Deoxy-sphingolipids, oxidative stress, and vitamin C correlate with qualitative and quantitative patterns of small fiber dysfunction and degeneration

Maike F. Dohrn, Christina Dumke, Thorsten Homemann, Stefan Nikolind, Angelika Lampert, Volker Espenkott, Jan Vollert, Annabelle Ouwenbroek, Martina Zanella, Jörg B. Schulz, Burkhard Gessa, Roman Rolke

Abstract

Defined by dysfunction or degeneration of Aδ and C fibers, small fiber neuropathies (SFNs) entail a relevant health burden. In 50% of cases, the underlying cause cannot be identified or treated. In 100 individuals (70% female individuals; mean age: 44.8 years) with an idiopathic, skin biopsy-confirmed SFN, we characterized the symptomatic spectrum and measured markers of oxidative stress (vitamin C, selenium, and glutathione) and inflammation (transforming growth factor beta, tumor necrosis factor alpha), as well as neurotoxic 1-deoxy-sphingolipids. Neuropathic pain was the most abundant symptom (95%) and cause of daily life impairment (72%). Despite the common use of pain killers (64%), the painDETECT questionnaire revealed scores above 13 points in 80% of patients. In the quantitative sensory testing (QST), a dysfunction of Aδ fibers was observed in 70% and of C fibers in 44%, affecting the face, hands, or feet. Despite normal nerve conduction studies, QST revealed Aβ fiber involvement in 46% of patients’ test areas. Despite absence of diabetes mellitus or mutations in SPTLC1 or SPTLC2, plasma 1-deoxy-sphingolipids were significantly higher in the sensory loss patient cluster when compared with those in patients with thermal hyperalgesia (P < 0.01) or those in the healthy category (P < 0.1), correlating inversely with the intraepidermal nerve fiber density (1-deoxy-SA: P < 0.05, 1-deoxy-SC: P < 0.01). Patients with arterial hypertension, overweight (body mass index > 25 kg/m²), or hyperlipidemia showed significantly lower L-serine (arterial hypertension: P < 0.001) and higher 1-deoxy-sphingolipid levels (arterial hypertension: P < 0.001, overweight: P < 0.001, hyperlipidemia: P < 0.01). Lower vitamin C levels correlated with functional Aβ involvement (P < 0.05). Reduced glutathione was lower in patients with Aδ dysfunction (P < 0.05). Idiopathic SFNs are heterogeneous. As a new pathomechanism, plasma 1-deoxy-sphingolipids might link the metabolic syndrome with small fiber degeneration.

Keywords: Small fiber neuropathy, Neuropathic pain, Idiopathic neuropathy, Quantitative sensory testing, 1-deoxy-sphingolipids

1. Introduction

Small fiber neuropathies (SFNs) comprise a symptom spectrum caused by dysfunction or degeneration of intraepidermal C and Aδ nerve fibers. Neuropathic pain, the leading manifestation of SFNs, is typically described as a burning, tingling, electrifying, pricking, or itching sensation on the surface of or closely underneath the skin. SFNs can also cause sensory deficits, including a reduced pinprick and temperature perception, as well as widespread autonomic dysfunction.
2. Materials and methods

2.1. Patient selection

All patients were examined at the Neuromuscular Outpatient Clinic, Department of Neurology of the RWTH Aachen University Hospital, Aachen, Germany. The study design conformed to the Declaration of Helsinki, and ethical approval was obtained before study initiation (EK 310/16). All parts of the study protocol were conducted by the same experienced examiners. For study participation, preexisting symptomatic treatments, for example neuropathic pain medications, were not paused.

After a preselection of 140 patients, we prospectively included 100 individuals in total. For inclusion, patients had to show the characteristic clinical picture and patient history, (2) evidence of either small fiber dysfunction using quantitative sensory testing (QST) or of small fiber degeneration in the skin biopsy, and (3) the exclusion of a large fiber polyneuropathy by nerve conduction studies (NCSs).5,10 Despite because of being difficult to objectify, SFNs have the potential to cause severe disability and a high psychological burden.

The causative spectrum of SFNs is heterogeneous ranging from inflammatory and metabolic to hereditary causes.4,7,18,19 Approximately half of all diagnosed SFNs remain idiopathic to date.4

As a potential risk factor, oxidative stress has previously been identified to be a key player in diabetic neuropathies2,6,29 and with hereditary sensory and autonomic neuropathy (HSAN) types 1A and C.34,39 In a small patient cohort, the oral substitution of L-serine was associated with an improvement of disease severity; long-term effects remain, however, to be studied in larger cohorts.12,14,31

In this study, we examined the phenotypic spectrum of idiopathic small fiber neuropathies in a cohort of 100 affected individuals and correlated the presence or absence of certain features with serum markers of inflammation, oxidative stress, and metabolic syndrome. As potentially influenceable contributors to the development of SFNs, they might become targets for future treatment approaches.

2.2. Patient history and painDETECT questionnaire

A detailed patient history was obtained using a standardized protocol for all study visits. We assessed the age at symptom onset, the first symptoms, the symptom dynamics during the disease course, the current pattern including sensory plus and minus symptoms and autonomic dysfunction, alleviating or worsening factors, and the symptom of highest subjective impact on quality of life. We further assessed a detailed social and family history and asked for former and current comorbidities.

The pain history was specified, focusing on pain characteristics, intensity, localization, radiation, dynamics, and influencing factors. We quantified neuropathic pain components using the painDETECT questionnaire with a 38-point global sum score.11 An overall score of 19 points or more indicates a neuropathic character of pain with a probability of 90%, whereas it is unlikely when less than 12 points are scored.

2.3. Bedside examinations

In a detailed bedside examination, we qualitatively assessed all sensory modalities, including perception of light touch, pinprick, and temperature to be reduced or normal. In case of any impairment, we further examined its distribution pattern, namely length-dependent or discontinuous, and the precise levels of perception compared to normal. To assess clinically significant large fiber involvement, we further screened all patients for a reduced vibration perception at medial malleoli, patella, and wrist levels, using a Rydel-Seiffer tuning fork (64 Hz) with a default scale from 0 to 8, for signs of afferent ataxia (gait pattern, Romberg manoeuvre, and heel–knee test), abnormalities in deep tendon reflexes, and reduction in muscle strength (range 0-5 according to medical research council).

2.4. Neurophysiological examinations

Nerve conduction studies were performed by the same experienced examiners in all 100 cases. Compound motor action potentials (CMAPs), motor nerve conduction velocities, distal motor latency, and F-waves were measured at the tibial nerve on one side. Sensory nerve action potentials (SNAPs) and sensory nerve conduction velocities were orthodromically measured for the sural nerve on both sides.

In 99 of the 100 patients, we assessed a complete quantitative sensory testing (QST) profile at one clinically affected site. In total, 17 affected hands and 82 affected feet were tested. If mirror image body areas such as both feet were affected, we assessed the patient’s most affected foot. Following the protocol of the German Research Network on Neuropathic Pain (DFNS),15,36 the same experienced examiners applied standardized stimuli on different skin areas to delineate the type of involved nerve fibers compared with a group of healthy control subjects, who were matched for age, sex, and body region.25,27 This enabled measuring quantitative thresholds of thermal and mechanical detection and pain, paradoxical heat sensations, thresholds to von Frey filaments, mechanical pain thresholds to pinprick stimuli and blunt pressure, stimulus/response functions for pinprick and dynamic mechanical alldynia, and pain summation (wind-up ratio).

