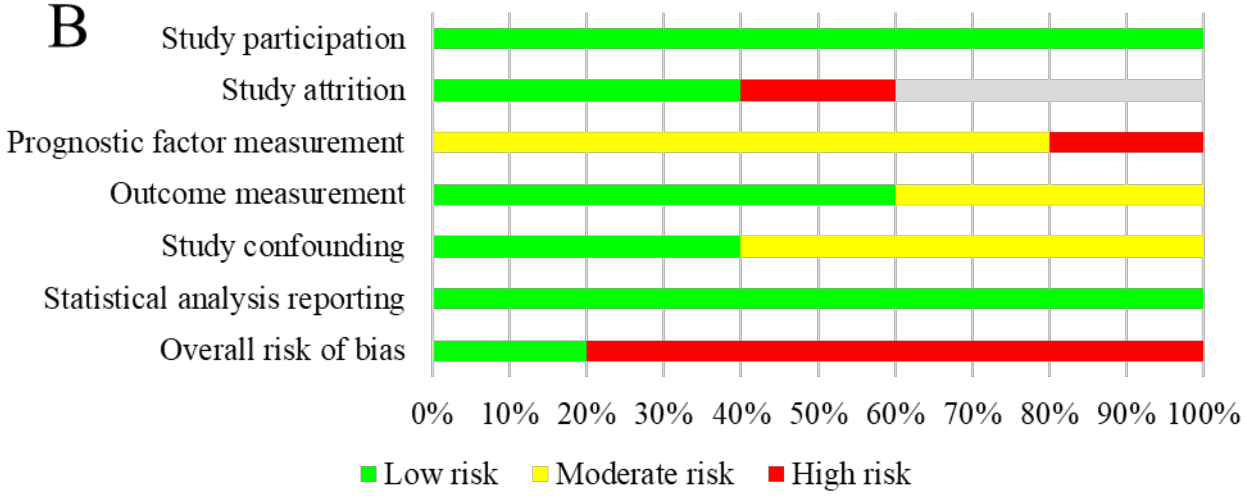
+ Low risk

? Moderate risk

– High risk

	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
Golabek et al. 2012[1]	+	NA	?	?	?	+	-	Yes
Lee et al. 2007[2]	+	-	?	+	?	+	-	Yes
Løwenstein et al. 2021[3]	+	+	-	+	+	+	-	Yes
Shin et al. 2016[4]	+	NA	?	?	?	+	-	Yes
Tai et al. 2009[5]	+	+	?	+	+	+	+	Yes

B



1: Diabetes definition and duration, as well as LUTS assessment were not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

2: Study design is not reported. Not all patients were included in the analysis, diabetes definition and treatment are not reported.

3: Definition of diabetes and type of it are not reported.

4: Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

5: Definition of diabetes is not reported.

Supplementary Figure 1 Panel B: Risk of bias assessment on study level [A] and across studies [B] assessing mean postvoid residual (mL) in diabetic female population.

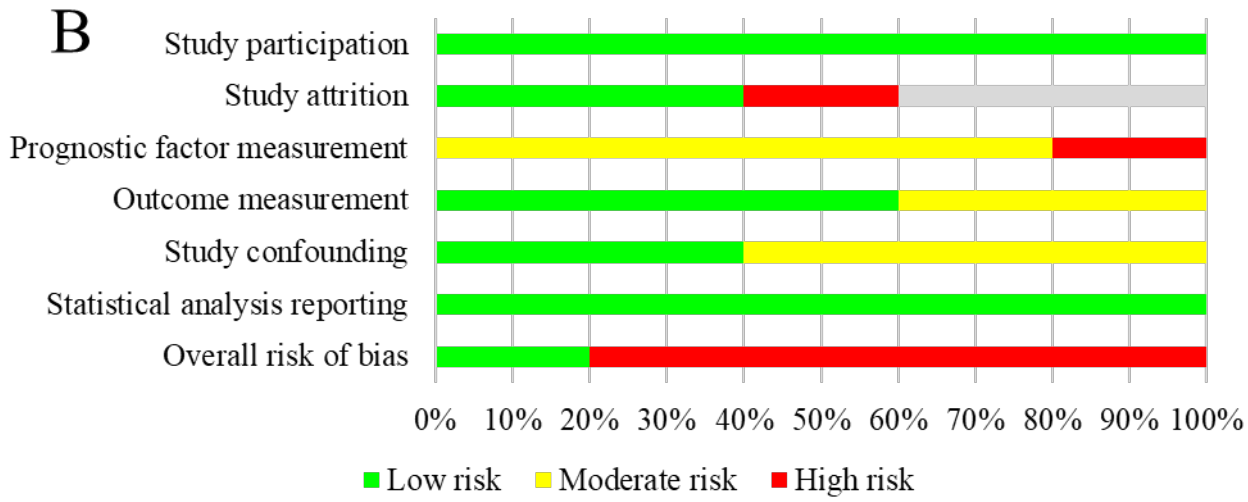
A

+ Low risk

? Moderate risk

– High risk

	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
Al Timimi et al. 2020[1]	+	+	-	-	?	-	-	Yes
Changxiao et al. 2014[2]	+	?	+	?	?	+	-	Yes
Gali et al. 2015[3]	+	-	?	?	+	+	-	Yes
Golabek et al. 2012[4]	+	NA	?	?	?	+	-	Yes
Lee et al. 2007[5]	+	-	?	+	?	+	-	Yes
Malik et al. 2020[6]	+	+	-	?	?	+	-	Yes
Shin et al. 2016[7]	+	NA	?	-	?	+	-	Yes
Tai et al. 2009[8]	+	+	?	+	+	+	+	Yes
Yenilmez et al. 2008[9]	+	?	?	?	-	+	-	Yes



1: HgA1c, diabetes duration are not reported. The measuring method of postvoid residual volume is not reported. The method (device) of the uroflowmetry parameters is not reported. Although it is reported that diabetes treatment was evaluated, but data could not be extracted. Statistical analysis is not reported.

2: Not all patients were included in the analysis. The measuring method of postvoid residual volume is not reported. Assessed confounding factor is caused by unreported BMI.

3: The study design is unknown, and not all patients were included in the analysis. The measuring method of postvoid residual volume is not reported. Diabetes definition is not reported.

4: Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

5: Study design is not reported. Not all patients were included in the analysis. Diabetes definition and treatment are not reported.

6: Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported.

7: Type of diabetes and LUTS assessment are not reported. The measuring method of postvoid residual volume is not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

8: Diabetes definition is not reported.

9: Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

Supplementary Figure 1 Panel C: Risk of bias assessment on study level [A] and across studies [B]] assessing mean Qmax (mL/sec) in diabetic female population.

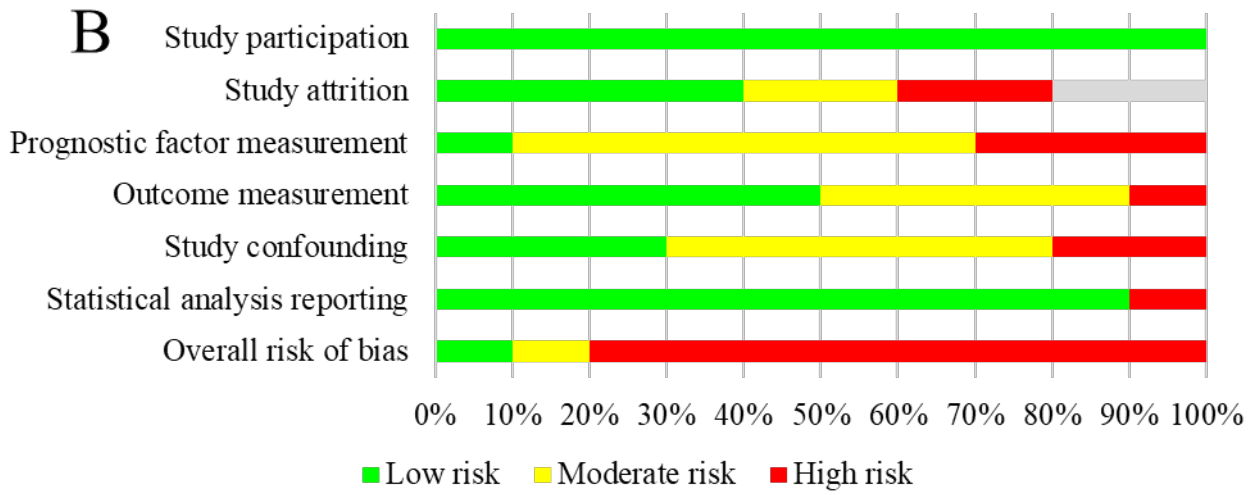
A

+ Low risk

? Moderate risk

– High risk

	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
Al Timimi et al. 2020[1]	+	+	-	-	?	-	-	Yes
Changxiao et al. 2014[2]	+	?	+	+	?	+	?	Yes
Gali et al. 2015[3]	+	-	?	+	+	+	-	Yes
Golabek et al. 2012[4]	+	NA	?	?	-	+	-	Yes
Lee et al. 2007[5]	+	-	?	+	?	+	-	Yes
Løwenstein et al. 2021[6]	+	+	-	+	+	+	-	Yes
Malik et al. 2020[7]	+	+	-	?	?	+	-	Yes
Shin et al. 2016[8]	+	NA	?	?	?	+	-	Yes
Tai et al. 2009[9]	+	+	?	+	+	+	+	Yes
Yenilmez et al. 2008[10]	+	?	?	?	-	+	-	Yes



1: HgA1c, diabetes duration are not reported. The method (device) of the uroflowmetry parameters is not reported. Although it is reported that diabetes treatment was evaluated, but data could not be extracted. Statistical analysis is not reported.

2: Not all patients were included in the analysis. Assessed confounding factor is caused by unreported BMI.

3: The study design is unknown, and not all patients were included in the analysis. The measuring method of postvoid residual volume, as well as diabetes definition are not reported.

4: Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI; and patients having Qmax lower than 12 mL/min were excluded.

5: Study design is not reported. Not all patients were included in the analysis. Diabetes definition and treatment are not reported.

6: Definition of diabetes and type of it are not reported.

7: Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported.

8: Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

9: Definition of diabetes is not reported.

10: Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

Supplementary Figure 1 Panel D: Risk of bias assessment on study level [A] and across studies [B]] assessing mean PdetQmax (cmH2O) in diabetic female population.

A

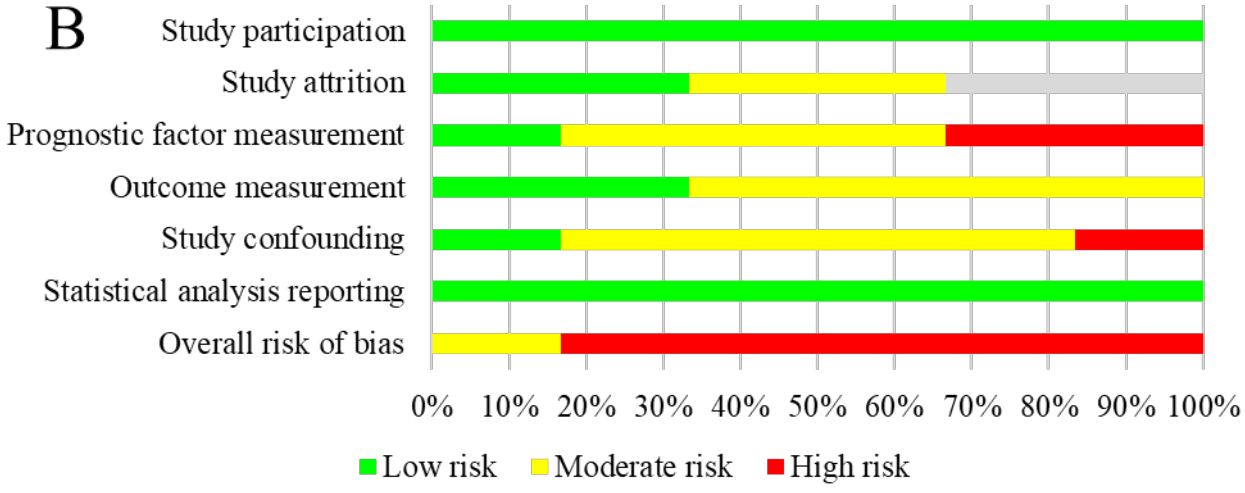
+ Low risk

? Moderate risk

– High risk

	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
Changxiao et al. 2014[1]	+	?	+	+	?	+	?	Yes
Golabek et al. 2012[2]	+	NA	?	?	?	+	-	Yes
Løwenstein et al. 2021[3]	+	+	-	-	+	+	-	Yes
Malik et al. 2020[4]	+	+	-	?	?	+	-	Yes
Shin et al. 2016[5]	+	NA	?	?	?	+	-	Yes
Yenilmez et al. 2008[6]	+	?	?	?	-	+	-	Yes

B



1: Not all patients were included in the analysis. Assessed confounding factor is caused by unreported BMI.

2: Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

3: Definition of diabetes and type of it are not reported.

4: Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported.

5: Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

6: Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

Supplementary Figure 1 Panel E: Risk of bias assessment on study level [A] and across studies [B] assessing mean first sensation (mL) in diabetic female population.

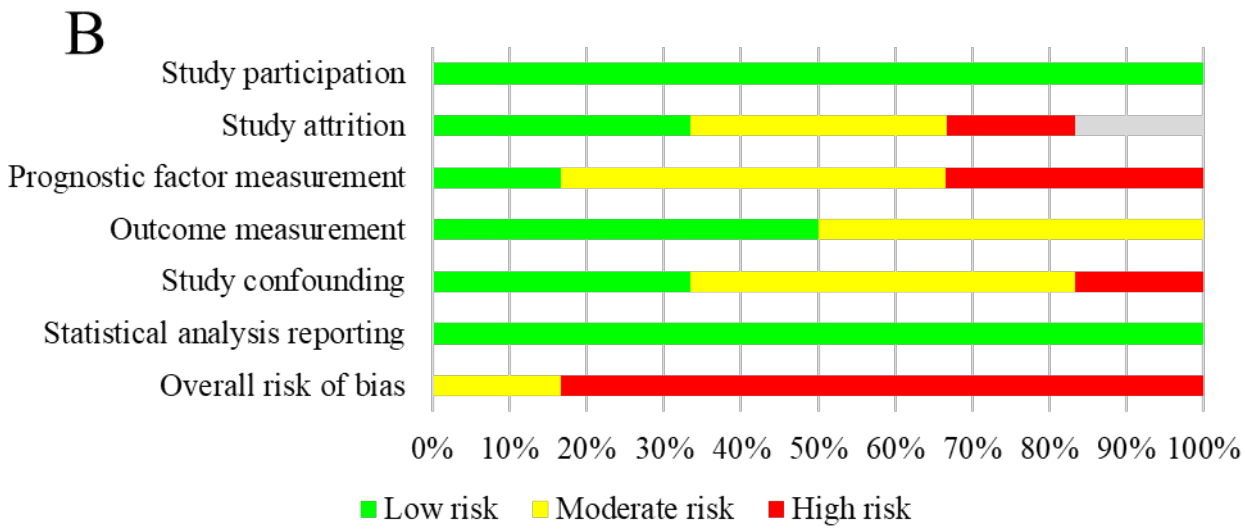
A

+ Low risk

? Moderate risk

– High risk

	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
Changxiao et al. 2014[1]	+	?	+	+	?	+	?	Yes
Gali et al. 2015[2]	+	-	?	+	+	+	-	Yes
Løwenstein et al. 2021[3]	+	+	-	+	+	+	-	Yes
Malik et al. 2020[4]	+	+	-	?	?	+	-	Yes
Shin et al. 2016[5]	+	NA	?	?	?	+	-	Yes
Yenilmez et al. 2008[6]	+	?	?	?	-	+	-	Yes



1: Not all patients were included in the analysis. Assessed confounding factor is that BMI was not reported.

2: The study design is unknown, and not all patients were included in the analysis. Diabetes definition is not reported.

3: Definition of diabetes and type of it are not reported.

4: Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported.

5: Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

6: Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

Supplementary Figure 1 Panel F: Risk of bias assessment on study level [A] and across studies [B] assessing mean cystometric capacity (mL) in diabetic female population.

A

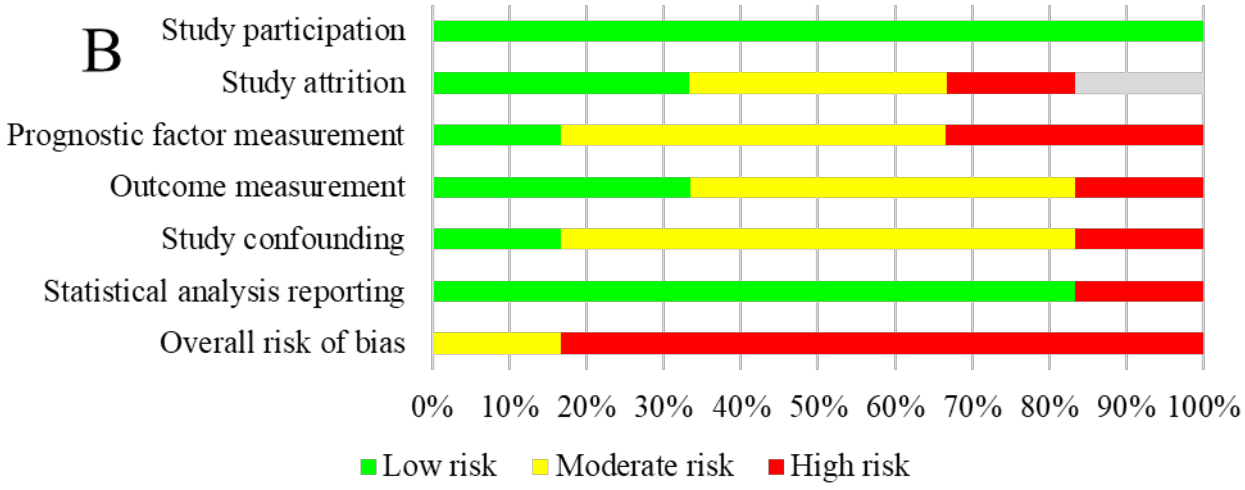
+ Low risk

? Moderate risk

– High risk

	Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
Al Timimi et al. 2020[1]	+	+	-	-	?	-	-	Yes
Changxiao et al. 2014[2]	+	?	+	+	?	+	?	Yes
Gali et al. 2015[3]	+	-	?	+	+	+	-	Yes
Golabek et al. 2012[4]	+	NA	?	?	?	+	-	Yes
Malik et al. 2020[5]	+	+	-	?	?	+	-	Yes
Yenilmez et al. 2008[6]	+	?	?	?	-	+	-	Yes

B



1: HgA1c, diabetes duration are not reported. The method (device) of the uroflowmetry parameters is not reported. Although it is reported that diabetes treatment was evaluated, but data could not be extracted. Statistical analysis is not reported.

