Increased levels of inflammatory factors are associated with severity of polyneuropathy in type 1 diabetes

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Conflict of Interest:
The authors declare that there is no conflict of interest associated with this manuscript.

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ABSTRACT
**Objective:** Distal symmetrical polyneuropathy (DSPN) is a severe common long-term complication of type 1 diabetes caused by impaired sensory-motor nerve function. As chronic low-grade inflammation may be involved in the pathogenesis of DSPN, we investigated the circulating levels of inflammatory markers in individuals with type 1 diabetes with and without DSPN. Furthermore, we determined to what extent these factors correlated with different peripheral sensory nerve functions.

**Design:** Cross-sectional study

**Patients:** The study included 103 individuals with type 1 diabetes with (n=50) and without DSPN (n=53) as well as a cohort of healthy controls (n=21).

**Measurements:** Circulating levels of various inflammatory markers (cytokines, chemokines and soluble adhesion molecules) were determined in serum samples by Luminex multiplexing technology. Peripheral sensory nerve testing e.g. vibration, tactile and thermal perception was assessed by standardised procedures.

**Results:** The cytokines IL-1α, IL-4, IL-12p70, IL-13, IL-17A, TNF-α, the chemokine MCP-1, and the adhesion molecule E-selectin were significantly increased in individuals with type 1 diabetes with DSPN compared to those without DSPN (p<0.001). These observations were independent of age, sex, BMI, disease duration and blood pressure. Additionally, higher serum concentrations of cytokines and chemokines were associated with higher vibration and tactile perception thresholds, but not with heat tolerance threshold.

**Conclusions:** Individuals with type 1 diabetes and concomitant DSPN display higher serum levels of several inflammatory markers. These findings support that systemic low-grade inflammation may play a role in the pathogenesis of DSPN

**INTRODUCTION**

Globally, diabetes is estimated to affect over 450 million individuals, and a substantial increase in prevalence is expected over the next decades ¹. Distal symmetrical polyneuropathy (DSPN) is the most common complication affecting up to 50% of individuals with long-term type 1 diabetes ². In the initial stages, DSPN is characterized by impairments of the sensory function caused by damages to small and partially myelinated nerve fibres. This typically presents as paraesthesia and numbness in a so-called stocking-glove distribution. The condition gradually affects a larger
proportion of the peripheral nerves and the symptoms advance in a length-dependent distal-to-proximal manner. Although the progression of DSPN is slow and subtle, it is currently inevitable, irreversible, and with no curative treatment options.

Despite intense research, the underlying pathogenic mechanisms of DSPN remain largely unclear. However, evidence points toward pro-inflammatory pathways, brought about by metabolic alterations associated with hyperglycaemia as a pivotal facilitator of neurotoxicity. These pathways include activation of protein kinase C as well as the polyol and hexosamine pathway, which contribute to oxidative stress and chronic low-grade inflammation.

Biomarkers of ongoing inflammatory processes include cytokines, chemokines and adhesion molecules, all of which are key players in the immune response. We hypothesized that individuals with type 1 diabetes and DSPN presented higher levels of circulating inflammatory markers compared to type 1 diabetes subjects without DSPN and healthy. Furthermore, we aimed to test if increased serum concentrations of inflammatory factors are associated with severity of peripheral sensory nerve dysfunction.

**MATERIALS AND METHODS**

**Study Population**

The cross-sectional study population consisted of participants recruited from the department of endocrinology, originally deriving from two clinical studies, performed at our research unit at Aalborg University Hospital, Denmark: Cohort 1 (total n=56); a cross-sectional study, investigating cardiovascular and autonomic complications in diabetic autonomic neuropathy, and cohort 2 (total n=48); baseline date from a double-blinded, randomized controlled trial (RCT) investigating the potential neuroprotective effects of the glucagon-like peptide-1 (GLP-1) agonist liraglutide (NCT-02138045). Both studies were conducted in accordance with the Declaration of Helsinki, local regulations and International Conference on Harmonization Good Clinical Practice guidelines. The protocol, amendments and informed consent form for both studies were approved by The North Denmark Region Committee on Health Research Ethics, Denmark (N-20170045 and N-20130077). Furthermore, the RCT study was approved by the Danish Health and Medicines Authority (EudraCT No.: 2013-004375-12). All subjects gave their informed consent before participation. Inclusion criteria for both studies included a verified diagnosis of type 1 diabetes as
well as Northern European descent and age above 18 years. The DSPN diagnosis was confirmed by standardized nerve testing including conduction velocity assessments in all participants in cohort 2, and in three participants in cohort 1. A complete list of inclusion and exclusion criteria is provided in the supplementary material (Supp. Table 1). Consequently, to perform this secondary analysis of similarly collected data, we pooled it, which provided the advantage of increased power of the statistical analysis.