2.5. Interpretation of nerve fiber involvement

When we analyzed each patient’s data set, we found both loss and gain in sensory nerve fiber function. For comparison, we used...
a control group of 100 age-matched, sex-matched, and area-matched healthy control subjects. As shown in previous studies, deficits in Aδ fiber function can typically be characterized by abnormal cold detection thresholds (CDTs). Dysfunction of C fibers is represented by elevated warm detection threshold (WDT) values. A combined loss of function of C and Aδ fibers is reflected by elevated cold pain thresholds (CPTs), heat pain thresholds (HPTs), pressure pain thresholds (PPTs), mechanical pain thresholds (MPTs), or decreased mechanical pain sensitivity (MPS). Aδ fiber deficits can be seen with increased mechanical detection thresholds (MDTs) and vibration detection thresholds (VDTs). Increased mechanical pain sensitivity (MPS) or the presence of dynamic mechanical allodynia (DMA) is consistent with the concept of central sensitization of the nociceptive system. Temporal summation (wind-up) of pain is assumed to be consistent with elevated wind-up ratios (WURs). Because these values cluster in their functional interpretation, we applied established statistical analyses to divide our study participants into QST-based subgroups, namely (1) sensory loss, (2) mechanical hyperalgesia, and (3) thermal hyperalgesia.

2.6. Assessment of sudomotor function

We measured the electrochemical skin conductance at both the patients’ palms and soles of the feet using the already established Sudoscan device. For that, patients stood upright, distributing their body weight equally to the plate electrodes. A not noticeable current of <4V was applied by default, stimulating sweat production. Results were given in percentiles and compared with those of healthy sex-matched, age-matched, and weight-matched controls.

2.7. Skin biopsies

A skin punch biopsy indicative of small fiber degeneration was an inclusion criterion for the study. We therefore did not obtain new specimens within this study protocol, but reassessed the preexisting neuropathological reports. In 61% of the cases, this specimens within this study protocol, but reassessed the inclusion criterion for the study. We therefore did not obtain new.

2.8. Biomarker analysis

Except for transforming growth factor beta (TGF-β), sphingolipids, fat, and amino acid profiles, all serum laboratory values were measured at the clinically validated laboratory of the RWTH Aachen University hospital, following the local standard procedures. This includes the analysis of tumor necrosis factor alpha (TNF-α), glutathione (reduced, oxidated, and overall levels), selenium, and vitamin C. Transforming growth factor beta was measured from EDTA blood specimens after being transferred on dry ice to the Synevo study service laboratory in Berlin, Germany. The plasma sphingoid base and amino acid profile, as well as plasma triglycerides, were analyzed at the Institute of Clinical Chemistry, University Hospital Zürich (Zürich, Switzerland). Before analysis, the extracted plasma sphingolipids were subjected to an acid/base hydrolysis to release the free sphingoid bases from the conjugated N-acyl chains and headgroups. The profiling included C16SO, C16SA, C17SO, C17SA, C18SO, C18SA, C19SO, C20SO, C20SA, sphingadiene, 1-deoxy-sphingosine (1-deoxySO), and 1-deoxy-sphinganine (1-deoxySA). Details on the procedure have been described earlier. The plasma profiles were compared with a group of 34 not age-matched or sex-matched healthy individuals. The molecular genetic analysis of the SP7LC1 and SP7LC2 genes was part of a standardized next-generation sequencing–based diagnostic panel for sensory neuropathies performed at the Institute for Human Genetics at the RWTH Aachen University Hospital. Using the same screening panel, we excluded known pathogenic variants in the GLA gene.

2.9. Statistical evaluation

The original data set was implemented into SPSS and Graphpad Prism7 softwares. To compare one group with another, we used the Student t test for normally distributed and the Mann-Whitney test for nonparametric data. Gaussian distribution was tested with the Kolmogorow–Smimow, D’Agostino & Pearson omnibus, and Shapiro–Wilks normality tests. Group comparisons were performed using 1-way ANOVA or with the Kruskal–Wallis test if non-parametric. The P levels were corrected for multiple comparisons with the Tukey–Kramer or Dunn post-test method. Linear regression analyses were performed to assess clinical, paraclinical, and score correlations. To compare individual QST parameters directly with each other and to correlate them with other numeric markers, we standardized them in comparison with healthy sex-matched and age-matched control values from identical body regions. All QST parameters with the exception of PHS, CPT, HPT, and VDT were normally distributed in log space and were transformed logarithmically before statistical analysis. We calculated z score values using the expression: $z = \frac{value_{patient} - mean_{controls}}{SD_{controls}}$. This procedure resulted in a QST profile presenting all parameters as standard normal distributions (zero mean and unit variance) independent of age, sex, and body region. $Z$ values greater than 0 indicate a gain of function when the patient is more sensitive to the tested stimuli compared with controls (hyperalgesia, allodynia, and hyperpathia), whereas $z$ scores less than 0 indicate a loss of function. To assign patients to sensory phenotypes, a published algorithm was used, which is based on sensory profiles from patients with neuropathic pain and healthy participants with induced mechanistic surrogate models.

2.10. Data availability

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

3. Results

3.1. Patient cohort

We examined 100 patients (70% female individuals, 30% male individuals; mean age at examination: 44.8 [20–77] years) with clinical and histological signs of SFNs and normal lower limb nerve conduction studies to exclude a significant large fiber polyneuropathy (Table 1). Unspecific clinical signs were not considered exclusion criteria so that distal pailhypoesthesia occurred in 10% and abolished Achilles tendon reflexes in 5% of the patients. The mean duration between symptom onset and
histological diagnosis was 5.9 years (5 months-30 years) and more than 10 years in 23.6% of the cohort. The age at onset was broadly distributed, mostly involving the third, fourth, and fifth decades of life; however, 12% of the included patients reported first symptoms before the age of 20 years. The distribution pattern was described as length-dependent in 58% and as diffuse in 41% of the patients. To be classified idiopathic, common disease causes such as diabetes mellitus were excluded before study participation. With overweight (35%), arterial hypertension (27%), and hyperlipidemia (9%) belonging to the most frequent comorbidities, however, a nondiabetic metabolic syndrome (combination of 3 of the above, following WHO definition) was diagnosed in 5%. Concomitant chronic pain syndromes were migraines (15%) and fibromyalgia (11%), and a postural tachycardia syndrome was found in 8%. The most common medications in our patient cohort addressed pain: a specific treatment of neuropathic pain had been administered in 49% (anticonvulsants [n = 39], antidepressants [n = 23], topic drugs [n = 1]), and unspecific pain killers were taken in 12% (NSAIDs [n = 28], opioids [n = 19]). Combinations were possible.

3.2. Small fiber neuropathy characteristics

3.2.1. Symptom history

Sensory plus signs, including paresthesias and neuropathic pain, were most frequently the first manifestation of SFNs in this collective (57%), whereas sensory minus signs such as numbness and thermohypoesthesia occurred in 23% and autonomic symptoms in 10%. Further unspecific complaints such as fatigue or generalized weakness were attributed to the SFN onset in another 65%. In 11% of the cases, patients reported not one but several first symptoms at the same time. A potentially triggering event in timely correlation with symptom onset was reported in 60% of the patients. This included psychological stress (25%), infections (15%), medications (8%), and others (17%). At the time of the clinical visit, 95% of the patients reported neuropathic pain, 88% paresthesia, and 77% numbness. With 96% of the examined cohort, the face placed first in the ranking of affected localizations, whereas 78% of the patients reported an (additional) hand and 72% a lower leg involvement (Fig. 1A). The whole arms and legs but not the trunk and face were affected in 28%. The face was affected in 33% and the trunk in 38% (Fig. 1B). Autonomic symptoms were present in 85% overall, the most frequent of which were reported dizziness, (impending) blackouts (57%), diarrhea (43%), and hyperhidrosis (47%). In 9 of these patients, a postural tachycardia syndrome had previously been diagnosed. A significant weight loss had not been noted in any of the cases. Ninety-two percent of the patients reported a relevant daily life impairment. The most common cause of such was neuropathic pain (72%).