2: Not all patients were included in the analysis. Assessed confounding factor is caused by unreported BMI.

3: The study design is unknown, not all patients were included in the analysis. Diabetes definition is not reported.

4: Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI.

5: Type of diabetes and definition of it are not reported. LUTS assessment is not reported. Diabetes treatment is partly reported.

6: Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

I.

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Urodynamics in Early Diagnosis of Diabetic Bladder Dysfunction in Women: A Systematic Review and Meta-Analysis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Conflict of interest: None declared

Background: Urodynamics can detect subtle voiding changes before cystopathy symptoms manifest. The aim of the present study was to assess urodynamic changes in diabetic women.

Material/Methods: A systematic search was performed on 04 November 2021 to identify studies reporting urodynamic parameters in diabetic women. Data were analyzed in a single-arm meta-analysis due to lack of sufficient studies with direct comparisons to healthy women. For data synthesis, a random-effects model with restricted maximum-likelihood estimation was applied. The calculated effect sizes were visualized in forest plots. Statistical heterogeneity was assessed using the I^2 measure and the χ^2 test. The risk of bias was assessed using the QUIPS tool. PROSPERO ID: CRD42021256275.

Results: Out of 1750 records, 10 studies were used in the analysis (n=2342 diabetic women). Pooled event rates showed that mean voided volume was 288.21 mL [95% confidence interval (CI): 217.35-359.06, I^2=98%], mean postvoid residual volume was 93.67 mL [95% CI: 31.35-155.99, I^2=100%], mean Q_{max} was 18.80 mL/sec [95% CI: 15.27-22.33, I^2=99%], mean $P_{det}Q_{max}$ is 30.13 cmH_2O [95% CI: 25.53-34.73, I^2=90%], mean first sensation of bladder filling was 178.66 mL [95% CI: 150.59-206.72, I^2=97%], and mean cystometric capacity was 480.41 mL [95% CI: 409.32-551.50, I^2=98%] in diabetic women.

Conclusions: Pooled results indicate that diabetic women tend to have a smaller voided volume, slower Q_{max} and $P_{det}Q_{max}$, larger postvoid residual, and higher first sensation of bladder filling and cystometric capacity compared to the general female population.

Keywords: **Diabetes Complications • Diabetes Mellitus • Diabetic Neuropathies • Meta-Analysis • Systematic Review • Urodynamics • Urology**

Abbreviations: **BMI** – body mass index; **BOO** – bladder outlet obstruction; **CENTRAL** – Cochrane Central Register of Controlled Trials; **CI** – confidence interval; **DC** – diabetic cystopathy; **κ** – Cohen's kappa; **LUTS** – lower urinary tract symptoms; **MCC** – maximum cystometric capacity; **mL** – milliliter; **OAB** – overactive bladder; **$P_{det}Q_{max}$** – maximal detrusor pressure at maximal flow rate; **PRISMA** – Preferred Reporting Items for Systematic reviews and Meta-Analyses; **Q_{ave}** – average flow rate; **Q_{max}** – maximum flow rate; **Q_{acc}** – urine flow acceleration (Q_{max}/TQ_{max}); **QUIPS** – Quality in Prognostic Studies; **REML** – restricted maximum likelihood; **TQ_{max}** – time to maximum flow rate; **UB** – underactive bladder

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/937166

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Background

Diabetic cystopathy (DC) is a well-recognized urological complication of diabetic autonomic neuropathy [1]. The classic triad of DC is decreased bladder sensation, increased bladder capacity, and impaired bladder emptying with postvoid residual volume [2-6]. Patients usually have overactive bladder or overflow incontinence, including urinary frequency, urgency, incontinence, and nocturia, which are listed among the lower urinary tract symptoms (LUTS) [7,8].

DC gradually progresses over time from an initial compensated [typically manifests as overactive bladder (OAB)] to a later decompensated [typically manifests as underactive bladder (UB)] phase that cause storage or voiding LUTS [9,10].

Uroflowmetry is a simple urodynamic diagnostic tool to measure voided volume and maximum flow rate. Cystometry measures pressure/volume relationship of bladder during the filling (storage) phase of the micturition cycle via a catheter. As urodynamics can detect subtle voiding changes even before the manifestation of LUTS, urodynamic evaluation can be useful in the early diagnosis of DC.

The normative reference values for bladder function by urodynamics in healthy women have been described earlier [11,12], but the literature on diabetic women is scant. Therefore, our aim was to assess urodynamic alterations in diabetic women.

Material and Methods

The study is reported as per the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) 2020 Statement [13] .

Study protocol

The study was in line with the protocol registered on 23 May 2021, and can be found under the registration number: CRD42021256275 in PROSPERO. A review protocol was not prepared.

Our primary aim was to compare the urodynamic parameters of diabetic women to those of healthy women, but the eligible studies according to our inclusion criteria did not provide sufficient raw data on healthy women. Since a direct comparison could not be implemented in diabetic and non-diabetic patients, we conducted a single-arm meta-analysis and positive event rates were pooled for statistical analysis. Furthermore, our intent was to detect changes in voiding in diabetic women with cystopathy compared to diabetic women without peripheral neuropathy by uroflowmetry, but the eligible studies did not contain sufficient raw data to conduct the analysis. Otherwise, we fully adhered to the study protocol.

Systematic Search

Two review authors (ÁRM and PP) independently carried out the systematic literature search in Embase, MEDLINE (via PubMed), the Cochrane Central Register of Controlled Trials (CENTRAL), and the Web of Science. The authors applied the following keywords: (urodynamic or uroflow* or uroflowmetry) and (diabetes or diab*) (**Supplementary Table 1**) on 04 November 2021. In each database, they applied "All text" or "All fields" in the search bar avoiding any filters or restrictions regarding publication year, language, and place of origin. They reviewed the included studies to find any articles previously missed in the original search.

Selection and Eligibility

The authors included case-control and cohort studies, as well as full-text articles and conference abstracts in the synthesis of quantitative and qualitative data to reduce selection bias. Case reports featuring single patients were excluded. In case of potentially overlapping study populations (based on authors, sites, patients and urodynamic parameters), those with better quality of data were included.

Eligible studies had to provide data on diabetic women, with or without voiding disorder, and urodynamic parameters [14], which include uroflowmetry and cystometry parameters [15,16]. Regardless of the method of measurement (with ultrasonography or via catheter), postvoid residual volume (in mL) was also included in the analysis. Studies that included both sexes were included if they performed subgroup analysis for diabetic women.

Articles examining patients after kidney transplantation or surgery affecting the genitourinary tract, patients with neurogenic bladder dysfunction and other neurological disease (eg, progressive neurological conditions such as Parkinson's disease, dementia, multiple sclerosis) except peripheral neuropathy; pregnant women and women within first 6 months postpartum, end-stage kidney disease, or kidney transplants were excluded, since they can influence urodynamic parameters per se, causing indistinguishable confounding factors.

The records were selected via a standard three-phased process including titles, abstracts, and full-texts independently by the 2 review authors (ÁRM and PP) with EndNote X9.1.1 software (2020 Clarivate™ Analytics, Philadelphia, PA, USA). They resolved any disagreements in any phase through consensus, and calculated the Cohen's kappa in each phase to test inter-rater reliability.

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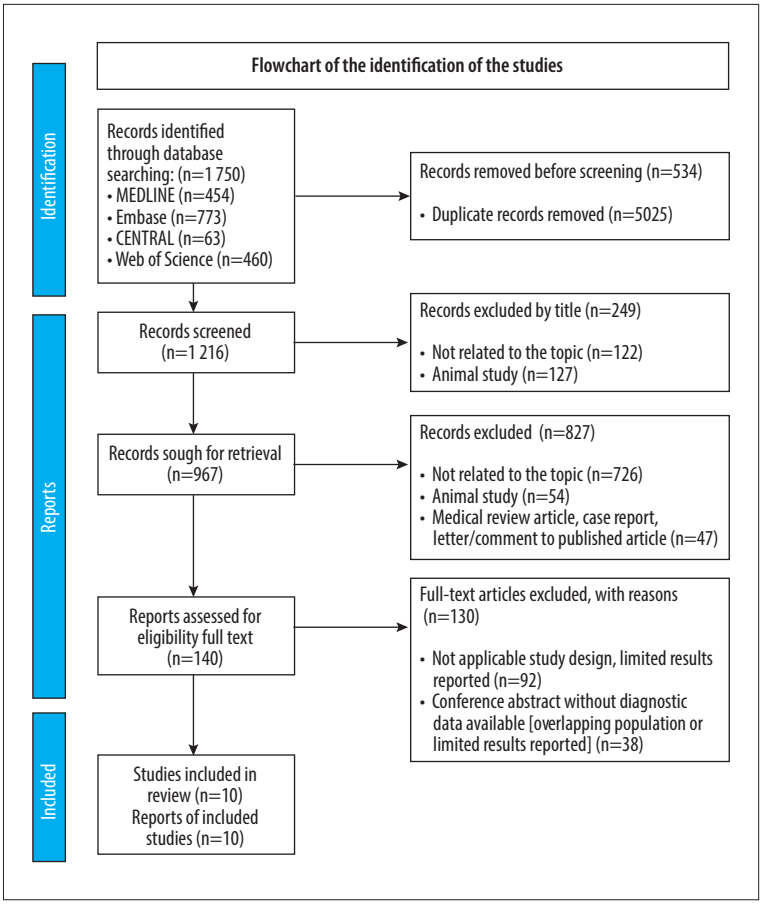

Figure 1. PRISMA flow diagram of the selection of the studies. The algorithm of the study selection; out of the 1750 records, 10 full-text articles were used in the final analysis. The figure was created using Microsoft® Word (version 16.54; 2019).

Data extraction

The 2 independent review authors (ÁRM and PP) extracted the data into a purpose-designed data collection table, with any disagreements resolved by consensus. The following data were extracted from each study: 1) study information (first author, year of publication, recruitment period, country of origin), 2) study design and methodology (retrospective versus prospective, inclusion and exclusion criteria, single versus multicenter study), 3) patient information (number of patients, mean age, definition of diabetes, type of diabetes, diabetes duration, body mass index (BMI), HgA1c values, diabetes treatment, diabetic complication), 4) LUTS assessment and symptoms, DC and peripheral neuropathy existence, and 5) urodynamic parameters of diabetic patients (voided volume, postvoid residual, Q_{max}, $P_{det}Q_{max}$, volume at first sensation, maximal cystometry capacity).

Statistical analysis

Cohen's kappa (κ) was calculated to test interrater reliability. It ranges from -1 to +1, where values ≤0 indicates no agreement (which are unlikely in practice), 0 represents the amount of agreement that can be expected from random chance, and 1 represents perfect agreement between the review authors. κ results should be interpreted as the followings: values ≤0 as indicating "no agreement", 0.01-0.20 as "none to slight", 0.21-0.40 as "fair", 0.41-0.60 as "moderate," 0.61-0.80 as "substantial", and 0.81-1.00 as "almost perfect" agreement [17].

For data synthesis we used the random-effects model with restricted maximum-likelihood (REML) estimation in all cases; means and 95% confidence intervals (CIs) were calculated. The calculated effect sizes were visualized in forest plots. Heterogeneity was tested using Cochrane's *Q* and the I^2 statistics. I^2 statistic represents the percentage of the total variability across studies: 30% to 60%, 50% to 90%, and 75% to 100% corresponded to "moderate", "substantial", and "considerable" degrees of heterogeneity, respectively, based on the Cochrane's handbook for Systematic Reviews of Interventions [18]. We considered the Q test significant if $P<0.1$. Statistical analyses were carried out using R statistical software (version 4.0.5) and package *meta* (version 4.18-1). We created a single-arm meta-analysis based on urodynamic parameters and the results are graphically presented in forest plots.

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Risk of bias assessment

The risk of bias of the studies were evaluated by 2 independent review authors (ÁRM and PP) using the Quality In Prognostic Studies (QUIPS) tool [19]. The result of the assessment was graphically demonstrated; any disagreements were resolved by consensus among the review authors.

To judge overall risk, the review authors (ÁRM and PP) described studies with a low risk of bias as those in which at least 5 of the 6 important bias domains (study participants, study attrition, prognostic factor measurement, outcome measurement, study confounding, and statistical analysis) were rated as having low risk of bias. If there was at least 1 domain rated as high risk, or more than 3 domains rated as moderate risk of bias, the overall risk of bias was deemed high. All other variations were determined as moderate risk of bias.

When the study design could not be identified, study attrition was not evaluated and was thus labeled as 'grey'. Grey means not applicable or not reported.

There were not enough studies in the analyses to evaluate publication bias by funnel plots and statistical tests.

Results

Study Selection

Out of 1750 records (MEDLINE, n=454; Embase, n=773; CENTRAL, n=63; and Web of Science, n=460), a total of 140 articles were assessed for eligibility by full text, of which 10 studies [20-29] were used in the quantitative synthesis. κ of the title selection was 0.99 (99.7% agreement), 0.98 (99.3% agreement) of the abstract selection, and 0.99 (99.8% agreement) of the full-text selection. The flowchart and reasons for exclusions on full-text assessment is illustrated in **Figure 1**.

Study Characteristics

The baseline characteristics of the included studies are reported in **Table 1**. Studies took place in 9 different countries, and were published between 2002 and 2020. Six studies were prospective [20,21,25,26,28,29], 2 were retrospective cohorts [23,27], and 2 studies did not provide sufficient information about study design [22,24].

Table 1. Baseline characteristics of the included studies.

First author	Country (centers) and recruitment period	Study design	Inclusion criteria
Al Timimi et al 2020 [20]	Iraq (single) 2018-2019	Prospective cross-sectional	Patients with T2DM at least 5 years with LUTS
Changxiao et al 2014 [21]	China (multi) 2010-2013	Prospective cross-sectional	Women aged ≥18 years with DM
Galí et al 2015 [22]	Italy (single) 2008-2010	NA	Patients with T2DM at least 5 years with moderate/ severe LUTS
Golabek et al 2012 [23]	Republic of Ireland (single) 2004-2008	Retrospective cohort	Diabetic female with OAB, defined as an involuntary rise in detrusor pressure of greater than 5 cm H2O during filling
Lee et al 2007 [24]	Taiwan (single) 2002-2003	NA	Women with T2DM with no concurrent neurologic disorder or medical conditions that could interfere with voiding function, without bladder dysfunction
Løwenstein et al 2021 [25]	Denmark (multi) 2016-2020	Prospective randomized	Adult women with symptoms of urinary incontinence, urgency and nocturia
Malik et al 2020 [26]	USA (multi) 2010-2014	Prospective	Female patients with urology-based voiding dysfunction and no neurologic disease
Shin et al 2016 [27]	South-Korea (single) 2008-2015	Retrospective cohort	Women without BOO who were diagnosed with SUI
Tai et al 2009 [28]	Taiwan (single) 2005-2007	Prospective	Women with T2DM, age 50-75 years
Yenilmez et al 2008 [29]	Turkey (single) 2004-2007	Prospective cross-sectional	Patients with T2DM and LUTS

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Table 1 continued. Baseline characteristics of the included studies.

First Author	LUTS					No. of patients with DC (%)	No. of patients with peripheral neuropathy (%)
	LUTS assessment	No. of patients with LUTS (%)	No. of patients with urge incontinence (%)	No. of patients with stress incontinence (%)	No. of patient with OAB (%)		
Al Timimi et al 2020 [20]	NA	71 (100%)	3 (4.2%)	3 (4.2%)	13 (18.3%)	31 (43.7%)	NA
Changxiao et al 2014 [21]	IUA/ICS	1525 (93%)	NA	NA	918 (55.9%)	1558 (95%)	NA
Galí et al 2015 [22]	IPSS, QoL, OAB-q, ICI-SF	19 (100%)	14 (73.7%)	4 (21%)	15 (79%)	NA	14 (73.7%)
Golabek et al 2012 [23]	NA	29 (100%)	15 (51%)	0	29 (100%)	29 (100%)	NA
Lee et al 2007 [24]	AUA-SI	47 (100%)	NA	NA	0	47 (100%)#	18 (38.3%)
Løwenstein et al 2021 [25]	ICIQ-UI SF, ICIQ-OAB	31 (100%)	NA	4 (12.9%)	4 (12.9%)	NA	4 (12.9%)
Malik et al 2020 [26]	NA	96 (100%)	10 (11%)	44 (45%)	21 (24%)	NA	NA
Shin et al 2016 [27]	NA	92 (100%)	0	92 (100%)	0	NA	NA
Tai et al 2009 [28]	AUA-SI IUSS	100 (36.7%)	49 (18%)	30 (11%)	NA	NA	52 (19.1%)
Yenilmez et al 2008 [29]	NA	45 (100%)	NA	NA	NA	0*	17 (37.7%)

Ten full-text articles were included with a total of 2342 diabetic female patients from 9 countries. The majority of the patients (2055 – 87.7%) had LUTS; 1620 diabetic patients had urodynamic measurements and thus were included in the meta-analysis. # Diabetic cystopathy was defined as postvoid residual volume greater than 100 mL. * Diabetic cystopathy was defined as an increase in bladder capacity (more than 500 mL), impaired bladder sensation and decrease bladder contractility. AUA-SI – American Urological Association Symptom Index; DC – diabetic cystopathy; DM – diabetes mellitus; ICIQ-OAB – International Consultation of Incontinence Questionnaire – Overactive bladder questionnaire; ICIQ-UI – International Consultation of Incontinence Questionnaire Urinary Incontinence Short Form; ICI-SF – International Consultation on Incontinence – Short form; IPSS – International Prostate Symptom Score; IUA/ICS: International Urogynecological Association/International Continence Society Standardization of Terminology Reports; IUSS – Indevus Urgency Severity Scale; LUTS – lower urinary tract symptoms; NA – not available data (not reported); OAB – overactive bladder (detrusor overactivity); OAB-q – Overactive Bladder Questionnaire; QoL – Quality of life Questionnaire; SUI – stress urinary incontinence; T2DM – type 2 diabetes mellitus.