In addition to participants with type 1 diabetes, a third cohort including 21 age- and gender-matched healthy controls was included (N-20090008).

Presented results are based on secondary analyses, and data from cohort 2 was based on baseline measurements obtained prior to the study intervention.

e conduction studies was evaluated by use of standardized neurophysiological testing by trained neurophysiologists, according to American Association of Electrodiagnostic Medicine.

Assessments of nerve conduction velocities, amplitudes and F-waves were per-
formed on the motor nerves (peroneal, tibial and ulnar nerves) and sensory nerves (sural, radial and median). To avoid influence of skin-temperature on the conduction velocity, appropriate warming measures were used to ensure that the testing room did not allow skin temperatures below 32°C. Spring-ring electrodes were used to record digital sensory nerve action potential. A plastic bar electrode was used for all other nerves. The results were processed according to reference values accepted by our EMG laboratories. To evaluate
the severity of large fibre neuropathy, a composite score consisting of numerical ratings from 5 components of the sural, peroneal and tibial nerve assessment was used (range 0–15), where a total of 3 points indicate DSPN.

*Standardized clinical nerve testing*

Standardized neurophysiological nerve testing studies was conducted and evaluated by trained neurophysiologists, according to American Association of Electrodiagnostic Medicine. Consequently, assessments of nerve conduction velocities, amplitudes and F-waves were performed on the motor nerves (peroneal, tibial and ulnar nerves) and sensory nerves (sural, radial and median). To avoid the influence of skin-temperature on the conduction velocity, appropriate heating and warming measures were used to ensure skin temperatures above 32°C. The results were processed according to reference values accepted by our EMG laboratories, which were used to verify the diagnose of DSPN according to the Toronto criteria.

*Quantitative Sensory Testing of Peripheral Nerves*

The sensory response to quantitative sensory testing of the peripheral nerves was investigated by three different tests. I) Vibration perception threshold was determined using a biothesiometer (Bio-Medical Instruments, Newbury, OH, USA), which allows for application of a vibration stimulus to the skin by gradually increasing amplitudes up to a maximum of 50 volts. The stimulus was applied three times consecutively to the dorsum of the first phalanx on both feet. Subjects were instructed to report when the vibration was first perceived, and the average of the three
readings was calculated. The final vibration perception threshold for analysis was derived as the average of readings from both feet. For investigation of II) tactile perception thresholds, Von Frey monofilaments (Optihair von Frey Filaments, MARSTOCK nervtest, Marburg, Germany) were used. Increasing forces (from 0.008g to 300g) were applied to the skin and subjects were instructed to report when the stimuli was first perceived. For cohort 1, tactile perception threshold was assessed on the planar side of the metatarsophalangeal joint of the big toe, and for cohort 2, at the skin of the forearm. III) Heat tolerance threshold was determined by application of thermal stimuli to the skin of the forearm by a thermofoil thermode stimulator (Pathway, Medoc Ltd, Ramat Yishai, Israel). The temperature was increased until participants reached their individual heat tolerance threshold or until a maximum of 52°C. The process was repeated three times and the average score used for further analyses.

In cohort 2, presence of DSPN was verified by nerve conduction velocity testing of the sensory and motor components of the median, ulnar, sural, radial, tibial and peroneal nerves as previously described.