3.2.2. Qualitative and quantitative assessment of neuropathic pain

Patients described the quality of pain to be burning (74%), pricking (55%), needling (29%), pulsing (24%), electric shock-like (22%), and nagging (15%). However, other descriptive terms were used in 36%. With a mean point score of 17.5 ± 7.0 (range: 0-32), the painDETECT was in linear regression with the NRS, which was highly significant (P < 0.001). Acknowledging that the painDETECT has not been validated as a follow-up questionnaire, its point values were significantly higher in patients with a progressive disease course compared with those with a stable disease course (P < 0.01), which was in accordance with the NRS values (P < 0.01).

3.2.3. Qualitative and quantitative assessment of nerve fiber dysfunction

Sensory deficits were reported in 99%, but subjectively involved areas did not overlap with our clinical examination results (Fig. 1C and D). Compared with age-matched, sex-matched, and area-matched healthy controls, the SFN cohort showed a highly significant thermal hypoesthesia (P < 0.001) (Fig. 2), which is a characteristic sign of C (warmth) and Aδ fiber (cold) dysfunction.

Most likely related to central sensitization, they further experienced a profound mechanical hyperalgesia, as well as allodynia and an increased number of paradoxical heat sensations. The vibration detection threshold, a typical parameter for protopathic Aβ nerve fiber function, was normal in this cohort fitting into the diagnosis of a pure SFN. However, the mechanical detection threshold, likewise a marker for epicritic Aβ fiber involvement, was significantly elevated as well. Such tactile hypoesthesia can be observed in patients with large fiber damage. However, in healthy subjects with central sensitization of the nociceptive system after

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Table 1

| Patient overview. |
|-------------------|
| Demographics      |
| Sex               |
| Male: 30; female: 70 |
| Age at examination, y |
| 44.8 ± 12.5 (20-77) |
| BMI, kg/m²         |
| 27.5 ± 5.6 (17-41) |

| Symptoms and disease course |
|-----------------------------|
| Age at onset, y             |
| 36 (6-57)                   |
| Disease duration, y         |
| 9.4 ± 9.6 (1-42)            |
| Duration until diagnosis, y |
| 5.9 ± 6.7 (0-30)            |
| Sensory plus symptoms       |
| 98%                         |
| Sensory minus symptoms      |
| 94%                         |
| Autonomic symptoms          |
| 85%                         |
| Length-dependent distribution |
| 58%                         |
| Progressive course          |
| 64%                         |

| Clinical signs of large fiber involvement |
|------------------------------------------|
| Abolished Achilles tendon reflexes      |
| 5%                                       |
| Polyphoesthesia at ankles                |
| 10%                                      |
| Daily life impairment caused by SFN      |
| 92%                                      |

| NRS [0-10]       |
| 4.13 ± 2.24 (0-10) |

| PainDETECT [0-38] |
| 17.54 ± 6.98 (0-32) |

| Paraclinical examinations |
|---------------------------|
| Signs of large fiber polyneuropathy in NCS |
| 0%                        |
| Signs of Aδ fiber dysfunction in QST* |
| 61% (46%)                  |
| Signs of Aβ fiber dysfunction in QST* |
| 84% (70%)                  |
| Signs of C fiber dysfunction in QST* |
| 61% (44%)                  |
| Sudomotor dysfunction      |
| 23%                        |
| Distal IFNFD, fiber/mm     |
| 3.35 ± 2.17 (0.1-10.5)    |
| Slightly reduced distal IFNFD |
| 23%                        |
| Moderately reduced distal IFNFD |
| 29%                        |
| Severely reduced distal IFNFD |
| 35%                        |
| Early signs of degeneration |
| 10%                        |
| No classification          |
| 3%                         |

| Laboratory |
|------------|
| 1-deoxy-SA, µmol/L |
| 0.07 ± 0.04 (0.016-0.222) |
| 1-deoxy-SA, µmol/L |
| 0.33 ± 0.22 (0.16-0.89) |
| Reduced glutathione, mg/L [150-460] |
| 265.9 ± 139.2 (34-765) |
| Vitamin C, mg/dL [4-20] |
| 10.99 ± 5.01 (9-25.1) |
| TGF-β, ng/ml [18.3-41.6] |
| 24.07 ± 7.33 (9.3-46.3) |
| TNF-α, ng/ml [<8.1] |
| 6.18 ± 2.22 (3.9-13.8) |

* Combining the test and control areas, test area only in parentheses.

BMI, body mass index; IFNFD, intraneural fiber density; NCS, nerve conduction studies; NRS, numeric rating scale; QST, quantitative sensory testing; SFN, small fiber neuropathy; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha.
intradermal capsaicin injections, a purely functional secondary hypoesthesia could be detected. This phenomenon might be explained possibly due to a functional switch at the spinal level based on C-fiber-induced primary afferent depolarization, resulting in presynaptic inhibition of low threshold mechanoreceptor input and an ensuing loss of tactile sensitivity.26 Interestingly, this finding was observed in 34% of our patients, indicating that such a functional shift of epicritic large fiber performance might be present in SFN, where C fiber input is disturbed as well. The preserved Aβ fiber function of deeper tissues (VDT) supports this assumption.

3.2.4. Histological assessment of nerve fiber degeneration
In the overall cohort, the mean distal intraepidermal nerve fiber density (IENFD) was 3.4/mm² ± 2.2/mm (0.1/mm-10.5/mm²). Depending on age-related and sex-related normative values,23 this reduction of IENFD was classified as slightly reduced in 23%, moderately reduced in 29%, and severely reduced in 35% of the cases (Table 1). In 10%, the IENFD was normal, but other signs of nerve fiber degeneration, such as swellings of the nerve endings (>1.5 μm), were observed. Histologically, a disturbed innervation of sweat glands was reported in 20% of the patients.

3.2.5. Sudomotor dysfunction
The presence or absence of histologically visible sweat gland degeneration did not correlate with the electrochemical skin conductance in 4% of 52 examined patients. Hands were more frequently (19%) affected by sudomotor dysfunction than feet (4%), and a combination of both occurred in 4%. This did not correlate with the subjective sensory involvement patterns described earlier (Fig. 1). Patients, who reported a subjective hypohidrosis (n = 14), did not show a higher rate of measurable sudomotor dysfunction in this test (X² test, P > 0.05) nor did patients who reported a predominant or first autonomic manifestation.

3.3. Phenotype clusters and diagnostic patterns

To determine SFN subphenotypes, we correlated different diagnostic parameters assessing potential patterns and clusters. Patients, who reported sensory plus symptoms as first SFN manifestation, continued to experience such plus symptoms significantly more often than patients with other first symptoms (P < 0.05). Patients with a progressive disease course were significantly more likely to experience pain (P < 0.001), and the disease duration correlated with the painDETECT score (P < 0.05). Patients with numbness experienced a significantly longer disease duration than those without (P < 0.05). No significant correlations were observed between the localization patterns (length-dependent, asymmetric, and diffuse) and the reported disease course (stable, slowly progressive, and relapsing–remittent).

