Quantitative Sensory Testing and Confocal Corneal Microscopy: Indications, Methodology, Interpretation, and Pitfalls

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Educational Objectives

1. Describe the methodology of confocal corneal microscopy and quantitative sensory testing.

2. Compare the diagnostic value, advantages, and limitations of confocal corneal microscopy and quantitative sensory testing.

Introduction

Neuropathic pain is pain that arises as a consequence of a lesion or disease affecting the somatosensory system; therefore, the diagnosis must be confirmed by tests that can demonstrate or exclude the neurological deficit [15,60]. In case of systemic neurological diseases such as large- and small-fiber polyneuropathies of different origin, assessment of intraepidermal nerve fiber density (IENFD), confocal corneal microscopy (CCM), and standardized quantitative sensory testing (QST) are often used as confirmatory tools for scientific research as well as for clinical diagnostics in a single patient.

The second reason to use tools such as QST is to analyze the underlying mechanism of pain, because similar combinations of different symptoms and signs can be found in different entities, several different combinations can occur in patients with the same disease entity, and even within the same patient, different mechanisms may be responsible for the generation of pain [9,8,19,39,47,51,66].

The aim of this chapter is the description of methodology of CCM and QST, including a comparison of their diagnostic value, advantages, and limitations.

Confocal Corneal Microscopy

Neuroanatomy

The eye is the only human organ that allows inspection of the central and peripheral nerves at once. The cornea is densely innervated by the ophthalmic branch of the trigeminal nerve. The innervation of the cornea can be divided into three structures: the subepithelial plexus, the stromal nerves, and the sub-basal plexus, which lies between the basal epithelium and Bowman’s membrane. The sub-basal plexus can be assessed by CCM, especially for the evaluation of nerve loss due to neurological diseases. It originates from stromal nerves penetrating Bowman’s membrane, branching into small fibers with a thickness between 0.2 and 10 μm, running parallel to the eye surface [4,3,43]. The corneal nerves are either thinly myelinated Aδ fibers or unmyelinated C fibers with low-threshold polymodal receptors for nociception and mechanical and cold stimuli [1,10,43,55].
Methods

Laser-scanning CCM uses a laser beam as a light source. This beam is scanned by a set of mirrors and focused on the microscope objective [25]. The reflected light is scanned by the same mirrors, while a detector aperture is responsible for preventing the light coming from points other than the observation specimen from reaching the photomultiplier. Stave et al. have invented a detachable system, the Rostock cornea module (RCM), which can be added to the commercially available Heidelberg retina tomograph to allow high-resolution corneal images [57].

For CCM, the examiner attaches the microscope to the central cornea after topical application of a local anesthetic and a drop of hypromellose. The microscope is linked to a retina tomograph, controlled by a digital video camera from the side. After calibration, pictures of the sub-basal nerve plexus can be continuously taken manually. The obtained pictures have a square dimension of 400 × 400 μm with a resolution of 384 × 384 pixels. The pictures with the best presentation of corneal nerve fibers are manually selected and stored. Analysis can be done either manually or automatically with available software. (See Fig. 1.)

The main CCM parameters analyzed [43] are (a) corneal nerve fiber length (NFL), defined as absolute length of nerves and branches per square millimeter (normal range around 18 [11–24] mm/mm²); (b) nerve fiber density (NFD), defined as the total number of major nerves per square millimeter (43 [31–45] no./mm²); and (c) nerve fiber branching (NFB), defined as the number of branch points per square millimeter (35 [25–55] no./mm²). NFL is the most reliable parameter, but NFD seems to be the most valid CCM parameter [2,43,44]. (See Fig. 2.)

