Prior Authorization Review Panel  
MCO Policy Submission

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Type of Submission – Check all that apply:

- [ ] New Policy
- [x] Revised Policy*
- [ ] Annual Review – No Revisions
- [ ] Statewide PDL

*All revisions to the policy must be highlighted using track changes throughout the document. Please provide any clarifying information for the policy below:

CPB 0774 Nerve Fiber Density Measurement

This CPB has been revised to state that measurement of nerve fiber density is considered experimental and investigational for diagnosis of endometriosis.

Update History since the last PARP Submission:

03/27/2019-This CPB has been updated with additional coding.

Name of Authorized Individual (Please type or print): Dr. Bernard Lewin, M.D.

Signature of Authorized Individual: [Signature]

Revised July 22, 2019
Nerve Fiber Density Measurement

Policy

*Please see amendment for Pennsylvania Medicaid at the end of this CPB.*

Aetna considers measurement of intra-epidermal nerve fiber density (IENFD) by skin biopsy medically necessary for the diagnosis of small-fiber neuropathy when all of the following criteria are met:

- Individual presents with painful sensory neuropathy; and
- There is no history of a disorder known to predispose to painful neuropathy (e.g., diabetic neuropathy, toxic neuropathy, HIV neuropathy, celiac neuropathy, inherited neuropathy); and
- Physical examination shows no evidence of findings consistent with large-fiber neuropathy, such as reduced or absent muscle-stretch reflexes or reduced proprioception and vibration sensation; and
- Needle electromyography (EMG) and nerve conduction velocity studies are normal and show no evidence of large-fiber neuropathy.

Aetna considers measurement of IENFD experimental and investigational for monitoring disease progression or response to treatment, or for the following indications and all other indications because its effectiveness for these indications has not been established (not an all-inclusive list):

- As a marker of pre-clinical asymptomatic small-fiber sensory neuropathy in hypothyroid persons

www.aetna.com/cpb/medical/data/700_799/0774.html
Diagnosis of endometriosis
Evaluation of hereditary transthyretin (TTR) amyloidosis and iatrogenic TTR amyloidosis
Evaluation of individuals with Ehlers-Danlos syndromes
Evaluation of individuals with Fabry disease
Evaluation of individuals with fibromyalgia
Evaluation of individuals with postural tachycardia syndrome
Evaluation of individuals with REM sleep behavior disorder

Aetna considers measurement of sweat gland nerve fiber density for the diagnosis of complex regional pain syndrome, small-fiber neuropathy and other indications experimental and investigational because its effectiveness has not been established.

See also CPB 0485 - Autonomic Testing / Sudomotor Tests (../400_499/0485.html).

Background
Intraepidermal nerve fiber density (IENF) testing identifies the density (number) of small nerve fibers from skin biopsy specimens for the diagnosis of small fiber neuropathy (SFN). IENF is often referred to as a skin biopsy test.

Small fiber neuropathy is a disease characterized by diminished nerve fiber density in the epidermis (outer layer) of the skin, resulting in painful symptoms, usually in the extremities, that may rarely become disabling. It may occur either independently or as the result of another disease, such as diabetes or alcohol abuse. Large nerve fiber neuropathy is nerve damage affecting the large nerve fibers. Symptoms include weakness, numbness, tingling or loss of balance.

IENF testing is the examination of a thin skin specimen which is obtained by a punch biopsy. The specimen is stained and prepared for examination by a pathologist, who evaluates the number and structural integrity of the small fibers. SFN is identified by a reduction of the intraepidermal nerve fiber density or structural abnormalities. The procedure is simple to perform, takes no more than five to ten minutes and causes little discomfort.

This test offers some unique advantages over nerve conduction velocity (NCV) tests and nerve biopsy. Unlike NCV, the IENF test can reveal damage present in smaller nerve fibers.
Small-fiber neuropathy (SFN), also known as small-fiber sensory/peripheral neuropathy, is a peripheral nerve disease that selectively afflicts small diameter myelinated and non-myelinated nerve fibers. It most commonly occurs in middle-aged and older people, and is characterized by painful burning feet with reduced pain and temperature perception, and in some cases autonomic dysfunction. Although SFN can be caused by metabolic disorders (e.g., diabetes, metabolic syndrome), viruses and infectious diseases (e.g., human immunodeficiency virus, herpes zoster), genetic abnormalities (e.g., Fabry’s disease, hereditary sensory and autonomic neuropathies), drugs and toxins (e.g., metronidazole, alcohol, and arsenic), and autoimmune diseases (e.g., vasculitis, Sjögren’s syndrome), the cause often remains a mystery because standard electrophysiological tests for nerve injury do not detect small-fiber function. Despite the magnitude of the symptoms, there are few objective methods to identify and quantify these neuropathies.

Diagnosis of SFN is made on the basis of clinical features, normal nerve conduction velocity studies (NCVS) and abnormal specialized tests of small nerve fibers, which include measurement of intra-epidermal nerve fiber density (IENFD) and quantitative sudomotor axon reflex for autonomic fibers. Unless an underlying disease is identified, treatment is usually symptomatic and directed towards alleviation of neuropathic pain (Hoitsma et al, 2004; Fink and Oaklander, 2006).

Measurement of IENFD is an objective diagnostic test of SFN. For a diagnostic test to be clinically useful, it should correspond well with clinically meaningful physical findings. Walk and co-workers (2007) performed a retrospective analysis of the concordance between foot IENFD and clinical findings in all patients seen at their institution with possible idiopathic SFN who underwent skin biopsy for IENFD determination. They found a high concordance between reduced foot IENFD and loss of pinprick sensitivity in this patient population. These findings indicated that IENFD determination is a clinically relevant objective test in patients undergoing evaluation for possible SFN.

Darby et al (2007) assessed the loss of autonomic nerve fibers in patients with clinical pure sensory SFN. These investigators performed skin punch biopsies in age-matched (n = 17) and sex-matched (n = 15) controls. Biopsies were taken 10 cm above the lateral malleolus, and thin sections were stained with hematoxylin and eosin and the panaxonal marker protein-gene-product (PGP) 9.5. Positively stained fibers, represented as dots, innervating the erector pili muscles, arterioles, and sweat glands (SG) were counted. The ratios between the number of nerve fibers and nuclei of each structure were calculated. The autonomic innervation was significantly reduced in the patients' group compared with controls in all the examined autonomic-innervated structures: SG (0.27 +/- 0.15 versus 0.66 +/- 0.37, p = 0.001), arterioles (0.38 +/- 0.32 versus 0.86 +/- 0.45, p = 0.002), and the erector pili muscle (0.58 +/- 0.27 versus
1.23 +/- 0.87, p = 0.036). These findings suggested that autonomic involvement occurs in patients with sensory SFN and that punch skin biopsy using thin sections is a simple and convenient method to detect these dermal autonomic small-fiber abnormalities.

Quattrini and colleagues (2007) quantified small nerve fiber pathological changes by means of IENFD measurement and corneal confocal microscopy (CCM) in patients with diabetic neuropathy (DN). A total of 54 subjects stratified for neuropathy, using neurological evaluation, neurophysiology, and quantitative sensory testing (QST), and 15 control subjects were studied. They underwent a punch skin biopsy to measure IENFD and CCM to quantify corneal nerve fibers. Intra-epidermal nerve fiber density, branch density, and branch length showed a progressive reduction with increasing severity of neuropathy, which was significant in patients with mild, moderate, and severe neuropathy. Corneal confocal microscopy also showed a progressive reduction in corneal nerve fiber density (CNFD) and branch density, but the latter was significantly reduced even in diabetic patients without neuropathy. Both IENFD and CNFD correlated significantly with cold detection and heat as pain thresholds. Intra-epidermal and corneal nerve fiber lengths were reduced in patients with painful DN compared with their painless counterparts. Both IENFD and CCM assessment accurately quantify small nerve fiber damage in diabetic patients. However, CCM quantifies small fiber damage rapidly and non-invasively and detects earlier stages of nerve damage compared with IENF pathology. This may make it an ideal technique to accurately diagnose and assess progression of DN.