Patients with C fiber dysfunction in the QST experienced significantly higher painDETECT scores (P < 0.001), which was mostly attributed to burning and pressure pain. Contrarily, patients with Aβ nerve fiber involvement reported significantly higher pain intensities when exposed to light touch (P < 0.01) and
3.4. Biomarkers and clinical correlations

To better understand potential etiologies and biomarker constellations, we measured several blood parameters and compared them within patient subgroups. 1-deoxy-SA and 1-deoxy-SO have both been previously described in association with diabetic and hereditary sensory and autonomic neuropathies, disturbing axonal outgrowth. Diabetes mellitus, known to increase plasma 1-deoxy sphingolipid levels, was ruled out in all patients before study inclusion. To further exclude a hereditary liability to 1-deoxy sphingolipid production, the patients were screened for molecular genetic mutations in the genes SPTLC1 and SPTLC2, revealing no pathogenic or likely pathogenic variants in any of the cases.

Compared with healthy controls (n = 34, 21 female individuals, 13 male individuals, mean age: 51 years; mean 1-deoxy-SA: 0.07 ± 0.03 μmol/L, range: 0.03-0.15 μmol/L; mean 1-deoxy-SO: 0.43 ± 0.17 μmol/L, range: 0.1-1.4 μmol/L), plasma 1-deoxy-SA and 1-deoxy-SO were not significantly elevated in the overall SFN cohort (mean 1-deoxy-SA: 0.07 ± 0.04 μmol/L, range: 0.02-0.2 μmol/L; mean 1-deoxy-SO: 0.33 ± 0.22 μmol/L, range: 0.16-0.89 μmol/L) and neither were other physiological sphingoid bases such as C18SO and C18SA. In patients with SFNs, however, both 1-deoxy-SA and 1-deoxy-SO were found to correlate inversely with the distal IENFD (Fig. 3A), meaning that patients with higher 1-deoxySL plasma levels showed a significantly lower IENFD (1-deoxy-SA: P < 0.05, r = −0.2; 1-deoxy-SO: P < 0.01, r = −0.3). Cluster members of the sensory loss category showed significantly higher 1-deoxy-SA levels compared with the thermal hyperalgesia or healthy (P < 0.01, P < 0.1) category. Furthermore, we observed an inverse correlation of these lipids with thermal pain perception in the painDETECT (1-deoxy-SA: P < 0.01, r = −0.3, 1-deoxy-SO: P < 0.05, r = −0.2), as well as with the C nerve fiber–specific QST marker WDT (1-deoxy-SA: P < 0.05, r = −0.2, 1-deoxy-SO: P < 0.05, r = −0.2). A linear regression was found between the body mass index and both 1-deoxy-sphingoid bases (1-deoxy-SA: P < 0.001, r = 0.4, 1-deoxy-SO: P < 0.01, r = 0.4), which was a unique finding different from the nontoxic C18 sphingolipids. Similarly, both 1-deoxy-SA and 1-deoxy-SO plasma levels were found to be significantly higher in patients with arterial hypertension (1-deoxy-SA: P < 0.01, 1-deoxy-SO: P < 0.01) (Fig. 3B). The 1-deoxy-sphingolipid bases correlated with triglyceride (1-deoxy-SA: P < 0.001, r = 0.7, 1-deoxy-SO: P < 0.001, r = 0.7) and cholesterol levels in plasma (1-deoxy-SA: P < 0.01, r = 0.2, 1-deoxy-SO: P < 0.05, r = 0.1), and an inverse correlation was observed with high-density lipoprotein (HDL) cholesterol (1-deoxy-SA: P < 0.01, r = −0.3, 1-deoxy-SO: P < 0.01, r = −0.3) (Fig. 3C).

Considering that 1-deoxy-sphingoid bases are derived from L-alanine instead of L-serine, a significant correlation was observed with the former (P < 0.001, r = 0.4) and an inverse correlation with the latter (P < 0.001, r = −0.4), as well as with the serine/alanine ratio (1-deoxy-SA: P < 0.0001, r = −0.6, 1-deoxy-SO: P < 0.001, r = −0.2) (Fig. 3D). Accordingly, a low serine/alanine ratio was observed in patients with lower HDL cholesterol levels (Fig. 3E) or arterial hypertension (Fig. 3F).

The mean vitamin C levels in this cohort were 11 ± 5 mg/dL (range: 0.9-25.1; reference: 4-20 mg/dL; supplementary Figure 1B, available as supplemental digital content at http://links.lww.com/PAIN/B562). We found a serum vitamin C deficiency in 10% of the patients. Leading symptoms, localization patterns, clinical course, or neuropathic pain severity did not significantly deviate from the rest of the cohort. However, patients...
### Table 2

|                | CDT (Aβ) | WDT (C)  | MDT (Aβ) | VDT (Aβ) | PainDETECT | NRS | Distal IENFD | Reduced glutathione | Vitamin C | TNF-α | TGF-β | 1-Deoxy-SA | 1-Deoxy-SO | L-alanine | L-serine | HDL | LDL | Triglyceride |
|----------------|----------|----------|----------|----------|------------|-----|--------------|---------------------|-----------|-------|-------|------------|-----------|-----------|----------|-----|-----|-------------|
| CDT (Aβ)       | <0.001   | <0.001   | <0.001   | 0.01     | 0.07       | 0.87| 0.89         | 0.004               | 0.50      | 0.15  | 0.13  | 0.07       | 0.37      | 0.10      | 0.14     | 0.14| 0.80| 0.10       |
| WDT (C)        | <0.001   | <0.001   | 0.001    | 0.07     | 0.32       | 0.22| 0.55         | 0.18                 | 0.57      | 0.44  | 0.04  | 0.03       | 0.17      | 0.48      | 0.02     | 0.44| 0.45| 0.02       |
| MDT (Aβ)       | <0.001   | <0.001   | <0.001   | <0.001   | 0.01       | 0.79| 0.01         | 0.83                 | 0.94      | 0.87  | 0.54  | 0.76       | 0.37      | 0.53      | 0.50     | 0.53|     |             |
| VDT (Aβ)       | <0.001   | <0.001   | 0.001    | <0.001   | 0.33       | 0.29| 0.43         | 0.25                 | 0.35      | 0.10  | 0.09  | 0.21       | 0.12      | 0.62      | 0.01     | 0.42| 0.41| 0.81       |
| PainDETECT     | 0.01     | 0.07     | 0.001    | 0.29     | <0.001     | 0.36| 0.60         | 0.70                 | 0.46      | 0.39  | 0.34  | 0.67       | 0.85      | 0.38      | 0.86     | 0.49| 0.69|             |
| NRS            | 0.07     | 0.32     | <0.001   | 0.43     | <0.001     | 0.02| 0.50         | 0.51                 | 0.52      | 0.23  | 0.77  | 0.60       | 0.42      | 0.48      | 0.54     | 0.73|     |             |
| Distal IENFD   | 0.87     | 0.22     | 0.60     | 0.25     | 0.36       | 0.92| 0.42         | 0.11                 | 0.59      | 0.003 | 0.04  | 0.004      | 0.62      | 0.87      | 0.54     | 0.14| 0.03|             |
| Reduced glutathione | 0.89 | 0.55     | 0.79     | 0.35     | 0.60       | 0.50| 0.42         | 0.83                 | 0.59      | 0.80  | 0.52  | 0.83       | 0.11      | 0.01      | 0.61     | 0.51| 0.50|             |
| Vitamin C      | 0.004    | 0.18     | 0.01     | 0.10     | 0.70       | 0.51| 0.11         | 0.83                 | 0.37      | 0.65  | 0.83  | 0.43       | 0.85      | 0.31      | <0.001   | 0.58| 0.07|             |
| TNF-α          | 0.50     | 0.57     | 0.83     | 0.09     | 0.46       | 0.52| 0.59         | 0.59                 | 0.37      | 0.51  | 0.27  | 0.95       | 0.08      | 0.05      | 0.53     | 0.51| 0.78|             |
| TGF-β          | 0.15     | 0.44     | 0.94     | 0.18     | 0.39       | 0.23| 0.003        | 0.80                 | 0.65      | 0.51  | 0.15  | 0.49       | 0.04      | 0.62      | 0.12     | 0.22| 0.93|             |
| 1-Deoxy-SA     | 0.13     | 0.04     | 0.87     | 0.21     | 0.34       | 0.77| 0.04         | 0.52                 | 0.83      | 0.27  | 0.15  | <0.001     | <0.001    | <0.001    | 0.005    | 0.14| <0.001|             |
| 1-Deoxy-SO     | 0.07     | 0.03     | 0.54     | 0.12     | 0.67       | 0.74| 0.004        | 0.83                 | 0.43      | 0.95  | 0.49  | <0.001     | <0.001    | 0.001    | 0.001    | 0.21| <0.001|             |
| L-alanine      | 0.37     | 0.17     | 0.76     | 0.62     | 0.85       | 0.60| 0.62         | 0.11                 | 0.85      | 0.08  | 0.04  | <0.001     | <0.001    | 0.40      | 0.13     | 0.87| <0.001|             |
| L-serine       | 0.10     | 0.48     | 0.37     | 0.01     | 0.38       | 0.42| 0.87         | 0.01                 | 0.31      | 0.05  | 0.62  | <0.001     | <0.001    | 0.001    | 0.20      | 0.88| 0.27|             |
| HDL            | 0.14     | 0.02     | 0.53     | 0.42     | 0.86       | 0.48| 0.54         | 0.61                 | <0.001    | 0.53  | 0.12  | 0.005      | 0.001     | 0.13      | 0.27      | 0.04| <0.001|             |
| LDL            | 0.80     | 0.45     | 0.50     | 0.41     | 0.49       | 0.54| 0.14         | 0.51                 | 0.58      | 0.51  | 0.22  | 0.14       | 0.21      | 0.87      | 0.88      | 0.04| 0.86|             |
| Triglyceride   | 0.10     | 0.02     | 0.52     | 0.81     | 0.69       | 0.73| 0.03         | 0.50                 | 0.07      | 0.78  | 0.93  | <0.001     | <0.001    | 0.27      | <0.001   | 0.86|     |             |