Clinical Data

In patients with polyneuropathy, CCM results correlate closely with electrophysiological and skin biopsy findings in most studies [30,43,69]; in some studies they also correlate with thermal QST parameters [58,59,69], and in a study of diabetic patients, they correlate with the degree of retinopathy [11]. However, the sensitivity of CCM to detect nerve loss in polyneuropathy appears to be higher than the assessment of IENFD or the measurement of thermal/mechanical thresholds (QST), particularly in diabetes mellitus [2,44,43,59,69] or in patients with sarcoidosis [16]. For other diseases there are only a few data available. For example, although there is one animal study [18], there are no CCM studies in cancer patients after chemotherapy, but our team’s initial experiences are promising (see Case 3 in Fig. 2), although chemotherapy-induced neuropathy mostly affected the Aβ-fiber system [5], as shown also in one of our case reports (Fig. 2).

In contrast to IENFD in patients with type I and II diabetes [2,11,17,26] as well as in sarcoidosis [14,16], corneal NFL, NFB, and NFD correlated significantly with the severity of neuropathy. For example, in one of the largest trials, with more than 100 patients, nerve fiber length was 8.05 ± 0.71 mm/mm² in patients with diabetes without polyneuropathy, and 5.48 ± 0.45, 3.01 ± 0.39, and 2.99 ± 0.34 mm/mm² in patients with slight, moderate, and highly increased neuropathy scores, respectively (control 11.21 ± 0.88 mm/mm²) [59]. Also, at very early stages and rapidly after diagnosis of diabetes, the CCM parameters (particularly NFB; see for example Fig. 2) are significantly reduced [29,69], whereas IENFD and QST data show no close correlation with the severity of the disease. Similar results were reported recently by Brines et al. for patients with sarcoidosis at different stages of severity [14]. This group also described, unlike...
for IENFD, a significant inverse correlation for NFL and NFD for the degree to which pain interferes with daily activities [14]. Diabetic patients with and without pain due to polyneuropathy also showed significant CCM differences in contrast to IENFD [56,61]. Comparisons between CCM and QST, using a validated protocol (see below), have not been published. Most studies only assessed the response to one or two stimuli (vibration, cold or heat), with conflicting results [59,69].

One of the presumably most important advantages of CCM seems to be that the corneal nerve density is actually more sensitive to treatment than the skin innervation as assessed by immunohistochemistry. After pancreas transplantation due to renal failure, the corneal nerves recovered within 6–12 months, in contrast to the skin nerves [58]. Comparable results were reported recently after successful treatment of sarcoidosis with ARA 290, an 11-amino-acid peptide derived from the structure of erythropoietin (EPO), which leads to significant relief of pain, symptoms and partial normalization of the corneal nerve fiber length and density [16].

However, there are several limitations for the routine use of CCM, including high costs and reduced availability in most centers. Furthermore, for research purposes, many methodological questions have not been answered. In fact, inter-reader reliability (when analyzing the same pictures) is high both in healthy people and in diabetic patients, but interobserver reliability (two persons examining the same eye) is lower, which may explain the remarkable differences of the reported cut-off values for the CCM parameters in different studies (see above). There is an unmet need for studies in elderly healthy subjects and in patients with CCM values at the outer limits of the normal range. Unfortunately, and in contrast to QST and IENFD, no normative database is available yet for CCM. And finally, to estimate the risk of false-positive results, we need studies that analyze the influence of non-neurological diseases, such as rheumatic disorders with Sicca syndrome or eye diseases and former eye surgery, on CCM parameters by comparing CCM, QST, and IENFD in these groups.

**Conclusions**

CCM is a promising and well-tolerated tool to detect loss of thin nerves at very early stages of systemic diseases and to monitor nerve recovery after successful