Umapathi and associates (2007) identified an early stage of DN by measuring injury to epidermal nerve fibers. These researchers compared IENFD at the ankle and thigh of 29 diabetic subjects who had no clinical or electrophysiological evidence of SFN or large-fiber neuropathy to that of 84 healthy controls. The mean ankle IENFD of diabetic subjects was 9.1 +/- 5.0 mm and that of controls, 13.0 +/- 4.8 mm (p < 0.001). The thigh IENFD did not differ significantly. The IENFD ratio (thigh IENFD divided by ankle IENFD) was 2.39 +/- 1.30 in diabetic subjects and 1.77 +/- 0.58 in controls (p < 0.001), indicating a length-dependent reduction of IENFD in diabetics. Ankle IENFD remained significantly lower and the IENFD ratio higher in diabetic subjects after adjusting for age. Two subjects had parasympathetic dysfunction, 2 had retinopathy, and 2 early nephropathy. Age, height, weight, duration of diabetes, and average HbA1c did not influence IENFD among diabetic subjects. These researchers used receiver operating characteristic (ROC) curves to describe and compare the utility of various threshold values of ankle IENFD and IENFD ratio for the diagnosis of early DN. The sensitivity and specificity of diagnosing DN using ankle IENFD of less than 10 mm were 72.4 % and 76.2 %, respectively. Thus, asymptomatic diabetics have a measurable, length-dependent reduction of distal epidermal nerves. Analogous to microalbuminuria in DN, reliable identification and quantitation of nascent DN may have potential therapeutic implications.
In a prospective study, Vlckova-Moravcova et al (2008) quantified IENFD and sub-epidermal nerve plexus densities (SENPD) by immunostaining in skin punch biopsies from the distal calf in 99 patients with clinical symptoms of painful sensory neuropathy and from 37 age-matched healthy volunteers. The clinical diagnosis was based on history and abnormal thermal thresholds on QST. In patients with neuropathy, IENFD and SENPD were reduced to about 50 % of controls. Elevated warm detection thresholds on QST correlated with IENFD but not with SENPD. Using ROC curve analysis of IENFD values, the diagnostic sensitivity for detecting neuropathy was 0.80 and the specificity 0.82. For SENPD, sensitivity was 0.81 and specificity 0.88. With ROC analysis of both IENFD and SENPD together, the diagnostic sensitivity was further improved to 0.92. The combined examination of IENFD and SENPD is a highly sensitive and specific diagnostic tool in patients suspected to suffer from painful sensory neuropathies but with normal values on clinical neurophysiological studies.

Sommers (2008) stated that the sensitivity and specificity of skin biopsy in detecting SFN is supported by new data. Skin innervation is affected in neuropathies formerly considered as the large-fiber type, such as porphyria and chronic inflammatory demyelinating neuropathy. New methods have been devised to complement histological evaluation of skin innervation by in-vivo microscopy and by neurophysiological assessment of small nerve fibers. Skin biopsies have been used to learn more about the pathophysiology of neuropathies, such as the discovery of reduced vascular endothelial growth factor expression in DN and the increase in cytokine expression in some painful SFN. Quantification of skin innervation has been used as a measure for treatment success in experimental studies and is presently used for follow-up in clinical trials. Skin biopsy in the diagnosis of neuropathy is moving from a method giving descriptive results to a tool that may be helpful in etiological diagnostics, as a follow-up in clinical trials, and in pathophysiological research.

Devigili et al (2008) stated that SFN is frequently encountered in clinical practice either as prevalent manifestation of more diffuse neuropathy or distinct nosologic entity. Due to their physiological characteristics, small nerve fibers can not be investigated by routine electrophysiological tests, making the diagnosis particularly difficult. Quantitative sensory testing to evaluate the psychophysical thresholds for cold and warm sensations and skin biopsy with quantification of somatic IENF have been used to ascertain the damage to small nerve fibers. These investigators screened 486 patients referred to their institutions and collected 124 patients with sensory neuropathy. Among them, they identified 67 patients with pure SFN using a new diagnostic "gold standard", based on the presence of at least two abnormal results at clinical, QST and skin biopsy examination. The diagnosis of SFN was achieved by abnormal clinical and skin biopsy findings in 43.3 % of patients, abnormal skin biopsy and QST findings in 37.3 % of patients, abnormal clinical and QST findings in 11.9 % of patients, whereas 7.5 % patients had abnormal results at all the examinations. Skin biopsy showed a diagnostic efficiency of 88.4 %,
clinical examination of 54.6 % and QST of 46.9 %. Receiver operating characteristic curve analysis confirmed the significantly higher performance of skin biopsy comparing with QST. However, these researchers found a significant inverse correlation between IENFD and both cold and warm thresholds at the leg. Clinical examination revealed pinprick and thermal hypoesthesia in about 50 % patients, and signs of peripheral vascular autonomic dysfunction in about 70 % of patients. Spontaneous pain dominated the clinical picture in most SFN patients. Neuropathic pain intensity was more severe in patients with SFN than in patients with large or mixed fiber neuropathy, but there was no significant correlation with IENFD. The etiology of SFN was initially unknown in 41.8 % of patients and at 2-year follow-up a potential cause could be determined in 25 % of them. Over the same period, 13 % of SFN patients showed the involvement of large nerve fibers, whereas in 45.6 % of them the clinical picture did not change. Spontaneous remission of neuropathic pain occurred in 10.9 % of SFN patients, while it worsened in 30.4 % of them.

Laaksonen et al (2008) examined the neurological and neurophysiological findings and neurological symptoms in 12 women with Fabry disease and studied the relationship between the subjective symptoms and the findings on the various tests -- neurography, vibratory and thermal QST, skin biopsy for measuring IENFD, heart rate variability and sympathetic skin response (SSR) tests for detecting autonomic dysfunction, pain-, depression- and somatic symptom-questionnaires and clinical examination. Only 2 women had no persistent symptoms or signs of polyneuropathy, 10 had symptoms of SFN. Neurological examination was normal in most patients; 5 patients had decreased IENFD or thermal hypoesthesia in QST. In QST, A-delta-fiber function for innocuous cold was more often impaired than C-fiber function.

Conventional NCVS were mostly normal. Carpal tunnel syndrome (CTS) incidence was increased, 25 % had symptomatic CTS. The authors concluded that heterozygous women carrying the gene for Fabry disease have symptoms and findings of small-fiber polyneuropathy more often than has previously been considered. The prevalence of CTS is also increased. While the clinical diagnosis of SFN is difficult, the diagnostic yield can be increased using a combination of thermal QST and IENFD measurements. The American Academy of Neurology's assessment on QST (Shy et al, 2003) stated that abnormalities on QST must be interpreted in the context of a thorough neurological examination and other appropriate testing, such as electromyography, nerve biopsy, skin biopsy, or appropriate imaging studies.

Teoh and associates (2008) compared simple tests of small nerve fiber function with IENFD in the evaluation of SFN. Patients with idiopathic SFN of the hands were prospectively studied. Evaluation involved clinical examination, NCVS, SSR and skin wrinkling stimulated by water and EMLA (eutectic mixture of local anaesthetics). Of 21 patients, 16 (76 %) had low IENFD, 15 (71 %) impaired water-induced wrinkling, 14 (67 %) impaired EMLA-induced wrinkling, and 9 (43 %)
abnormal SSR. The authors concluded that stimulated skin wrinkling was nearly as sensitive as IENFD in diagnosing SFN, whereas SSR was of less use. Stimulated skin wrinkling is a useful supportive test when IENFD or other tests of small nerve fiber function are not available.

Scherens and colleagues (2009) noted that dysesthesias of the lower limbs are a common complaint of patients and may be indicative of peripheral neuropathy. These investigators examined the prevalence and type of neuropathy in patients presenting with this complaint and compared the diagnostic performance of different diagnostic modalities. A total of 42 patients were recruited prospectively and underwent a clinical examination, NCVS, QST, and skin biopsy at the dorsum of the foot. All patients had a correlate for their dysesthesias in at least one diagnostic modality. Most patients (over 90 %) had signs of small fiber loss or dysfunction. In approximately 50 % of all patients large fibers were also affected. Nerve conduction velocity studies were abnormal in 23/42 patients (54.8 %). Cold or warm detection thresholds in QST were abnormal in 15/42 (35.7 %) patients. Decreased IENFD was found in 37 patients (88.1 %), including some patients with normal QST findings. Nearly all patients with pathological QST had a reduced IENFD, indicating a high positive predictive value (93 %) of QST in screening for reduced IENFD as correlate for neuropathy. Thus, in all patients with lower limb dysesthesias of unknown origin, the non-invasive methods of NCVS and QST should be used and potentially complemented by skin biopsy.