Using *p*-values derived from age-matched, sex-matched, and area-matched healthy controls, we correlated all GST parameters with each other (shown here are only CDT, WDT, MDT, and VDT), with the pain questionnaire painDETECT, the numeric rating scale, and several blood markers. Representational parameters for Aβ, C, and Aβ nerve fiber dysfunction showed a high tendency to cluster with each other. Another significant correlation was observed between the numeric rating scale, depicting the momentary pain level, and the painDETECT score, which is an indicator for neuropathic pain. The painDETECT further correlated with both parameters of Aβ and Aβ nerve dysfunction and with markers of (neural) sensitization. 1-Deoxy-sphingolipid bases were found to correlate with L-alanine and triglycerides. An inverse correlation was found with WDT, IFNα, L-serine, and HDL cholesterol. Significant correlations are shown in bold, and correlation coefficients / added whenever *p*-values were < 0.001.  

CDT, cold detection threshold; HDL, high-density lipoprotein; IENFD, intraepidermal nerve fiber density; LDL, low-density lipoprotein; MDT, mechanical detection threshold; NRS, numeric rating scale; GST, quantitative sensory testing; TGF-β, transforming growth factor β; TNF-α, tumor necrosis factor α; VDT, vibration detection threshold; WDT, warm detection threshold; 1-deoxySA, 1-deoxy-sphinganine; 1-deoxySO, 1-deoxy-sphingosine.
with an Aβ nerve fiber dysfunction showed significantly lower vitamin C levels in serum ($P < 0.05$) than those with a pure C and A6 neuropathy (Fig. 4A). Moreover, patients with sensory loss showed significantly lower vitamin C levels than patients with thermal hyperalgesia or mechanical hyperalgesia ($P < 0.01$). Vitamin C levels were significantly lower in patients with arterial hypertension ($P < 0.05$). The IENFD did not correlate with vitamin C levels.

To assess further oxidative stress markers, we measured serum levels of selenium (89.2 ± 19.2 μg/L) (supplementary Figure 1A, available as supplemental digital content at http://links.lww.com/PAIN/B562) and glutathione (supplementary Figure 1C, available as supplemental digital content at http://links.lww.com/PAIN/B562) in its reduced (mean 265.9 ± 139.2 mg/L) and oxidized (mean 113.9 ± 36.4 mg/L) forms, as well as its overall (mean 381.6 ± 135.5 mg/L) amount. Of these, reduced glutathione was below the reference level (150-460 mg/L) in 22% of the patients. These patients did not differ from the rest of the cohort regarding quality, severity, localization, and course of symptoms. However, reduced glutathione values were significantly lower in patients with A6 nerve fiber dysfunction ($P < 0.05$) (Fig. 4B).

As markers of inflammation, we measured TNF-α ($n = 90$) and TGF-β ($n = 80$) levels in serum, identifying elevated TNF-α levels in 16.7% and reduced TGF-β levels in 12.5% of the examined patients. One further patient had elevated TGF-β in serum. Comparing these patients with the overall SFN cohort, we did not find specifically correlating subphenotypes such as a relapsing course or a diffuse localization reminding of other inflammatory findings. Neither did one of the 2 parameters correlate with any of the characteristics of metabolic syndrome. However, TGF-β values were significantly lower in serum of patients with a moderately and severely reduced distal IENFD compared with those with a normal IENFD (Fig. 4C).

4. Discussion

In this study, we characterized a cohort of 100 patients with idiopathic SFNs, assessed common and variable symptoms, and correlated these with several markers for metabolic syndrome, oxidative stress, and systemic inflammation. This is the largest study on patients with idiopathic SFNs that has been published in the literature so far. Except for skin biopsies that had to show positive results as an inclusion criterion and were therefore obtained before study participation, all examinations were performed by the same experienced investigators. The main limitation of this study was, indeed, the lack of a control group that showed negative results for biopsy. We were thereby not able to calculate sensitivities or specificities for the used diagnostic tests, which has, albeit, been done before.8 The purpose of this study was rather to characterize patients with idiopathic small fiber neuropathies, to determine subphenotypes, and to correlate these with potential biomarker constellations.

In concordance with the literature,4,18,19,22,40,45 our data showed that first manifestations, leading symptoms, progression patterns, and localizations vary distinctly. These results reflect the symptomatic heterogeneity of SFN even within strictly preselected, idiopathic patients.

Neuropathic pain was the most common first manifestation of SFNs with a tendency to become even more frequent with a progressive course and more severe with a longer disease duration. It was significantly associated with daily life impairment. The painDETECT score was significantly higher in patients with C fiber dysfunction in the QST, but did not correlate with the IENFD in distal skin biopsies. This supports the hypothesis that neuropathic pain, the key symptom of SFN, might be more closely related to small fiber dysfunction than degeneration. Reported by Woolf, abnormal sensory afferent fiber input is the prerequisite for an impaired peripheral nociceptive drive to spinal cord projection neurons to the brain.48 Such wide dynamic range (WDR) projection neurons may sensitize on this ongoing input, resulting in a facilitated synaptic transmission and lowered threshold of these central neurons.

