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Review

Novel Insights into Sensorimotor and Cardiovascular Autonomic Neuropathy from Recent-Onset Diabetes and Population-Based Cohorts

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The most prevalent chronic complications of diabetes are diabetic neuropathies, among which distal sensorimotor polyneuropathy (DSPN) and cardiovascular autonomic neuropathy (CAN) are the best studied. Their major clinical sequelae such as foot ulcers, neuropathic pain, and orthostatic hypotension are associated with lower quality of life and increased risk of mortality. Here we discuss the recent insights into DSPN and CAN focusing on two prospective cohorts; that is, the German Diabetes Study (GDS) including recent-onset diabetes patients and the population-based Cooperative Health Research in the Region of Augsburg, Germany (KORA) surveys. The insights from these studies investigating novel tools for early detection and prediction of (pre) diabetic neuropathy as well as biomarkers of oxidative stress and inflammation should ultimately culminate in improving the health care of patients affected by this serious condition.

Highlights

Recent-onset type 2 diabetes (T2D) is characterized by an array of abnormalities in nerve function and morphology so that diabetic neuropathy is not a 'late' but an 'early' complication with roles for blunted cardiorespiratory fitness, insulin resistance, and hepatic steatosis in suppressed cardiovagal tone.

An increased risk of distal sensorimotor polyneuropathy (DSPN) and cardiovascular autonomic neuropathy (CAN) is found in people with prediabetes, particularly in those with combined impaired fasting glucose and impaired glucose tolerance.

Pro- and antioxidant biomarkers may be implicated in the development of DSPN, whereas studies for CAN are missing.

Subclinical inflammation precedes the onset of DSPN in the general elderly population, possibly involving both innate and adaptive immunity.

Lifestyle intervention and multifactorial cardiovascular risk intervention (CVRI) may exert favorable effects on nerve function, while efforts to develop and establish novel treatment strategies targeting specific pathomechanisms should be intensified.

Background

Despite their major impact on morbidity and prognosis, distal sensorimotor polyneuropathy [**DSPN** (see Glossary)] and cardiovascular autonomic neuropathy (**CAN**) (Box 1) remain frequently underdiagnosed and undertreated in patients with type 1 diabetes (T1D) and type 2 diabetes (T2D) [1,8,9]. The prevalence of both DSPN and CAN increases with increasing duration of diabetes, and the traditional view holds that DSPN and CAN are late complications of T1D and T2D caused by poor glycemic control. However, evidence has accumulated indicating that the development of both DSPN and CAN starts before the onset of diabetes [2]. DSPN and CAN are most likely to be caused by a complex interplay between metabolic, immune, lifestyle, and genetic factors [1,10,11]. In addition to glycemic control, age, obesity, dyslipidemia, hypertension, impaired kidney function, low physical activity, and smoking appear to contribute to the risk of DSPN and/or CAN in older individuals and in those with prediabetes, T1D, or T2D [2,12–17].

The questions of when the development of DSPN and CAN begins and which risk factors underlie their pathophysiology has important clinical implications for prevention, screening, and public health: (i) because of the high number of individuals who are elderly and/or have prediabetes; and (ii) because current treatment options mostly address the symptoms of DSPN and CAN rather than their underlying pathomechanisms [1,10]. Given that the improvement of prevention, diagnosis, and both pharmacological and nonpharmacological therapy represents a major challenge in medical care, especially for older individuals without and with

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Box 1. Diabetic Neuropathy in Context

Diabetic neuropathies are the most prevalent chronic complications of diabetes, comprising a heterogeneous group of conditions that affect different parts of the nervous system and present with diverse clinical manifestations among which DSPN and diabetic autonomic neuropathies, particularly CAN, are by far the most studied [1]. Most common among diabetic neuropathies is chronic DSPN, affecting around 30% of people with diabetes. DSPN represents a major health problem as it may present with excruciating neuropathic pain and is responsible for an increased risk of mortality as well as substantial morbidity, resulting from foot ulceration, amputations, and impaired quality of life [2]. A simple definition of DSPN for clinical practice is the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes [1].