Loseth and associates (2008) examined if neuropathy in diabetic patients with normal NCVS could be detected by measurements of thermal thresholds and quantification of IENFD, and assessed differences in parameters between patients with and without neuropathic symptoms. A total of 22 patients with and 37 patients without sensory symptoms suggesting distal neuropathy were included. Measurements of warm and cold perception thresholds and skin biopsy for quantification of IENFD were performed distally on the leg. Reference data were used to normalize test results for age and height or gender of individual patients by calculating the Z-scores. Intra-epidermal nerve fiber density was significantly reduced in both symptomatic and asymptomatic patients compared to controls (p < 0.001), and in patients with symptoms compared to those without (p = 0.01). Thermal thresholds were significantly elevated (more abnormal) in patients with symptoms compared to controls (p < 0.01), but only for cold perception threshold (CPT) (p < 0.001) in the asymptomatic group. When comparing symptomatic and asymptomatic patients, there was no statistically significant difference in thermal thresholds. Depletion of IENFs in skin biopsy was the most frequent abnormal finding in the subgroup of patients with neuropathic symptoms (36 %) followed by abnormal CPT (27 %). The authors concluded that patients with diabetes and normal NCVS had significantly lower IENFD and higher CPT than controls, whether they had symptoms of polyneuropathy or not. In patients with neuropathic symptoms, abnormal IENFD predominated and thus, seemed to be the most sensitive tool of detecting small diameter nerve fiber involvement.
Gorson et al (2008) described a syndrome of generalized small fiber gangionopathy (SFG) with early involvement of the face, trunk or proximal limbs. The investigators conducted a retrospective case review including skin biopsies from four neuromuscular centers. Patients with preexisting diseases associated with ganglionopathies were excluded. The investigators studied 12 men and 11 women, with an average age of 50 years. Neuropathic pain developed over days in eight and over months in the other patients. The face (n = 12), scalp (n = 10), tongue (n = 6), trunk (n = 15) and acral extremities (n = 21) were involved. Symptoms began in the hands or face before the legs in 10. The pain was characterized as burning (n = 22), prickling (n = 13), shooting (n = 13) or allodynic (n = 11). There was loss of pinprick sensation in affected regions in 19, with minimal or no loss of large fiber sensibility. Laboratory findings included abnormal glucose metabolism in 6 patients, Sjogren syndrome in 3 and monoclonal gammopathy, sprue and hepatitis C infection in 1 each, with the remainder idiopathic. Sensory nerve action potentials were normal in 12 and were reduced in the hands but normal in the legs in 6. Skin biopsy in 14 of 17 showed reduced nerve fiber density in the thigh equal to or more prominent than in the calf; 2 of 7 patients improved with immune therapies, 13 symptomatically with analgesic medications and the remainder had little improvement; 10 considered the pain disabling at the last follow-up (mean of 2 years).

Gemignani et al (2010) reported the features of non-length dependent SFN and compared them to those with distal length-dependent SFN. In a series of 224 consecutive neuropathy patients, the investigators evaluated 44 patients with SFN diagnosed in the presence of both symptoms and signs; 11 were classified as non-length dependent SFN. Disease associations were Sjogren's syndrome (2 patients), impaired glucose tolerance, rheumatoid arthritis, hepatitis C virus, Crohn's disease, and idiopathic (5 patients). In the 33 patients with distal SFN, the age of onset was significantly older and more had impaired glucose metabolism (16/33). In both groups, pain was mainly characterized as burning, but patients with non-length dependent SFN more often reported an "itchy" quality and allodynia to light touch.

Khan and Zhou (2012) sought to characterize non-length-dependent small-fiber sensory neuropathy (NLD-SFSN), noting that it is not as well characterized as length-dependent small-fiber sensory neuropathy (LD-SFSN). The investigators compared 63 patients with NLD-SFSN with 175 patients with LD-SFSN for their demographics and disease associations. The investigators found that age was younger in those with NLD-SFSN (45.5 ± 13.1 years) than in those with LD-SFSN (55.1 ± 11.4 years, p < 0.001). Forty-six of 63 (73.0 %) patients were women in the NLD-SFSN group, whereas 84 of 175 (48.0 %) were women in the LD-SFSN group (p < 0.001). Disease associations were identified in 26 of 63 (41.3 %) patients with NLD-SFSN, including diabetes or prediabetes in 10 (15.9 %), connective tissue diseases in 6 (9.5 %), thyroid dysfunction in 4 (6.3 %), sarcoidosis in 3 (4.8 %), vitamin B(12) deficiency in 2 (3.2 %),
and paraproteinemia in 1 (1.6 %). The investigators found that immune-mediated conditions were present in 9 of 63 (14.3 %) patients with NLD-SFSN and 6 of 175 (3.4 %) patients with LD-SFSN (p = 0.012).

Nerve fiber density measurement has been used as a research endpoint in clinical studies. Jacobs and Cheng (2011) assessed the efficacy of an oral combination of L-methylfolate, methylcobalamin, and pyridoxal 5'-phosphate for improving ENFD in the lower extremity of patients with diabetic peripheral neuropathy (DPN). Eleven consecutive patients with type 2 diabetes with symptomatic DPN were assessed for ENFD at the calf by means of skin punch biopsy and then placed on twice daily oral combination L-methylfolate, methylcobalamin, and pyridoxal 5'-phosphate. After approximately 6 months of treatment, patients underwent follow-up biopsy. At the end of their treatment, 73 % of patients showed an increase in calf ENFD, and 82 % of patients experienced both reduced frequency and intensity of paresthesias and/or dysesthesias.

The European Federation of Neurological Societies' guidelines on the use of skin biopsy in the diagnosis of peripheral neuropathy (Lauria et al, 2005) noted that for diagnostic purposes in peripheral neuropathies, a 3-mm punch skin biopsy at the distal leg and quantifying the linear density of IENF in at least three 50-micrometer thick sections per biopsy, fixed in 2 % periodate-lysine-paraformaldehyde or Zamboni's solution, by bright-field immunohistochemistry or immunofluorescence with anti-PGP 9.5 antibodies is recommended (level A recommendation). Quantification of IENFD closely correlated with warm and heat-pain threshold, and appeared more sensitive than sensory NCVS and sural nerve biopsy in diagnosing sensory SFN. Diagnostic efficiency and predictive values of this technique were very high (level A recommendation). Confocal microscopy may be particularly useful to investigate myelinated nerve fibers, dermal receptors and dermal annex innervation.

The Australia and New Zealand Horizon Scanning Network (Purin et al, 2007) assessment on skin biopsy diagnosis of peripheral neuropathy noted that "[a]lthough the evidence for the use of skin biopsy to diagnose SFN was mainly from small scale studies, the technique appears to perform well".

The American Academy of Neurology practice parameter Evaluation of Distal Symmetric Polyneuropathy (England et al, 2009) recommends that autonomic testing should be considered in the evaluation of patients with polyneuropathy to document autonomic nervous system dysfunction. Such testing should be considered especially for the evaluation of suspected autonomic neuropathy and distal small fiber sensory polyneuropathy. In addition, it states that
for symptomatic patients with suspected polyneuropathy, skin biopsy is a validated technique for determining IENFD and may be considered for the diagnosis of distal symmetric polyneuropathy, especially small fiber sensory polyneuropathy.

Torvin Moller and associates (2009) stated that Fabry disease is an X-linked inherited lysosomal disorder with dysfunction of the lysosomal enzyme alpha-galactosidase A causing accumulation of glycolipids in multiple organs including the nervous system. Pain and somatosensory disturbances are prominent manifestations of this disease. Until recently, disease manifestations in female carriers of Fabry disease have been questioned. To explore the frequency of symptoms and the functional and structural involvement of the nervous system in female patients, these investigators examined the presence of pain, manifestations of peripheral neuropathy and nerve fiber density in skin biopsies in 19 female patients with Fabry disease and 19 sex- and age-matched controls. Diaries, quantitative sensory testing, neurophysiologic tests and skin biopsies were performed. Daily pain was present in 63 % of patients, with a median VAS score of 4.0. Tactile detection threshold and pressure pain threshold were lower and cold detection thresholds increased in patients. Sensory nerve action potential amplitude and maximal sensory conduction velocity were not different, whereas there was a highly significant reduction in IENFD. There were no correlations between pain VAS score, quantitative sensory testing, and IENFD.