General Characteristics of Diabetic Women

We included 10 studies to the quantitative synthesis that reported on a total of 2342 diabetic patients, including 2055 patients (87.7%) with LUTS. The majority of the patients had type 2 diabetes. In 7 studies, the type of diabetes was reported [20-24,28,29], while in 3 studies [25-27] it was not, although they had small number of patients. The mean age of the study populations ranged between 52.75±9.2 and 64.7±11.1 years, the mean duration of diabetes ranged between 8.04±0.69 and 12.42±7.3 years, the mean BMI ranged between 22.8±2.4 and 33.2±7.8 kg/m^2, and the mean HgA1c ranged between 6.05±2.38 and 9.1±2.6%. The demographic characteristics of diabetic patients are presented in **Table 2**.

Urodynamic Parameters of Diabetic Women

The urodynamic parameters of the diabetic women were assessed qualitatively. These data are represented in **Table 3**.

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Table 2. Demographic characteristics of women with diabetes of the included studies.

Study	Definition of diabetes	Number of patients (percentage of T2DM in%)	Mean age (years) ±SD	Mean diabetes duration (years) ±SD	Mean BMI (kg/m²) ±SD	HgA1c (%) ±SD	Diabetes treatment number of patients with treatment (percentage in %)		
							Oral antidiabetic agent	Insulin	Diet and exercise
Al Timimi et al 202 0[20]	WHO (Alberti and Zimmet 1998[#]) criteria	71 (100%)	62±13	12.2±4.1	28.9±4.57	NA	NA	NA	NA
Changxiao et al 2014 [21]	ADA	1640 (95)*	52.75±9.2	8.04±0.69	NA	6.8±1.87	1107 (67.5)	384 (23.4%)	103 (6.2%)
Galí et al 2015 [22]	NA	19 (100)	63.1±10.0	11.9±5.2	28.8±2.3	9.1±2.6	11 (57.9%)	8 (42.1%)	0
Golabek et al 2012 [23]	NA	29 (89)	53.84±16.0	NA	NA	6.05±2.38	NA	NA	NA
Lee et al 2007 [24]	NA	47 (100)	63.6±9.3	12.42±7.3	25.3±2.7	7.7±1.4	NA	NA	NA
Løwenstein et al 2021 [25]	NA	31 (not reported)	64.7±11.1	11.1±10.1	31.8±5.5	6.7 (6.4-7.2)***	15 (47%)	3 (9.7%)	NA
Malik et al 2020 [26]	NA	96 (not reported)	57.6±12.2	10.3±8.5	33.2±7.8	7.5±2.1	NA	13 (13%)	NA
Shin et al 2016 [27]	ADA	92 (not reported)	58.34±8.25	9.24±7.63	NA	7.27±1.43	NA	NA	NA
Tai et al 2009 [28]	NA	272 (100)	63.1±9.8	11.6±8.5	22.8±2.4	7.3±1.2	234 (86%)	16 (5.9%)	4 (1.5%)
Yenilmez et al 2008 [29]	NA	45 (100)	60.1±1.4[§]	11.4±1.0**§	28.8±0.8**,§	7.3±0.3**,§	NA	13 (35%)**	NA

[#] Diabetes was defined according to Alberti et al 1998 (Alberti, K. G. M. M., Zimmet, P.Z. 1998 Definition, diagnosis and classification of diabetes mellitus and its complications Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO Consultation. *Diabetic Medicine* 15 (7): 539-553); * Of 1640 patients, 918 had uroflow measurement and thus were included in the meta-analysis; ** Data of 37 diabetic women after uroflowmetry without major complication after the procedure; *** Data presented as median, 25th percentile (first figure in the brackets), and 75th percentile (second figure in the brackets); [§] Data presented as mean and standard error. ADA – American Diabetes Association; BMI – Body mass index; NA – not available data (not reported); SD – standard deviation; T2DM – type 2 diabetes mellitus; WHO – World Health Organization.

Voided Volume

The pooled event rates show that mean voided volume in diabetic women (n = 471) was 288.21 mL [95% CI: 217.35-359.06] with a considerable level of heterogeneity (I^2=98%) (**Figure 2 A**).

Postvoid Residual Volume

The pooled event rate represents that mean postvoid residual volume in diabetic women (n=1589) was 93.67 mL [95% CI: 31.35-155.99] with a considerable level of heterogeneity (I^2=100%) (**Figure 2B**).

Q_{max}

The mean Q_{max} in diabetic women (n=1620) was 18.80 mL/sec [95% CI: 15.27-22.33] with a considerable level of heterogeneity (I^2=99%) (**Figure 2C**).

$P_{det}Q_{max}$

The mean $P_{det}Q_{max}$ in diabetic women (n=1211) was 30.13 cm-H_2O [95% CI: 25.53-34.73] with a considerable level of heterogeneity (I^2=90%) (**Figure 2D**).

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Table 3. Baseline characteristics of urodynamic parameters of diabetic women in the included studies.

First Author	Mean voided volume (mL) ±SD	Mean postvoid residual volume (mL) ±SD	Mean Q_{max} (mL/sec) ±SD	Mean $P_{det}Q_{max}$ (cmH_2O) ±SD	Mean first sensation of bladder filling (mL) ±SD	Mean cystometric capacity (mL) ±SD
Al Timimi et al 2020 [20]	NA	127±15	14±1.3	NA	NA	426±414
Changxiao et al 2014 [21]	NA	323±79.7	9.6±7.1	32.4±13.2	238.1±58.3	624±117.4
Galí et al 2015 [22]	NA	12.1±14	19.8±3	NA	165.5±55.3	380±78
Golabek et al 2012 [23]	414.59±154.87	5 (0-35)**	22.331±9.99	40.69±22	NA	447±118.95
Lee et al 2007 [24]	239.4±173.6	104.9±59.1	15.2±1.2	NA	NA	NA
Løwenstein et al 2021 [25]	327 (293-348)*	NA	27.6±11.1	22.5±10.8	139±119	NA
Malik et al 2020 [26]	NA	99±46	19±15	27±18	174±179	493±284
Shin et al 2016 [27]	274.73±131.92	33.24±55.63	23.55±10.26	26.78±15.4	173.4±75.84	NA
Tai et al 2009 [28]	199.5±85.2	74.3±30.5	13.9±7.2	NA	NA	NA
Yenilmez et al 2008 [29]	NA	55.4±11#	24.8±1.3#	34.1±1.5#	166±10#	495±23#

Of 2342 patients, 1620 had urodynamic measurements and thus were included in the meta-analysis. * Data presented as median, and range in brackets; # Data presented as mean and standard error; ** Data presented as median, 25th percentile (first figure in brackets), and 75th percentile (second figure in brackets). NA – data not available (not reported); $P_{det}Q_{max}$ – maximal detrusor pressure at maximal flow rate; Q_{max} – maximum flow rate; SD – standard deviation.

First Sensation of Bladder Filling

The mean first sensation of bladder filling in diabetics (n=1201) was 178.66 mL [95% CI: 150.59-206.72] with a considerable level of heterogeneity (I^2=97%) (**Figure 2E**).

Cystometric capacity

Th mean maximum cystometric capacity (MCC) in diabetic women (n=1178) was 480.41 mL [95% CI: 409.32-551.50] with a considerable level of heterogeneity (I^2 = 98%) (**Figure 2F**).

Risk of bias assessment

A summary of the risk of bias assessment is visually presented in **Supplementary Figure 1**.

In the analysis of female diabetic patients' urodynamic parameters, the majority of the studies had a high overall risk of bias [20,22-27,29]. The main reasons include confounding factors, such as unreported 1) diabetes definition, 2) diabetes duration, 3) HgA1c, 4) diabetes treatment, 5) BMI, 6) LUTS assessment, 7) device and methodology of the uroflowmetry measurement, 8) measuring method of postvoid residual volume, and 9) statistical analysis. Existence of pyuria was a confounding factor as well. One study had moderate overall risk of bias because not all patients were included in the urodynamic analysis, and an assessed confounding factor was that BMI was not reported [21]. One study was reported as having low overall risk of bias [28].

Discussion

Summary of Evidence

Lower mean voided volume, Q_{max} and $P_{det}Q_{max}$, as well as higher mean postvoid residual volume, first sensation of bladder filling, and cystometric capacity in the diabetic group was detected compared to healthy women [11,12].

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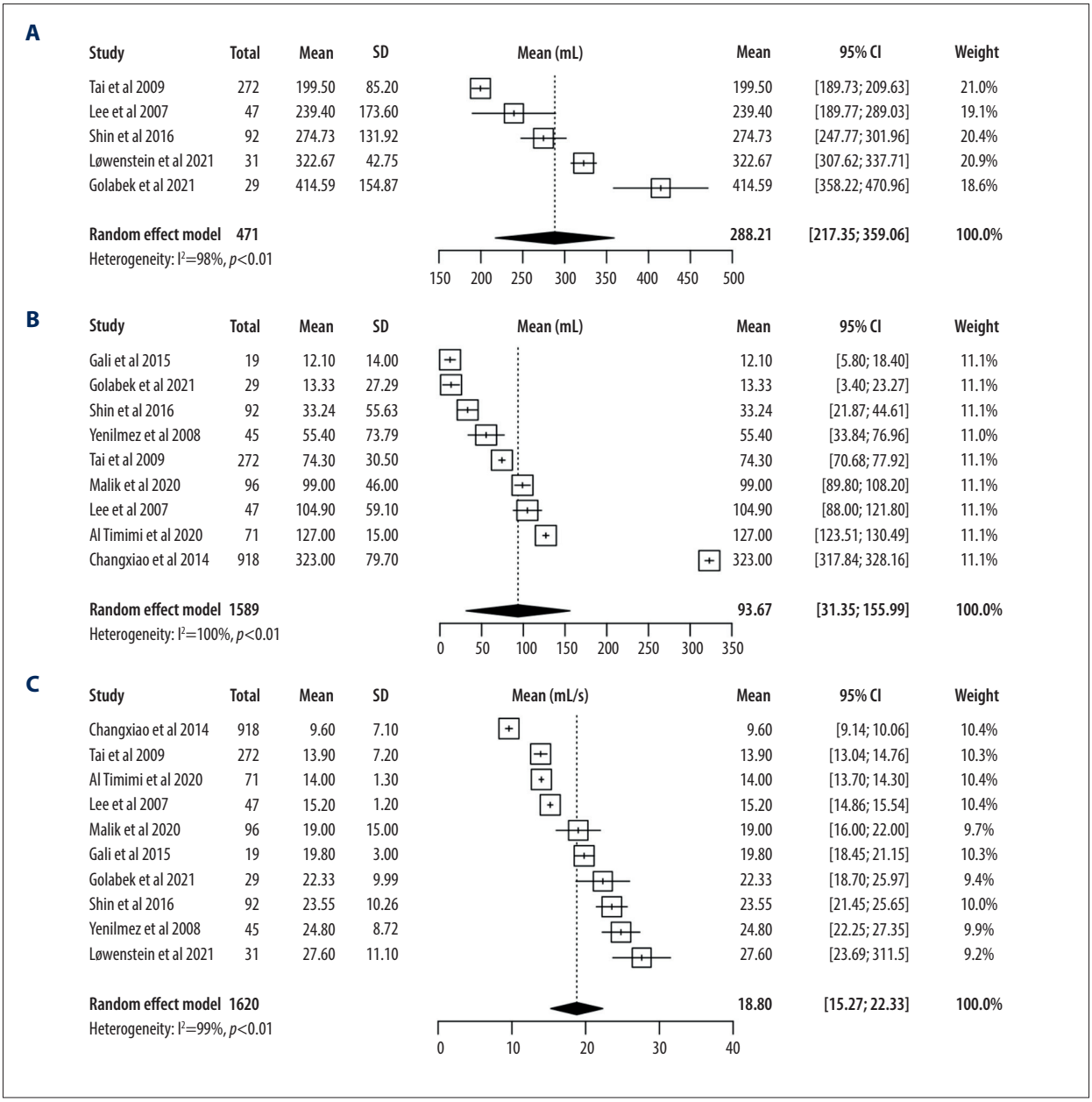
Diabetic Women

Voided Volume

Lower voided volume in diabetes is a surprising finding, because higher voided volumes are generally expected in diabetic patients with higher fluid turnover; and even much higher volumes in autonomic neuropathy [1,5,30]. However, in diabetic patients, according to the literature [31,32], the residual urine is larger as the autonomic neuropathic bladder cannot completely empty the bladder, and there will be residue in it. On the other hand, if we add the emptied amount and the residue volume, there is a clearly higher bladder capacity in diabetic patients [31]. This phenomenon could be explained by the selection of different stages and duration of diabetes. This clinical heterogeneity is also indicated by the statistical heterogeneity.

Q_{max} and $P_{det}Q_{max}$

Q_{max} is always lower in patients with an autonomic neuropathic bladder [33] due to impaired detrusor muscle function, which agrees with our findings. A smaller voided volume always has a lower Q_{max} [34,35], but in this case the bladder also contains residual volume, and this should be taken into account during contraction, so the value is even worse.



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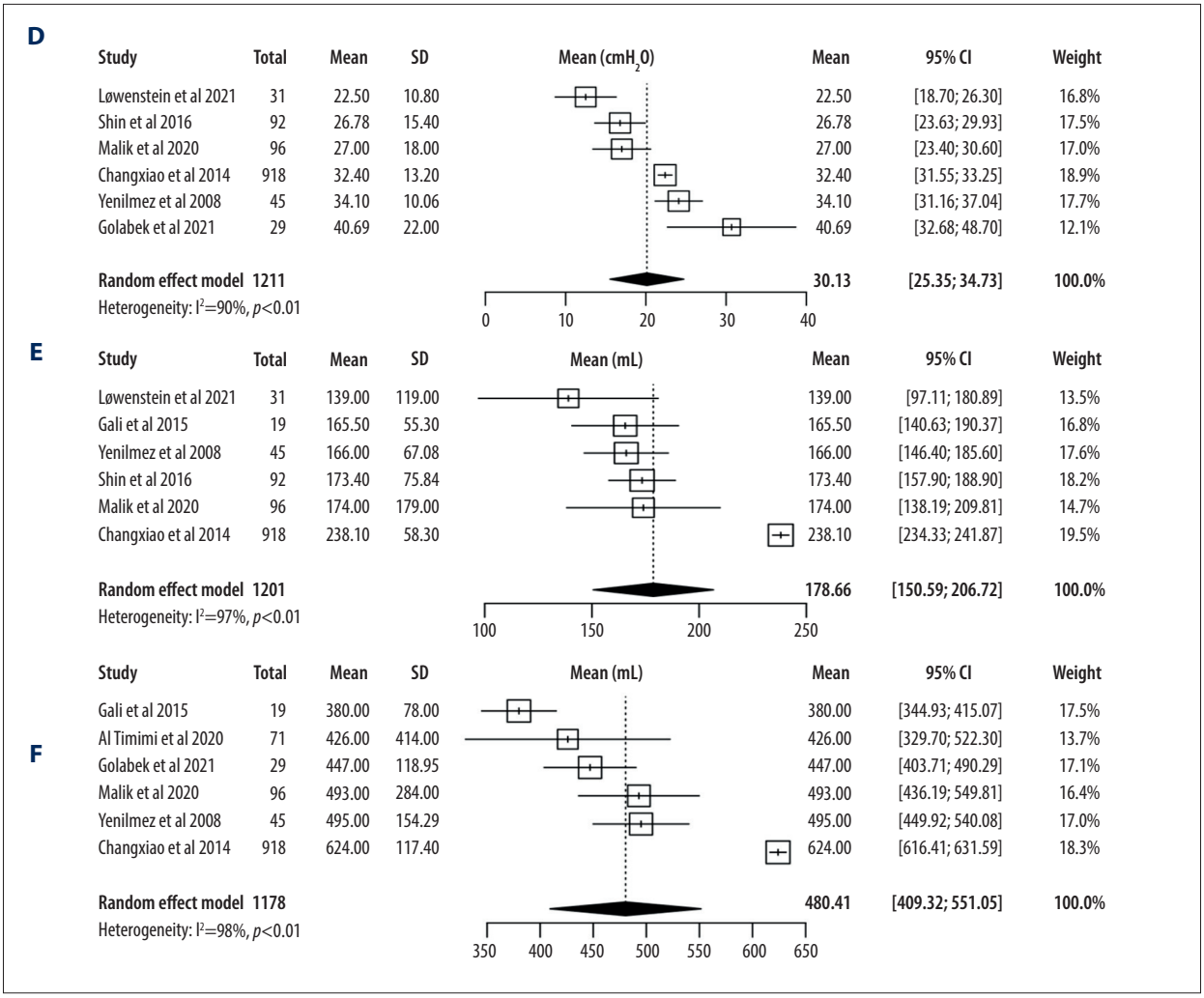

Figure 2. Forest plots of the urodynamic parameters of diabetic women. (**A**) Represents the pooled mean voided volume of diabetic women (n=471). (**B**) Shows the pooled mean postvoid residual volume of diabetic women (n=1589). (**C**) Demonstrates the pooled mean Q_{max} values in diabetic women (n=1620). (**D**) Presents the pooled mean $P_{det}Q_{max}$ values in diabetic women (n=1211). (**E**) Reveals the pooled mean first sensation of bladder filling in diabetic women (n=1201). (**F**) Unveils the pooled mean maximum cystometric capacity (MCC) in diabetic women (n=1178). Statistical analyses were carried out using R statistical software (version 4.0.5) and package "meta" (version 4.18-1).