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**Table 2.** Case reports: corneal confocal microscopy (CCM) pictures, quantitative sensory testing (QST) results, and clinical and electrophysiological data from three patients with moderate polyneuropathy of different origin. MDT, mechanical detection threshold; VDT, vibration detection threshold.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Clinical Symptoms</th>
<th>Neurography</th>
<th>QST</th>
<th>CCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>32-year-old male with diabetes mellitus type 1</td>
<td>Painless, only moderate numbness (feet, normal reflexes, VDT 6/8)</td>
<td>Normal</td>
<td>Normal, with moderate thermal/mechanical loss within normal range</td>
<td><img src="image1" alt="CCM image" /></td>
</tr>
<tr>
<td>33-year-old female with HIV infection for 5 years</td>
<td>None</td>
<td>Normal</td>
<td>Normal values, only blunt pressure hypoalgesia</td>
<td><img src="image2" alt="CCM image" /></td>
</tr>
<tr>
<td>63-year-old male after chemotherapy with vincristine (liver cancer)</td>
<td>Burning pain in all limbs, VDT 3/8, loss of ankle jerk reflex</td>
<td>Normal</td>
<td>Isolated severe mechanical hypoesthesia (MDT/VDT)</td>
<td><img src="image3" alt="CCM image" /></td>
</tr>
</tbody>
</table>
curative treatment. For both purposes, CCM is more sensitive than IENFD and presumably QST, particularly in diabetic polyneuropathy and sarcoidosis. For other neuropathies, the current data are too limited to allow a valid conclusion. Furthermore, CCM does not differentiate which fiber system is affected. Given the lack of valid normative data, including older healthy persons and patients with other disorders that may affect corneal sensitivity, the validity of CCM changes cannot yet be estimated.

**Quantitative Sensory Testing**

Quantitative sensory testing (QST) is a noninvasive psychophysical tool to assess human responses to painless or painful stimuli. In contrast to CCM and IENFD, QST results reflect the functionality of, and not structural changes in, the nervous system [7,32,33].

**Methods**

Over the last few decades, QST has been developed to complement the clinical neurological examination by more precise assessment of somatosensory aberrations in an area previously determined by a bedside sensory examination [7].

Generally, stimuli of increasing or decreasing intensity are applied, and the patient reports by pressing a button or by verbal statement when a sensation firstly appears or disappears. A threshold is defined as a mean from a series of trials during the continuous stimulus application (method of limits), or alternatively by a series of predefined stimuli (method of levels), which is independent of the reaction time, but takes longer compared to the method of limits [64]. For all sensory qualities, negative (hypoesthesia, hypoalgesia) and positive (alldynia, hyperalgesia, hyperesthesia) phenomena can be assessed. The different QST parameters of the German Research Network for Neuropathic Pain (DFNS) are listed in Table I, and detailed information about their assessment has been recently published [32,33]. This QST protocol includes different detection and pain thresholds as well as stimulus-response curves. There are 13 different tests; therefore, the examination of one of the most painful body areas as well as the contralateral or any other control area takes approximately 1 hour. Thermal and mechanical

<table>
<thead>
<tr>
<th>Type of Stimulus</th>
<th>DFNS Protocol</th>
<th>Testing Device</th>
<th>Axon Type</th>
<th>Central Pathway</th>
<th>Mechanism of Hyperalgesia/Alldynia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thermal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold</td>
<td>CDT</td>
<td>Computer-controlled thermoster</td>
<td>Aδ</td>
<td>Spinothalamic</td>
<td>–</td>
</tr>
<tr>
<td>Warmth</td>
<td>WDT</td>
<td>Aδ, C</td>
<td>C</td>
<td>Spinothalamic</td>
<td>–</td>
</tr>
<tr>
<td>Cold pain</td>
<td>CPT</td>
<td>Aδ, C</td>
<td>C</td>
<td>Spinothalamic</td>
<td>Central and peripheral sensitization, reduced inhibition</td>
</tr>
<tr>
<td>Heat pain</td>
<td>HPT</td>
<td>Aδ, C</td>
<td>Aδ</td>
<td>Spinothalamic</td>
<td>Peripheral sensitization</td>
</tr>
<tr>
<td><strong>Mechanical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Static punctuate (blunt)</td>
<td>MDT</td>
<td>Calibrated von Frey filaments</td>
<td>Aδ</td>
<td>Lemniscal</td>
<td>–</td>
</tr>
<tr>
<td>Vibration</td>
<td>VDT</td>
<td>Graded tuning fork, vibrometer</td>
<td>Aδ</td>
<td>Lemniscal</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cutaneous punctuate (sharp)</td>
<td>MPT, MPS</td>
<td>Calibrated sharp metal probes (pinpricks)</td>
<td>Aδ, C</td>
<td>Spinothalamic</td>
<td>Central sensitization</td>
</tr>
<tr>
<td>Blunt pressure</td>
<td>PPT</td>
<td>Pressure algometer</td>
<td>Aδ, C</td>
<td>Spinothalamic</td>
<td>Unknown</td>
</tr>
<tr>
<td>Dynamic mechanical</td>
<td>DMA</td>
<td>Brush, cotton wool, Q-Tip</td>
<td>Aδ, C</td>
<td>Lemniscal</td>
<td>Central sensitization</td>
</tr>
<tr>
<td>Wind-up</td>
<td>WUR</td>
<td>Calibrated sharp metal probes (pinpricks)</td>
<td>Aδ, C</td>
<td>Spinothalamic</td>
<td>Central sensitization, reduced inhibition</td>
</tr>
</tbody>
</table>