Nebuchennykh and co-workers (2010) examined involvement of large and small nerve fibers in patients with hypothyroidism and symptoms and signs of polyneuropathy. A total of 16 patients with established diagnosis of hypothyroidism were extracted from a patient population participating in a "polyneuropathy study". In addition, 7 patients with other additional potential causes of polyneuropathy than hypothyroidism were investigated. The patients underwent neurological examination, routine blood tests, nerve conduction studies (NCS), QST and skin biopsies with assessment of IENFD. A total of 63 % of the patients with "pure" hypothyroidism had abnormalities on NCS, 25 % had reduced IENFD and 31 % had abnormalities on QST. Four patients (25 %) met criteria for small fiber polyneuropathy, the other (75 %) were classified as having mixed fiber polyneuropathy. There were no differences in the amount of abnormalities on NCS, QST and skin biopsy between patients with hypothyroidism and those with hypothyroidism and other potential causes of polyneuropathy. The authors concluded that the majority of patients with hypothyroidism had involvement of both large and small nerve fibers. However, some patients had isolated small fiber polyneuropathy. Patients with "pure" hypothyroidism had essentially the same degree of peripheral nerve fiber involvement as those with other additional causes of polyneuropathy.
Magri and colleagues (2010) assessed by means of IENFD in 18 untreated patients with hypothyroidism, either overt (OH) or subclinical (SH), who did not complain of neurological symptoms; 15 healthy, age-matched, controls were also studied. A nerve conduction study was performed. Skin biopsy was performed using the skin of upper thigh and distal leg. Nerve fiber density was measured using an immunofluorescence technique. The density of innervation was calculated by counting only fibers crossing the basement membrane. Electroneurographic parameters were similar in patients and controls. When compared with healthy controls, patients with OH or SH showed a significantly lower IENFD. As assessed by the proximal/distal fiber density ratio, the hypothyroid neuropathy was length-dependent. When individually considered, an abnormally reduced IENFD was observed in 60% of patients with OH at the distal leg and in 20% at the proximal site. In patients with SH, an abnormal IENFD was found at the distal leg in 25% of cases and at the proximal thigh in 12.5% of cases. The authors concluded that the results of this study provided the first direct demonstration of reduced IENFD in patients with OH or SH. In all patients, the IENFD reduction was length-dependent. They stated that these findings suggested that a considerable number of untreated hypothyroid patients may have preclinical asymptomatic small-fiber sensory neuropathy. The findings of this small study needs to be validated by well-designed studies.

Sweat glands, innervated by the autonomic nerves, are involved with regulation of body temperature and hydration. Symptoms of autonomic neuropathy may entail abnormal sweating or temperature regulation, among others (e.g., gastroparesis, incomplete bladder emptying, irregular bowel movements, irregular heart rate, postural hypotension, sexual dysfunction, and urinary urgency). Both sweat gland nerve fiber density (SGNFD) and IENFD can be reduced in generalized SFN, but in some autonomic neuropathies (e.g., Ross syndrome), only the SGNFD is reduced (Sommer et al, 2002).

Hilz et al (2004) assessed cutaneous nerve fiber loss in conjunction with temperature and sweating dysfunction in familial dysautonomia (FD). In 10 FD patients, the investigators determined warm and cold thresholds at the calf and shoulder, and sweating in response to acetylcholine iontophoresis over the calf and forearm. Punch skin biopsies from calf and back were immunostained and imaged to assess nerve fiber density and neuropeptide content. Mean temperature thresholds and baseline sweat rate were elevated in the patients, while total sweat volume and response time did not differ from controls. The average density of epidermal nerve fibers was greatly diminished in the calf and back. There was also severe nerve loss within the subepidermal neural plexus (SNP) and deep dermis. The few sweat glands present within the biopsies had had reduced innervation density. Substance P immunoreactive (-ir) and calcitonin gene related peptide-ir (CGRP-ir) were virtually absent, but vasoactive intestinal peptide-ir (VIP-ir) nerves were present in the SNP. Empty Schwann cell sheaths were observed. Temperature perception was more impaired than sweating. Epidermal nerve fiber
density was found to be profoundly reduced in FD. Decreased SP and CGRP-ir nerves suggest that the FD gene mutation causes secondary neurotransmitter depletions. Empty Schwann cell sheaths and VIP-ir nerves suggest active denervation and regeneration.

Gibbons et al (2009) evaluated a novel method to quantify the density of nerve fibers innervating sweat glands in healthy control and diabetic subjects, and compared the results to an unbiased stereological technique, and identified the relationship to standardized physical examination and patient-reported symptom scores. A total of 30 diabetic and 64 healthy subjects had skin biopsies performed at the distal leg and distal and proximal thigh. Nerve fibers innervating sweat glands, stained with protein gene product 9.5, were imaged by light microscopy. Sweat gland nerve fiber density was quantified by manual morphometry. As a gold standard, 3 additional subjects had biopsies analyzed by confocal microscopy using unbiased stereological quantification. Severity of neuropathy was measured by standardized instruments including the Neuropathy Impairment Score in the Lower Limb (NIS-LL) while symptoms were measured by the Michigan Neuropathy Screening Instrument. Manual morphometry increased with unbiased stereology (r = 0.93, p < 0.01). Diabetic subjects had reduced SGNFD compared to controls at the distal leg (p < 0.001), distal thigh (p < 0.01), and proximal thigh (p < 0.05). The SGNFD at the distal leg of diabetic subjects decreased as the NIS-LL worsened (r = -0.89, p < 0.001) and was concordant with symptoms of reduced sweat production (p < 0.01). In summary, the authors described a novel method to quantify the density of nerve fibers innervating sweat glands. The technique differentiates groups of patients with mild diabetic neuropathy from healthy control subjects and correlates with both physical examination scores and symptoms relevant to sudomotor dysfunction. The validity of this novel technique needs to be confirmed by well-designed studies.

Gibbons et al (2010) evaluated 36 diabetic and 72 healthy control subjects who underwent detailed neurologic examinations and punch skin biopsies. Physical exam findings were quantified by neuropathy impairment score in the lower limb. Skin biopsies quantified IENFD and SGNFD by a manual, automated, and semiquantitative method. The automated and manual SGNFD correlated with the IENFD at the same site (r = 0.62, p < 0.05 automated method, r = 0.67, p < 0.05 manual method). As neuropathy worsened, the SGNFD at the distal leg declined (automated counting r = -0.81, p < 0.001; manual counting r = -0.88, p < 0.001). The semi-quantitative method displayed poor inter- and intra-reviewer reliability and correlated poorly with standard neuropathy evaluation scores.

Loiavenbruck et al (2010) suggested a concomitant loss of sweat gland volume and sweat gland nerve fiber length in neuropathy, with greater loss of sweat gland nerve fibers in anhidrotic skin, possibly exceeding collateral reinnervation. The investigators studied 10 neuropathy patients in whom anhidrosis was found by thermoregularory sweat testing (TST) and 10 matched controls.
Skin biopsies were taken from both anhidrotic and sweating skin and immunohistochemical staining was done for nerves and basement membrane. For each biopsy, total tissue volume, total SG volume, and total SGNF length were measured. Sweat gland nerve fiber length per biopsy volume, sweat gland (SG) volume per biopsy volume (SG%), and SGNF length per SG volume were calculated. Sweat gland nerve fiber length per biopsy volume was reduced in anhidrotic site biopsies of patients compared with controls; SG% was decreased and SGNF length per SG volume increased in patients compared with controls.

To evaluate the loss of autonomic nerve fibers in patients with clinical pure small-fiber sensory neuropathy, Dabby et al (2007) performed skin punch biopsies in 17 and 15 age- and sex-matched controls. Biopsies were taken 10 cm above the lateral malleolus, and 5-mum sections were stained with hematoxylin and eosin and the panaxonal marker protein gene product (PGP) 9.5. Positively stained fibers, represented as dots, innervating the erector pili muscles, arterioles, and SG were counted. The ratios between the number of nerve fibers and nuclei of each structure were calculated. The autonomic innervation was significantly reduced in the patients’ group compared with controls in all the examined autonomic-innervated structures: SG (0.27 +/- 0.15 versus 0.66 +/- 0.37, p = 0.001), arterioles (0.38 +/- 0.32 versus 0.86 +/- 0.45, p = 0.002), and the erector pili muscle (0.58 +/- 0.27 versus 1.23 +/- 0.87, p = 0.036).