At maximum flow, the detrusor pressure ($P_{det}Q_{max}$) is lower in diabetes due to the dysfunction and abnormal innervation.

Lee at al [24] reports lower Q_{max} in the diabetic group without bladder dysfunction (n=135, Q_{max}: 19.9±0.7 mL/s), which is even more profound with bladder dysfunction (n=47, Q_{max}: 15.2±1.2 mL/s) compared to healthy women (n=197, Q_{max}: 25.8±8.4 mL/s).

Postvoid Residual Volume, First Sensation of Bladder Filling, and Cystometric Capacity

Autonomic neuropathy reduces the sensation of the bladder [36-38], so it is well understood that the onset of the first urge to urinate in diabetic patients occurs at higher bladder volumes.

Bladder capacity is always higher during cystometry and is markedly higher in diabetic patients [36,39].

Lee at al [24] reported that 1.6% of diabetic women had bladder capacity >500 mL and 0% in the healthy control group; 25.8% of patients had voiding dysfunction in the diabetic group and 3.5% in the healthy population; and 14.8% of diabetic patients and 1.5% of healthy people have postvoid residual >100 mL.

Non-Diabetic Women

Haylen et al [34] found that Q_{max} and Q_{ave} are strongly related to voided volume. Afraa et al [40] found Q_{max} values ranging between 23 and 32 mL. Wyman et al [12] included 3090

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healthy women ranging in age from 19 to 91 years from 24 studies in their meta-analysis of normative reference values for bladder function parameters. They found 334 mL [95% CI: 299-350] for mean voided volume, 12 mL [95% CI: 4-20] for mean postvoid residual volume, and 28 mL/s [95% CI: 27-30] for mean Q_{max}. Sorel et al [11] included 1416 adult patients to their systematic review. They found 338 mL [SD: 161] for mean voided volume, 23.5 mL [SD: 10] for mean Qmax, and 15.5 mL [SD: 25] for mean postvoid residual volume. Mahfouz et al [41] found 175 mL for first sensation of bladder filling, and normal maximum cystometric capacity of 300-500 mL.

These findings agree with our results.

Diabetic Adults with and without LUTS

As diabetes progresses, LUTS also appears [36,42] which suggests more pronounced bladder damage, so it is understandable that in diabetes with LUTS, higher voided volumes suggest greater bladder capacity compared to diabetics without LUTS. The same more pronounced damage is indicated by increased detrusor muscle weakness in diabetic patients with LUTS compared to those without voiding symptoms. Higher detrusor pressure at maximum flow only fits into this pattern if it indicates a LUTS outflow disturbance. The rate of residual urine is higher in diabetics who also have LUTS.

Non-Invasive Urodynamic Measurements in Routine Diabetes Follow-Ups

Translational research takes scientific discoveries made in the laboratory and transforms them into new treatments [43]. Therefore, the sooner the early signs of DC are discovered, the earlier the therapeutic modifications can be initiated. Uroflowmetry can highlight the progressive nature of diabetes – starting with storage changes, then developing voiding dysfunction due to detrusor overdistension, to the decompensated phase. As early alterations in voiding patterns can be seen during the urodynamic examination before bothersome urinary symptoms are recognized by patients, urodynamics, mostly uroflowmetry, can contribute to early diagnosis of DC. Therefore, the inclusion of routine uroflow measurements to the current guidelines of diabetes management is crucial.

Although progression of DC is believed be related to the duration of diabetes, and poor metabolic status; animal studies raised the question of whether changes in bladder function begin soon after its onset [43,44]. Previous studies have suggested that DC is not the prime urodynamic finding in diabetics. Kaplan et al [6] found that detrusor overactivity was the most common finding. Kebapci et al [45] came to the conclusion that classic CD occurs in only 44% of women with type 2 diabetes followed for a mean of 13.85 years; more common findings are detrusor overactivity, stress, and urge incontinence.

Although, summary mean estimates of bladder function parameters for diabetic women were calculated, heterogeneity between the studies was high for all outcomes. Therefore, this precludes generalization of these estimates to all diabetic women. Further research is needed to determine reference values within specific subgroups.

Strength of the Study and Limitations

To the best of our knowledge, this is the first meta-analysis that synthetizes quantitative data about urodynamic measurements of female patients with diabetes. Nonetheless, the strength of our meta-analysis is the use of a comprehensive and precise search strategy and data extraction.

The main limitation is that we could not directly compare diabetic and non-diabetic women, since there were insufficient studies directly comparing diabetic and non-diabetic patients. The lack of definition of diabetic cystopathy is also a limitation, and only 2 studies reported it [24,29].

Conclusions

Implication for Practice

Diabetes is an important independent risk factor for LUTS. Urodynamics can detect early alterations in voiding function, which might help to apply interventions to delay or prevent the onset of diabetes to limit difficulties in voiding.

Uroflowmetry may be considered in current diabetes guidelines. Regular uroflow measurements can contribute to the early recognition of DC.

Implication for Research

Due to the limitations, our findings should be verified by future comparative studies in people with diabetes. To carry out more accurate analyses, it is important to compare larger number of patients with different stages and duration of diabetes, with different metabolic status as well.

Acknowledgments

László Szabó is the guarantor of this study and had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

Supplementary Materials

Supplementary Table 1. Search strategy of diabetes and urodynamics.

MEDLINE
1. "urodynamic"
2. " uroflow*"
3. "uroflowmetry"
4. "diabetes"
5. "diab*"
6. (#1 OR #2 OR #3) AND (#4 OR #5)

("urodynamical"[All Fields] OR "urodynamically"[All Fields] OR "urodynamics"[MeSH Terms] OR "urodynamics"[All Fields] OR "urodynamic"[All Fields] OR "uroflow*"[All Fields] OR ("uroflowmetries"[All Fields] OR "uroflowmetry"[All Fields])) AND ("diabete"[All Fields] OR "diabetes mellitus"[MeSH Terms] OR ("diabetes"[All Fields] AND "mellitus"[All Fields]) OR "diabetes mellitus"[All Fields] OR "diabetes"[All Fields] OR "diabetes insipidus"[MeSH Terms] OR ("diabetes"[All Fields] AND "insipidus"[All Fields]) OR "diabetes insipidus"[All Fields] OR "diabetic"[All Fields] OR "diabetics"[All Fields] OR "diabets"[All Fields] OR "diab*"[All Fields])
Search Date: November 4, 2021
Number of Results: 454

EMBASE
1. "urodynamic"
2. " uroflow*"
3. "uroflowmetry"
4. "diabetes"
5. "diab*"
6. (#1 OR #2 OR #3) AND (#4 OR #5)

(urodynamic OR uroflow* OR uroflowmetry) AND (diabetes OR diab*)
Search Date: November 4, 2021
Number of Results: 773

Cochrane Central Register of Controlled Trials (CENTRAL)
1. "urodynamic"
2. " uroflow*"
3. "uroflowmetry"
4. "diabetes"
5. "diab*"
6. (#1 OR #2 OR #3) AND (#4 OR #5)

(urodynamic OR uroflow* OR uroflowmetry) AND (diabetes OR diab*)
Search Date: November 4, 2021
Number of Results: 63

Web of Science Core Collection
1. "urodynamic"
2. " uroflow*"
3. "uroflowmetry"
4. "diabetes"
5. "diab*"
6. (#1 OR #2 OR #3) AND (#4 OR #5)

ALL FIELDS: ((urodynamic or uroflow* or uroflowmetry)) AND ALL FIELDS: ((diabetes or diab*))
Search Date: November 4, 2021
Number of Results: 460

Total number of records: 1750

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

QUIPS Tool – Risk of Bias Assessment

QUIPS assess risk of bias in studies of prognostic factors.

Concerns about risk of bias and applicability were rated as "low", "moderate" or "high" in six domains 1) study participation, 2) study attrition, 3) prognostic factor measurement, 4)outcome measurement, 5) study confounding, as well as 6) statistical analysis and reporting.

To judge overall risk, the review authors (ÁRM and PP) described studies with a low risk of bias as those in which at least five of the six important bias domains were rated as having low risk of bias. If there was at least one domain rated as high risk, or more than three domains rated as moderate risk of bias, the overall risk of bias was deemed high. All other variations were determined as moderate risk of bias.

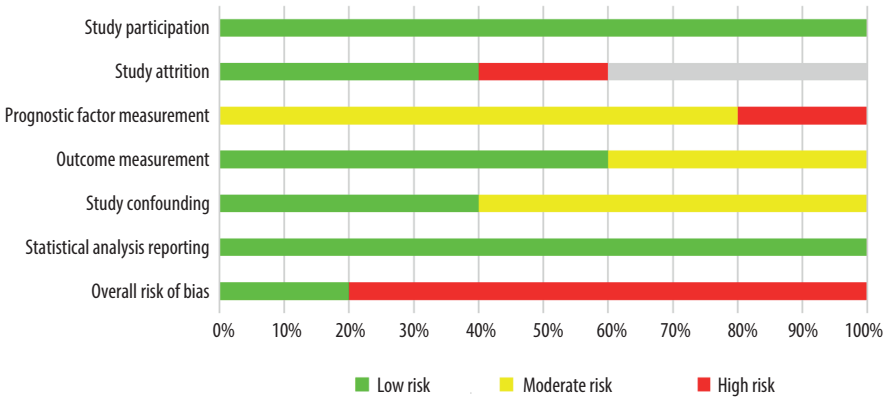
Abbreviations: QUIPS – quality in prognostic studies.

A A1

+ High risk
? Moderate risk
– Low risk

		Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
1	Golabek et al.2012	+	NA	?	?	?	+	-	Yes
2	Lee et al.2007	+	-	?	+	?	+	-	Yes
3	Løwenstein et al 2021	+	+	-	+	+	+	-	Yes
4	Shin et al 2016	+	NA	?	?	?	+	-	Yes
5	Tai et al 2009	+	+	?	+	+	+	+	Yes

A2



Supplementary Figure 1A. Risk of bias assessment on study level [**A1**] and across studies [**A2**] assessing mean voided volume (mL) in diabetic female population. **1:** Diabetes definition and duration, as well as LUTS assessment were not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **2:** Study design is not reported. Not all patients were included in the analysis, diabetes definition and treatment are not reported. **3:** Definition of diabetes and type of it are not reported. **4:** Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **5:** Definition of diabetes is not reported.

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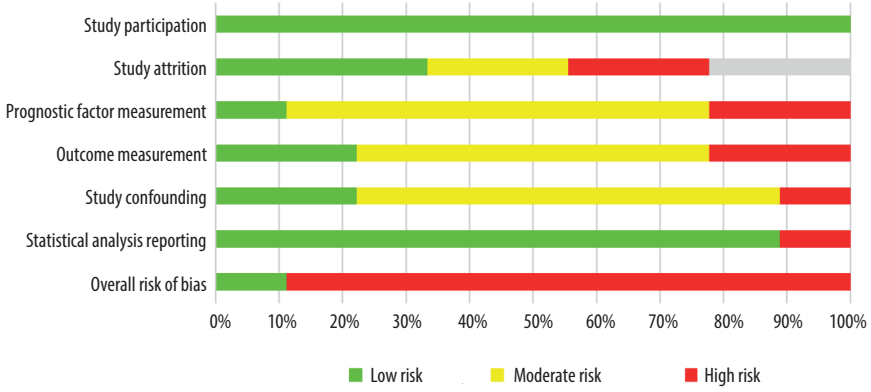
B **B1**

High risk
Moderate risk
Low risk

		Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
1	Al Timimi et al 2020	+	+	-	-	?	-	-	Yes
2	Changxiao et al 2014	+	?	+	?	?	+	-	Yes
3	Gali et al 2015	+	-	?	?	+	+	-	Yes
4	Golabek et al 2012	+	NA	?	?	?	+	-	Yes
5	Lee et al 2007	+	-	?	+	?	+	-	Yes
6	Malik et al 2020	+	+	-	?	?	+	-	Yes
7	Shin et al 2016	+	NA	?	-	?	+	-	Yes
8	Tai et al.2009	+	+	?	+	+	+	+	Yes
9	Yenilmez et al 2008	+	?	?	?	-	+	-	Yes

B2



Supplementary Figure 1B. Risk of bias assessment on study level [**B1**] and across studies [**B2**] assessing mean postvoid residual (mL) in diabetic female population. **1:** HgA1c, diabetes duration are not reported. The measuring method of postvoid residual volume is not reported. The method (device) of the uroflowmetry parameters is not reported. Although it is reported that diabetes treatment was evaluated, but data could not be extracted. Statistical analysis is not reported. **2:** Not all patients were included in the analysis. The measuring method of postvoid residual volume is not reported. Assessed confounding factor is caused by unreported BMI. **3:** The study design is unknown, and not all patients were included in the analysis. The measuring method of postvoid residual volume is not reported. Diabetes definition is not reported. **4:** Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **5:** Study design is not reported. Not all patients were included in the analysis. Diabetes definition and treatment are not reported. **6:** Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported. **7:** Type of diabetes and LUTS assessment are not reported. The measuring method of postvoid residual volume is not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **8:** Diabetes definition is not reported. **9:** Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

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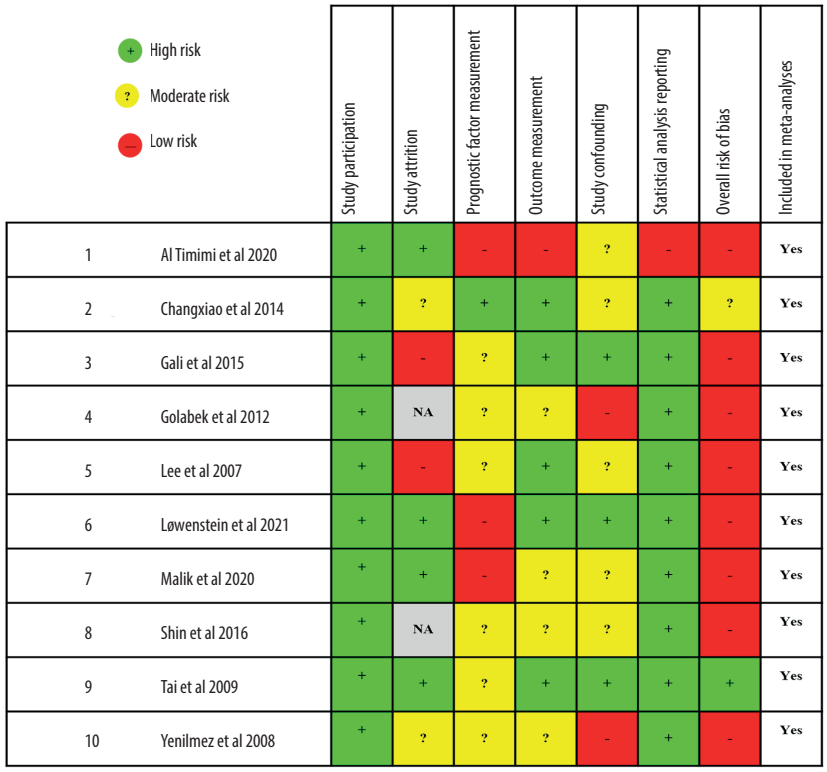
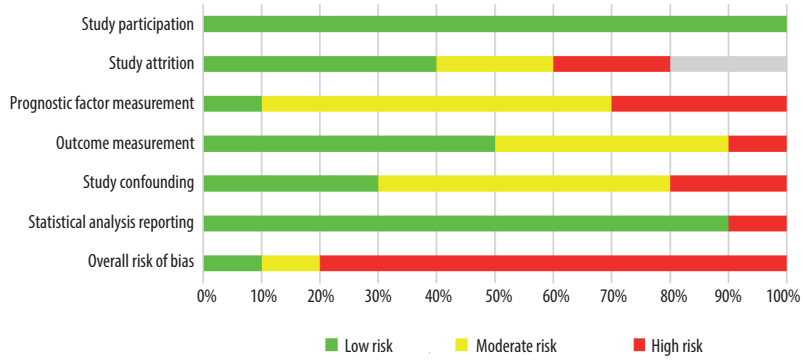
C **C1**



C2



Supplementary Figure 1C. Risk of bias assessment on study level [**C1**] and across studies [**C2**]] assessing mean Qmax (mL/sec) in diabetic female population. **1:** HgA1c, diabetes duration are not reported. The method (device) of the uroflowmetry parameters is not reported. Although it is reported that diabetes treatment was evaluated, but data could not be extracted. Statistical analysis is not reported. **2:** Not all patients were included in the analysis. Assessed confounding factor is caused by unreported BMI. **3:** The study design is unknown, and not all patients were included in the analysis. The measuring method of postvoid residual volume, as well as diabetes definition are not reported. **4:** Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI; and patients having Qmax lower than 12 mL/min were excluded. **5:** Study design is not reported. Not all patients were included in the analysis. Diabetes definition and treatment are not reported. **6:** Definition of diabetes and type of it are not reported. **7:** Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported. **8:** Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **9:** Definition of diabetes is not reported. **10:** Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