Serum Concentrations of Inflammation Markers

Fasting blood samples were collected from the cubital vein of study participants. Subsequently, samples were centrifuged, and isolated serum was aliquoted in appropriate volumes. Aliquots were stored at -80°C and thawed just prior to analyses. Concentrations of the cytokines interleukin (IL) 1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17A, interferon (IFN) α and γ, and tumor necrosis factor α (TNF-α), as well as chemokines C-X-C motif chemokine 10 (CXCL10), monocyte chemoattractant protein 1 (MCP-1), C-C motif chemokine 3 (CCL3), CCL4, granulocyte-macrophage colony-stimulating factor (GM-CSF), and lastly soluble cell adhesion molecules (CAM) intracellular CAM-1 (ICAM-1), E-selectin, and P-selectin were analysed in duplicates using the Inflammation 20-Plex Human ProcartaPlex™ Panel (Invitrogen™, Thermo Fischer Scientific, Cat. No. EPX200-12185-901) according to the instructions of the manufacturer. Readings below detection level were replaced with values equal to the detection level value divided by the square root of 2. Readings with a value of zero or with a coefficient of variation (CV) above 20% between duplicates were excluded from the dataset (Supp. Table 2). To account for possible short-term rises in circulating inflammatory markers not related to diabetes, readings above 3 standard deviations (SD) from the sample mean were excluded from the dataset.
Table 2). Serum samples from all three cohorts were measured together to limit inter-plate variability.

**Statistics**
We performed secondary analyses on similarly collected data. Data distribution was assessed using Shapiro-Wilks Test of Normality. In the attempt of achieving normal distribution, non-normally distributed data was log-transformed prior to further analyses. For comparisons among groups, one-way ANOVA or the non-parametric Kruskal-Wallis Test by Ranks were employed as appropriate based on distribution of the data. Post hoc analyses were performed using either Bonferroni Correction or Dunn’s Multiple Comparison Test.

Associations between levels of inflammation markers and the presence of DSPN were adjusted for age, sex, body mass index (BMI), disease duration and blood pressure using binominal logistic regression with DSPN as the dependent variable. Outliers were excluded from the data set prior to regression analyses. Based on the logistic regression analyses, receiver operating characteristic (ROC) curves were plotted in order to evaluate the sensitivity, specificity, and area under curve (AUC) of inflammation markers as biomarkers of DSPN. Correlations between serum concentrations of inflammatory markers and functional measures of peripheral nerve function were investigated by Spearman’s Rank Correlation Coefficient.

*p-values* ≤ 0.05 were considered statistically significant in all analyses. All statistical analyses were performed using STATA software (StataCorp LLC, version 15.1).

**RESULTS**

**Study Population**
In total, cohorts 1 and 2 contained 104 individuals with type 1 diabetes. Of these, 56 participants from cohort 1 were allocated to the group without presence of DSPN (hereafter referred to as the T1D group) and 48 participants from cohort 2 were allocated to the group with DSPN (hereafter referred to as the T1D+DSPN group). Furthermore, one subject was excluded from the data set due to daily treatment with Methotrexate. The final distribution of subjects in groups was hereafter: Healthy (n=21), T1D (n=53), and T1D+DSPN (n=50). Demographic and clinical characteristics of the three groups are presented in Table 1.
Associations between Circulating Inflammatory Markers and Diabetic Polyneuropathy

By Luminex multiplexing technology, we successfully measured thirteen inflammatory markers in serum samples; six cytokines (IL-1α, IL-4, IL-12p70, IL-13, IL-17A, and TNF-α), four chemokines (CXCL10, MCP-1, CCL3, and CCL4), and three adhesion molecules (ICAM-1, E-selectin and P-selectin). IL-1α, IL-4, IL-13, and CCL4 were detected in 68%, 64%, 48%, and 67% of the samples, respectively. The remaining nine detectable markers were detected in 100% of the samples (Supp. Table 2).

For all cytokines, the serum concentrations were significantly higher in subjects with type 1 diabetes and presence of DSPN compared to subjects without DSPN ($p<0.001$) (Figure 1A). One of the four chemokines, MCP-1, was significantly elevated in the T1D+DSPN group ($p<0.001$) (Figure 1B). The remaining three showed no differences between groups. Likewise, the concentration of one out of three detectable adhesion molecules, namely E-Selectin, was significantly higher in the T1D+DSPN group ($p<0.001$), whereas the remaining two revealed no differences between groups (Figure 1C). All eight inflammatory markers that were present in significantly higher concentrations in sera from DSPN subjects remained significantly associated with DSPN after adjustments for age, sex, BMI, disease duration and blood pressure (Table 2). Sensitivity and specificity of these eight inflammatory markers in recognizing presence of DSPN were all above 75%, with IL-12p70 having the largest AUC (0.93) (Figure 2).