Donadio and colleagues (2010) reported on the first direct analysis of skin sympathetic fibers including structure and function in pure autonomic failure (PAF) and multiple system atrophy (MSA) to ascertain different underlying autonomic lesion sites which may help differentiate between the 2 conditions. The authors studied eight patients with probable MSA (mean age of 60 ± 5 years) and 9 patients fulfilling diagnostic criteria for PAF (64 ± 8 years). They underwent head-up tilt test (HUTT), microneurographic search for muscle and skin sympathetic nerve activities from peroneal nerve and punch skin biopsies from finger, thigh and leg to evaluate cholinergic and adrenergic autonomic dermal annexes innervation graded by a semiquantitative score. MSA and PAF patients presented a comparable neurogenic orthostatic hypotension during HUTT and high failure rate of microneurographic trials to record sympathetic nerve activity, suggesting a similar extent of chronic dysautonomia. In contrast, they presented different skin autonomic innervation in the immunofluorescence analysis. MSA patients showed a generally preserved skin autonomic innervation with a significantly higher score than PAF patients showing a marked post-ganglionic sympathetic denervation. In MSA patients with a long disease duration, morphological abnormalities and/or a slightly decreased autonomic score could be found in the leg reflecting a mild postganglionic involvement.

Donadio et al (2012) took punch skin biopsies from the thigh and lower leg of 28 patients with various types of autonomic neuropathy for quantitative evaluation of skin autonomic innervation. Results were compared with scores obtained from 32 age-matched healthy controls and 25
patients with somatic neuropathy. The autonomic cut-off score was calculated using the receiver operating characteristic curve analysis. Skin biopsy disclosed a significant autonomic innervation decrease in autonomic neuropathy patients versus controls and somatic neuropathy patients. The investigators reported that autonomic innervation density was abnormal in 96 % of patients in the lower leg and in 79 % of patients in the thigh. The abnormal findings disclosed by routine autonomic tests ranged from 48 % to 82 %.

Kharkar et al (2012) evaluated the use of commercially available standard biopsy methods to detect intradermal axon pathology in CRPS-I, and to ascertain if these structural changes can explain quantitative sensory testing (QST) findings in CRPS-I. The investigators retrospectively reviewed charts and laboratory data from an outpatient clinic. Skin biopsies from 43 patients with CRPS-I were stained with PGP 9.5, and epidermal nerve fiber density, sweat gland nerve fiber density and morphological abnormalities were evaluated. Thirty-five patients had quantitative sensory testing. Alterations in skin innervation were seen in approximately 20 % of CRPS-I patients with commercial processing. There were no patient characteristics, including duration of disease, that predicted a decreased epidermal nerve fiber density (ENFD). There was no consistent relationship between QST changes and ENFD measured by standard commercial skin biopsy evaluation procedures. The authors noted that commercial processing of tissue does not utilize stereologic quantitative analysis of nerve fiber density. Biopsy material is utilized from a proximal and distal source only, and differences in denervation of a partial nerve territory may be missed. The functional attributes of small fibers cannot be assessed. The authors posited that the negative results indicate that CRPS-I may be associated with changes in the ultramicroscopic small fiber structure that cannot be visualized with commercially available techniques. The authors posited, alternatively, that functional rather than structural alterations of small fibers or pathological changes at a more proximal site such as the spinal cord or brain may be responsible for the syndrome.

Tzatha and Chin (2014) quantified epidermal sensory and sweat gland nerve fiber densities in skinbiopsies of 11 patients with benign fasciculations and no other known cause for neuropathy. The investigators found that 9 of the 11 patients (82 %) had significantly reduced epidermal or sweat gland nerve fiber densities at the calf or thigh, in comparison with control values.

Sommer et al (2002) said that Ross syndrome consists of segmental hyperhidrosis with widespread anhidrosis, Adie syndrome, and areflexia. The cause of this disorder is unknown. Selective degeneration of cholinergic fibers or of neural crest-derived structures has been suggested. The authors presented clinical and skin biopsy data of 4 patients, providing evidence of reduced cholinergic sweat gland innervation in hypohidrotic skin by morphometric analysis. The authors concluded that these findings indicated a selective degenerative process of the cholinergic sudomotor neurons.
Provitera et al (2014) quantified sudomotor innervation in skin biopsy of 29 patients with multiple system atrophy (MSA) (19 male and 10 female; age of 60.0 ± 7.7 years) and 29 age- and sex-matched healthy subjects. Samples were obtained from thigh and leg and, in 20 out of the 29 cases, also from fingertip. Dysautonomic complaints were evaluated by SCOPA-AUT, a self-administered questionnaire. Sudomotor function was evaluated in a subgroup of patients by the silastic imprint test. Skin samples were processed by indirect immunofluorescence using pan-neuronal and selective cholinergic markers. Total length of sudomotor nerves was measured on digital confocal images using a semiautomated morphometric approach. Measurements of sudomotor nerve density (total length of nerve per volume of glandular tissue) favorably correlated to values obtained using a stereologic unbiased method. Sudomotor nerve density was lower in patients compared to controls in all the examined sites (0.9 ± 0.2 versus 1.9 ± 0.4 nm/μm(3), p < 0.001, in fingertip; 0.7 ± 0.2 vs 1.9 ± 0.5 nm/μm(3), p < 0.001, in thigh; 0.6 ± 0.2 vs 1.8 ± 0.4 nm/μm(3), p < 0.001, in leg). The authors concluded that their data support the hypothesis that postganglionic impairment occurs in MSA and may contribute with the coexisting degeneration of central structures to the development of dysautonomic disorders in this condition.

Chao et al (2014) performed skin biopsies on the distal leg of familial amyloid polyneuropathy (FAP) patients with a follow-up duration of 3.8 ± 1.6 years. Sudomotor innervation was stained with 2 markers: protein gene product 9.5 (PGP 9.5), a general neuronal marker, and vasoactive intestinal peptide (VIP), a sudomotor nerve functional marker, followed by quantitation according to sweat gland innervation index (SGII) for PGP 9.5 (SGIIPGP 9.5) and VIP (SGIIVIP). There were 28 patients (25 men) with Ala97Ser transthyretin and late onset (59.9 ± 6.0 years) disabling neuropathy. Autonomic symptoms were present in 22 patients (78.6 %) at the time of skin biopsy. The SGIIPGP 9.5 and SGIIVIP of FAP patients were significantly lower than those of age- and gender-matched controls. The reduction of SGIIVIP was more severe than that of SGIIPGP 9.5 (p = 0.002). Patients with orthostatic hypotension or absent sympathetic skin response at palms were associated with lower SGIIPGP 9.5 (p = 0.019 and 0.002, respectively). SGIIPGP 9.5 was negatively correlated with the disability grade at the time of skin biopsy (p=0.004) and was positively correlated with the interval from the time of skin biopsy to the time of wheelchair usage (p = 0.029). The investigators concluded that this study documented the pathological evidence of sudomotor denervation in FAP. SGIIPGP 9.5 was functionally correlated with autonomic symptoms, autonomic tests, ambulation status, and progression of disability.

Using a stereologic approach, Liu et al (2015 measured the density of nerve fibers innervating sweat gland (SG) fragments in patients with diabetes mellitus (DM) and healthy controls using protein gene product (PGP), tyrosine hydroxylase (TH), and vasoactive intestinal peptide (VIP) to determine which marker best detected differences between the groups. Factors associated with
SG nerve fiber (SGNF) innervation were assessed and the change in SG innervation over a 1-year time period was determined. The investigators assessed 92 control subjects and 2 groups of subjects with DM totaling 97 subjects in this cross-sectional study. Intraepidermal nerve fiber density and SG innervation were determined from leg skin biopsies that were immunohistochemically stained for ubiquitin hydrolase, VIP, and TH. Factors associated with SG innervation were assessed and 15 subjects were longitudinally followed for 1 year. SGNF innervation was reduced in subjects with DM compared with controls. Lower SG innervation values were associated with increasing glycated hemoglobin A1c, body mass index (BMI), men compared with women, and tobacco use, but not diabetes type or age. Sex, A1c, and BMI remained significant in multivariate modeling. The investigators reported that SG innervation measured by VIP+ fibers is a more sensitive marker for neuropathy than either PGP or TH. Fifteen subjects with DM followed for 1 year showed a significant decrease in SGNF innervation but not intraepidermal nerve fiber density. The investigators concluded that stereologic measurement of SG innervation is feasible to assess postganglionic autonomic nerve fiber densities. SG innervation was reduced in subjects with DM compared with control subjects and was associated with sex, A1c, and BMI in multivariate modeling. VIP+ SGNF is more severely reduced in DM than TH+ or PGP9.5+-based assessments. Progression of diabetic polyneuropathy was detected by SGNF over a 1-year time period.