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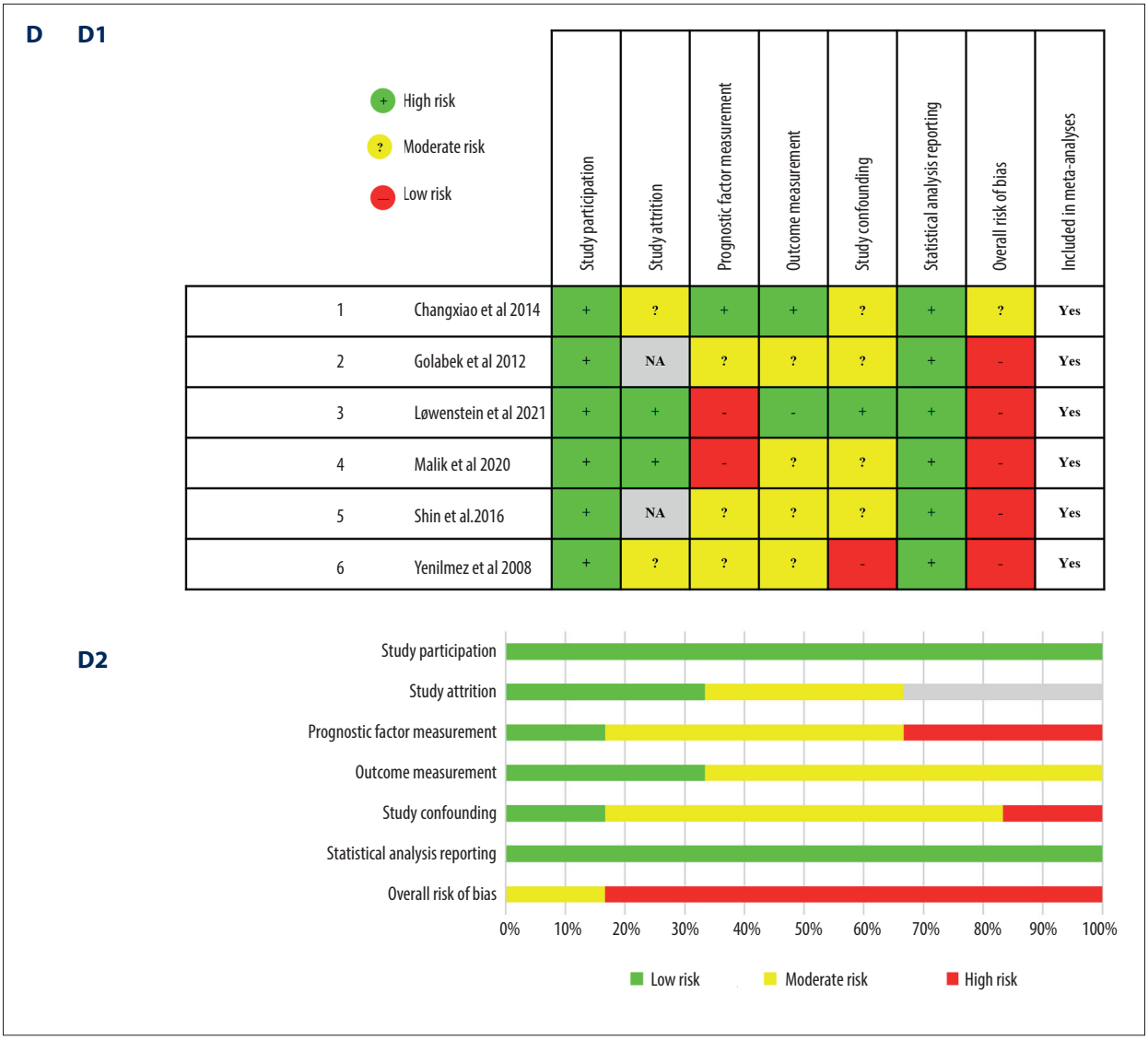
D **D1**



Supplementary Figure 1D. Risk of bias assessment on study level [**D1**] and across studies [**D2**] assessing mean $P_{det}Q_{max}$ (cmH_2O) in diabetic female population. **1:** Not all patients were included in the analysis. Assessed confounding factor is caused by unreported BMI. **2:** Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **3:** Definition of diabetes and type of it are not reported. **4:** Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported. **5:** Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **6:** Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

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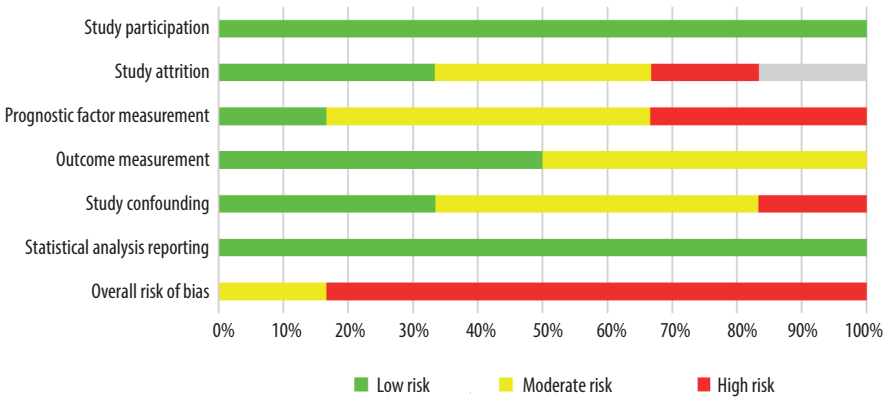
E **E1**

E2



Supplementary Figure 1E. Risk of bias assessment on study level [**E1**] and across studies [**E2**] assessing mean first sensation (mL) in diabetic female population. **1:** Not all patients were included in the analysis. Assessed confounding factor is that BMI was not reported. **2:** The study design is unknown, and not all patients were included in the analysis. Diabetes definition is not reported. **3:** Definition of diabetes and type of it are not reported. **4:** Type of diabetes and definition of it, as well as LUTS assessment are not reported. Diabetes treatment is partly reported. **5:** Type of diabetes and LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **6:** Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

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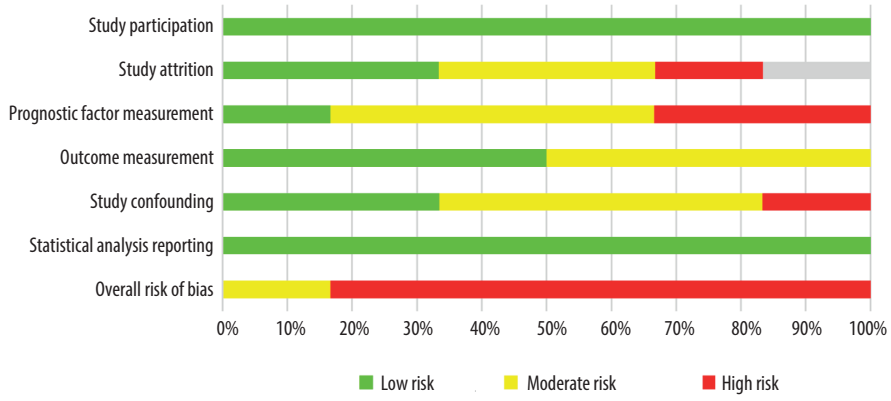
F F1

+ High risk
? Moderate risk
– Low risk

		Study participation	Study attrition	Prognostic factor measurement	Outcome measurement	Study confounding	Statistical analysis reporting	Overall risk of bias	Included in meta-analyses
1	Al Timimi et al 2020	+	+	-	-	?	-	-	Yes
2	Changxiao et al 2014	+	?	+	+	?	+	?	Yes
3	Gali et al 2015	+	-	?	+	+	+	-	Yes
4	Golabek et al 2012	+	NA	?	?	?	+	-	Yes
5	Malik et al 2020	+	+	-	?	?	+	-	Yes
6	Yenilmez et al 2008	+	?	?	?	-	+	-	Yes

F2



Supplementary Figure 1F. Risk of bias assessment on study level [**F1**] and across studies [**F2**] assessing mean cystometric capacity (mL) in diabetic female population. **1:** HgA1c, diabetes duration are not reported. The method (device) of the uroflowmetry parameters is not reported. Although it is reported that diabetes treatment was evaluated, but data could not be extracted. Statistical analysis is not reported. **2:** Not all patients were included in the analysis. Assessed confounding factor is caused by unreported BMI. **3:** The study design is unknown, not all patients were included in the analysis. Diabetes definition is not reported. **4:** Diabetes definition and duration, as well as LUTS assessment are not reported. Assessed confounding factors are caused by unreported treatment of diabetes and BMI. **5:** Type of diabetes and definition of it are not reported. LUTS assessment is not reported. Diabetes treatment is partly reported. **6:** Not all patients were included in the analysis. Diabetes definition and LUTS assessment are not reported. Existence of pyuria is a confounding factor. Diabetes treatment is partly reported.

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CLINICAL ARTICLE

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Urine flow acceleration in healthy children: A retrospective cohort study

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Abstract

Aims: To establish normal reference values of urine flow acceleration (Q_{acc}) in healthy children, as there is a lack of nomograms for normative reference values of Q_{acc} by voided volumes in the pediatric population so far. Q_{acc} might be an early indicator of autonomic neuropathy in children and adolescents.

Methods: Data were retrospectively collected from healthy children who underwent uroflowmetry between 1990 and 1992. Exclusion criteria were voided volume less than 20 ml, and postvoid residual more than 15%. Baseline characteristics and uroflowmetry parameters were collected from girls and boys aged between 6 and 18 years. Voided volume, voiding time, time to maximum flow rate, and maximum and average flow rates of urine were measured, and Q_{acc} was calculated. Postvoid bladder diameter was measured by ultrasonography and converted to volume.

Results: Uroflowmetry parameters of 208 children (≤18 years old, 45.2% girls, mean age 9.68 ± 3.09 years) who performed 404 micturition were analyzed. Median voided volume, voiding time, time to Q_{max}, Q_{ave}, Q_{max}, Q_{acc}, and postvoid residual volume were 130 [20–460] ml, 10 [3–56] s, 3 [1–14] s, 11.7 [2.5–36.6] ml/s, 20.5 [5–50] ml/s, 6 [0.81–25] ml/s^2, and 1.83 [0–38.62] ml, respectively. Q_{acc} nomograms were given in centile forms for girls and boys separately, which show an inversely proportional correlation between voided volumes.

Conclusions: These are the first nomograms for normative reference values of Q_{acc} in the pediatric population (girls and boys separately) by voided volumes in centile forms. These may be useful to interpret abnormal Q_{acc} values and diagnose lower urinary tract diseases over a wide range of voided volumes.

Abbreviations: BOO, bladder outlet obstruction; BPH, benign prostate hyperplasia; CAD, cardiovascular autonomic dysfunction; DC, diabetic cystopathy; DM, diabetes mellitus; GFR, glomerular filtration rate; ml, milliliters; LUT, lower urinary tract; Q_{ave}, average flow rate; Q_{max}, maximum flow rate; Q_{acc}, urine flow acceleration (Q_{max}/TQ_{max}); SD, standard deviation; STROBE, Strengthening The Reporting of Observational Studies in Epidemiology; TNF, tumor necrosis factor; TQ_{max}, time to maximum flow rate.

KEYWORDS
children, normative reference values, pediatrics, urine flow acceleration, urodynamics, uroflowmetry

1 | INTRODUCTION

Uroflowmetry is an essential, noninvasive, easy-to-use, widely accessible, and quick urodynamic diagnostic tool in the evaluation of voiding function.[1–4] Urine flow rate measurements are generally used to determine lower urinary tract (LUT) dysfunction. Normative reference values of bladder function in healthy adult women[5,6] have been widely studied, although there are quite a few studies in the healthy pediatric population.[7,8] We have previously established normal reference values for maximum and average urine flow in children,[7] which have been adopted by the International Continence Society and recommended in the 2nd edition of the Book of Incontinence[9] for those who study urine flow in children. The uroflow patterns of children with LUT symptoms[10–13] are still under evaluation. In our previous study,[7] we did not establish normal reference values for urine flow acceleration (Q_{acc}), although a difference was observed in children with diabetes mellitus.[14]

Acceleration of the detrusor muscle contraction characterizes the bladder function. It is a calculated urodynamic value, which is the ratio of maximum flow rate (Q_{max}) and time to maximum flow rate (TQ_{max}), measured in ml/s^2; and refers to the increased flow rate in a period of time from the beginning of urination to the peak value. Only a few studies have addressed Q_{acc}[14,15] so far.

Q_{acc} may be increased in children with urgent and frequent micturition, as well as with urge incontinence. Possible damage in the detrusor muscle function may also impair Q_{acc}, therefore a change or a decrease in the acceleration of the detrusor muscle contraction can be an early sign of the damage in the muscle and/or of the innervation. Q_{acc} may be changed in outflow obstruction, such as in benign prostate hyperplasia (BPH). Q_{acc} has been found to be superior to Q_{max} in the diagnosis of bladder outlet obstruction (BOO) in adult men with BPH.[15] Furthermore, Q_{acc} may be reduced if a patient has autonomic neuropathy (e.g., diabetic cystopathy [DC]). DC is a well-recognized urological complication of diabetic autonomic neuropathy[16] with the classic triad of decreased bladder sensation, impaired bladder emptying with postvoid residual volume, and increased bladder capacity.[17–21] Patients generally have the symptoms of overactive bladder or overflow incontinence, including urinary frequency, urgency, incontinence, and nocturia; which are listed among the LUT symptoms. DC might develop over time, but in the beginning, only mild symptoms can be observed in children.

Until now, Q_{acc} was a less frequently adapted parameter, but as previous studies suggest that it might indicate the deviation of detrusor muscle function earlier than other uroflowmetry parameters (e.g., Q_{max}, Q_{ave}), therefore it seems to be a better indicator of diabetic autonomic neuropathy than cardiovascular dysfunction tests (Ewing tests).[22]

Since there is no consensus on the cut-off values of Q_{acc} in the pediatric population which limits the use of Q_{acc}, our aim was to establish normal ranges of it in both genders by voided volumes.

2 | MATERIALS AND METHODS

The study is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) 2004 Statement[23] (the STROBE checklist of our work is available in Supporting Information: Table 1). The study was approved by the Heim Pál National Paediatric Institute's Local Ethics Committee (registration number: KUT-37/2021, Date: 23 July 2021). The data were obtained from a previous uroflow study[7] carried out between 1990 and 1992 at Child Health Centre, Borsod Abaúj-Zemplén County Teaching Hospital, Miskolc, Hungary. Some parts of the data have been published previously,[7] but acceleration values have not been reported so far. The research and methodology used for data collection are explained in detail in the previous study.[7]

2.1 | Study design and eligibility

A single-center, retrospective cohort study was conducted with 270 healthy children and adolescents. Our study protocol was based on the previously designed study.[7] Age (years), gender (boys/girls), weight (kg), and height (cm) were collected. Each child urinated multiple times (spontaneously in calm conditions, at first desire to void after 15 ml/kg liquid consumption, and at the maximal sensation of bladder fullness). The reliability of the tests was not examined as the children's first urination occurred upon arrival at the Urodynamics

Laboratory, and the second urination occurred at the first urge to urinate after 15 ml/kg liquid consumption. Thus, almost everyone voided a different volume during the second urination. There were no children with Q_{acc} values below 10th percentile or above 90th percentile.

Eligibility criteria were healthy children aged between 6 and 18 years, without any acute or chronic (neurological, gastrointestinal, pulmonological, cardiac, urological, nephrological, etc.) disease or medicine consumption. Exclusion criteria therefore were the following conditions: (1) acute febrile condition (≥38°C core temperature) in the past 7 days; (2) acute or chronic urinary tract or kidney disease: renal insufficiency (glomerular filtration rate [GFR] ≤ 60 ml/min per 1.73 m^2), urinary tract infection; (3) urological disease: bladder cancer, urolithiasis, urethral stricture, posterior urethral valve, meatal stenosis, previous genitourinary surgery, conditions causing urinary outflow problems (phimosis, hypospadias, vesicoureteral reflux); (4) neurological disorders (multiple sclerosis, transient ischemic attack, transverse myelitis, myelocele, meningomyelocele, previous spinal cord operation, or operation which might injure the sacral nerve plexus etc.); (5) medicines taken which can cause neuropathy: cytostatic agents, immunosuppressive agents (tumor necrosis factor [TNF] -alfa inhibitors), cardiovascular medicines (statins, digoxin, amiodaron), antimicrobial agents (nitrofurantoin, linezolid, voriconazole, itraconazole, antituberculotics, metronidazole, fluoroquinolone), anti-ulcerative agents (cimetidin), neuropsychological agents (levodopa, phenytoin); (6) psychiatric disorders that prevent participation/collaboration in the study; (7) constipation; (8) patients who are pregnant or gave birth in the last 12 months; (9) voided volume less than 20 ml, and (10) postvoid residual more than 15% of voided volume.[8,24] We did not enroll those children who had LUT symptoms as well.

During spontaneous urination, children voided smaller volumes (20–50 ml) with lower Q_{ave} and Q_{max} values. In 7.9% of the total micturition, either Q_{max} or Q_{ave} was less than 5 ml/s. According to the normal nomograms,[7] the values of Q_{ave} 2.5 ml/s and Q_{max} 5 ml/s are above 5% (between 5 and 10 percentile values), which means they are within the normal range. The first step of the study's protocol, children were asked to void spontaneously regardless of the need for urination. After that these children voided when they felt the first sensation of bladder filling (after drinking 15 ml/kg liquid), which was clearly larger volumes with higher Q_{max} and Q_{ave} values. So they did not void small amounts all the time, which means they did not have small bladder capacity. Therefore, we think these small voided volumes with lower Q_{max} and Q_{ave} values are acceptable. Given there were only a few numbers of voiding, which does not change the normal values of Q_{acc}, we did not exclude these urinations from the analysis to reduce selection bias. These data confirm our hypothesis that at spontaneous voiding, healthy younger children void smaller amounts of volumes with lower Q_{max} and Q_{ave} values.

