Diagnostic Accuracy of the Water-Immersion Wrinkle Test for the Diagnosis of Small-Fiber Neuropathy

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INTRODUCTION

Small-fiber neuropathies (SFN) selectively affect peripheral small-diameter, thinly myelinated (A-delta) and unmyelinated (C) fibers. Among the conditions that typically involve these fibers are diabetes mellitus (the most frequent); systemic or familial amyloidosis; neuropathy associated with chronic alcohol use; Sjögren syndrome; acquired immunodeficiency syndrome; Fabry disease; hereditary sensory neuropathies; among others. This neuropathy may be characterized by sensory symptoms (loss or reduction of perception of cold and/or heat, altered nociceptive sensation [pain]) and may be accompanied by unpleasant symptoms (paresthesias such as tingling, pins and needles, burning) and dysautonomia. These manifestations negatively affect the physical and mental quality of life of millions of people worldwide.[1–4]

The diagnosis of SFN is often challenging and is based on a combination of clinical evaluation, functional tests, and morphometric examinations.[5–6] Skin biopsy with quantification of intraepidermal nerve fibers, evaluation of thermal thresholds with quantitative sensory testing (QST), and in some cases nociceptive evoked potentials are considered reference standard tests for diagnostic confirmation.[6] However, these techniques are time-consuming to analyze, are not available in primary care settings or routine neurology clinics, and require highly specialized equipment and specifically trained staff.

In terms of functional assessment, QST measures thermal, vibratory, and pain thresholds, evaluating the sensory function of A-delta, C, and A-beta fibers. It is a noninvasive technique that assesses the increase in threshold for the stimuli mentioned in the presence of neuropathy. However, it has some limitations: as a psychophysical test, it is subject to bias; abnormal results have no localizing value to differentiate peripheral from central lesions; and it is time-consuming and available only in specialized centers. Reported sensitivity ranges from 60–85%. The German Research Network on Neuropathic Pain developed a standardized QST protocol that includes seven different tests and measures 13 parameters. Normative reference values are available for both sexes, different age groups, and various body regions such as the face, hand, and foot. QST parameters have been shown to be region-specific, age-dependent, and less sensitive in older patients.[7–16]

Another important neurophysiologic test is the sympathetic skin response (SSR), used to evaluate pre- and post-ganglionic sympathetic activity by measuring changes in voltage at the skin surface after electrical stimulation. This test allows a broader evaluation of autonomic involvement in SFN and is routinely performed in patients with suspected dysautonomia, either due to signs/symptoms or the underlying condition.[17–20]

The diagnostic accuracy of clinical examination alone in SFN has been reported as 54.6%, with a sensitivity of 62.6% and a negative predictive value of 53.7%. To facilitate diagnosis in clinical practice—given the subjectivity of symptoms and the often near-normal physical

exam—tools have been developed such as the Utah Early Neuropathy Scale (UENS), which detects subtle sensory abnormalities. This scale has demonstrated 92% sensitivity and 94% inter-rater reliability for early identification of SFN. One study tested seven different clinical neuropathy scales and their ability to detect neuropathy in patients newly diagnosed with impaired glucose tolerance and found that all tests could distinguish patients with neuropathy from controls with high diagnostic performance. The Modified Toronto Clinical Neuropathy Score (mTCNS) achieved the best diagnostic performance, with 98% sensitivity and 97% specificity. UENS achieved 85% sensitivity and 97% specificity.[5; 21–25]

Nevertheless, given the advantages and limitations of the tests mentioned above, there is a need for tools that can help physicians select/identify patients who require second-level diagnostic testing. The Water-Immersion Wrinkle Test (WIWT) has been used as an indicator of acral sympathetic dysfunction for over 80 years; it is a simple bedside semiologic test that contributes to the noninvasive diagnosis of distal SFN, particularly with autonomic involvement. In the sympathetic cutaneous terminals of distal finger pads, water penetrates through the sweat pores, generating electrolyte changes that induce discharges. These trigger vasoconstriction of the glomus bodies (arteriovenous structures attached to the dermis), reducing their volume and generating wrinkles due to dermal retraction. Elevated water temperature accelerates the process; a 1997 study evaluated wrinkle generation at different temperatures and observed that at low temperatures (20 °C) the time required for wrinkles to appear is approximately 10 minutes and at higher temperatures (45 °C) they appear at approximately 3 minutes.[26] Currently, the standardized technique requires 30 minutes with water at 40 °C. Normally, three or more digital wrinkles are expected according to the wrinkle grading scale. In patients with sensory neuropathy and reduced intraepidermal nerve fiber density, sensitivity of 71% (58%–82%) and specificity of 72% (56%–85%) have been observed. Several studies have reported sensitivity and specificity in the ranges of 70%–90% and 70%–80%, respectively.[27–33]