The heart receives input from both the sympathetic and parasympathetic systems regulating heart rate, rhythm, and contractility. Cardiac parasympathetic activity is mediated through the vagus nerve, which originates in the medulla oblongata, while sympathetic innervation to the heart originates mainly from the right and left stellate ganglia [3]. The autonomic nervous system regulates both the cardiovascular system and energy balance and is disturbed in diabetes and obesity. The sympathetic nervous system is a key player linking the development and progression of cardiovascular disease with obesity [4]. CAN, defined as the impairment of cardiovascular autonomic control in the setting of diabetes after exclusion of other causes, affects around 20% of individuals with diabetes when detected by cardiovascular reflex tests based on HRV and blood pressure response to standing up [5]. HRV reflects fluctuations in autonomic inputs to the heart and is associated with increased mortality in various conditions including diabetes and cardiovascular disease [6,7].

diabetes, this review aims to highlight new developments in detection, pathophysiology, and treatment options of DSPN and CAN in the elderly general population and in patients recently diagnosed with T1D or T2D.

Early Detection of Neuropathy in Recent-Onset Diabetes

The German Diabetes Study (GDS) is a prospective cohort study describing the impact of subphenotypes on the course of the disease and aims to identify prognostic factors and mechanisms underlying the development of related comorbidities. This study comprises intensive phenotyping including detailed assessment of nerve function and morphology as well as biomarkers focusing on **subclinical inflammation** and **oxidative stress** within 12 months after clinical diagnosis and thereafter at 5-year intervals for 20 years [18].

The diagnosis of DSPN by clinical examination is subjective and may show unsatisfactory reproducibility even among proficient examiners. Hence, objective measures are needed to accurately determine early nerve pathology indicating incipient stages of DSPN, which may be more amenable to intervention than advanced clinical alterations. Small-fiber pathology may be quantified in skin biopsies by assessing intraepidermal nerve fiber density (IENFD) using the pan-neuronal marker protein gene product 9.5 (PGP9.5), while growth-associated protein 43 (GAP-43) can be used as a marker for regenerating nerve fibers [19]. In patients with T2D from the GDS baseline cohort, IENFD was reduced compared with controls by approximately one-quarter using both PGP9.5 and GAP-43 [19–21], while the GAP-43:PGP9.5 ratio did not differ between the groups suggesting that cutaneous nerve regeneration is not compromised in the early course of diabetes [19]. The findings for the various skin biopsy biomarkers in patients with recent-onset T2D from the GDS baseline cohort are summarized in Table 1 [19–22].

An emerging noninvasive technique to quantify corneal nerve fiber pathology is **corneal confocal microscopy (CCM)** assessing the corneal sub-basal nerve plexus localized between the basal epithelium and Bowman's membrane [23]. Patients with T2D from the GDS baseline cohort showed an early loss of small nerve fibers that was detected by both CCM and skin biopsy. More recently, in the same cohort, spatial point pattern analysis (SPPA) of the distribution of corneal nerve branching points revealed increased clustering rather than a random distribution of early corneal nerve fiber loss. The detection of the latter was substantially

Table 1. Biomarkers Derived from Skin Biopsy in Patients Recently Diagnosed with T2D from the Baseline Cohort of the GDS[a]

Biomarker	Trait	Localization	Parameter (unit)	Finding	Refs
PGP9.5	Pan-neuronal	Epidermal Dermal	IENFD (fibers/mm) DNFL ($\mu m/mm^2$)	↓[b] ↔	[22]
GAP-43	Nerve growth	Epidermal Dermal	IENFD (fibers/mm) DNFL ($\mu m/mm^2$)	↓ ↔	[19]
GAP-43:PGP9.5 ratio	Nerve regeneration	Epidermal Dermal	IENFD ratio DNFL ratio	↔ ↔	[19]
SOD2	Antioxidative defense	Dermal	Area (%)	↑	[21]
PAR	DNA repair	Dermal	Area (%)	↔	
CD31	Endothelial cells	Dermal	Area (%)	↔	[21]
Langerhans cells	Dendritic immune cells	Epidermal	Cells/mm^2	↓	[20]

[b]↓ reduced, ↑ increased, ↔ not different versus matched controls.