Associations between Circulating Inflammatory Markers and Peripheral Nerve Function

To explore possible associations between inflammation markers and the functionality of different sensory nerve fibres in diabetes, serum concentrations of the markers were correlated with three types of sensory stimuli perception abilities (Table 3). For vibration perception threshold, significant and positive correlations were found for four out of six cytokines (IL-1α, IL-4, IL-12p70, and IL-17A) in the T1D+DSPN group. In the T1D group, IL-1α was also correlated with vibration perception threshold. Likewise, tactile perception threshold was positively correlated with IL-1α in type 1 diabetes both with and without DSPN. Additionally, IL-4 and IL-17A showed a positive correlation with tactile perception threshold in the T1D+DSPN group. One (CXCL10) and two (MCP-1 and CCL3) chemokines were positively correlated with tactile perception threshold in the T1D and T1D+DSPN groups, respectively. No significant correlations between adhesion molecules and vibration perception threshold or tactile perception threshold were
observed. Regarding heat tolerance threshold, IL-1α in the T1D group and P-selectin in the T1D+DSPN groups revealed positive correlations. These results suggest that the serum levels of several inflammatory factors correlate with severity of DSPN in individuals with type 1 diabetes.

**DISCUSSION**

In this hypothesis-generating study, we showed that individuals with type 1 diabetes and DSPN displayed increased levels of systemic inflammatory markers compared to diabetes without DSPN and healthy controls. Furthermore, we showed associations between peripheral sensory testing and specific inflammatory markers, indicating an association between inflammation and severity of damage of tactile neurones.

*Serum Concentrations of Cytokines*

IL-1α, IL-4, IL-13, and CCL4 were detected in less than 70% of the samples, and thus the results regarding levels of these biomarkers, should be interpreted cautiously. The remaining biomarkers were detected in 100% of the samples, which is specified (Supp. Table 2). Nevertheless the performance for each of these biomarkers were evaluated by AUROC, and it evident that the ability to categorize the presence or non-presence of DSPN by the measured biomarkers are very good despite unequal numbers of samples. In the present study, all detected cytokines (IL-1α, IL-4, IL-12p70, IL-13, IL-17A and TNF-α) were significantly increased in individuals with type 1 diabetes and confirmed DSPN compared to subjects without DSPN. These cytokines exert primarily pro-inflammatory effects except for IL-13 and IL-4, which also possess anti-inflammatory properties under some circumstances. The observed increase of pro- and anti-inflammatory cytokines may be a sign of compensatory mechanisms of the immune system aimed at balancing the immune activity. However, such protective mechanisms may fail in the presence of factors such as chronic oxidative stress, consequently leading to a pathological and systemic inflammatory state. It is noteworthy that of all analysed inflammatory factors, only IL-13 was significantly different between the type 1 diabetes group and healthy controls, indicating that the systemic inflammatory state is only modestly changed in long-term type 1 diabetes without DSPN.

In line with the present study, previous studies showed increased pro-inflammatory cytokines in the circulation of both type 1 and type 2 diabetes with DSPN. The most well-established cytokine
associated with diabetic DSPN is TNF-α. Our finding of increased TNF-α in DSPN therefore confirms previous reports. Regarding the investigated interleukins, some have been reported in previous studies to be I) elevated in type 1 diabetic subjects suffering from complications (IL-1α), II) associated with DSPN when present in a frequent genetic polymorphism variant (IL-4), and III) increased in type 2 diabetic subjects with DSPN (IL-12). Taken together, our observation that increased serum levels of inflammatory cytokines are increased in the circulation of DSPN patients, support that chronic low-grade inflammation is associated with the presence of DSPN and may even play a causal role. However, the cross-sectional nature of our data does not allow for investigation of causation. Moreover, it may be speculated that neural degradation in DSPN and subsequent release of damage-associated molecular patterns (DAMPs) may perpetuate an inflammatory response thereby contributing to a chain reaction that sustain a chronic low-grade inflammatory environment.

**Serum Concentrations of Chemokines**

Out of the four chemokines detected in the present study, MCP-1 was significantly increased in type 1 diabetes with confirmed DSPN. TNF-α is known to induce fibroblast production of MCP-1, and it is possible that the increased TNF-α concentration found in type 1 diabetes and DSPN may have induced the observed rise in circulating MCP-1 levels. Furthermore, MCP-1 upregulation has been observed directly in injured peripheral nerves. Whether or not serum concentrations of MCP-1 and concentrations in injured peripheral nerves correlate remain unclear.