JUSTIFICATION

SFN presents diagnostic challenges due to the complexity of symptoms and the variety of available tests. While advanced tests such as skin biopsy, QST, or SSR are useful to confirm the diagnosis, simpler and more accessible tools such as WIWT offer a valuable contribution to initial screening. A comprehensive approach that combines functional, structural, and clinical tests is essential for the effective diagnosis and management of this condition. We propose to estimate the diagnostic accuracy of the Water-Immersion Wrinkle Test and to compare its sensitivity and specificity with routine tests such as QST and SSR. The SFN diagnosis will combine the clinical suspicion of SFN by neuromuscular neurology specialists and at least one clinical scale indicating small-fiber involvement. We propose to use UENS and mTCNS due to their high sensitivity and specificity.

OBJECTIVES

Primary Objective

- To determine the sensitivity and specificity of the WIWT for diagnosing SFN against the composite clinical reference at baseline.

Secondary Objectives

1. To estimate the ROC area under the curve (AUC) of the continuous WIWT score versus the composite reference at baseline.
2. To estimate positive and negative predictive values for WIWT at the observed SFN prevalence at baseline.
3. To assess inter-rater reliability of WIWT wrinkle scoring using ICC(2,1), with two rating sessions 48 hours to 7 days apart.
4. To assess intra-rater reliability of WIWT wrinkle scoring using ICC(3,1), with a second reading 48 hours to 7 days later.
5. To compare diagnostic performance of WIWT with QST and SSR at baseline, including AUC and sensitivity/specificity.

MATERIALS AND METHODS

Study design

This prospective diagnostic-accuracy study will be conducted at Hospital Británico, Buenos Aires, Argentina.

Population

All patients evaluated by the Neurophysiology Service, Neuromuscular Diseases team, Hospital Británico, between November 1, 2024 and November 1, 2025 inclusive, with diagnostic suspicion of small-fiber neuropathy by a neuromuscular neurology specialist.

Sample size

Based on epidemiological data on pure SFN primarily from a study conducted in the Netherlands[34] that reported an incidence of 12 cases per 100,000 person-years and a prevalence of 53 cases per 100,000 persons, and using an estimated sensitivity and specificity from the literature (80% and 70%, respectively), the required sample size was calculated.

Therefore, the necessary sample size for this study is approximately 30 subjects. This estimate provides a solid basis for the evaluation of the diagnostic test and allows a precise estimation of sensitivity and specificity in the study population. It should be noted that this calculation does not include specific adjustments for low disease prevalence; however, as a

national referral neuromuscular service, the effective prevalence in our institution may be higher than population estimates and may not require low-prevalence adjustments. Moreover, the prevalence described above may be underestimated and higher in the general population.[39]

Selection criteria

Inclusion
• Age > 18 years.
• Suspicion of small-fiber neuropathy based on history and/or physical examination by a neuromuscular neurology specialist.
• Sensory and motor nerve conduction studies within normal limits.
• Prior QST performed, regardless of result.
• Prior SSR performed, regardless of result.

Exclusion
• Patients under 18 years of age.
• Individuals who do not wish to participate in the study or who decline informed consent.
• Patients with any contraindication to any of the aforementioned studies.
• Neurophysiological studies demonstrating large-fiber involvement.

Source of information, data collection, and processing

Our hospital cares for patients with neuromuscular diseases. All information required for this study will be stored in an electronic database accessible only to the investigators and sub-investigators and may be verified by IRB members when deemed necessary. Demographic data and variables of interest will be obtained from the Hospital Británico electronic medical record. The information obtained will be maintained during the analysis of this work in an anonymized electronic case report form (CRF), where each patient will be coded using the first letter of the last name and first name plus a combination of the first, third, and fifth (if present) digits of the patient's medical record number, plus a sequential letter according to alphabetical order, so that any investigator/author can return to the original medical record if relevant information is needed, while preventing direct identification of the patient.

Selection and inclusion will take place after patients are informed, their questions are answered, and informed consent is obtained.