improved by combining SPPA parameters with a standard CCM measure such as corneal nerve fiber length [24]. However, CCM and skin biopsy did not detect nerve pathologies in the same patients, suggesting a patchy manifestation pattern of small-fiber neuropathy in various organs, possibly due to distinct underlying pathophysiological processes. Since nerve function assessed by **nerve conduction studies**, **quantitative sensory testing**, and **cardiovascular autonomic function tests** was also impaired, parallel involvement of small and large nerve fibers in the early development of DSPN was suggested [22]. These findings are at variance with the notions that small-fiber damage precedes large-fiber damage in the development of DSPN [25] and that CCM correlates with DSPN severity [23], but it has to be considered that the GDS baseline cohort includes only patients with recent-onset diabetes. While this criterion is almost ideal when addressing the development of DSPN in diabetes, comparing the extent of correlations of CCM indices with DSPN measures between populations with recent-onset and longer-term diabetes appears difficult due to their distinct clinical profiles.

Since the development and progression of diabetic neuropathy in any single patient cannot be completely predicted by hyperglycemia or other traditional risk factors, the search for genetically determined factors remains of major interest [26]. Shunting of glycolytic intermediates into the pentose phosphate pathway has been suggested to protect against hyperglycemia-induced microvascular damage. Transketolase (TKT), an enzyme involved in the pentose phosphate pathway, is activated by its cofactor thiamine and diverts metabolic intermediates from glycolysis to the pentose phosphate pathway. Nine SNPs of the *TKT* gene were genotyped in patients from the GDS baseline cohort. After adjusting for multiple testing, associations of SNPs with neuropathic symptoms and reduced thermal detection thresholds were found, which were primarily driven by male sex and the presence of T2D. These findings suggest a role for pathways metabolizing glycolytic intermediates in early DSPN [27].

Several mechanisms have been suggested as being responsible for CAN and, among these, insulin resistance appears to play a crucial role [28]. In the GDS baseline cohort, cardiorespiratory fitness was reduced in recently diagnosed T2D but preserved in T1D, while heart rate variability (HRV) was decreased in both diabetes types. However, a strong association of cardiorespiratory fitness with cardiac autonomic function was found primarily in T1D, supporting the therapeutic concept of promoting physical activity and achieving physical fitness, especially in the early course of diabetes [29]. Furthermore, lower cardiovagal activity *per*

Glossary

Adaptive immune system: one of the two main subsystems of the immune system (see also 'Innate immune system') in vertebrates, also called the acquired immune system; main functions include recognition of 'non-self' antigens, antigen-specific B and T cell responses, and the development of an immunological memory.

Cardiovascular autonomic function tests: reflex tests evaluating different functions of the autonomic nervous system such as changes in heart rate and blood pressure after various stimuli.

Cardiovascular autonomic neuropathy (CAN): neuropathy attributable to damage to the autonomic nerve fibers innervating the heart and blood vessels leading to abnormalities in heart rate control and vascular dynamics.

Corneal confocal microscopy (CCM): noninvasive technique that provides *in vivo* imaging of corneal nerve fibers.

Distal sensorimotor polyneuropathy (DSPN): symmetric distal polyneuropathy with mainly sensory and less pronounced motor nerve involvement.

Innate immune system: one of the two main subsystems of the immune system (see also 'Adaptive immune system') in vertebrates, which is evolutionarily older; main functions include recruiting immune cells to sites of tissue damage or infection along gradients of cytokines and chemokines, phagocytosis, and antigen presentation to activate the adaptive immune system.

Nerve conduction studies: objective assessment of the electrical conduction of large motor and sensory nerves representing the gold standard to confirm peripheral neuropathy.

Oxidative stress: imbalance between reactive oxygen species and antioxidant defense mechanisms that detoxify and counterbalance the effects of prooxidant molecules.