**Serum Concentrations of Cell Adhesion Molecules**

We found a significant increase in soluble E-selectin in type 1 diabetes subjects with DSPN compared to both absence of DSPN and healthy controls. In a previous prospective study, 28 subjects with diabetes were followed over a five-year period with measurements of peripheral nerve conduction velocity and serum concentrations of various CAMs at baseline and follow-up. The results of that study showed that elevated serum levels of E- and P-selectin significantly predicted the deterioration rate of peripheral nerve function. However, conflicting results of E-selectin levels in diabetes have been reported in different cross-sectional clinical studies ranging from reports of elevated levels, no detectable differences, to decreased levels compared to non-diabetic subjects.
Although the pathogenesis of DSPN remain largely elusive, it has become widely accepted that pathological changes in the vasa nervorum, is important in the cascade of events leading to the common symptoms associated with DSPN. From the endothelial surface, E-selectin causes arrest of circulating leucocytes thereby facilitate migration from the blood stream into the tissue. In chronic conditions, this may ultimately cause endothelial damage resulting in local hypoxia, which may lead to peripheral nerve damage associated with DSPN.

Several previous studies reported an increase in the serum concentration of soluble ICAM-1 in individuals diabetes both with and without microvascular complications. However, adjustments for influencing factors were only included in some. In our study, a significant association between DSPN and an increased ICAM-1 serum level was found. However, this association was no longer statistically significant after adjustment for influencing factors.

**Influencing Factors**

Age, sex, BMI and blood pressure have previously been shown to be associated with systemic inflammation, DSPN susceptibility or both. Furthermore, disease duration is a plausible influencing factor as well, due to the prolonged exposure to hyperglycaemia. In the present study, however, the differences found for all inflammatory markers between T1D and T1D+DSPN groups, except ICAM-1 and CCL3, remained statistically significant after correction for the adjustment factors mentioned. This supports the assumption that the observed differences in serum concentrations between groups were directly related to the presence of DSPN. Furthermore, ROC curves of these inflammation markers revealed high AUCs, ranging from 0.88 (MCP-1) to 0.93 (IL-12p70), which further supports the hypothesis that elevated serum levels of these substances may be biomarkers of DSPN.

**Correlations between Inflammatory Markers and Peripheral Nerve Function**

Our findings that vibration and tactile perception threshold, but not heat tolerance threshold, are inversely associated with the concentrations of circulating cytokines and chemokines suggest that the level of serum inflammation is a determinant of DSPN severity. The results support a possible preferential neurotoxicity in tactile responsive Aβ-fibres in contrast to the small non-myelinated C-fibres, which convey heat stimuli from free nerve endings in the epidermis to the spinal cord.
This finding, however, contradicts with current literature, which suggests that non-myelinated fibres are susceptible to toxic factors in the extracellular environment due to the lack of protection from surrounding myelin sheaths⁴. Therefore, data should be interpreted with caution, especially because heat tolerance threshold was measured on the forearm as opposed to the foot, where neuropathic alterations would be expected to be more prominent²⁷.

**Limitations**

This study was conducted based on secondary analysis, and therefore some limitations are present. Firstly, a prospective design would have been preferred in order to predict the causality between low-grade inflammation and DSPN. However, by pooling existing data deriving from these clinically well-characterized cohorts, we could perform analysis of the serum samples from all three cohorts at the same time by measuring samples together in random order and thereby limit the inter-plate variability of the used assays. Secondly, the T1D+DSPN cohort was verified with abnormal nerve conduction studies according to the Toronto criteria, whereas the diagnose of no-neuropathy in the T1D cohort was based on the lack of symptoms and clinical suspicion of the presence of DSPN. It could therefore be argued that the presence of DSPN in the T1D group is underestimated and thereby possibly skewing the results. However, the presence of patients with neuropathy in Study 1, would only strengthen our findings, and thus the presented results remain valid. Lastly, even though the presence of exclusion criteria limit the external generalizability because the cohorts are vulnerable to selection bias, it could also be argued that the exclusion criteria emphasizes that the raised level of inflammatory markers in type 1 diabetes with DSPN, was not induced by concomitant elevated blood pressure, other neurological disorders or impaired kidney function.