Since Q_{max} and Q_{ave} values have already been presented (Miskolc Nomograms[7]), and our current study is focused on Q_{acc} values, we did not wish to publish Q_{max} and Q_{ave} values again. In our previous study[7] Q_{ave} and Q_{max} values were divided into three body surfaces (<0.92 m^2, between 0.92 and 1.42 m^2, and >1.42 m^2) and not into age (basically younger children have lower body surfaces). As we did not want to deviate from the original study protocol, we planned to have a subgroup analysis based on body surfaces. Unfortunately, due to the low number of children in each subgroup—according to the sample size calculation—data were not enough to make valid analyses.

2.2 | Measurements and outcomes

Urinary bladder function was assessed by uroflowmetry, and postvoid residual volume was detected by ultrasonography. All examinations were performed by L.Sz. (nephrologist and urodynamic specialist). Data were collected using a targeted questionnaire, each measurement was documented by LSz. Uroflowmetry was performed using a Uroflow-cystometer (X0002; Metripond) which determined Q_{max}, Q_{ave}, and TQ_{max}. Voided volume (ml), voiding time (s), average and maximum urinary flow rate (ml/s), and time to maximum urinary flow (s) were measured; urine flow acceleration (ml/s^2) was calculated. Q_{max} and Q_{ave} were defined according to the International Children's Continence Society.[25] Voided volume was measured manually using a graduated cylinder; boys voided in a standing, girls in a sitting position. Ultrasound was accomplished before and after micturition. Scans were obtained with a real-time ultrasound scanner (Hitachi EUB 40.5 MHz transducer) using a direct scanning technique. Postvoid bladder diameter (mm) was measured by ultrasonography and converted to bladder residual volume (ml). Manual data collection was turned into a digital form which was carried out by Á.R.M., data were tabulated and encoded. Data validation was conducted by P. P., M. F., and Sz.K. All patients' data are stored securely.

Different uroflow parameters can only be compared if voided volumes are the same since uroflow parameters highly depend on voided volumes. Therefore, children were allocated to one of the two groups by gender, and then Q_{acc} was determined by voided volumes.

TABLE 1 Baseline characteristics and uroflowmetry parameters of girls/boys/all children included in the study

	Age (years) $n = 94/114/208$	Weight (kg) $n = 93/113/206$	Height (cm) $n = 79/98/177$	Body surface (m^2) $n = 80/99/179$	Voided volume (ml) $n = 169/235/404$	Voiding time (s) $n = 169/235/404$	Time to Q_{max} (s) $n = 169/235/404$	Q_{ave} (ml/s) $n = 169/235/404$	Q_{max} (ml/s) $n = 169/235/404$	Q_{acc} (ml/s^2) $n = 169/235/404$	Postvoid residual (ml) $n = 169/235/404$
Median	10/9.9/10	32/32/32	140/141.5/141	1.18/1.18/1.18	130/140/130	10/10/10	3/4/3	12.5/11.1/11.7	23/19/20.5	7.25/5.4/6	1.40/1.90/1.83
Standard error	0.3/0.3/0.21	1.22/1.28/0.89	2.0/2.05/1.44	0.03/0.02/0.02	6.86/6.09/4.55	0.52/0.43/0.33	0.15/0.14/0.10	0.47/0.35/0.28	0.62/0.50/0.39	0.31/0.25/0.20	0.48/0.40/0.30
Minimum value	3/3/3	14/15/14	92/89/89	0.6/0.6/0.6	20/20/20	3/3/3	1/1/1	2.5/2.5/2.5	5/5/5	1.12/0.81/0.81	0/0/0
Maximum value	16/17/17	71/78/78	170/179/179	1.8/1.8/1.80	375/460/460	48/56/56	12/14/14	34/36.6/36.6	50/50/50	19.5/25/25	38.62/29.1/38.62

Note: Out of the 208 children, 114 are male, 94 are female. Altogether, 404 (235, 169) micturition was performed.

2.3 | Statistical analysis

All descriptive statistic calculations were carried out with MS Excel (version 16.52, Microsoft Corporation [2019]). The quantile method was used to establish the 3–97th percentile levels with SPSS (version 25.0 statistical software package: IBM Corporation). For presentation, the nomograms were expressed in centile forms, prepared for girls and boys separately. The centile curves of acceleration by voided volume were estimated by using lmsChartMaker Pro 2.3 software (Medical Research Council, UK 1997–2006; Cole and Green 1994; Cole and Pan 2004) based on the LMS method.[26] Normal nomograms of Q_{acc} in the pediatric population were visualized in percentile forms and graphically as well. When data show normal distribution mean ± standard deviation (SD), in other cases, median [range] was used. Data imputation has not been applied.

The sample size (n) was estimated by the following equations[27]

$$n = \frac{z^2 \times p(1-p)}{e^2},$$

$$n' = \frac{n}{1 + \frac{z^2 \times p(1-p)}{e^2 \times N}},$$

where z, z score ($z = 1.96$ for 95% confidence level); e, margin of error; N, population size of healthy children aged between 6 and 18 years; p, population proportion by using with confidence level = 95%, margin error = 5%, population proportion = 85% (healthy children were selected for the nomogram construction, proportion value was set by considering all the illnesses, diseases, medications and health status conditions that can influence uroflowmetry parameters in children) and population size = 1 827 520 (Hungarian Central Statistical Office, data for healthy children aged between 6 and 18 years in Hungary: https://www.ksh.hu/docs/hun/xstadat/xstadat_eves/i_zoi002a.html). The estimation revealed that a sample size of at least 196 healthy children (aged between 6 and 18 years) would be necessary for the study.

3 | RESULTS

3.1 | Study selection and baseline characteristics

Out of 270 healthy children, 208 were enrolled in the analysis and performed 404 micturition total. 62 children were excluded due to the following reasons: 33 children

were excluded due to voided volume of less than 20 ml, and 29 children were excluded due to a postvoid residual volume of more than 15% of voided volume.

Data quality tables are presented in Supporting Information: Table 2.

The baseline characteristics of girls/boys and all patients and uroflowmetry parameters are presented in Table 1. Out of 208 children, 94 are female, and 114 are male. The mean age of the total population is 9.68 ± 3.09 years, the median weight is 32 (14–78) kg, the mean height is 138.76 ± 19.21 cm, and the mean body surface is 1.19 ± 0.28 m^2. The Median voided volume is 130 [20–460] ml, the median voiding time is 10 [3–56] s, the median time to Q_{max} is 3 [1–14] s, the median Q_{ave} is 11.7 [2.5–36.6] ml/s, median Q_{max} is 20.5 [5–50] ml/s, median Q_{acc} is 6 [0.81–25] ml/s^2, and median postvoid residual volume 1.83 [0–38.62] ml.

Out of the 94 girls with 169 voidings, micturition total, the mean age is 9.71 ± 2.95 years, median weight is 32 [14–71] kg, mean height is 138.13 ± 17.84 cm, and mean body surface is 1.18 ± 0.27 m^2. The median voided volume is 130 [20–375] ml, the median voiding time is 10 [3–48] s, the median time to Q_{max} is 3 [1–14] s, the median Q_{ave} is 12.5 [2.5–34] ml/s, median Q_{max} is 23 [5–50] ml/s, median Q_{acc} is 7.25 [1.12–19.5] ml/s^2, and median postvoid residual volume 1.40 [0–38.62] ml.

Out of the 114 boys with 235 micturition total, the mean age is 9.65 ± 3.21 years, the median weight is 32 [15–78] kg, the mean height is 139.27 ± 20.32 cm, and the mean body surface is 1.20 ± 0.29 m^2. The median voided volume is 140 [20–460] ml, the median voiding time is 10 [3–56] s, the median time to Q_{max} is 4 [1–14] s, the median Q_{ave} is 11.11 [2.5–36.6] ml/s, median Q_{max} is 19 [5–50] ml/s, median Q_{acc} is 5.4 [0.81–25] ml/s^2, and median postvoid residual volume 1.9 [0–29.10] ml.

3.2 | Normative urine flow acceleration nomograms

Figures 1 and 2 demonstrate the nomograms of Q_{acc} by voided volumes in girls and boys, respectively. Supporting Information: Table 3 (Panel A and B) represents the 3% to 97% centile values of normal Q_{acc} in girls and boys, respectively.

4 | DISCUSSION

We have established nomograms for normative reference values of Q_{acc} in the pediatric population (girls and boys separately) by voided volumes in centile forms. We found an inversely proportional correlation between voided volumes and Q_{acc} parameters.

The diversity of Q_{acc} by percentiles is huge. We recommend that values between the 25 and 75th percentiles should be accepted as normal, which in boys is between 3.9 and 8 ml/s^2 for a voided volume of 150 ml. If we get values below 25% or above 75%, we recommend further examinations. Theoretically, a Q_{acc} value above 75% could be due to overactive bladder dysfunction.

Up to 350 ml of voided volume, higher Q_{acc} values were observed in girls than in boys. This phenomenon might be explained by the shorter and straighter urethra in the female population. Above 350 ml of voided volume, Q_{acc} values below at the 50th percentile were minimally lower in girls than in boys. A relatively small number of cases may play a role in the latter.

None of the children had BOO, as can be suggested based on the shape of the uroflow curve. According to the Miskolc Nomograms,[7] none of the children had plateau-shaped flow curves. Furthermore, reduced flow parameters can be observed in BOO (Q_{max} and Q_{ave} values are below 5%). Both Q_{max} and Q_{ave} values in the Miskolc Nomograms were above the 10th percentile. These findings can be confirmed with pressure/flow urodynamic tests (and video urodynamics), but due to their invasive nature, they are not performed routinely. We have not examined Q_{acc} in BOO so far, but according to Wen et al.,[15] Q_{acc} might be a better indicator than Q_{max} in the examination of BOO.

Micturition represents complex neuromuscular mechanisms involving structures of the LUT and nervous system. Voiding symptoms refer to a disease involving several factors of dysregulation; although the absence of voiding symptoms is not a guarantee of normal micturition.

Hubeaux et al.[28] found that almost one-third of the women without voiding complaint has been actually shown to have abnormal uroflowmetry.

Mattiasson and Teleman investigated the urethral motor function in incontinence women. Q_{acc} has been significantly increased in patients with incontinence: Q_{acc} was 13 ± 17.8 (2.2); 20 ± 18.9 (2.8); and 32 ± 24.9 (4.9) degrees (mean ± SD; [SE]) for incontinence, naive incontinence, and no incontinence, respectively.[29]

Cucchi concluded that all of the obstructed patients have lower values of acceleration than the controls, but patients with detrusor instability (today, the correct name is overactive bladder) tend to show higher Q_{acc} than those with stable bladders.[30] He also found that the measurement of Q_{acc} is a simple and reliable test to aid in the diagnosis of detrusor instability in stress-incontinent women.[31]

Q_{acc} might have a role in the detection of detrusor muscle dysfunction, as well. Karasu et al. found[32] higher values of Q_{acc} in intrinsic sphincter deficiency stress

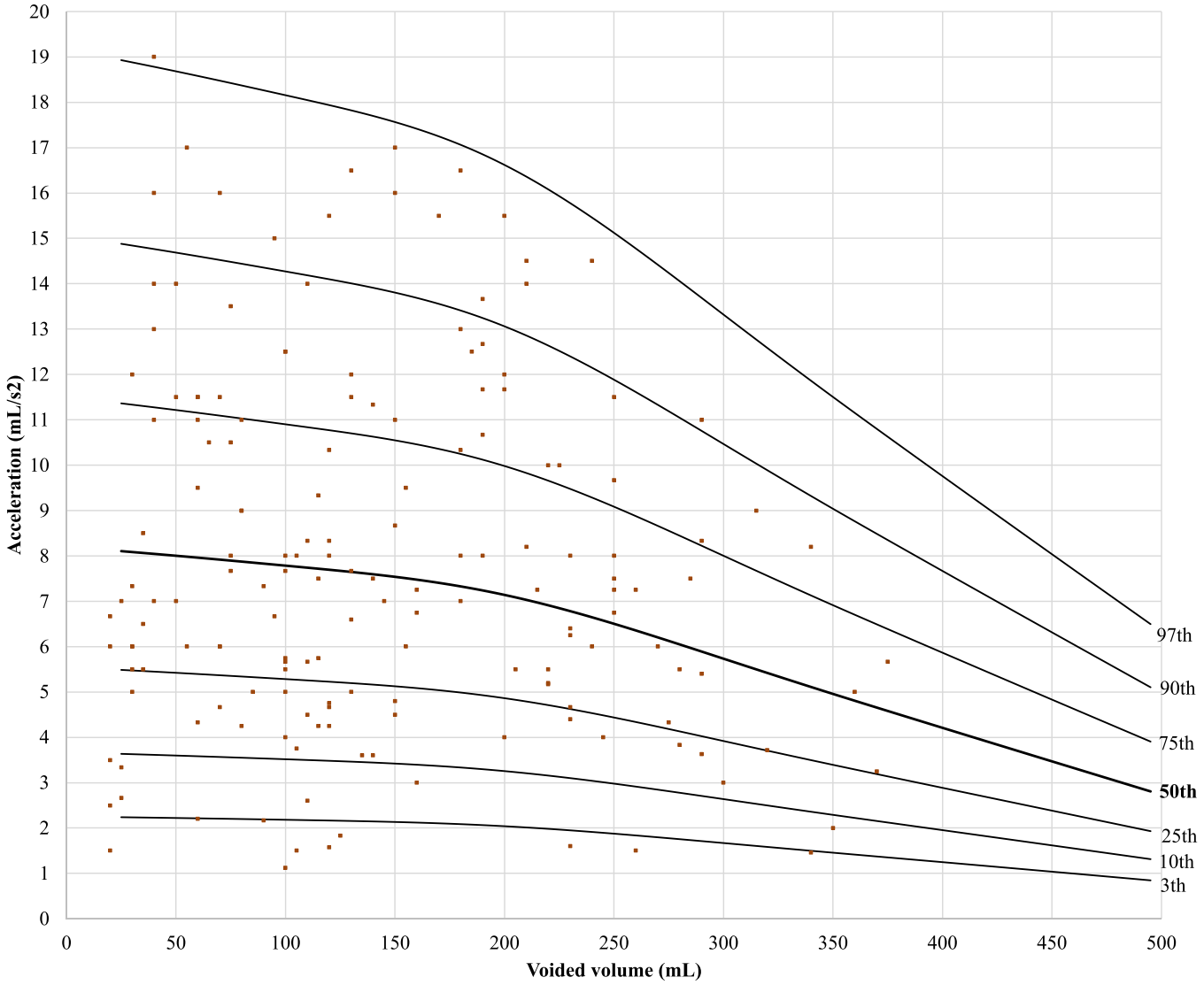

FIGURE 1 Urine flow acceleration (Q_{acc}) nomogram for girls. Q_{acc} is demonstrated by voided volumes in the female population ≤18 years of age.

incontinent women than stress incontinent ones alone, and they hypothesized that Q_{acc} might be a more reliable parameter for urethral resistance and tonus. Wen et al.[15] reported the Q_{acc} is superior to Q_{max} in diagnosing bladder outlet obstruction in men with BPH.

Acceleration of urine flow might provide a finer diagnosis of the relationship between abnormalities of the LUT and diabetes mellitus (DM)[33] as well.

We believe that Q_{acc} might be used primarily to detect damage to the detrusor muscle (especially to detect reduced detrusor contraction caused by DM). We consider that Q_{acc} is a better indicator of diabetic autonomic neuropathy than cardiovascular dysfunction tests (Ewing tests), because in our previous study[14] Q_{acc} levels were significantly decreased not only in diabetic children with cardiovascular autonomic dysfunction (CAD), but also in diabetic patients without CAD.

Theoretically, it can be assumed that Q_{acc} values do not decrease in the case of a reduced Q_{max}—if there is a urethral stricture in the background. While in the event of hypocontractility there might be a decrease. But as the disease progresses, the detrusor muscle transforms, and its contractility deteriorates, therefore reduced Q_{acc} values appear. Thus, Q_{acc} examinations might be suitable even as a replacement for invasive UPP, but we have not performed such tests so far.

By the evaluation of uroflow acceleration, there will be more biological indicators to assess the etiology of the urinary problems and the effect of different diseases and treatments for voiding function to get the appropriate and precise treatment for patients.