Furthermore, the IENFD did not correlate with any other symptom symptom categories; neither did sweat gland innervation correlate with subjective hypohydrosis or measured sudomotor function. Contrarily, these symptom patterns were more precisely reflected by clinical examinations and QST, further supporting that SFN symptoms are better explained by small fiber dysfunction than degeneration. Skin biopsies, required to show small fiber degeneration as an inclusion criterion in this study, have previously been discussed as a silver standard for SFN.45 Throughout the literature, they range notably in sensitivity (58%-94%) and specificity (64%-92%).1,8,23,30 depending on the respective IENFD cutoff values. As a potential confounder, skin biopsies constitute a histological snapshot, representing a very local and momentary degeneration status only, additionally taking into account that one third of the biopsies was not evaluated at our center. The functional significance of small fiber dysfunction rather than degeneration is also supported by the fact that disease duration did not correlate with reduced IENFD, whereas longer courses were associated with more frequently reported sensory loss. It is conceivable that a shift from plus to minus symptoms occurs with disease progression.

Autonomic symptoms did not correlate with objective parameters such as body mass index or electrochemical skin conductance. Being subjectively experienced and situation-dependent, objective measures for autonomic symptoms are known to be limited. Interestingly, the Sudoscan showed an abnormal electrochemical skin conductance in only 27% of the examined patients. In comparison with diabetic neuropathies,3 this number is relevantly lower. This strengthens our hypothesis that certain etiologies might correlate with clinical patterns and that diabetic SFN might differ from idiopathic ones to some extent.

Fibromyalgia was a concomitant diagnosis in 11% of our patients, which due to overlapping symptoms and discussed disease mechanisms merits to be mentioned as a potential limitation. Contrarily, one could argue that the described patient collective is representative for what has been observed throughout the literature: patients with SFN tend to have a higher prevalence of fibromyalgia, and patients with fibromyalgia frequently show signs of small fiber pathology. Whether these are completely distinct diagnoses or whether there is a spectrum with some overlap seems to be controversial and is not in the focus of this work.

Known to be neurotoxic in hereditary sensory and autonomic neuropathy (HSAN) type 1,34 1-deoxy-sphingolipids have previously been demonstrated to inhibit axonal outgrowth. Even without mutations in SPTLC1 or SPTLC2, 2 genes encoding for the serine palmitoylCoA-transferase, the synthesis of sphingoid bases can be shifted toward an overproportioned use of L-alanine instead of L-serine, which results in the lack of 1 hydroxyl group in position 1 that is essential for further metabolism and degradation. This is favored when L-serine is lacking13,28 or L-alanine, the main gluconeogenic amino acid, is overabundant in conditions such as diabetes mellitus.2 In the patient cohort studied here, plasma 1-deoxy sphingolipids were significantly higher in the sensory loss cluster and showed a significantly
inverse correlation with the distal IENFD. Looking at features of metabolic syndrome other than diabetes mellitus, 1-deoxy-sphingoid bases were significantly higher in SFN patients with arterial hypertension, hypercholesterolemia, and overweight. Considering that 1-deoxy sphingolipids have been previously described as markers of the metabolic syndrome and that metabolic syndrome is a risk factor of other prediabetic axonal neuropathy subtypes as well, we hypothesized that they promote nerve fiber degeneration and might therefore be held responsible for a subgroup of SFNs so far considered idiopathic. In this patient collective, diabetes mellitus was ruled out by measuring the percentage of glycated haemoglobin. We admit that although HbA1c levels were all within the range of normal, an impaired glucose tolerance was not explicitly excluded by oral glucose tolerance testing.

Plasma 1-deoxy-sphingolipids correlated inversely with L-serine/L-alanine ratios in plasma. A functional lack of the former and overabundance of the latter, potentially associated with changes in hepatic metabolism, might be a possible factor contributing to the development of SFNs that merits further investigation in the future. The inverse association between plasma serine/alanine and 1-deoxy-sphingolipid levels was also reported recently in the context of the rare eye disease macular telangiectasia type 2 and in cancer. Patients with primary serine biosynthetic defects often manifest with intellectual disability, microcephaly, ichthyosis, seizures, and peripheral neuropathy. In addition, these patients showed significantly elevated plasma 1-deoxy-sphingolipids. Increasing serine availability by oral supplementation in the context of a therapeutic intervention has already been shown to improve the course of HSAN1. Consequently, 1-deoxy-sphingolipids might not only contribute to pathophysiological understanding of SFN, but also become a therapeutic target in the future.

Vitamin C is an essential cofactor for collagen formation and myelination. As an antioxidant, it functions as an electron donor in

Figure 3. 1-Deoxy sphingolipid bases and characteristics of metabolic syndrome. Within this idiopathic small fiber neuropathy cohort (n = 100), plasma levels of the neurotoxic sphingoid base 1-deoxy-sphinganine (1-deoxySA) correlated inversely with the intraepidermal nerve fiber density measured in skin biopsies from the distal lower limbs (A). Representing markers of metabolic syndrome, 1-deoxySA levels were significantly higher in patients with arterial hypertension (B) and correlated inversely with HDL cholesterol levels in plasma (C). Considering that 1-deoxy-sphingolipids are derived from alanine instead of serine, we found a significant correlation between 1-deoxySA levels and the serine/alanine ratio in plasma (D). Accordingly, serine/alanine ratios were significantly lower in patients with low HDL cholesterol levels (E) or arterial hypertension (F). Altogether, this suggests that 1-deoxySA levels increase by a misbalance in L-serine/L-alanine levels, which is most likely associated with (beginning) metabolic syndrome. HDL, high-density lipoprotein; IENFD, intraepidermal nerve fiber density.
redox reactions, thereby playing a protective role in obesity and metabolic syndrome. In a recent study including 120 patients with postherpetic neuralgia, vitamin C levels correlated negatively with several sensory plus symptoms, suggesting a protective effect on C and Aβ nerve fibers. In our idiopathic SFN cohort, we found reduced vitamin C levels in 10 patients. Overall vitamin C levels correlated inversely with the mechanical detection threshold, which is a marker for Aβ nerve fiber dysfunction. Accordingly, a negative correlation between the sensory loss cluster and vitamin C levels was shown. Interlinking neuropathy and vitamin C with its protective role in metabolic syndrome and oxidative stress, vitamin C levels were significantly lower in patients with arterial hypertension. No such correlation was observed with plasma lipids and other oxidative stress markers such as selenium and glutathione. By contrast, serum levels of reduced glutathione were significantly lower in patients with signs of Aδ nerve fiber dysfunction, adding to the hypothesis that oxidative stress might primarily contribute to the development of small fiber neuropathy, whereas lower vitamin C levels might promote myelinated fiber involvement, independently.

Similar to oxidative stress, inflammation is an important mechanism of nerve damage. Transforming growth factor beta is one of the most common anti-inflammatory cytokines down-regulating TNF-α levels and favoring the maturation of regulatory T cells. In this cohort, we found reduced TGF-β serum levels in 10 patients and significantly lower levels in patients with a moderately or severely reduced IENFD (Fig. 4). There was no significant correlation with TNF-α levels, which were elevated in 15 patients overall. Reduced TGF-β serum levels have previously been described in patients with complex regional pain syndrome, another disorder defined partly by neuropathic pain, whereas serum TNF-α level was found to be elevated and its gene expression upregulated in skin biopsies obtained from patients with SFNs. Of interest, these proinflammatory serum constellations did not cluster in patients with a relapsing disease course or diffuse and discontinuous distribution, which were both assumed possible features of autoimmune neuropathy. Similar to 1-deoxy-sphingolipids and vitamin C, TNF-α and TGF-β have previously been described to play a role in obesity and metabolic syndrome. In this cohort, however, they did not correlate with any of the aforementioned metabolic markers. We conclude that inflammation might be (partially) responsible for or contribute to the pain pattern at least in a subgroup of idiopathic SFNs.