**Clinical Relevance**

The increasing recognition of neuro inflammation as a potential key player in the pathogenesis of DSPN has recently paved the way for novel experimental therapeutic strategies aimed at dampening inflammatory immune processes²⁸,²⁹. Studies conducted in animal models of DSPN have shown promising results including lower circulating levels of pro-inflammatory cytokines as well as improved nerve conduction velocity after treatment with drugs with anti-inflammatory properties³⁰. In a recent clinical study, we investigated the neuronal function in type 1 diabetes individuals with confirmed DSPN in response to 26 weeks of treatment with the GLP-1 receptor
agonist liraglutide. Liraglutide elicited a significant reduction in IL-6 and showed numerical reductions of other cytokines. However, no improvements in neuronal function was observed following liraglutide treatment. From this it may be speculated whether the increased circulating level of inflammatory cytokines observed in the present study play a causal role in DSPN or whether the increased level is an epiphenomenon. Currently no studies have directly evaluated cytokine-neutralizing strategies to treat DSPN. Our previous study showing a lowering effect of liraglutide on systemic cytokines yet without improving nerve function suggests that cytokines are not causal. However, this may be because the progression of polyneuropathy had reached a late state level of irreversibility in this cohort. Hence, new clinical studies testing anti-inflammatory medications and in particular cytokine-neutralizing drugs to treat DSPN are highly warranted to prove causality of inflammation in neuropathy.

Concluding Remarks
In the present study, we showed that individuals with type 1 diabetes and concomitant DSPN display increased levels of circulating inflammatory markers compared to healthy controls and subjects with type 1 diabetes and no signs of DSPN. These elevations were independent of age, sex, BMI, and disease duration. Furthermore, we observed that decreased vibration perception of the dorsum of the foot, and to a lesser extent tactile perception, were associated with increased levels of several inflammatory markers and particularly with that of IL-1α. These findings support that systemic low-grade inflammation may play a role in the pathogenesis of DSPN. Further studies, including a prospective study design, are wanted to elucidate the causality of a pro-inflammatory state of the blood and DSPN development and progression in diabetes. If such a causality can be confirmed, anti-inflammatory therapeutics may be applied to prevent the development of DSPN in diabetes.
REFERENCES


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### TABLE 1
Demographic and clinical characteristics among groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>T1D</th>
<th>T1D+DSPN</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51.3 ± 6.4</td>
<td>42 (28 – 57)</td>
<td>50.5 ± 8.7</td>
<td>0.002</td>
</tr>
<tr>
<td>Sex (% of females)</td>
<td>28.6</td>
<td>54.7</td>
<td>20.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 (23.7 – 28.0)</td>
<td>25.9 ± 3.8</td>
<td>27.5 (25.0 – 30.5)</td>
<td>0.019</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>19.0</td>
<td>26.5</td>
<td>22.0</td>
<td>0.601</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>-</td>
<td>20 (13 – 31)</td>
<td>32.4 ± 9.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (% (mmol/mol))</td>
<td>33 (33 – 37)</td>
<td>61 (53 – 71)</td>
<td>63.5 (58 – 72)</td>
<td>0.227</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>128.9 ± 14.7</td>
<td>130 (120 – 149)</td>
<td>140.0 ± 15.0</td>
<td>0.021</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>75.8 ± 10.9</td>
<td>73.6 ± 8.1</td>
<td>78.3 ± 8.5</td>
<td>0.005</td>
</tr>
<tr>
<td>Pulse (beats/min)</td>
<td>66.0 ± 6.8</td>
<td>69.8 ± 9.1</td>
<td>74.8 ± 11.3</td>
<td>0.014</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>5.4 ± 0.9</td>
<td>4.4 ± 0.9</td>
<td>4.5 (3.9 – 4.7)</td>
<td>0.729</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.2 ± 0.5</td>
<td>0.8 (0.6 – 1.1)</td>
<td>0.8 (0.6 – 1.1)</td>
<td>0.635</td>
</tr>
<tr>
<td>Vibration perception (V)</td>
<td>-</td>
<td>12 (9.5 – 22.5)</td>
<td>32.5 ± 13.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tactile perception (g)</td>
<td>-</td>
<td>0.7 (0.4 – 1.6)</td>
<td>0.6 (0.16 – 1.4)</td>
<td>0.120</td>
</tr>
<tr>
<td>Heat tolerance (°C)</td>
<td>-</td>
<td>48.9 (46.1 – 50.1)</td>
<td>49.0 (46.8 – 49.6)</td>
<td>0.916</td>
</tr>
</tbody>
</table>

