

## STUDY PROTOCOL AND STATISTICAL ANALYSIS PLAN

**Official Title:** Comparison of Superficial Pelvic Floor Shear Wave Elastography Between Healthy Women and Women With Pudendal Neuralgia

**NCT Number:** NCT07458737 **Document Date:** August 22, 2023 **Version:** 1.0

---

**Sponsor:** Comenius University

**Responsible Party:** Comenius University (Sponsor)

**Principal Investigator:** Prof. Dr. Magdaléna Hagovská, PhD. (Pavol Jozef Šafárik University in Košice)

**Study Director/ Faculty Contact:** [prof. MD. Jan Svihra, PhD] (Comenius University)

**Collaborating Institution:** Pavol Jozef Šafárik University in Košice

---

### Document Summary:

This document outlines the clinical protocol and the predefined statistical analysis plan for the observational case-control study investigating the biomechanical properties of the superficial pelvic floor muscles using shear wave elastography. The study was approved by the Ethics Committee of the Košice Self-Governing Region (Ref. No. 6808/2023/ODDZ) on August 22, 2023.

### 1. Title page

**Official title** (Comparison of Superficial Pelvic Floor Shear Wave Elastography Between Healthy Women and Women With Pudendal Neuralgia).  
NCT07458737 (Retrospectively registered)

**Version and date** (Version 1.0 (August 22, 2023))

**Principal Investigator** - prof. Dr. Magdalena Hagovská, PhD.

Primary Completion: February 25, 2026

Study Completion: March 2, 2026

## 2. Background & Objectives

**Background:** Knowledge regarding the elastometric properties of the superficial pelvic floor muscles (sPFM) in clinical populations remains limited. In particular, few comparative studies have examined sPFM stiffness in healthy women and those with chronic pelvic pain conditions such as pudendal neuralgia (PN).

**Objective:** The primary aim was to compare superficial pelvic floor stiffness using shear wave elastography (SWE) between healthy women and those with PN. The secondary objective was to assess the impact of PN-related pelvic floor disorders on quality of life and sexual distress.

## 3. Methods

**Study Design:** This observational case–control study compared stiffness (kilopascals, kPa) of the *m. ischiocavernosus*, *m. bulbospongiosus*, and superficial transverse perineal muscles in women with PN and healthy controls. Imaging was performed using a Consona N8 ultrasound system (3.0–13.0 MHz). SWE measurements utilised shell analysis to compare two distinct sites within each muscle: the area of lowest stiffness (a-sites) and the area of highest stiffness (b-sites). Clinical impact was assessed using the Pelvic Floor Impact Questionnaire–Short Form 7 (PFIQ-7) and the Female Sexual Distress Scale–Revised (FSDS-R). Between-group comparisons were performed using the Mann–Whitney U test.

### Participants

A total of 94 potential participants were assessed for eligibility, including 49 women with PN (potential cases) and 45 healthy volunteers (potential controls). Based on the inclusion and exclusion criteria, 5 cases were excluded (3 because of refusal and 2 because of botulinum toxin therapy) and 1 control was excluded because of refusal. The final sample included in the analysis consisted of 88 participants: 44 cases with PN and 44 healthy controls.

### Inclusion and Exclusion Criteria

#### Inclusion Criteria

For the PN group, inclusion required a confirmed diagnosis based on the five **Nantes diagnostic criteria** (Labat et al., 2008):

1. Pain located in the anatomical territory of the pudendal nerve
2. Pain exacerbated by sitting
3. Absence of pain that awakens the patient at night
4. No objective sensory loss on clinical examination
5. Positive response to a diagnostic anaesthetic pudendal nerve block

Additional requirements for both groups included an age of  $\geq 18$  years and, for the PN group, a symptom duration of at least 3 months.

**Inclusion Criteria (Healthy controls)** Women aged  $\geq 18$  years No history of chronic pelvic pain No history/symptoms of pelvic floor dysfunction (as per screening/interview)

## Exclusion Criteria

Participants were excluded from the study if they met any of the following criteria:

1. History of psychiatric, neurological (other than PN), or serious internal, orthopedic, or oncological diseases
2. Botulinum toxin therapy within the last 12 months
3. Pelvic surgery within the last 12 months
4. Incomplete or incorrectly completed questionnaires
5. Refusal to participate or withdrawal of informed consent

## Ultrasound and SWE Protocol

### *Equipment and Quality Standards*

Imaging was performed using the **Consona N8 ultrasound system** (Shenzhen Mindray Bio-Medical Electronics Co., Ltd.) equipped with SWE software. A high-frequency **L13-3N linear array transducer** (frequency range 3.0–13.0 MHz) was used. Tissue stiffness was quantified in kilopascals (kPa), with the display scale set from **0 to 800 kPa**. To ensure measurement validity, two real-time quality indicators were applied:

1. **Reliability index:** A value of **>90%** (represented by a green reliability map) was required for a measurement to be considered valid.
2. **Motion stability index:** Measurements were recorded only when stability reached a level of **4–5 stars**, indicating minimal probe or tissue motion.