Quantitative sensory testing: psychophysical tests to assess large and small sensory nerve fiber function; examples include measurements of the detection thresholds of precisely calibrated vibratory, thermal, or painful stimuli.

se rather than higher sympathetic predominance as derived from spectral analysis of HRV recordings was linked to insulin resistance in both T1D and T2D, while in T1D lower residual β-cell function (i.e., lower C-peptide response to glucagon) was associated with higher, possibly compensatory, parasympathetic tone [30]. By contrast, lower cardiac vagal activity and baroreflex sensitivity were associated with increased hepatic fat content in T2D but not T1D, suggesting that hepatic steatosis may be relevant in the early development of parasympathetic CAN in T2D [31]. However, it should be taken into account that spectral analysis of HRV can give information on vagal and sympathetic activity only in relative terms whereas techniques such as microneurography allow direct measurement, but this is not feasible in large-scale studies. It is of particular clinical interest that interventions known to enhance insulin sensitivity, such as weight loss and physical training, also enhance vagal activity (see Implications for Prevention and Treatment). Collectively, these studies suggest that diabetic neuropathy is not a 'late' but an 'early' complication of diabetes, but prospective studies are required to better define the precise temporal sequence of these alterations.

Reactive oxygen species: highly reactive chemical species containing oxygen, which can exert harmful effects at high concentrations by damaging nucleic acids, proteins, and lipids.
Subclinical inflammation: low-grade but chronic increase in the systemic levels of multiple biomarkers of inflammation (with CRP as the most frequently measured biomarker in clinical practice).
Sudomotor function: function of sweat gland innervation.

Neuropathy in the Elderly Population with Prediabetes and Diabetes

The past two decades witnessed considerable interest in the relation of fasting glucose and 2-h post-load glucose with cardiovascular morbidity and mortality [32]. However, whether impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are associated with higher risk of both DSPN and CAN is a matter of ongoing debate [33,34]. This question was addressed in the frame of the KORA (Cooperative Health Research in the Region of Augsburg, Germany) surveys. Participants aged ≥55 years were phenotyped for possible DSPN [35] by the Michigan Neuropathy Screening Instrument (MNSI) examination part and for CAN by indices derived from resting supine for 5-min HRV recordings through cross-sectional (S) and follow-up (F) population-based surveys (S1: 1984–1985, S2: 1989–1990, S3: 1994–1995, S4: 1999–2001, S4 follow-up F4: 2006–2008, FF4: 2013–2014) and the KORA-A study (participants with diabetes from S2, S3, and the KORA Myocardial Infarction Registry and 1:1 matched controls) (Table 2; [36–40]). It is satisfactory to apply the aforementioned tools in an epidemiological setting, but they have their limitations. While the MNSI cannot be used to confirm DSPN [35], HRV recordings do not represent the 'gold standard' for the diagnosis of CAN [5]. Nonetheless, these studies demonstrated that DSPN and painful DSPN were slightly more prevalent in persons with IFG than in those with normal glucose tolerance (NGT) but were markedly higher in participants with IGT than in those with IFG. The prevalence of DSPN in individuals with both IFG and IGT reached the levels found in patients with newly detected and known diabetes (KORA F4). Moreover, using a combination of linear and nonlinear HRV measures, higher prevalence rates of CAN were noted not only in individuals with known diabetes but also in

Table 2. Prevalence of DSPN, Painful DSPN, and CAN in Persons with NGT, IFG, IGT, Newly Detected Diabetes (New DM), or Known Diabetes (Known DM) Participating in the KORA Studies[a]

Survey	Definition	Neuropathy	NGT (%)	IFG (%)	IGT (%)	IFG + IGT (%)	New DM (%)	Known DM (%)	Refs
S2 + S3	MNSI >2	DSPN	7.4	11.3	13.0	n.a.	n.a.	28.0	[36]
F4	Bilateral reduction of VPT and/or T/PS in the feet	DSPN	11.1	5.5	14.8	23.9	16.1	22.0	[37]
S2 + S3	MNSI >2 and pain in the feet	Painful DSPN	1.2	4.2	8.7	n.a.	n.a.	13.3	[38]
AMIR	MNSI >2 and pain in the feet	Painful DSPN	3.7	5.7	14.8	n.a.	n.a.	21.0	[39]
S4	Reduced HRV in two or more of four indices	CAN	4.5	8.1	5.9	11.4	11.7	17.5	[40]

Abbreviations: AMIR, Augsburg Myocardial Infarction Registry; MNSI, clinical examination part; n.a., not available; T/PS, touch/pressure sensation (10-g monofilament); VPT, vibration perception threshold (tuning fork).

those with combined IFG and IGT, similar to participants with newly detected diabetes compared with persons with NGT (KORA S4) (Table 2). These findings were corroborated by smaller, clinic-based studies showing higher rates of CAN, elevated heat and current perception thresholds, and abnormal circadian blood pressure regulation in individuals with IGT than in those with NGT [41,42]. However, it has to be conceded that one population-based survey did not find an increased rate of DSPN in individuals with prediabetes [43].