Definition and operationalization of variables

After signing consent, data will be obtained from the history and physical examination, including sensory testing of all four limbs to detect alterations in perception induced by cotton, pinprick, and vibration (medial malleolus, distal radial bone), as well as joint-position sense testing at the distal metacarpophalangeal joints of the thumbs and the metatarsophalangeal joints of the hallux. The presence and distribution of sensation and negative and positive signs will be recorded through comparative evaluation of affected and

unaffected skin areas to differentiate the quality of altered sensation and define dermatomal, mono/multineuropathic, and polyneuropathic distribution. Pallesthesia will be quantified using a graduated 128-Hz tuning fork. Cutaneous sensory signs will be assessed by asking the patient to keep their eyes closed and to report sensation induced by tactile stimuli and gentle brushing with cotton and a flat-tip brush (dynamic allodynia), punctate cutaneous stimulation with a pin (punctate allodynia), prick with a disposable needle (hyperalgesia), cooling/heating with tubes of cold/hot water (thermal allodynia), and superficial and deep mechanical sensation through digital pressure on the skin and underlying tissue (static allodynia and hyperalgesia). Altered sensation (e.g., spread, increase and/or persistence, electric-shock sensation) in affected areas (soles of the feet, dorsum of the feet, legs, fingertips, palms, forearms) compared with expected sensation in unaffected areas will be classified as allodynia or hyperalgesia depending on the stimulus used. Sensory signs will be graded as +2, +1 (gain of function), 0, −1, −2 (loss of function) to allow comparison with findings from QST, SSR, and WIWT. These sensory parameters, as well as muscle strength (Medical Research Council—MRC—scale) and deep tendon reflexes of all four limbs, will be included as variables. Signs and symptoms of dysautonomia will also be recorded based on history, physical exam, the patient's medical record, and an orthostatic hypotension test measuring blood pressure supine and after 3 minutes standing. In all patients, routine tests such as laboratory studies, neuroimaging, etc., performed to determine the underlying etiology of the neuropathy will also be included as study variables.

All patients with suspected SFN (clinical suspicion plus abnormality on >1 neuropathy rating scale—UENS and mTCNS) will undergo neurophysiological studies such as: sensory and motor nerve conduction studies; QST; routine SSR. Studies such as nerve conduction, QST, and SSR are part of essential standards of care for patients with suspected SFN. In addition to the above, patients included will be offered WIWT, a bedside semiologic test, at no additional cost.

Each operator and observer will be blinded to every other study. A single investigator with experience will perform WIWT. Operators performing QST and SSR will be blinded to each other and to WIWT. QST and SSR operators have extensive experience in their field.

Test methods

Water-Immersion Wrinkle Test (WIWT):

Technique: Patients will be seated upright with both hands immersed in water in a comfortable position. The proposed immersion time will be 15 minutes and the temperature will be maintained at 40 °C, verified with a thermometer available in the service. Cutaneous wrinkles will be graded using a published and validated scale.[27] Wrinkles will be considered abnormal if absent or severely reduced (Grades 0–2).

Wrinkle grading scale:
• Grade 0: Absence of wrinkles.
• Grade 1: Barely perceptible wrinkles with the fingertip not completely smooth.

• Grade 2: Two or fewer superficial wrinkle lines on the fingertip.
• Grade 3: Three or more deep wrinkle lines on the fingertip.
• Grade 4: Wrinkles that completely distort the fingertip pulp.

Data collection and assessment: Wrinkles will be evaluated at all times by a trained examiner. The examiner will assign the rating at the end of the test and will be blinded to QST and SSR results. The examiner's final result will be the sum of grades for digits 2, 3, 4, and 5. Using 12 points as the threshold (Grades 3 and 4 considered normal), a score equal to 12 points (3 points per finger) is the lower limit of normal. Scores below 12 points will be considered abnormal. Both hands will be assessed and the scores summed, using 24 points as the lower limit of normal.

Inter- and intra-observer variability of the wrinkle score will be assessed using four investigators trained in wrinkle assessment, who will receive photographs of subjects with different degrees of wrinkling. Photographs of the fingertips will be taken with the rear camera of an iPhone 13 (12 MP), at a distance of 10 cm and with a black background, set to 3024 × 4032 pixels and saved in JPEG format. In each test session, the images will be arranged in random order and distributed to the investigators, who will evaluate the digital photos. Each rater will score the same group of photos on two different days. The minimum time between assessments will be 48 hours and the maximum 7 days. Grades for digits 2, 3, 4, and 5 of both hands will be summed, and the normal/abnormal thresholds defined above will be used.