Stiffness was visualised using a colour-coded map, where red indicated high stiffness, green represented medium stiffness, and blue indicated soft tissue. Measurements were obtained by placing a **circular region of interest (ROI) with a diameter of 2 mm** in a uniformly green area on the reliability index map. **Shell analysis** was used to identify and compare two specific locations within each muscle: the site of maximum stiffness (**b-site**) and the site of minimum stiffness (**a-site**).

Primary outcome reported in ClinicalTrials.gov refers to the b-sites (maximum stiffness)."

### *Muscle Visualization Technique*

Ultrasound assessments were performed by a single experienced examiner to eliminate inter-observer variability. In accordance with the protocol described by **Kadah (2024)**, the following approaches were used:

- **M. bulbospongiosus:** The transducer was placed in the sagittal plane at the midline. It was then tilted and rotated approximately  $5^\circ$  laterally until the muscle fibres were clearly visualised.
- **M. ischiocavernosus:** From the previous position, the transducer was moved laterally and tilted  $10^\circ$ – $20^\circ$  towards the pubic bone to align with the muscle fibres.

- ***M. transversus perinei superficialis***: The transducer was placed in the transverse plane and tilted towards the anal opening on both the left and right sides of the perineum.

Static images were obtained three times for each muscle at rest, and the median values were used for statistical analysis.

## Clinical Assessments and Questionnaires

### Standardised Questionnaires

1. **Pelvic Floor Impact Questionnaire–Short Form 7 (PFIQ-7)**: This 21-item instrument assesses the impact of pelvic floor disorders across three domains: bladder (Urinary Impact Questionnaire, UIQ-7), bowel (Colorectal–Anal Impact Questionnaire, CRAIQ-7), and vaginal symptoms (Pelvic Organ Prolapse Impact Questionnaire, POPIQ-7). Items are scored from 0 to 3, with total scores ranging from 0 to 300; higher scores indicate a greater negative impact on quality of life (Barber et al., 2005). The Slovak version was validated with a Cronbach’s alpha of 0.951.
2. **Female Sexual Distress Scale–Revised (FSDS-R)**: A 13-item questionnaire measuring sexually related personal distress. Responses are recorded on a 5-point Likert scale (0 = Never to 4 = Always), with a total score range of 0–52. Higher scores reflect greater levels of distress (Derogatis et al., 2008). The Slovak version was validated with a Cronbach’s alpha of 0.941.

## 4. Statistical Analysis Plan - SAP

### Sample Size

The sample size was estimated for a two-sided comparison of two independent groups (case–control study) with  $\alpha = 0.05$  and power = 80%. Given the absence of directly comparable prior data for superficial pelvic floor elastography in PN, a conservative medium effect size (Cohen’s  $d \approx 0.6$ ) was assumed. Under these assumptions, the required sample size was approximately 42 women per group. Allowing for incomplete measurements or participant exclusions, a target recruitment of 45–50 participants per group was considered appropriate.

### Statistical Analysis

Statistical analyses were performed using **IBM SPSS Statistics for Macintosh, version 30.0** (IBM Corp., Armonk, NY). Data normality was assessed using the **Shapiro–Wilk test**. Because the muscle stiffness values (kPa) and clinical scores did not follow a normal distribution, non-parametric tests were used.

Continuous variables are presented as **median and interquartile range (IQR)**. Between-group differences (PN vs. healthy) were evaluated using the **Mann–Whitney U test**. For categorical data, **Fisher’s exact test** was used to compare frequencies between groups. The relationship between muscle stiffness and clinical outcomes (PFIQ-7, FSDS-R) was assessed using **Spearman’s correlation coefficient (r)**. All tests were two-tailed, and the significance level was set at  $p < .05$ .

## 6. Ethics

This observational case–control study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (**STROBE**) guidelines. The study was carried out at a specialised outpatient physiotherapeutic clinic between March 2025 and february 2026. Ethical approval was granted by the Ethics Committee of the Košice Self-Governing Region, Slovakia (**Ref. No. 6808/2023/ODDZ, 22.8.2023**). The study adhered to the Declaration of Helsinki, and all participants provided written informed consent prior to enrolment. The study was retrospectively registered at ClinicalTrials.gov (registration date: 6 February 2026).