The European Federation of Neurological Societies (EFNS) and the Peripheral Nerve Society (PNS)'s guideline on the use of skin biopsy in the diagnosis of SFN (2010) stated that the quantification of sudomotor nerve fibers is technically challenging because of the complex 3-dimensional structure of the sweat glands. Different methods have been proposed but none has been standardized (Lauria et al, 2005). A novel method using an unbiased stereologic technique has been recently proposed (Gibbons et al, 2009). The authors examined blindly 30 diabetic neuropathy patients and 64 healthy subjects finding a significant difference between groups. The density of sweat gland nerve fibers at the distal leg of diabetic patients decreased as the Neuropathy Impairment Score in the Lower limbs worsened (p < 0.001) and was concordant with symptoms of reduced sweat production (p < 0.01). In a further work, the authors reported a significant correlation between the stereologic unbiased method and a new automated technique for quantification of sudomotor nerve fibers, and showed that the descriptive semi-quantitative approach has a poor inter- and intra-observed reliability (Gibbons et al, 2010).

The EFNS/PNS guideline noted that morphometric data on sweat gland innervation density in healthy subjects and in patients with SFN are limited and further studies are warranted. The descriptive semi-quantitative approach should not be used to quantify sweat gland innervation (level B recommendation). The unbiased stereologic technique recently proposed could be a helpful tool (level B recommendation). The guideline also stated that the reliability of already tested or new methods to quantify the density of nerve fibers in the sub-epidermal dermis and
autonomic structures (e.g., sweat gland nerve, erector pili muscle, and vessels) should be confirmed by further studies in patients with homogeneous types of peripheral neuropathy, including SFN. Correlative studies between skin biopsy, autonomic tests, and non-conventional neurophysiologic tools are also warranted.

Kharkar et al (2012) stated that accumulating experimental and clinical evidence supports the hypothesis that complex regional pain syndrome type I (CRPS-I) may be a small fiber neuropathy. These researchers evaluated the use of commercially available standard biopsy methods to detect intra-dermal axon pathology in CRPS-I, and examined if these structural changes can explain quantitative sensory testing (QST) findings in CRPS-I. Skin biopsies from 43 patients with CRPS-I were stained with PGP 9.5, and ENFD, sweat gland nerve fiber density, as well as morphological abnormalities were evaluated. A total of 35 patients had QST. Alterations in skin innervation were seen in approximately 20 % of CRPS-I patients with commercial processing. There were no patient characteristics, including duration of disease, which predicted a decreased ENFD. There was no consistent relationship between QST changes and ENFD measured by standard commercial skin biopsy evaluation procedures. The authors concluded that the negative results indicate that CRPS-I may be associated with changes in the ultramicroscopic small fiber structure that cannot be visualized with commercially available techniques. Alternatively, functional rather than structural alterations of small fibers or pathological changes at a more proximal site such as the spinal cord or brain may be responsible for the syndrome.

Also, an UpToDate review on “Etiology, clinical manifestations, and diagnosis of complex regional pain syndrome in adults” (Abdi, 2013) does not mention measurement of sweat gland nerve fiber density as a diagnostic tool.

Gibbons et al (2013) defined the neuropathology, clinical phenotype, autonomic physiology and differentiating features in individuals with neuropathic and non-neuropathic postural tachycardia syndrome (POTS), a disorder of orthostatic intolerance characterized by excessive tachycardia of unknown etiology. A total of 24 subjects with POTS and 10 healthy control subjects had skin biopsy analysis of IENFD, QST and autonomic testing. Subjects completed quality of life, fatigue and disability questionnaires; they were divided into neuropathic and non-neuropathic POTS, defined by abnormal IENFD and abnormal small fiber and sudomotor function. Overall, 9 of 24 subjects had neuropathic POTS and had significantly lower resting and tilted heart rates; reduced parasympathetic function; and lower phase 4 Valsalva maneuver overshoot compared with those with non-neuropathic POTS (p < 0.05). Neuropathic POTS subjects also had less anxiety and depression and greater overall self-perceived health-related quality of life scores than non-neuropathic POTS subjects. A sub-group of POTS patients (cholinergic POTS) had abnormal proximal sudomotor function and symptoms that suggest gastro-intestinal and genito-
urinary parasympathetic nervous system dysfunction. The authors concluded that POTS subtypes may be distinguished using small fiber and autonomic structural and functional criteria. Patients with non-neuropathic POTS have greater anxiety, greater depression and lower health-related quality of life scores compared to those with neuropathic POTS. They stated that these findings suggested different pathophysiological processes underlie the postural tachycardia in neuropathic and non-neuropathic POTS patients. The findings have implications for the therapeutic interventions to treat this disorder.

Haensch et al (2014) evaluated the correlation between C-fiber involvement shown by skin biopsy and adrenergic cardiac MIBG-uptake in POTS patients. Skin biopsies of 84 patients with POTS were examined by Protein Gene Product 9.5 (PGP9.5) immunohistochemistry and were compared to MIBG myocardial scintigraphy imaging data. Mean IENFD was in the lower normal age-adjusted range, 7.2 ± 2.9 /mm (normal greater than or equal to 7/mm), and it was slightly below the normal range in 45 % of POTS patients; MIBG-uptake was reduced in 21 %. Low IENFD correlated with reduced cardiac MIBG uptake (r = 0.39, p = 0.001). The authors concluded that a subset of neuropathic POTS patients might harbor mild SFN with abnormalities of unmyelinated nerve fibers in the skin associated with reduced myocardial post-ganglionic sympathetic innervation. The clinical value of IENFD in the management of patients with POTS needs to be further investigated.

An UpToDate review on “Postural tachycardia syndrome” (Freeman and Kaufmann, 2014) does not mention measurement of epidermal nerve fiber density as a management tool.

Evaluation of Fibromyalgia

Caro and Winter (2014) stated that a subset of patients with fibromyalgia (FM) exhibit a large fiber demyelinating peripheral polyneuropathy akin to that seen in chronic inflammatory demyelinating polyneuropathy (CIDP). It has been suggested that this demyelinating process is likely to be immune mediated. Because it is known that similar large fiber neuropathic lesions may be associated with a cutaneous SFN, these researchers determined the prevalence of SFN, as measured by ENFD, in a series of patients with FM and clinically healthy control subjects. A total of 41 consecutive patients with FM and 47 control subjects underwent a 3-mm punch skin biopsy at the proximal thigh and distal leg near the ankle, for analysis of the ENFD. Patients with FM who had clinical evidence of a disorder known to be associated with SFN were excluded. The patients with FM also underwent pinwheel testing and vibratory testing for hypesthesia and serologic testing for a series of cytokine, circulating immune complex, and complement measurements. All patients with FM had evidence of stocking hypesthesia. The ENFD of patients with FM was lower than that of control subjects at both the calf (mean ± SD 5.8 ± 2.8 versus 7.4 ± 1.9; P = 0.0002) and thigh (9.3 ± 3.2 versus 11.3 ± 2.0; p = 0.0007). There was an
inverse correlation between calf ENFD and age at the time of skin biopsy in patients with FM (r = -0.29, p = 0.03) but not in control subjects; however, analysis of covariance showed that this relationship could not be explained by aging alone. Serologic evaluation showed an inverse correlation between calf ENFD in patients with FM and the interleukin-2 receptor (IL-2R) level (r = -0.28, p = 0.04). However, an inverse correlation between thigh ENFD and serum IL-2R levels did not reach significance (p = 0.08). Analysis of thigh-to-calf ENFD ratios suggested that the ENFD decline in FM is affected by both a diffuse and a length-dependent process. The authors concluded that the calf and thigh ENFD in patients with FM is significantly diminished compared with that in control subjects. Advancing age alone cannot explain this finding. Calf ENFD was inversely correlated, although weakly, with serum levels of IL-2R. They stated that these findings suggested that SFN is likely to contribute to the pain symptoms of FM; that pain in this disorder arises, in part, from a peripheral immune-mediated process; and that measurement of ENFD may be a useful clinical tool in FM.