### TABLE 1 LEGEND

Results displayed as either mean ± SD or median (1\textsuperscript{st}-3\textsuperscript{rd} quartiles) based on distribution of the data. Displayed p-values are derived from analyses of differences between the T1D and T1D+DSPN groups. Healthy controls (n=21), T1D (n=53), and T1D+DSPN (n=50). Boldface font indicates statistical significance (p ≤ 0.05).
TABLE 2
Odds ratio for associations between inflammation markers and presence of DSPN among subjects with diabetes

<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>UNADJUSTED</th>
<th>ADJUSTED</th>
<th>p-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
</tr>
<tr>
<td>IL-1α</td>
<td>29.10 (6.12 – 138.43)</td>
<td>&lt;0.001</td>
<td>19.12 (3.22 – 113.56)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.23 (1.12 – 1.35)</td>
<td>&lt;0.001</td>
<td>1.24 (1.09 – 1.41)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>1.09 (1.05 – 1.14)</td>
<td>&lt;0.001</td>
<td>1.12 (1.05 – 1.20)</td>
<td>0.001</td>
</tr>
<tr>
<td>IL-13</td>
<td>1.83 (1.33 – 2.52)</td>
<td>&lt;0.001</td>
<td>1.80 (1.20 – 2.70)</td>
<td>0.005</td>
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<tr>
<td>IL-17A</td>
<td>1.20 (1.09 – 1.31)</td>
<td>&lt;0.001</td>
<td>1.15 (1.03 – 1.28)</td>
<td>0.010</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.07 (1.03 – 1.10)</td>
<td>&lt;0.001</td>
<td>1.05 (1.01 – 1.10)</td>
<td>0.007</td>
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<table>
<thead>
<tr>
<th>CHEMOKINES</th>
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<th>p-value</th>
<th>p-value</th>
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<tr>
<td></td>
<td>OR (95% CI)</td>
<td></td>
<td>OR (95% CI)</td>
<td></td>
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<tr>
<td>CXCL10</td>
<td>1.09 (1.00 – 1.20)</td>
<td>0.058</td>
<td>1.02 (0.90 – 1.16)</td>
<td>0.796</td>
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<tr>
<td>MCP-1</td>
<td>1.02 (1.01 – 1.03)</td>
<td>&lt;0.001</td>
<td>1.01 (1.00 – 1.03)</td>
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<tr>
<td>CCL3</td>
<td>1.03 (1.00 – 1.06)</td>
<td>0.041</td>
<td>1.03 (0.99 – 1.07)</td>
<td>0.128</td>
</tr>
<tr>
<td>CCL4</td>
<td>1.00 (1.00 – 1.03)</td>
<td>0.901</td>
<td>0.99 (0.96 – 1.03)</td>
<td>0.705</td>
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<tr>
<th>ADHESION</th>
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<td></td>
<td>OR (95% CI)</td>
<td></td>
<td>OR (95% CI)</td>
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<tr>
<td>ICAM-1</td>
<td>1.02 (1.00 – 1.04)</td>
<td>0.011</td>
<td>1.01 (0.99 – 1.03)</td>
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<tr>
<td>E-selectin</td>
<td>1.25 (1.12 – 1.40)</td>
<td>&lt;0.001</td>
<td>1.26 (1.10 – 1.43)</td>
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<tr>
<td>P-selectin</td>
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<td>0.213</td>
<td>1.00 (1.00 – 1.00)</td>
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TABLE 2 LEGEND