5. Conclusions

Metabolic syndrome, oxidative stress, and inflammation are closely interlinked. Each of these, and especially a combination, can contribute to nerve damage. In this study, we showed that neurotoxic 1-deoxy-sphingolipids and vitamin C, TNF-α and TGF-β have previously been described to play a role in obesity and metabolic syndrome. In this cohort, however, they did not correlate with distinct clinical patterns in this cohort.

For patients, families, and caregivers, idiopathic SFNs entail a relevant daily life burden. To improve both cure and care for these patients, 3 main problems need to be addressed in the future: the lack of pathophysiological understanding, the lack of specific treatment, and the lack of diagnostic gold standards, all increasing the risk of chronification. Further studies are needed to fill these gaps.

Figure 4. Serum vitamin C, reduced glutathione, and TGF-β in correlation with subphenotypes. Serum levels of vitamin C were significantly lower in patients with a disturbed Aβ nerve function (A) and reduced glutathione significantly lower in individuals with Aδ involvement (B). Normative values are indicated by horizontal lines. Patients with a more pronounced nerve fiber degeneration, indicated by a lower intraepidermal nerve fiber density in distal skin biopsies, tended to show lower TGF-β levels in serum, which correlated significantly (C). TGF-β, transforming growth factor β.
Conflict of interest statement
AO, CD, MZ, SN, TH, and VE have no conflicts of interest to declare. ALa has a research agreement with Hoffmann – La Roche and has received speaker fees or honoraria for counseling services from Grünenthal. BG received financial support from Pfizer, Grifols, and Bayer for conference contributions. JBS serves at advisory boards for Biogen and Roche. JV has received consultancy fees from Vertex Pharmaceuticals. MFD received financial reimbursement for consulting and advisory board activities and travel support to attend scientific meetings by Akcea Therapeutics Inc., Alnylam Pharmaceuticals Inc., Amicus Therapeutics, and Pfizer Pharmaceuticals. MFD further received research funding by Pfizer Pharmaceuticals (ASPIRE 2018). RR has received speaker fees or honoraria for counseling services from the following companies: Aristo Pharma, Grünenthal, Lilly & Company, Pfizer, Tilray Germany, and Spectrum Therapeutics.

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Appendix A. Supplemental digital content
Supplemental digital content associated with this article can be found online at http://links.lww.com/PAIN/S562.

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References
[1] Baron R, Maier C, Attal N, Binder A, Bouhassira D, Cruccu G, Finnerup NB, Haanpää M, Hansson P, Höllermann P. Peripheral neuropathic pain: a mechanism-related organizing principle based on sensory profiles. PAIN 2017;158:261.
[2] Bertea M, Rütli MF, Othman A, Marti-Jaun J, Hersberger M, von Eckardstein A, Brown RH, Hommerm T, Eichler FS. Oral L-serine supplementation reduces production of neurotoxic deoxysphingolipids in mice and humans with hereditary sensory autonomic neuropathy type 1. J Clin Invest 2011;121:4735–45.
[3] Casellini CM, Parson HK, Richardson MS, Nevoret ML, Vink AI. Sudoscan, a noninvasive tool for detecting diabetic small fiber neuropathy and autonomic dysfunction. Diabetes Techno Ther 2013;15:948–53.
[4] de Greef B, Hoeijmakers JG, Faber CG, Lauria G, Mercier L. Small-fiber neuropathies—advances in diagnosis, pathophysiology and management. Nat Rev Neurosci 2012;8:369.
[5] Hoitsma E, Reulen J, de Baets M, Drent M, Spaans F, Faber C. Small-fiber neuropathy: a common and important clinical disorder. J Neurosci 2004;24:227–119.
[6] Hube L, Dohrn MF, Karsai G, Himsharn S, Van Damme P, Schulz JB, Weis J, Hommerm T, Claes KG. Metabolic syndrome, neurotoxic 1-deoxysphingolipids and nervous tissue inflammation in chronic idiopathic axonal polyneuropathy (CIAP). PLoS One 2012;7:e107583.
[7] Jin HY, Park TS. Role of inflammatory biomarkers in diabetic peripheral neuropathy. J Diabetes Investig 2019;10:1016.
[8] Laconis DJM, Medicine NOJotAAoE. Small-fiber neuropathies—advances in diagnosis, pathophysiology and management. Nat Rev Neurosci 2012;8:369.
[9] Lee M. Transforming growth factor beta superfamily regulation of adipose tissue biology in obesity. Biochim Biophys Acta Mol Basis Dis 2018;1864:11607.
[10] Magerl W, Krumova EK, Baron R, Tölle T, Treede R-D, Muellg C. Reference values for the painDETECT project—far more than a screening tool on neuropathic pain. Curr Med Res Opin 2016;32:1033–57.
[11] Маер С, Ролке Р, Габриел Н, Нейман Р, Френоц Ф, Актель А, Бирбауер Н, Биркин Ф. Неопатия 1-деоксисфинголипидов и нервное ткань воспаление в хронической идиопатической амнерической амнеопатии (CAIP). PLoS One 2012;7:e107583.
[12] Maier C, Attal N, Birbaumer N, Birklein F, Gierthmühlen W, Weis J, Schulz J, Hornemann T. Elevation of plasma 1-deoxysphingolipids in type 2 diabetes mellitus: a susceptibility to neuropathy? Eur J Neurol 2015;22:806–14, e55.
[13] Dohrn MF, Lampert A, Ueckeyer N, Kurth ULI. Neuropathic pain syndromes and channelopathies. Internist 2019;60:90–7.
[14] Fabry V, Gerdalit A, Acket B, Cintas P, Brousseau U, Vero-Coste E, Euvard SM, Pavvy-Le Tron A. Which method for diagnosing small fiber neuropathy? Front Neurosci 2020;11:342.
[15] Ferreira C, Gooberd S, Soldatos A, Byers H, Ghaouarhlai-van der Vlugt J, Beers-Stef F, Groden C, van Karnebeck C, Gahl W, Vaz F. Deoxysphingolipid precursors indicate abnormal sphingolipid metabolism in individuals with primary and secondary disturbances of serine availability. Mol Genet Metab 187;124:2049.
[16] Freeman R, Gerdalit JS, Faber CG, Gibbons C, Haroutounian S, Laura G, Levine T, Malik RA, Singleton JR, Smith AM. Idiopathic distal sensory polyneuropathy: ACTTION diagnostic criteria. Neurology 2020;95:1005–14.
[17] Freynhagen R, Tollé TR, Goeckel U, Baron R. The painDETECT project far more than a screening tool on neuropathic pain. Curr Med Res Opin 2016;32:1033–57.
[18] Fridman V, Surinavanyarayanan S, Novak P, David W, Macklin EA, McKenna-Yasek D, Walsh K, Aziz-Bose R, Oaklander AL, Brown R. Randomized trial of L-serine in patients with hereditary sensory and autonomic neuropathy type 1. Neurology 2019;92:e3599.
[19] Gantner ML, Eade K, Wallace M, Handzik MK, Fallon R, Trombley J, Bonelli R, Giles S, Harkins-Perry S, Heeren TF, Serine and lipid metabolism in macular disease and peripheral neuropathy. N Engl J Med 2019;381:1422–33.
[20] Gess B, Baets J, De Jonghe P, Peilby MM, Parreyson D, Young P, Ascorbic acid for the treatment of Charcot-Marie-Tooth disease. Cochrane Database Syst Rev 2015;12:CD011952.
[21] Gess B, Röhr D, Feldrich R, Sereda MW, Klettner I, Humburg A, Nowitzki J, Stricker J-K, Halffter H, Young P, Sodium-dependent vitamin C transporter 2 deficiency causes hypomyelination and extracellular matrix defects in the peripheral nervous system. J Neurosci 2011;31:17180–92.
[22] Hoeijmakers JG, Faber CG, Lauria G, Mercier L, Waxman SG. Small-fiber neuropathies—advances in diagnosis, pathophysiology and management. Nat Rev Neurosci 2012;8:369.
[23] Hoitsma E, Reulen J, de Baets M, Drent M, Spaans F, Faber C. Small fiber neuropathy: a common and important clinical disorder. J Neurosci 2004;24:227–119.
[24] Hube L, Dohrn MF, Karsai G, Himsharn S, Van Damme P, Schulz JB, Weis J, Hommerm T, Claes KG. Metabolic syndrome, neurotoxic 1-deoxysphingolipids and nervous tissue inflammation in chronic idiopathic axonal polyneuropathy (CIAP). PLoS One 2012;7:e107583.
[25] Jin HY, Park TS. Role of inflammatory biomarkers in diabetic peripheral neuropathy. J Diabetes Investig 2019;10:1016.
[26] Laconis DJM, Medicine NOJotAAoE. Small-fiber neuropathies—advances in diagnosis, pathophysiology and management. Nat Rev Neurosci 2012;8:369.
[27] Hoitsma E, Reulen J, de Baets M, Drent M, Spaans F, Faber C. Small fiber neuropathy: a common and important clinical disorder. J Neurosci 2004;24:227–119.
[28] Hube L, Dohrn MF, Karsai G, Himsharn S, Van Damme P, Schulz JB, Weis J, Hommerm T, Claes KG. Metabolic syndrome, neurotoxic 1-deoxysphingolipids and nervous tissue inflammation in chronic idiopathic axonal polyneuropathy (CIAP). PLoS One 2012;7:e107583.
[29] Jin HY, Park TS. Role of inflammatory biomarkers in diabetic peripheral neuropathy. J Diabetes Investig 2019;10:1016.
abnormalities in 1236 patients with different neuropathic pain syndromes. PAIN 2010;150:439–50.