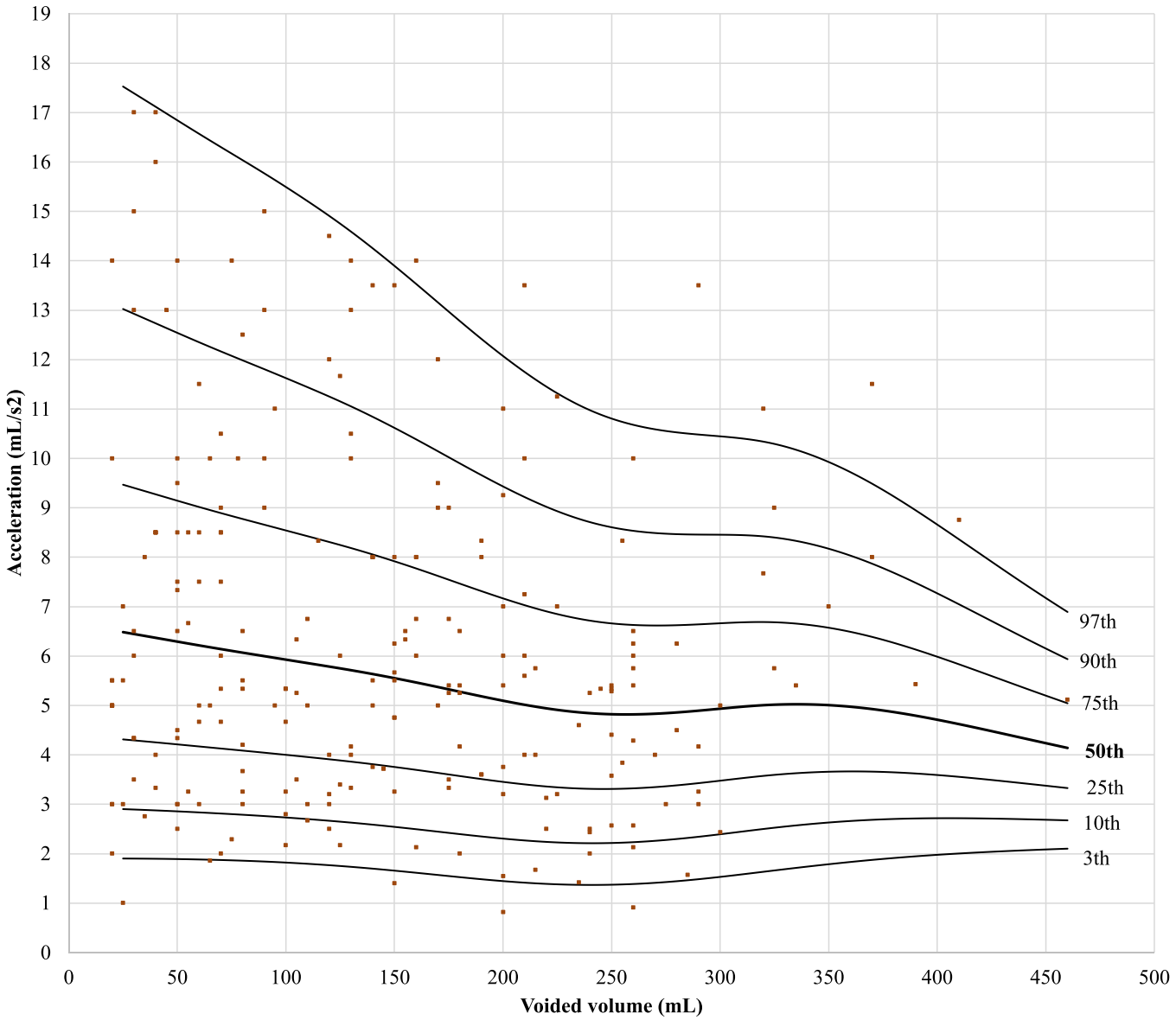

FIGURE 2 Urine flow acceleration (Q_{acc}) nomogram for boys Q_{acc} is demonstrated by voided volumes in the male population ≤18 years of age

Since there are only a few studies evaluating Q_{acc} values in adults and pediatric populations with different diseases; furthermore, normative reference values of Q_{acc} are lacking, we found it important to establish normal ranges of Q_{acc} in both genders by voided volumes in children.

4.1 | Strength of the study and limitations

The strength of this study is the novelty of the evaluation of normal reference values of $Q_{acc,}$ which includes a relatively large number of children with Q_{acc} calculation.

The main limitation of this study is the possible selection bias due to the retrospective design.

5 | CONCLUSION

Normal reference values for urinary flow acceleration were established in percentile forms in children. As the acceleration of urine flow can provide a finer diagnosis of abnormalities of the LUT and various chronic diseases (diabetes mellitus etc.), our results could form a basis for studies about the diagnostic significance of uroflow parameters in different diseases in children.

5.1 | Implication for practice

As soon as studies—comparing the Q_{acc} values of healthy children and patients with different diseases—identify

diagnostic cut-off values, the use of the normal reference values of Q_{acc} can be easily translated to everyday clinical practice. According to the currently available literature, Q_{acc} is an important tool to aid in the diagnosis of LUT symptoms. By establishing normative reference values, interpreting the different Q_{acc} parameters might help clinicians evaluate different diseases.

5.2 | Implication for research

Since we evaluated the Q_{acc} patterns of healthy asymptomatic pediatric populations, we formed the basis of future prospective studies. Prospective studies comparing healthy children and pediatric populations of different diseases with or without LUT symptoms will be needed to establish cut-off values to differentiate normal and abnormal uroflow patterns (voided volume, voiding time, Q_{ave}, Q_{max}, TQ_{max}).

AUTHOR CONTRIBUTIONS

Study concept and design: László Szabó and Ágnes Rita Martonosi. *Acquisition of data*: Ágnes Rita Martonosi, Mária Földi, Szabolcs Kiss, and Piroska Pázmány. *Analysis and interpretation of data*: Ágnes Rita Martonosi and Piroska Pázmány. *Drafting of the manuscript*: Ágnes Rita Martonosi. *Critical revision of the manuscript for important intellectual content*: Piroska Pázmány, Szabolcs Kiss, Mária Földi, and László Szabó. *Statistical analysis*: Annamária Zsákai and Ágnes Rita Martonosi. *Study supervision*: László Szabó.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This current study was approved by the Heim Pál National Paediatric Institute's Local Ethics Committee (registration number: KUT-37/2021 Date: July 23, 2021) The previous study was approved by the Borsod-Heves-Nógrád Counties Regional Scientific Ethics Committee (registration number: 02-01-1990), where uroflow examinations were performed. In this study, no patients were involved in the design, conduct, or interpretation of the study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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III.

BMJ Open

INvesTigating the Abnormality of detrusor ConTractility by uroflowmetry in diabetic children (INTACT Trial): protocol of a prospective, observational study

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ABSTRACT

Introduction Bladder emptying abnormalities and cardiovascular autonomic dysfunction are manifestations of autonomic dysfunction in people with diabetes mellitus (DM), which are major causes of morbidity and mortality. Since they can reduce the quality of life, they are urgent to be addressed before resulting in complications. As uroflowmetry might determine autonomic neuropathy earlier than cardiovascular autonomic dysfunction symptoms occur, our aim is to detect early abnormalities in bladder muscle function in children with DM. We investigate the diagnostic accuracy of uroflowmetry. As a secondary aim, we compare the prevalence of uroflowmetry abnormalities to the appearance of measures of cardiovascular autonomic neuropathy. Finally, as an ancillary study, we examine the association of uroflowmetry with the appearance of peripheral neuropathy. These three aims, we feel, will put our results regarding uroflowmetry into an overall context of nerve disease early in the course of type 1 DM. To our knowledge, such an approach has heretofore not been performed.

Methods and analysis This will be a prospective, observational, single-centre clinical study. Patients with DM fulfilling the inclusion criteria and healthy controls will have uroflowmetry examination, cardiovascular autonomic dysfunction tests (heart rate response to deep breathing, to Valsalva manoeuvre, blood pressure and heart rate response to standing up, and to sustained handgrip) and nerve conduction test. The autonomic nervous system function will be examined by the reproducible and standardised cardiovascular reflex tests described by Ewing *et al*. During the examination, electrocardiogram (ECG) and blood pressure values will be recorded continuously. Heart rate response to deep inspiration will be executed to investigate the parasympathetic nervous system. Peripheral neuropathy will be evaluated by nerve conduction test. After a pilot period, when the first 50 diabetic and 50 healthy children will be assessed, sample size calculation will be carried out. The primary objective of this trial is to evaluate the diagnostic accuracy (sensitivity, specificity, positive and negative predictive value) of uroflowmetry. To do so, we compare uroflowmetry to the gold standard neuropathy tests, which are cardiovascular autonomic dysfunction tests (heart rate response to deep breathing, to Valsalva manoeuvre, blood pressure and heart rate response to standing up and to sustained handgrip).

Ethics and dissemination Ethics approval was obtained from the Scientific and Research Ethics Committee of the Heim Pál National Paediatric Institute in Budapest, Hungary (registration number KUT-37/2021). Results will be submitted for publication in a peer-reviewed journal.

Trial registration number NCT05247840.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Assessment of metabolic status, autonomic and peripheral neuropathy simultaneously in diabetic children.
⇒ Selection of the widest possible paediatric age group (5–18 years) who can cooperate in performing the tests.
⇒ There are no drugs used in the study; therefore, no adverse and serious adverse events are expected.
⇒ Compliance is expected to be worse among younger study participants.
⇒ Uroflowmetry examinations might have potential confounding factors ((1) uroflow values are highly dependent on voided volumes; (2) psychological excitement can affect the sensation of bladder fullness, and thus voided volumes).

INTRODUCTION

Diabetes and urological problems are common and have a prominent effect on the quality of life. Autonomic neuropathy, a major cause of morbidity and mortality in diabetes, can manifest in genitourinary, cardiovascular, gastrointestinal and sudomotor symptoms etc.[1]

Diabetic cystopathy (DC), the most common urological complication of diabetic autonomic neuropathy[2] occurs in 25%–90%

of patients.[3] It can impair the detrusor muscle function, leading to lower urinary tract (LUT) problems.[4 5] The classic triad of DC is decreased bladder sensation, impaired bladder emptying and increased bladder capacity causing overactive bladder, urge or overflow incontinence and nocturia.[6–11] Assessment of DC includes urodynamic measurements[12] and LUT symptoms questionnaires.[13]

Cardiovascular autonomic neuropathy (CAN), which occurs in 2.5%–90% of patients with diabetes,[14] is associated with abnormalities of vascular dynamics and heart rate control. The clinical symptoms might vary from tachycardia, orthostasis to myocardial infarction.[14] Diagnosis is based on sympathetic and vagal autonomic function examinations. The gold standard tests are cardiac autonomic reflex tests, including heart rate, blood pressure and sudomotor responses.[15]

Recent studies suggest that uroflowmetry might determine autonomic neuropathy earlier than CAN symptoms occur.[16–18] Since DC can reduce the quality of life, it is urgent to be addressed before resulting in complications.

Uroflowmetry is a non-invasive, widely accessible, quick and easy-to-use urodynamic diagnostic tool to evaluate voiding function[19] and to determine LUT dysfunction. As uroflowmetry might detect subtle voiding modifications in neuropathic patients before LUT symptoms manifest,[16] it might be a useful tool in the early diagnosis of dysfunction of the detrusor muscle.

In addition to autonomic neuropathy, peripheral neuropathy is one of the most bothersome complications of type 2 diabetes mellitus (DM), with a global prevalence of 35.78% (in Europe 48.14%) among adult patients.[20] The most common form of it is symmetric generalised polyneuropathy which is a well-known microvascular complication of type 2 DM.

Since the diagnosis of autonomic and peripheral neuropathy is complex and might be inconvenient for children, our aim is to detect early abnormalities in bladder muscle function in diabetic children in an easy and painless way before the manifestation of autonomic or peripheral neuropathy.

The main objective of this trial is to evaluate the diagnostic accuracy (sensitivity (SE), specificity (Sp), positive and negative predictive value) of uroflowmetry. To do so, we compare uroflowmetry to the gold standard neuropathy tests, which are cardiovascular autonomic dysfunction tests (heart rate response to deep breathing, to Valsalva manoeuvre, blood pressure and heart rate response to standing up and to sustained handgrip) and in parallel, we evaluate peripheral nerve conduction test in diabetic children and healthy controls.

METHODS AND ANALYSIS

Study design

It is a prospective, observational, single-centre clinical trial. The study protocol is constructed in accordance with the Standard Protocol Items: Recommendation for Interventional Trials (SPIRIT) 2013 Statement[21]; for the checklist, see online supplemental file 1.

Patient enrolment

Inclusion criteria

Patients with diabetes

Children aged 5–18 years (boys, girls) with type 1, type 2 and monogenic DM who are treated at the Endocrinology Department and Outpatient Clinic of Heim Pál National Paediatric Institute (HOGYI, Budapest, Hungary) will be enrolled. The definition of diabetes is based on the American Diabetes Association criteria.[22]

Healthy controls

Healthy volunteer children aged 5–18 years (boys and girls) without any acute or chronic disease will be enrolled and the same tests will be performed on them as on diabetic children. Control subjects will be recruited by the HOGYI's Volunteer Recruiting Programme in kindergartens and schools.

Exclusion criteria

Patients with diabetes

Diabetic children with the following conditions will be excluded from the study:

1. Acute febrile condition (≥38°C core temperature) in the past 7 days.
2. Acute or chronic urinary tract or kidney disease: renal insufficiency (GFR ≤60 mL/min per 1.73 m^2[23]), urinary tract infection.
3. Urological disease: bladder cancer, urolithiasis, urethral stricture, posterior urethral valve, meatal stenosis, previous genitourinary surgery, conditions causing urinary outflow problems (phimosis, hypospadias, vesicoureteral reflux).
4. Cystic fibrosis-related diabetes.
5. Neurological disorders (multiple sclerosis, transient ischaemic attack, transverse myelitis, myelocele, meningomyelocele, previous spinal cord operation, or operation which might injure the sacral nerve plexus).
6. Medicines taken which can cause neuropathy[24]:
   - Cytostatic agents: cyclophosphamide, platinum-based antineoplastic agents, vinca alkaloids, epothilones, taxanes, proteasome inhibitors and immunomodulatory drugs.[25]
   - Immunosuppressive agents: TNF-alfa inhibitors (adalimumab, infliximab, etanercept), interferon.
   - Cardiovascular medicines: statins, digoxin, amiodaron.
   - Antimicrobial agents: nitrofurantoin, linezolid, voriconazole, itraconazole, antituberculotics, metronidazole, fluoroquinolone.
   - Antiulcerative agent: cimetidin.
   - Neuropsychological agents: levodopa, phenytoin.
7. Psychiatric disorders that prevent participation/collaboration in the study.

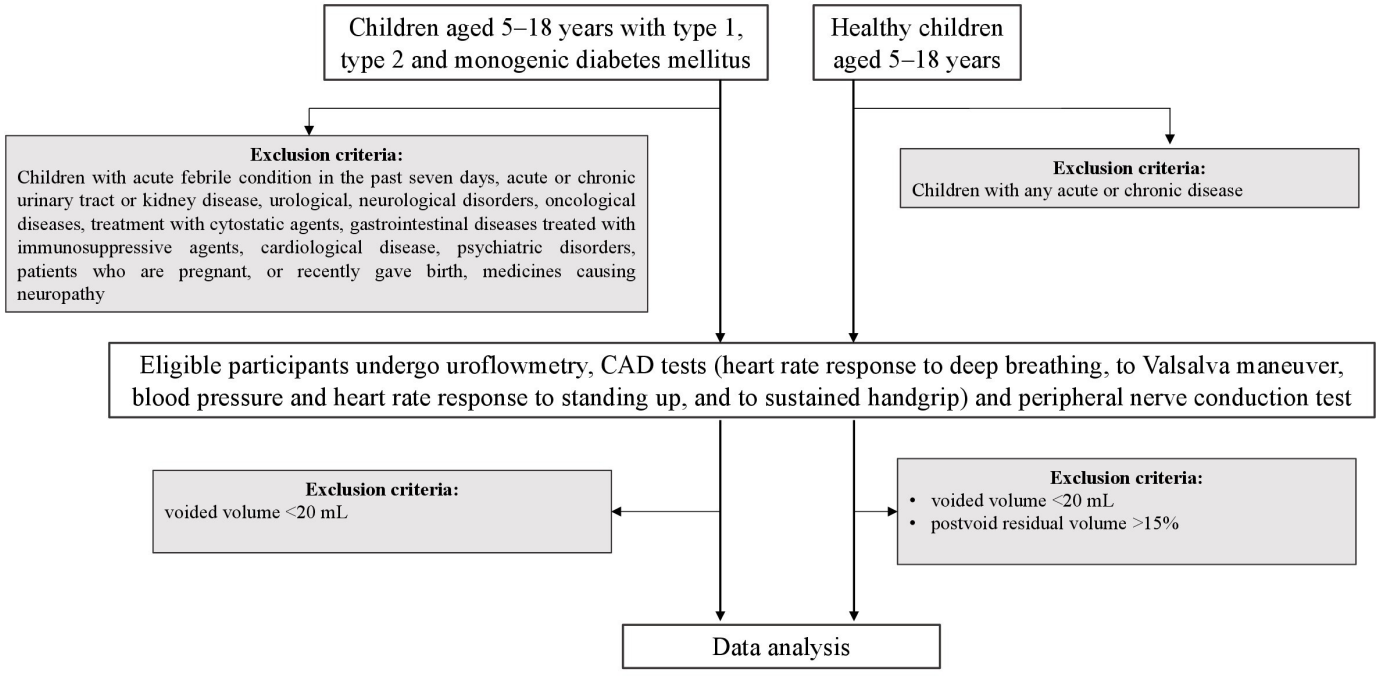

Figure 1 Flow chart of the participants according to the SPIRIT 2013 statement. CAD, cardiovascular autonomic dysfunction; SPIRIT, Standard Protocol Items: Recommendation for Interventional Trials.

8. Constipation (defined according to the Rome IV criteria[26]).
9. Voided volume <20 mL.
10. Patients who are pregnant or gave birth in the last 12 months.
11. Lack of consent of the patient or legal representative; the patient or legal representative withdraws his or her voluntary consent during the study.

Healthy controls

Children with voided volume <20 mL, and postvoid residual volume >15% will be excluded.

All patients who meet the inclusion criteria will be informed of the possibility of taking part in the INvesTigating the Abnormality of detrusor ConTractility Trial. Informed consent will be signed by the patient, or in the event of incapacitated status, by the patient's legal guardian; for details, see online supplemental file 2. The consent will be obtained by the steering committee (SC) members. Those enrolled in the study will be monitored yearly regardless they reach the age limit of 18 years during the recruitment period. The flow chart of the participants according to the SPIRIT 2013 statement is demonstrated in figure 1.