*Abbreviations:* CDT, cold detection threshold; CPT, cold pain threshold; DFNS, German Research Network for Neuropathic Pain; DMA, dynamic mechanical allodynia; HPT, heat pain threshold; MDT, mechanical detection threshold; MPS, mechanical pain sensitivity; MPT, mechanical pain threshold; PPT, pressure pain threshold; VDT, vibration detection threshold; WDT, warm detection threshold; WUR, wind-up ratio.
stimuli of different qualities and intensities are applied in the testing area in descending or ascending order of magnitude [7,33,40,50]. Thus, both small- and large-fiber function as well as the function of the corresponding central pathways are assessed (Table I). Unfortunately, there is only one parameter (pressure pain threshold [PPT]), which gives information about the deeper tissue (muscle and fascia). PPT has been shown to be decreased predominantly in complex regional pain syndrome (CRPS), but also after peripheral nerve injury or in polyneuropathy [24,39,53]. Use of a different device (Somedic) than in the one used according to the DFNS protocol (Wagner) permits the assessment of static joint-associated hyperalgesia in the hands or feet, which was shown to be more specific for the diagnosis of CRPS [40].

In general, for the assessment of pain sensitivity a stimulus-response curve is obtained by the patient’s response to mechanical (for example pinprick stimuli) or thermal stimuli of different intensity below and above the individual threshold in a balanced order [33,50]. This paradigm is especially valuable for assessment of positive signs like allodynia and hyperalgesia. The DFNS approach implies a subthreshold assessment of painful stimuli in contrast to the application of suprathreshold application, for example of tonic stimuli. The perceptual correlate of dorsal horn neuron wind-up [38,46] is assessed using the temporal summation of pain stimuli, known as the wind-up ratio (WUR), which is a quotient of the pain intensity after the stimulus series and the single stimulus. However, neither WUR nor any of the pain thresholds can replace the assessment of the function of the descendent noxious inhibitory system using conditioned pain modulation or other models [48].

Although there are some correlations between certain QST parameters, the analysis of the total sensory profile is helpful for the better understanding of possible mechanisms (see Fig. 3). For example, abnormal cold detection threshold (CDT) may represent Aδ-fiber impairment, if additionally paradoxical heat sensation (PHS) occurs. This phenomenon is presumably generated by impaired central inhibition, either by Aδ-cold-fiber loss or by effects on inhibitory thalamic centers [20,27]. The co-occurrence of abnormal CDT and PHS thus underlines the plausibility of a QST result in a single case. However, remarkably, the occurrence of PHS in combination with normal thermal thresholds can be
interacted as a sign of central compensation of the peripheral loss, as reported in children with diabetes mellitus without clinical symptoms of polyneuropathy [13].

Validation
The protocol established by the DFNS has been validated [21,50] and is now used widely in German and European consortia (Europain, Neuropain). Normative data are the first prerequisite, not only for the hitherto controversially discussed use of QST (and other tools) in the diagnostics of a single case [7,28], but also if QST results are to be used to compare data from different patients, obtained by different investigators and also across laboratories.

Therefore, the first (partly completed) task of the DFNS/Europain group was to build a normative reference database for QST data, allowing the use of correction factors for gender; different age groups, including children; and different body areas, including the face, upper and lower limbs, and trunk [12,37,39,45,50].