[28] Muthusamy T, Cordes T, Handzik MK, You L, Lim EW, Gengatharan J, Pinto AF, Badur MG, Kolar MJ, Wallace M. Serine restriction alters sphingolipid diversity to constrain tumour growth. Nature 2020;586:790–5.

[29] Mwinyi J, Boström A, Fehrer I, Othman A, Waeb er G, Marti-Soler H, Vollenweider P, Marques-Vidal P, Schröth HB, Von Eckardstein A. Plasma 1-deoxysphingolipids are early predictors of incident type 2 diabetes mellitus. PLoS One 2017;12:e0175776.

[30] Nebuchennykh M, Løseth S, Lindal S, Mellgren S. The value of skin biopsy with recording of intraepidermal nerve fiber density and quantitative sensory testing in the assessment of small fiber involvement in patients with different causes of polyneuropathy. J Neurol 2009;256:1067.

[31] Othman A, Bianchi R, Alecu I, Wei Y, Porretta-Serapiglia C, Lombardi R, Chiorazzi A, Meregalli C, Oggoni N, Cavaletti G. Lowering plasma 1-deoxysphingolipids improves neuropathy in diabetic rats. Diabetes 2015;64:1035–45.

[32] Othman A, Rütti MF, Ernst D, Saely CH, Rein P, Drexl H, Porretta-Serapiglia C, Lauria G, Bianchi R, von Eckardstein A. Plasma deoxysphingolipids: a novel class of biomarkers for the metabolic syndrome? Diabetologia 2012;55:421–31.

[33] Passage E, Norreel JC, Noack-Fraissignes P, Sanguedolce V, Pizant J, Thirion X, Robaglia-Schlupp A, Pellissier JF, Fontes M. Ascorbic acid treatment corrects the phenotype of a mouse model of Charcot-Marie-Tooth disease. Nat Med 2004;10:396–401.

[34] Penno A, Reilly MM, Houlden H, Laurà M, Rentsch K, Niederkofler V, Stoeckli ET, Nicholson G, Eichler F, Brown RH. Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids. J Biol Chem 2010;285:11178–87.

[35] Peters MJ, Bakkers M, Merkies IS, Hoeijmakers JG, van Raak EP, Faber C. Incidence and prevalence of small-fiber neuropathy: a survey in the Netherlands. Neurology 2013;81:1356–60.

[36] Pop Busui R, Sima A, Stevens M. Diabetic neuropathy and oxidative stress. Diabetes Metab Res Rev 2006;22:257–73.

[37] Reimer M, Forstenpointner J, Hartmann A, Otto JC, Vollert J, Gierthmühlern J, Klein T, Hüllmann P, Baron R. Sensory bedside testing: a simple stratification approach for sensory phenotyping. PAIN Rep 2020;5:e820.

[38] Rolke R, Baron R, Mai er CA, Töte L, Treede R-D, Beyer A, Binder A, Birbaumer N, Birkl ein F, Böteführ I. Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): standardized protocol and reference values. PAIN 2006;123:231–43.

[39] Roththier A, Auer-Grumbach M, Janssens K, Baets J, Penno A, Almeida-Souza L, Van Hoof K, Jacobs A, De Vriendt E, Schlotter-Weigel B. Mutations in the SPTLC2 subunit of serine palmitoyltransferase cause hereditary sensory and autonomic neuropathy type I. Am J Hum Genet 2010;87:513–22.

[40] Sopacua M, Højemakers JGJ, Merkies ISJ, Lauria G, Waxman SG, Faber CG. Small-fiber neuropathy: expanding the clinical pain universe. J Peripher Nerv Syst 2019;24:19–33.

[41] Üçeyler N, Eberle T, Rolke R, Birkl ein F, Sommer C. Differential expression patterns of cytokines in complex regional pain syndrome. PAIN 2007;132:195–205.

[42] Üçeyler N, Kafse W, Riediger N, He L, Necula G, Toyka K, Sommer C. Elevated proinflammatory cytokine expression in affected skin in small fiber neuropathy. Neurology 2010;74:1806–13.

[43] Vollert J, Magerl W, Baron R, Binder A, Enax-Krumova EK, Geisslinger G, Gierthmühen J, Henrich F, Hüllmann P, Klein T. Pathophysiologial mechanisms of neuropathic pain: comparison of sensory phenotypes in patients and human surrogate pain models. PAIN 2018;159:1090–102.

[44] Vollert J, Maier C, Attal N, Bennett DL, Bouhassira D, Enax-Krumova EK, Finnerup NB, Freynhagen R, Gierthmühen J, Haanpää M. Stratifying patients with peripheral neuropathic pain based on sensory profiles: algorithm and sample size recommendations. PAIN 2017;158:1446.

[45] Voortman M, Fritz D, Vogels O, Eftimov F, van de Beek D, Brouwer MC, Drent M. Small fiber neuropathy: a disabling and underrecognized syndrome. Curr Opin Pulm Med 2017;23:447.

[46] Wang L-K, Lin Y-T, Hung K-C, Chang C-Y, Wu Z-F, Hu M-L, Chen J-Y. Plasma vitamin C Concentrations were negatively associated with tingling, prickling or pins and needles sensation in patients with postherpetic neuralgia. Nutrients 2020;12:E2384.

[47] Weis J, Katona I, Müller-Newen G, Sommer C, Necula G, Hendrich C, Ludolph A, Sperfeld A-D. Small-fiber neuropathy in patients with ALS. Neurology 2011;76:2024–9.

[48] Woolf C. Evidence for a central component of post-injury pain hypersensitivity. Nature 1983;306:686–8.

[49] Ziegler D, Sohr CG, Nourooz-Zadeh J. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. Diabetes Care 2004;27:2178–83.