Patient and public involvement

Patients will be involved in the design and conduct of this research. During the feasibility stage, the priority of the research question, choice of outcome measures, methods of recruitment and the trial will be informed by a discussion with patients and the patient's legal guardian through a focus group session and a structured interview. Once the trial is published, participants will be informed of the results through a consultation.

Data collection

The questionnaire used in the study is shown in online supplemental file 3.

Baseline characteristics

All parameters below will be recorded:

1. Date of birth, age (years), gender (boys/girls), race (white/black/Indian/Asian/other).
2. Weight (kg), height (cm) and body surface calculated by Mosteller formula,[27] body mass index (BMI) and BMI percentiles.
3. Diet, alcohol consumption, smoking habits.
4. Regular medicine consumption (drug active ingredient).
5. Physical status, vital parameters (axillary temperature (°C), respiratory rate (respirations/min), oxygen saturation measured by pulse oxymetry, heart rate (beats/min), non-invasive blood pressure (mm Hg) and capillary refill time (sec)).

Clinical symptoms

Urge to urinate (urgency), daytime urine incontinence, nocturnal urination, nocturnal enuresis, frequency of bowel movement, consistency of stool.

Diabetes anamnesis

Type of diabetes, time of diagnosis, treatment (oral antidiabetics, diet, insulin), method of insulin administration (subcutaneous injection, pump), use of sensor–pump, the total number of diabetic ketoacidosis, hemoglobin

A1C value (%), fasting glucose value (mmol/L), postprandial glucose value (mmol/L).

Fluid balance in the past 48 hours

Documentation of 48-hour consumed liquid and outflow fluid flow.

Laboratory parameters

Urine rapid test (Medi-Test Combi 9+leuko), C reactive protein (mg/L), white blood cell count (G/L), absolute neutrophil count, absolute lymphocyte count, red blood cell count (T/L), haemoglobin (g/L and conversion: mmol/L), haematocrit (%), platelet count (G/L), glucose (mmol/L and conversion: mg/dL) blood urea nitrogen (mmol/L and conversion: mg/dL), creatinine (µmol/L and conversion: mg/dL, carbamide (mg/dL and conversion: mmol/L), estimated glomerular filtration rate (mL/min), aspartate aminotransferase/glutamic-oxaloacetic transaminase (U/L), alanine transaminase/glutamic pyruvic transaminase (U/L), gamma-glutamyl transferase (U/L), lactate dehydrogenase (U/L), alkaline phosphatase (U/L), Sodium (mmol/L), Potassium (mmol/L), Chloride (mmol/L), Calcium (mmol/L), albumin (g/L), total protein concentration (g/dL) and C-peptide (ng/mL) will be collected. Cut-off values will be determined by the Department for Laboratory Medicine of HOGYI, Budapest, Hungary.

Body composition analysis

Body composition analysis (InBody)—which determines the impedance by age, gender, body type or ethnicity—will be executed. Total body water, lean body mass, dry lean mass, skeletal muscle mass and body fat mass will be measured, and basal metabolic rate and body fat percentage will be calculated. The device will be calibrated according to the prescribed instructions for use by a skilled technician.

Uroflowmetry parameters

Uroflowmetry parameters will be recorded at spontaneous voiding and at the first sensation of bladder filling after 15 mL/kg liquid consumption. Urinary bladder function will be assessed by uroflowmetry, and postvoid residual volume will be detected by ultrasonography (SonoSite M Turbo). Uroflowmetry will be performed using a uroflow-cystometer (UroDoc Frytech), which determines Q_{max}, Q_{ave} and TQ_{max}. Voided volume (in mL), voiding time (in sec), average and maximum urinary flow rate (Q_{ave} and Q_{max} in mL/s), and time to maximum urinary flow (TQ_{max} in s) will be measured; urine flow acceleration (Q_{acc} in mL/s^2) will be calculated. Q_{max} and Q_{ave} are defined according to the International Children's Continence Society.[28] The voided volume will be measured by the uroflow-cystometer device; boys void in a standing, girls in a sitting position. Postvoid bladder diameter (mm) will be measured by ultrasonography and converted to bladder residual volume (mL). The device will be calibrated according to the prescribed instructions for use by a skilled technician. The examinations will take approximately 10 min.

Cardiovascular autonomic dysfunction tests

Cardiovascular autonomic dysfunction will be assessed by five reproducible and standardised cardiovascular reflex tests described by Ewing *et al*. Three of the five tests assess parasympathetic function: heart rate response to deep breathing, to standing and the Valsalva manoeuvre. Two tests evaluate sympathetic function, which are blood pressure responses from lying to standing and at sustained handgrip. Each of these five tests is assigned a score of 0 for normal, 0.5 for borderline and 1 for abnormal results. The sum of these 5 scores—which is the Ewing score—is used to assess the severity of cardiovascular autonomic dysfunction. Patients with an Ewing score ≥2 form the cardiovascular autonomic dysfunction+group, and those with less than 2 form the cardiovascular autonomic dysfunction—group.[14 15 29–31]

During the examination, ECG and blood pressure values will be recorded continuously with the reflex tests, as well as a 1 min rhythm strip to calculate the SD of the normal-to-normal interval.

ECG recording will be executed using 1, 6 and 12 leads. The captured ECG will be taken from a recording it creates a moment and from the amount of data stored in 1 min, averaged default value calculation is implemented as the followings: mean heart rate, heart rate distribution, P wave duration, PR and RR interval, QRS duration, ST segment, QT duration, corrected QT interval (QTc) according to the Bazett's formula. Normative values will be reported on the ECG record, as well as the heart rate of the patient. The characteristics of cardiovascular autonomic dysfunction tests are demonstrated in table 1.

Peripheral neuropathy examination

Peripheral neuropathy will be evaluated by a nerve conduction test. The device measures motor conduction in the lower extremities. It operates at two dedicated frequencies in order to perform a thick myelin sheath cordless fibre (5 Hz) and thin myelinated nerve fibre (2000 Hz) examination. The device will be calibrated according to the prescribed instructions for use by a skilled technician.

The duration of the cardiovascular autonomic dysfunction examinations is about 15–20 min, and the peripheral neuropathy examination (based on the patient's attention) takes 5–10 min per limb.

Recruitment period

Recruitment period of the entire study: the planned starting date of the study is March 2022, and the planned completion date is March 2028. During the first year period, all the previously diagnosed and treated patients with diabetes, as well as the newly diagnosed ones, will be examined. The metabolic status will be evaluated in parallel with autonomic and peripheral neuropathy. After this period patients will be followed up for 5 years (regardless they reach the age of 18 years), and the same

Table 1 Characteristics of the cardiovascular autonomic dysfunction tests

Name of the cardiovascular test	Characteristics of the test	Autonomic nerve system
Heart rate response to deep breathing	The child breathes deeply in a sitting position. The maximum and minimum heart rates during each breathing cycle are measured, and the mean of the differences during three successive breathing cycles are taken to give the maximum-minimum heart rate.	Parasympathetic function
Heart rate response to standing up	The child lies on a couch and then stands up unassisted. An immediate increase in heart rate happens immediately, maximal amount is at about the 15th beat after starting to stand, followed by a relative bradycardia, maximal around the 30th beat. This will be calculated as the 30:15 ratio, which means the ratio of the longest R-R interval around the 30th beat to the shortest R-R interval around the 15th beat.	Parasympathetic function
Valsalva manoeuvre	The child blows into a mouthpiece at a pressure of 40 mm Hg for 15 s. Normally the heart rate increases, followed by a rebound bradycardia. During the manoeuvre, the ratio of the longest and shortest R-R interval will be measured. The Valsalva ratio will be calculated as the mean ratio from three consecutive Valsalva manoeuvres.	Parasympathetic function
Blood pressure response to standing up (orthostatic hypotension)	The blood pressure (in mm Hg) is measured in a lying and standing position using a blood pressure metre (Omron M2 Intellisense). The difference in systolic blood pressure is considered to be the extent of the change in postural blood pressure. Systolic pressure is considered to be abnormal when a fall >20 mm Hg after standing up is observed.	Sympathetic function
Blood pressure response to sustained handgrip	Handgrip is maintained at 30% of the maximum voluntary contraction using a handgrip dynamometer up to a maximum of 5 min, and the blood pressure is measured each minute. The difference between the diastolic blood pressure just before the release of the handgrip, and before starting, is taken as the measure of response.	Sympathetic function

tests will be performed on the same individuals who were previously enrolled in the study, and all data (listed in the Data collection section) will be collected from them. During the 5-year follow-up period, data will be regularly collected every year. The follow-up will be continued even if we find abnormalities either in the peripheral or autonomic neuropathy examinations.

Withdrawal of a subject from the study

Patients will not be included in the per-protocol analysis if: (1) during the trial any exclusion criteria meet and (2) data required for the primary endpoints are missing.

Sample size calculation and power analyses

The trial will start with a pilot period when the first 50 diabetic and 50 healthy children will be assessed. This will be followed by a short evaluation period, during which the principal investigator and the study team could make adjustments to the study protocol to ensure feasibility. Based on the preliminary data of the pilot period, the investigators plan to carry out a sample size calculation in order to decide the timing of the interim analyses. Based on the estimated number of items, auditing trial conduct is planned after the first year and every year, on the basis which the SC will suggest changes if necessary.

Data analysis plan and outcomes

The primary endpoint is the diagnostic accuracy (SE, Sp, negative and positive predictive values) of the uroflowmetry test compared with the cardiovascular autonomic dysfunction tests in the detection of autonomic neuropathy. The secondary endpoints are the existence of peripheral and autonomic neuropathy in diabetic children in parallel with the metabolic status (prevalence and incidence of peripheral and autonomic neuropathy), differences in metabolic status (weight, height, body surface, BMI, laboratory parameters, body composition), fluid turnover and clinical symptoms of patients with diabetes comparing to healthy children.

All descriptive statistic calculations and analyses will be carried out with MS Excel (V.16.52, Microsoft Corporation (2019) and SPSS (V.24, IBM). Results will be characterised as either false positive, true positive, false negative or true negative. Using these data, sensitivities, specificities and ORs will be calculated. Basic statistical parameters, that is, mean, SD, SE of the mean, median, minimal and maximum values of the variables for the groups of diabetic children and members of the control groups, will be estimated. The centile distribution of the variables will be estimated by using lmsChartMaker Pro V.2.3 software (Medical Research Council, UK 1997–2006) based on the LMS method.[32 33] First, in the statistical analysis, we will measure the values of the above listed and detailed examinations (see Clinical symptoms–Peripheral neuropathy examination sections) and evaluate whether there is a significant difference between patients with diabetes and healthy controls: in the case of continuous variables Student's t-test and analysis of variance analysis for normally distributed variables, while Mann-Whitney U and Kolmogorov-Smirnov tests in the case of not normally distributes variables; furthermore χ^2 analysis will be used for testing distributions' homogeneity in the case of variables having discrete probability distribution. The statistical relationship and association between the variables will be tested by correlation, regression and contingency table analyses and Kendall's tau test. Univariate and multivariate analyses will be performed to assess the prognostic variables that affect the urinary bladder functions of diabetic children. As a second phase of the analysis, after

executing basic and comparative statistical analyses of the studied variables, the best cut-off values will be determined to differentiate early neuropathy from healthy controls (by estimating SE, Sp, positive and negative predictive value, likelihood ratio positive, likelihood ratio negative and characteristic of receiver operative curves). In the third phase, we will assess the diagnostic accuracy of each diagnostic test to differentiate early neuropathy (by estimating SE, Sp, predictive values, likelihood ratios, area under the receiver operating characteristic curve, overall accuracy and diagnostic OR). Significance will be set at p=0.05 level in the statistical analyses. We are not planning any statistical methods to handle missing data; data imputation will not be carried out. For each endpoint, a data quality table will be created in order to assess the extent of the potential bias provided by the missing data.

For dividing children into nutritional status subgroups, age-dependent cut-off values will be used. The following subgroups will be made during statistical analyses: (1) ages (5–9, 10–14, >14 years); (2) BMI (BMI percentiles and <18.5, 18.5–25, 25–30, 30–35, >35 kg/m^2, respectively, at the age 18 years); (3) type of diabetes (type 1, type 2, monogenic DM); (4) time of diagnosis (<5, 6–9, >10 years); (5) treatment (oral antidiabetics, diet, insulin); (6) method of insulin administration (subcutaneous injection, pump) and (7) number of total diabetic ketoacidosis (0–1, 2–5, 6–9, >10 x); (8) HgA1c value (<5.7, 5.7–6.4, >6.4%).

ETHICS AND DISSEMINATION

Ethical and legal considerations

The study will be conducted following the Declaration of Helsinki. It will be managed in compliance with the study protocol, Good Clinical Practice, designated standard operating procedures, and Hungarian laws and regulations. This protocol, in its current version, was approved by the Local Scientific and Research Ethics Committee of the HOGYI Medical Research Council (ethical approval number KUT-37/2021). The study was registered in ClinicalTrials.gov Protocol Registration and Results System under the registration number NCT05247840 on 18 February 2022.

Safety monitoring plan, trial administrative organisation, committees and boards

The corresponding centre and designer of the trial is the HOGYI, Budapest, Hungary. The study was designed by the SC and Independent Expert Committee (IEC).

The SC will be led by LS (principal investigator, paediatrician and specialist in nephrology and urodynamics). SC members will be: ÁRM (paediatrician), PP (physician) and SK (physician). Primary supervision of the study will be provided by the SC; it will make decisions concerning all important questions (eg, premature termination of the study, drop-outs during the study). All recommendations will be filed. SC serves as a data monitoring committee as well. If the study expands to multicentric, so does the number of SC members.

The IEC will include two paediatric endocrinologists (ZsV and ZsA), a paediatric neurologist (BR), a paediatric oncologist (GyP) and a paediatric gastroenterologist (AK). The task of the IEC is to monitor the trial regularly, to improve adherence to the protocol and to provide recommendations to the SC. An early quality assessment check will be performed on the first 50 patients. The IEC will perform an independent assessment of the trial-related documents and activities, with the aim of ensuring the respect of subjects' rights, safety and well-being and to guaranteeing the plausibility of the clinical data. The IEC will report to the SC. The SC will discuss all the information and—if differences would be expected to have any bearing on the interventions and outcomes of the study—the study needs to be reassessed and the IEC will make recommendations regarding either re-evaluation of the extension of the recruitment period or the number of study centres, power calculation or termination of the trial.

Furthermore, the study centre will include a patient representative. To comply with current ethical regulations, the study will have an independent physician and a safety manager as well.

Since there are no unknown drugs or therapy are used in the study, no adverse or serious adverse events are expected. In the trial, IEC will examine safety variables after every 25 patients have completed. Investigators will report adverse or serious adverse events on a separate form which will be sent to the IEC. The SC will discuss and, if the adverse effect is confirmed, it will be reported to the relevant institutional and national ethical committee.

Data management

The SC members will perform the examinations listed in the section Data collection. Data will be handled by the SC. Manual data collection will be turned into a digital form by a study data manager. Insufficient data will be filtered when digitised from the paper. At the end of each examination, before releasing the patient, a data quality and validity check will be performed. Once the data have been digitised, another data check will be executed. The principal investigator (LS) will ensure that the digitalised data are accurate, complete and legible. Any missing, implausible or inconsistent recordings will be referred back to the investigator who performed the examinations. Patients with missing information will be asked via a phone call to fulfil the missing data. All changes will be documented. All data will be retained for 10 years.

Data confidentiality

Manually collected data will be stored safely in the corresponding centre (HOGYI) and only be accessible to the SC members. Digitisation will only take place under the supervision of the PI. When digitised, data are separated, tabulated and encrypted, and patient data are stored

securely. Digitised data will be anonymised as well (the patent's personal data will be coded and stored separately for identification). There are no other contractual agreements that limit such access for SC members. Other participants (study manager, IEC members) do not have access to the final trial dataset. The study protocol is free for public access.

Dissemination policy

Any changes or deviations in the study protocol will be updated on ClinicalTrials.gov. The changes will be reported to the research ethic committee as well.

Authorships will be implemented according to the International Committee of Medical Journal Editors recommendations.[34] Results will be submitted for publication in a peer-reviewed journal.

Centres

The study will start as a single-centre trial, but additional centres are planned to be involved in the future.

DISCUSSION

To our best knowledge, this is the first prospective clinical trial evaluating early signs of neuropathy by simultaneously uroflowmetry, cardiovascular autonomic dysfunction tests and peripheral nerve conduction test in paediatric patients with diabetes and healthy controls.

CAN is one of the most studied forms of autonomic neuropathy, which is a frequent and early complication of diabetes. Although the progression of autonomic neuropathy is believed to be related to the duration of diabetes and poor metabolic status; animal studies have raised the question of whether changes in bladder function begin to occur soon after its onset.[35 36] Previous studies have suggested that DC is not the prime urodynamic finding in diabetics. Kaplan *et al*[37] found that detrusor overactivity was the most common finding. Kebapci *et al*[38] came to the conclusion that classic DC occurs in only 44% of adult women with type 2DM, followed for a mean of 13.85 years; more common findings are detrusor overactivity, stress and urge incontinence.

Therefore, the sooner the early signs of DC are discovered, the earlier the therapeutic modifications can be initiated (tight glycaemic control), which can improve the quality of life. Uroflowmetry can highlight the progressive nature of diabetes—starting with storage changes, then developing voiding dysfunction due to detrusor overdistension, to the decompensated phase. As early alterations in voiding patterns can be seen during the urodynamic examinations before bothersome urinary symptoms are recognised by patients, urodynamics, mostly uroflowmetry, can contribute to the early diagnosis of DC. Therefore, the inclusion of routine uroflow measurements in the current guidelines of diabetes management is crucial.

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