Today the DFNS protocol allows an intrasubject side-to-side comparison, which is an important way to increase the sensitivity of QST to detect moderate loss of function in a single patient, for example in unilateral diseases such as peripheral nerve injury and in cases when the QST parameter values in the affected area and the corresponding unaffected control area are both within the normal range [24,39,50]. This database, along with PC-based software such as Equista, is also used to transform the raw data and to correct for gender, age, and body area. In order to present the whole somatosensory profile in a comparable dimension, Z-transformation is done (except for PHS and DMA, which are generally not present in the group of healthy subjects), adjusting the data for age, gender, and test site:

\[ Z\text{-value} = \frac{\text{value patient} - \text{mean reference data}}{\text{standard deviation reference data}} \]

A Z-score of “0” represents a value corresponding to the mean of the healthy control subjects. A Z-score of 0 ± 1.96 represents the range including 95% of the healthy control subject data. A Z-value above “0” indicates a gain of function, i.e., the patient is more sensitive to the tested stimuli compared with controls (hyperesthesia, hyperalgesia), while Z-scores below “0” indicate a loss of function, referring to a lower sensitivity of the patient (hypoesthesia, hypoalgesia) (Fig. 3).

Last but not least, for valid and clinically useful data allowing any comparison over time or a different examiner, the QST has to be performed in a standardized manner, using standardized instructions and stimuli applied in a standardized way by trained investigators. Therefore, the DFNS and the Europain group have initiated a certification process for QST laboratories for verification of standardized instructions, adequate equipment, and training procedures and the accuracy of interpretation of results using the reference database [7,23].

Limitations
Like all psychophysical methods, QST requires the active participation of the patient, which should be considered during evaluation [7,28,64] (Table II). However, other accepted investigations also demand the patient’s cooperation and are susceptible to the patient’s effort. Appropriate precise data analysis, including specific algorithms, should prove the consistency of the QST results. For example, feigning normal subjects showed a larger variance of the thermal thresholds compared to truthful normal and neuropathic subjects [68].

QST is not validated for discrimination between abnormalities due to neuropathy or other non-neuropathic origin, as distinct loss of IENFD can concur with normal detection thresholds. However, the sensitivity can be increased by interpretation of the whole sensory profile. In contrast, pain-induced functional hypoesthesia in non-neuropathic pain syndromes usually falls within the normal range of healthy subjects and can be detected only by group comparison [22,28,51,67].

Some limitations depend on the protocol used. Using the DFNS protocol, the upper limit for stimulus intensity to avoid skin damage does not allow assessment of thermal hypoalgesia as a sensory minus sign, except for cases with an abnormal side-to-side difference compared to the unaffected contralateral area, because the 95% confidence intervals of healthy subjects for cold and heat pain threshold come close to the cutoff values of 0° and 50°C. Other limitations are listed in Table II.

Clinical Use
QST can be used to detect sensory impairment, such as in cases of suspected small-fiber neuropathy (SFN). A pure small-fiber neuropathy is characterized by isolated impairment of the thinly myelinated Aδ fibers and unmyelinated C fibers and is typically accompanied by dyesthesias or paresthesias and normal results in the
nerve conduction studies [35]. This subtype of peripheral neuropathy can also be an early sign of neuropathy of other origin [35] and can be identified by abnormally increased thermal detection thresholds in QST with a sensitivity of 36–85% in comparison to the invasive assessment by skin biopsy [42].

A comprehensive analysis of a cohort of over 1200 patients with different neuropathic pain syndromes including polyneuropathy, peripheral nerve lesions, postherpetic neuralgia, trigeminal neuralgia, central pain, and CRPS revealed remarkable differences in sensory profiles within the same kind of neuropathic pain or disease entity with a combination of sensory loss and gain for the different modalities. To better characterize the abnormalities in the somatosensory function, a classification based on the sensory findings was proposed (LoGa) [39]. It differentiates between normal sensory profiles (L0G0) on the one hand and sensory profiles characterized by hypoesthesia to thermal stimuli (L1), mechanical stimuli (L2), or both (L3), with or without concomitant hyperalgesia to thermal stimuli (G1), mechanical stimuli (G2), or both (G3). For example a mixed loss without any hyperalgesia was the most frequent combination of sensory signs in polyneuropathy and was present in 24% of patients (Fig. 4).