Results presented as odds ratio (OR) for presence of DSPN for every unit increase (pg/mL for cytokines and chemokines, ng/mL for adhesion molecules) in circulating inflammation marker with and without adjustments for age, sex, BMI, disease duration, and systolic as well as diastolic blood pressure in people with type 1 diabetes. 95% confidence interval (CI) and corresponding p-value are likewise shown. Boldface font indicates statistical significance (p ≤ 0.05).
<table>
<thead>
<tr>
<th>CYTOKINES</th>
<th>Vibration perception</th>
<th>T1D</th>
<th>T1D+DSPN</th>
<th>Tactile perception</th>
<th>T1D</th>
<th>T1D+DSPN</th>
<th>Heat tolerance</th>
<th>T1D</th>
<th>T1D+DSPN</th>
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<tr>
<td>IL-1α</td>
<td>0.328</td>
<td>0.018</td>
<td>0.448</td>
<td>0.002</td>
<td>0.486</td>
<td>&gt;0.001</td>
<td>0.319</td>
<td>0.033</td>
<td>0.318</td>
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<td>IL-4</td>
<td>0.074</td>
<td>0.608</td>
<td>0.309</td>
<td>0.035</td>
<td>0.021</td>
<td>0.887</td>
<td>0.346</td>
<td>0.017</td>
<td>0.204</td>
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<tr>
<td>IL-12p70</td>
<td>0.074</td>
<td>0.614</td>
<td>0.322</td>
<td>0.024</td>
<td>0.136</td>
<td>0.355</td>
<td>0.173</td>
<td>0.234</td>
<td>0.123</td>
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<tr>
<td>IL-13</td>
<td>0.141</td>
<td>0.318</td>
<td>0.260</td>
<td>0.089</td>
<td>0.108</td>
<td>0.452</td>
<td>0.137</td>
<td>0.374</td>
<td>0.016</td>
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<td>IL-17A</td>
<td>0.020</td>
<td>0.886</td>
<td>0.392</td>
<td>0.005</td>
<td>0.121</td>
<td>0.397</td>
<td>0.304</td>
<td>0.034</td>
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<td>TNFα</td>
<td>0.109</td>
<td>0.461</td>
<td>0.250</td>
<td>0.086</td>
<td>0.150</td>
<td>0.315</td>
<td>0.235</td>
<td>0.108</td>
<td>0.209</td>
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<th>T1D+DSPN</th>
<th>Tactile perception</th>
<th>T1D</th>
<th>T1D+DSPN</th>
<th>Heat perception</th>
<th>T1D</th>
<th>T1D+DSPN</th>
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<tbody>
<tr>
<td>CXCL10</td>
<td>0.355</td>
<td>0.009</td>
<td>0.078</td>
<td>0.593</td>
<td>0.316</td>
<td>0.023</td>
<td>-0.023</td>
<td>0.874</td>
<td>0.237</td>
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<tr>
<td>MCP-1</td>
<td>0.293</td>
<td>0.035</td>
<td>0.317</td>
<td>0.025</td>
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<td>CCL3</td>
<td>-0.031</td>
<td>0.828</td>
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<td>0.337</td>
<td>0.315</td>
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<tr>
<td>CCL4</td>
<td>0.047</td>
<td>0.752</td>
<td>0.001</td>
<td>0.994</td>
<td>0.235</td>
<td>0.112</td>
<td>-0.253</td>
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<table>
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<th>ADHESION MOLECULES</th>
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<th>T1D+DSPN</th>
<th>Tactile perception</th>
<th>T1D</th>
<th>T1D+DSPN</th>
<th>Heat perception</th>
<th>T1D</th>
<th>T1D+DSPN</th>
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<tbody>
<tr>
<td>ICAM-1</td>
<td>-0.015</td>
<td>0.916</td>
<td>-0.047</td>
<td>0.750</td>
<td>0.203</td>
<td>0.158</td>
<td>-0.063</td>
<td>0.671</td>
<td>0.251</td>
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<tr>
<td>E-selectin</td>
<td>0.226</td>
<td>0.111</td>
<td>0.249</td>
<td>0.081</td>
<td>0.105</td>
<td>0.467</td>
<td>-0.008</td>
<td>0.958</td>
<td>-0.039</td>
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</table>
TABLE 3 LEGEND

Results presented as Spearman’s rho ($r_s$) and corresponding $p$-value for correlations between inflammatory markers and measures of peripheral sensory nerve function. T1D (n=53), T1D+DSPN (n=50). Boldface font indicates statistical significance ($p \leq 0.05$).
FIGURE LEGENDS

FIGURE 1 LEGEND
Serum concentrations of cytokines (A), chemokines (B) and cell adhesion molecules (C) in healthy controls (n=21), T1D (n=53), and T1D+DSPN (n=50) displayed as boxplots. Circles indicate outliers. * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

FIGURE 2 LEGEND
Receiever operating characteristic curves of inflammation markers for recognizing presence of DSPN after adjustment for age, sex, BMI, disease duration, and systolic as well as diastolic blood pressure in people with type 1 diabetes.