Quantitative Sensory Testing (QST):

Assessment areas: all four limbs are tested. For thermal threshold detection, four attempts are taken and averaged. For vibratory threshold detection, six attempts are taken and averaged. Routine selected areas include:

• Thenar eminence of both hands (for thermal thresholds)
• Dorsal aspect of both feet (for thermal thresholds)
• Palmar surface of the distal phalanx of the index finger of both hands (for vibratory thresholds)
• Plantar surface of the first metatarsophalangeal joint (for vibratory thresholds)

Determination of thermal thresholds: A device delivering controlled thermal stimuli is used, such as the Medoc™ Thermal Sensory Analyzer (TSA-II), which has a contact probe (30 × 30 mm) to measure thermal perception thresholds. Stimuli are administered with a controlled increase or decrease in temperature at a rate of 1 °C/s, starting from a baseline temperature of 32 °C. Maximum and minimum temperatures evaluated are 50 °C and 16 °C, respectively. The following parameters are evaluated:

• Warm detection threshold (WDT): the temperature at which the patient perceives the onset of a change toward warmth.

• Cold detection threshold (CDT): the temperature at which the patient perceives the onset of a change toward cold.

The method of limits is used to determine these thresholds, asking the patient to indicate the exact moment at which a change in temperature is perceived or pain appears.

In the QST protocol, vibration testing is performed to measure the vibration perception threshold. This test is performed with a Medoc™ Vibration Sensory Analyzer (VSA-3000).

Procedure: The device generates vibration at a controlled frequency. The patient indicates when the vibratory stimulus is felt and when it disappears.
Criteria: Vibration is considered abnormal when thresholds exceed normative reference values ($Z > +1.96$ or $Z < -1.96$).

Sympathetic Skin Response (SSR):

The patient must be at rest and in a warm environment to avoid vasoconstriction that may alter results. The skin must be clean and free of oils or creams. Surface electrodes are placed on the skin in the area of interest, typically on the palm of the hand or the sole of the foot. Surface electrodes with conductive gel are used to ensure good conductivity. A series of brief, low-intensity electrical stimuli is applied to a specific nerve to elicit a sudomotor response. Stimulation is performed at standardized frequency and duration. The change in voltage on the skin surface is recorded before, during, and after stimulation. Electromyography (EMG) recording equipment is used. A surface electrode on the left palm with a reference electrode on the dorsum is standardized. Data are analyzed to determine the amplitude and latency of the sympathetic skin response and compared with reference values. These are:

• Stimulus intensity: 19.5 mA
• Latency: < 1800 ms
• Amplitude: > 900 µV

Statistical analysis

The diagnostic tests used for the detection of SFN will be compared, focusing primarily on WIWT and comparing its accuracy against QST and SSR in patients with and without neuropathy. The reference standard will be a combination of neurological assessments and validated quantitative scales (UENS and mTCNS). Statistical methods for evaluating diagnostic accuracy will be conducted in accordance with the STARD (Standards for Reporting of Diagnostic Accuracy) guidelines.[40]

Analysis objectives
• Compare the sensitivity and specificity of WIWT versus QST and SSR.
• Evaluate intra- and inter-observer correlation for WIWT reproducibility.
• Estimate diagnostic performance (accuracy) of each test compared against the reference standard using the area under the ROC curve (AUC).

Statistical methods

Nonparametric tests (such as Mann–Whitney) or the unpaired t-test, as appropriate, will be used to compare scores obtained in the different diagnostic tests between patients with and without SFN. Mann–Whitney U will be used when the data are not normally distributed (assessed using normality tests such as Shapiro–Wilk), and the unpaired t-test will be used when the data are normally distributed. A two-sided p value < 0.05 will be considered statistically significant.

The diagnostic accuracy of WIWT, QST, and SSR will be evaluated using the area under the ROC curve (AUC). The following metrics will be calculated:
Sensitivity: proportion of true positives.
Specificity: proportion of true negatives.
Positive Predictive Value (PPV) and Negative Predictive Value (NPV): estimated to provide additional evaluation of clinical performance.

Intra- and inter-observer correlation for WIWT will be calculated using the intraclass correlation coefficient (ICC) to assess reproducibility of ratings between different observers and within the same observer at different times.

Data will be recorded in Microsoft Excel® 2016 and analyzed with StatsDirect® Statistical Analysis.

Ethical considerations

The collected information will be confidential, and the participant's name will not appear in any record nor be disclosed, safeguarding confidentiality in accordance with Personal Data Protection Law No. 25.326. Written informed consent will be requested from all participants enrolled in the study.

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