In a controlled study, Kosmidis and colleagues (2014) examined if IENFD is reduced in the skin of FM patients, as observed in patients with painful SFSN. These researchers prospectively studied 46 FM patients (5 men and 41 women), aged 29 to 76 (mean of 52.5) years, diagnosed according to the ACR 2010 criteria, and 34 controls (18 women and 16 men) aged 19 to 84 (mean of 31.7) years. Intra-epidermal nerve fiber density was measured using published guidelines and immune markers were sought immunocytochemically. In 30 FM patients, pain intensity was assessed with the Neuropathic Pain Symptom Inventory (NPSI), a scale validated for neuropathic pain. A total of 15 of 46 (32.6 %) FM patients had reduced IENFD [range of 0.6 to 12.5 fibers/mm (mean of 4.83 SD: 2.5)], compared to healthy controls [2.8 to 11.5 fibers/mm (mean of 7.35, SD: 1.85)] (p < 0.0001). No significant correlation was noticed between NPSI scores and IENFD. No difference in the Langerhans cells, the major Antigen Presenting Cells (APCs) in the epidermis, or in IL-6 staining, was noted between FM and controls. Intra-epidermal nerve fiber density was equally reduced in a subset of FM patients who also had another autoimmune disease. The authors concluded that this was one of the largest series of FM patients demonstrating a significant reduction of IENFD in their skin biopsies. The findings indicated that in a subset of FM patients, the pain syndrome is, at least partially, of neuropathic origin. They stated that skin biopsy may prove a useful tool and a potential biomarker in future studies of FM patients.

Furthermore, and UpToDate review on “Clinical manifestations and diagnosis of fibromyalgia in adults” (Goldenberg, 2015) states that “Laboratory testing and other studies -- FM does not cause any abnormalities in routine clinical laboratory testing or imaging. However, abnormalities have been identified in research studies using specialized neuroimaging (e.g., functional magnetic resonance imaging [MRI]) and other techniques that reveal distinctions between patients with FM and control subjects. Research studies have also found that a subset of
patients with FM have abnormalities on skin biopsies suggestive of small fiber neuropathic changes; the meaning of these findings is uncertain, and such testing is not useful in clinical practice”.

**Diagnosis of Fabry Disease**

van der Tol and associates (2016) noted that Fabry disease (FD) is an X-linked lysosomal storage disorder caused by an α-galactosidase A enzyme deficiency due to pathogenic variants in the α-galactosidase A gene (GLA). An increasing number of individuals with a GLA variant, but without characteristic FD features, have been identified. A definite diagnosis of FD has important consequences for treatment and counseling. These investigators evaluated the diagnostic value of QST and IENFD for patients with an uncertain FD diagnosis. All patients with a GLA variant who initially presented at the Academic Medical Center with an uncertain FD diagnosis were included. A biopsy of an affected organ in a patient or family member showing FD characteristic storage was used as a reference standard for a diagnosis of FD. All patients underwent a comprehensive QST protocol and IENFD assessment that was compared to age and gender-matched healthy controls. Sensitivity and specificity were calculated for a combination of greater than or equal to 1 abnormal QST modality and an abnormal IENFD. A total of 26 patients participated (non-classical FD n = 18, 9 males; no FD n = 5, 3 males; uncertain n = 3, 1 male). Of the patients classified as non-classical FD, 28 % had greater than or equal to 1 abnormal QST modality and 83 % had an abnormal IENFD. From the patients without FD, 20 % had greater than or equal to 1 abnormal QST modality, and IENFD was abnormal in 25 % (1 not available). Sensitivity was 28 % and specificity 80 %. The authors concluded that in this study cohort, QST and IENFD could not reliably distinguish patients with FD from those without FD.

**Evaluation of Individuals REM Sleep Behavior Disorder**

Schrempf and colleagues (2016) stated that idiopathic rapid eye movement (REM) sleep behavior disorder (iRBD) has been increasingly acknowledged to be an initial specific manifestation of alpha-synucleinopathies such as Parkinson’s disease (PD), multiple system atrophy (MSA) and dementia with Lewy bodies (DLB). Recent findings suggested that cutaneous abnormalities like SFN and alpha-synuclein deposition might reflect brain pathology and might function as early biomarkers in PD. This was the 1st study to examine if iRBD patients already suffer from distinctive cutaneous features. These researchers examined skin punch biopsies from the distal leg of 18 iRBD patients and 22 age- and sex-matched controls using immunohistochemistry and microscopy. Further clinical evaluation included structured interviews, clinical motor and non-motor questionnaires and rating scales (e.g., Unified Parkinson's disease rating scale [UPDRS], non-motor symptoms questionnaire [NMS-Quest] and Beck Depression Inventory [BDI], Epworth Sleepiness Scale [ESS], evaluation of cognitive and
olfactory functioning) as well as blood samples. Intra-epidermal nerve fiber density was reduced in iRBD patients compared to controls (p = 0.037), whereas the axon swelling ratio did not differ between groups. Patients with iRBD reported non-motor symptoms more frequently than controls (UPDRS I, NMS-Quest). Olfaction and daytime sleepiness differed between both groups, whereas there were no differences regarding cognition. The authors concluded that these in-vivo findings demonstrated SFN in iRBD patients that were associated with non-motor symptoms indicating that peripheral abnormalities may occur early in iRBD. However, they stated that the prognostic value has to be further investigated in longitudinal studies.

Evaluation of Individuals with Ehlers-Danlos Syndromes

Cazzato and colleagues (2016) examined the involvement of small nerve fibers in Ehlers-Danlos syndrome (EDS). Subjects were patients diagnosed with EDS who underwent clinical, neurophysiologic, and skin biopsy assessment. These investigators recorded sensory symptoms and signs and evaluated presence and severity of neuropathic pain according to the Douleur Neuropathique 4 (DN4) and ID Pain questionnaires and the Numeric Rating Scale (NRS). Sensory action potential amplitude and conduction velocity of sural nerve was recorded. Skin biopsy was performed at distal leg IENFD obtained and referred to published sex- and age-adjusted normative reference values. This study included 20 adults with joint hypermobility syndrome/hypermobility EDS, 3 patients with vascular EDS, and 1 patient with classic EDS. All except 1 patient had neuropathic pain according to DN4 and ID Pain questionnaires and reported 7 or more symptoms at the Small Fiber Neuropathy Symptoms Inventory Questionnaire. Pain intensity was moderate (NRS greater than or equal to 4 and less than 7) in 8 patients and severe (NRS greater than or equal to 7) in 11 patients. Sural nerve conduction study was normal in all patients. All patients showed a decrease of IENFD consistent with the diagnosis of SFN, regardless of the EDS type. The authors concluded that SFN is a common feature in adults with EDS; and skin biopsy could be considered an additional diagnostic tool to investigate pain manifestations in EDS. This was a small (n = 20) study; and composed of all women except for 1 man. Thus, despite the finding of SFN in all patients, these researchers could not exclude sex-related differences. These preliminary findings need to be validated by well-designed studies.

Evaluation of Hereditary Transthyretin (TTR) Amyloidosis and Iatrogenic TTR Amyloidosis

Masuda and colleagues (2017) examined early skin denervation in hereditary transthyretin (TTR) amyloidosis and iatrogenic TTR amyloidosis. These investigators examined IENFD and clinical findings in 32 patients with hereditary TTR amyloidosis, 11 asymptomatic mutation carriers, 6 patients with iatrogenic TTR amyloidosis, and 23 healthy volunteers. IENFD values were reduced in patients with the V30M mutation (1.9 ± 2.1 per 1 mm), patients with non-V30M
mutations (5.8 ± 3.2 per 1 mm), and patients with iatrogenic TTR amyloidosis (3.5 ± 1.8 per 1 mm) compared with healthy volunteers (11.8 ± 3.2 per 1 mm) (p < 0.01). Skin denervation also occurred, even in pre-symptomatic V30M mutation carriers (5.0 ± 2.2 per 1 mm). The IENFD was correlated with disease duration (ρ = -0.533, p = 0.002) and various peripheral neuropathy parameters such as sensory impairment in the Kumamoto clinical score (ρ = -0.575, p = 0.001), heat-pain detection threshold (ρ = -0.704, p < 0.001), and sural sensory nerve action potential (ρ = 0.481, p = 0.005). TTR amyloid deposits frequently occurred in connective tissues and vessels of the dermal reticular layer in patients with hereditary TTR amyloidosis and those with iatrogenic TTR amyloidosis. The authors concluded that patients with hereditary TTR amyloidosis and those with iatrogenic TTR amyloidosis may show early skin denervation even in the pre-symptomatic stage. They stated that IENFD may thus be useful for early diagnosis, after the use of disease-modifying therapies, and may serve as a biomarker in clinical trials for hereditary and iatrogenic TTR amyloidosis. These researchers also noted that to clarify whether TTR genotypes affect skin denervation, further studies with large numbers of patients are needed. Furthermore, they stated that non-fibrillar cytotoxic TTR deposits were reportedly found in patients with pre-symptomatic and early stages of hereditary TTR amyloidosis, further studies including longitudinal evaluation of skin samples are needed.