Another example of the superior diagnostic value of QST (especially regarding thermal testing) for the detection of SFN compared to nerve conduction studies is diabetic polyneuropathy, both in clinically asymptomatic children with diabetes mellitus type I and in adult patients with diabetes mellitus type 2 [13,31,63]. This is of special importance because impairment of small-fiber but not large-fiber function is correlated to pain intensity, although it may occur in pain-free patients as well [31,41,49]. Other neuropathic syndromes for which QST may give additional useful information are peripheral nerve injury, CRPS (including differentiating between types I and II) [24,40], Fabry disease [36], spinal cord lesion [66], fibromyalgia [62], and painful paresthesias of unknown origin for non-invasive screening for SFN [51] and many others (reviewed in [7,28,32]), although the assessment of IENFD and CCM seems to be more sensitive.

Further indications for QST might be treatment monitoring for topical applied drugs such as lidocaine [34] or capsaicin (unpublished data), because both drugs lead to a partial block of Aδ and C fibers, and if they do not, the application was not adequate.

Sensory profiling by QST can probably improve outcomes in clinical studies if patients’ clinical phenotypes are carefully characterized, and profiles of

<table>
<thead>
<tr>
<th>Limitations</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychophysical approach; requires the active participation of the patient (risk of false-positive findings)</td>
<td>Special relevance in cases of limited verbal communication, impaired cognition, severe psychiatric diseases (e.g., schizophrenia or dissociative disorder), decreased awareness due to tiredness or medication (e.g., benzodiazepines), suspected malingering</td>
</tr>
<tr>
<td>Testing can only be done in a single body area and a single time</td>
<td>Choose the testing area after clinical examination; if needed, test in several areas</td>
</tr>
<tr>
<td>Limited sensitivity to detection loss of function in neuropathy (risk of false-negative findings)</td>
<td>For example, in children with diabetes [13] or individuals with enhanced limb cortical representation; sensitivity can improved by bilateral assessment in systemic neuropathy (side-to-side comparison), and by using the face or upper limb as a control area</td>
</tr>
<tr>
<td>Only assessment of the whole pathway is possible</td>
<td>No differentiation between peripheral or central origin of loss or hyperalgesia (assess, for example, the face as a control area in case of polyneuropathy and suspected lateral radiculopathy)</td>
</tr>
<tr>
<td>Cut-off values are required to avoid skin damage</td>
<td>In some case, minus signs cannot be assessed because the 95% confidence intervals of healthy subjects come close to the cut-off values</td>
</tr>
<tr>
<td>Time-consuming (about 1 hour for 2 areas)</td>
<td>Possible concentration problems or tiredness</td>
</tr>
<tr>
<td>Contralateral area is inappropriate as a control</td>
<td>In patients with systemic or suspected bilateral neurological disorder, choose the face or hand as a control to enhance sensitivity and specificity</td>
</tr>
</tbody>
</table>
Conclusions

In contrast to IENFD in skin biopsy and CCM, QST is a psychophysical assessment of the response of a patient or healthy subject to painless or painful stimuli, which means it describes the function of different nerves and corresponding pathways and not the structural changes. QST is the only method to differentiate between different types of nerve dysfunction (affecting C, Aδ, or Aβ fibers or mixed fibers) and is also the only one to assess a gain of sensory function (e.g., hyperalgesia, allodynia). For individual diagnostics, QST should not be used as a stand-alone method, but in case of (suspected) polyneuropathy, thermal QST threshold testing using a validated protocol is regarded as complementary in the detection of small-fiber involvement and should be included in the diagnostic algorithms of these patients, especially in research studies. Using side-to-side comparison is the first choice to detect unilateral local neuropathy.

References


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