Measurement of Nerve Fiber Density for Diagnosis of Endometriosis

In a prospective, case-control study, Liutkeviciene and colleagues (2019) examined if there is any difference between NFD in eutopic endometrium in women with and without endometriosis. The trial was conducted between October 2013 and December 2015. The study included 60 reproductive age women undergoing laparoscopy for suspected endometriosis, pelvic pain, or infertility and not currently receiving hormonal treatment for at least 3 months prior to laparoscopy. Immunohistochemical NFD in endometrial curetting using anti-gene product 9.5 was compared with surgical diagnosis. The nerve fibers were detected in eutopic endometrium in women with and without endometriosis. PGP9.5-positive nerve fibers were found in 26 (43 %) cases: 16 (50 %) in women with endometriosis and 10 (36 %) in women without endometriosis. The mean NFD was higher in the group with endometriosis (0.53 per mm2 ± 0.68) than without endometriosis (0.48 per mm2 ± 0.89), but no statistically significant difference was observed. Test specificity was 64.3 %, sensitivity 50 %, positive predictive value (PPV) of 61.5 %, negative predictive value (NPV) of 52.9 %, and overall accuracy of 56.7 %. The authors concluded that the detection of PGP9.5-positive nerve fibers in eutopic endometrium could not be used as a reliable diagnostic test of diagnosing endometriosis.
CPT Codes / HCPCS Codes / ICD-10 Codes

Information in the [brackets] below has been added for clarification purposes. Codes requiring a 7th character are represented by "+":

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>There are no specific codes for Intra-Epidermal Nerve Fiber Density Measurement or sweat gland nerve fiber density measurement:</td>
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Other CPT codes related to the CPB:

<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10004</td>
<td>Fine needle aspiration biopsy, without imaging guidance; each additional lesion (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>11104</td>
<td>Punch biopsy of skin (including simple closure, when performed); single lesion</td>
</tr>
<tr>
<td>11105</td>
<td>Punch biopsy of skin (including simple closure, when performed); each separate/additional lesion (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>88305</td>
<td>Level IV Surgical pathology, gross and microscopic examination, nerve, biopsy</td>
</tr>
<tr>
<td>+ 88314</td>
<td>Special stain including interpretation and report; histochemical stain on frozen tissue block (List separately in addition to code for primary procedure)</td>
</tr>
<tr>
<td>88341 - 88344</td>
<td>Immunohistochemistry or immunocytochemistry, per specimen</td>
</tr>
<tr>
<td>88356</td>
<td>Morphometric analysis; nerve</td>
</tr>
<tr>
<td>95860 - 95872</td>
<td>Electromyography</td>
</tr>
<tr>
<td>95907 - 95913</td>
<td>Nerve conduction studies</td>
</tr>
<tr>
<td>95921 - 95923</td>
<td>Testing of autonomic nervous system function</td>
</tr>
<tr>
<td>95937</td>
<td>Neuromuscular junction testing (repetitive stimulation, paired stimuli), each nerve, any 1 method</td>
</tr>
<tr>
<td>95943</td>
<td>Simultaneous, independent, quantitative measures of both parasympathetic function and sympathetic function, based on time-frequency analysis of heart rate variability concurrent with time-frequency analysis of continuous respiratory activity, with mean heart rate and blood pressure measures, during rest, paced (deep) breathing, Valsalva maneuvers, and head-up postural change</td>
</tr>
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</table>

Other HCPCS codes related to the CPB:

<table>
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<tr>
<th>Code</th>
<th>Code Description</th>
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</thead>
<tbody>
<tr>
<td>G0461</td>
<td>Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain</td>
</tr>
<tr>
<td>G0462</td>
<td>each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)</td>
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</table>

ICD-10 codes covered if selection criteria are met:

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<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
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</thead>
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<tr>
<td>G60.8</td>
<td>Other hereditary and idiopathic neuropathies</td>
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ICD-10 codes not covered for indications listed in the CPB (not all-inclusive):
<table>
<thead>
<tr>
<th>Code</th>
<th>Code Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>E00.0 - E00.9</td>
<td>Congenital iodine-deficiency syndrome other hypothyroidism [as a marker of pre-clinical asymptomatic small-fiber sensory neuropathy]</td>
</tr>
<tr>
<td>E03.0 - E03.1</td>
<td></td>
</tr>
<tr>
<td>E08.40 - E08.49</td>
<td>Diabetes mellitus due to underlying condition with neurological complications</td>
</tr>
<tr>
<td>E09.40 - E09.49</td>
<td>Drug or chemical induced diabetes mellitus with neurological complications</td>
</tr>
<tr>
<td>E10.40 - E10.49</td>
<td>Type 1 diabetes mellitus with neurological complications</td>
</tr>
<tr>
<td>E11.40 - E11.49</td>
<td>Type 2 diabetes mellitus with neurological complications</td>
</tr>
<tr>
<td>E13.40 - E13.49</td>
<td>Other specified diabetes mellitus with neurological complications</td>
</tr>
<tr>
<td>E75.21</td>
<td>Fabry (-Anderson) disease</td>
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<tr>
<td>E85.0 - E85.9</td>
<td>Amyloidosis [hereditary transthyretin (TTR) amyloidosis] [iatrogenic TTR amyloidosis]</td>
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<tr>
<td>G13.0</td>
<td>Paraneoplastic neuromyopathy and neuropathy</td>
</tr>
<tr>
<td>G13.1</td>
<td>Other systemic atrophy primarily affecting central nervous system in neoplastic disease</td>
</tr>
<tr>
<td>G47.52</td>
<td>REM sleep behavior disorder</td>
</tr>
<tr>
<td>G60.0</td>
<td>Hereditary motor and sensory neuropathy</td>
</tr>
<tr>
<td>G60.2</td>
<td>Neuropathy in association with hereditary ataxia</td>
</tr>
<tr>
<td>G61.1</td>
<td>Serum neuropathy</td>
</tr>
<tr>
<td>G62.0</td>
<td>Drug-induced polyneuropathy</td>
</tr>
<tr>
<td>G62.2</td>
<td>Polyneuropathy due to other toxic agents</td>
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<tr>
<td>G62.82</td>
<td>Radiation-induced polyneuropathy</td>
</tr>
<tr>
<td>G63</td>
<td>Polyneuropathy in diseases classified elsewhere</td>
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<td>G90.50 - G90.59</td>
<td>Complex regional pain syndrome</td>
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<tr>
<td>G90.9</td>
<td>Disorder of the autonomic nervous system, unspecified [Postural tachycardia syndrome]</td>
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<tr>
<td>M79.7</td>
<td>Fibromyalgia</td>
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<tr>
<td>N80.0 - N80.9</td>
<td>Endometriosis</td>
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<tr>
<td>Q79.6</td>
<td>Ehlers-Danlos syndrome</td>
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<tr>
<td>R00.0</td>
<td>Tachycardia, unspecified [Postural tachycardia syndrome]</td>
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</tbody>
</table>
The above policy is based on the following references:


43. Goldenberg DL. Clinical manifestations and diagnosis of fibromyalgia in adults. UpToDate [online serial]. Waltham, MA: UpToDate; reviewed July 2015.


Amendment to
Aetna Clinical Policy Bulletin Number: 0774
Nerve Fiber Density Measurement

There are no amendments for Medicaid.