Both in the general population and in diabetes patients, besides age, modifiable cardiovascular risk factors such as body mass index (BMI), waist circumference, and low physical activity as well as peripheral arterial disease were associated with DSPN and neuropathic pain, suggesting an interplay between DSPN and both cardiovascular risk factors and macroangiopathy in the lower limbs, which is important for screening scores (Box 2; [40,44–46]) and may constitute a preventive and interventional target for neuropathy [7,38,39]. Reduced HRV was associated with obesity and smoking in men but not in women, suggesting that sex may modulate the impact of these risk factors on CAN [47].

Oxidative Stress

Oxidative stress refers to an imbalance between **reactive oxygen species** and antioxidant defense. Oxidative stress is an important pathomechanism linking hyperglycemia and subclinical inflammation and the onset of diabetic complications [10]. However, it is also triggered by obesity-related metabolic derangements (e.g., hyperlipidemia) and environmental risk factors (e.g., air pollution), so oxidative stress is most likely to represent a central mediator between multiple risk factors on the one hand and diabetes and its comorbidities on the other hand [48].

Recent studies implicated systemic levels of prooxidant biomarkers such as myeloperoxidase (MPO), methylglyoxal, and superoxide anion and extracellular superoxide dismutase (SOD) 3, reflecting antioxidative defense in the onset of DSPN (Box 3) [16,21,49,50]. Thus, there is emerging evidence that higher levels of prooxidant biomarkers are linked with higher risk of DSPN. The interpretation of systemic levels of SOD3 appear less straightforward. Lower SOD3 levels as an indicator of reduced antioxidative defense would represent a plausible observation in the early course of DSPN, whereas higher SOD3 levels could reflect an antioxidative counterregulation to excessive superoxide that is, however, insufficient to prevent DSPN. At present, data are too limited to conclude whether higher or lower levels of biomarkers reflecting antioxidative defense are associated with higher risk of DSPN.

Box 2. Screening Tools for Peripheral and Autonomic Neuropathy

Since screening for both DSPN and CAN is being underused, simple screening scores have been proposed for practical purposes. The base model for DSPN comprised age, height, weight, pain or discomfort in the feet and/or legs, and duration of diabetes, while the clinical model additionally included diastolic blood pressure and serum creatinine levels. This simple tool could be helpful in differentiating between a high and low likelihood of having DSPN [44]. The proposed score for CAN comprised heart rate, BMI, hypertension, smoking, serum creatinine, and use of drugs suppressing HRV [40]. This screening score could be used in clinical practice, if equipment for measuring HRV is not readily available, but further validation is needed.

A useful indicator test assessing **sudomotor function** on the basis of a color change from blue to pink is the Neuropad, which has high sensitivity (65–100%) and moderate specificity (32–79%) for the diagnosis of DSPN in diabetes patients. These diagnostic properties are also applicable to individuals with recent-onset diabetes [45]. In the elderly population, the test showed moderately high sensitivity (77%) for the diagnosis of DSPN among diabetes subjects and modest sensitivity (58%) for those with prediabetes, while its specificity was rather low in individuals with diabetes (36%) or prediabetes (33%). The relatively high sensitivity suggests that the test may be used as a screening tool, while its lower specificity implies that results would need confirmation by established diagnostic modalities [46].

Box 3. Biomarkers of Oxidative Stress, DSPN, and CAN

Systemic levels of MPO and methylglyoxal as prooxidant biomarkers and extracellular SOD3 as a component of antioxidative defense have recently been investigated in the development of DSPN. In the older individuals from the population-based KORA F4/FF4 cohort (age range 62–81 years, approximately 60% with prediabetes or T2D), serum levels of MPO, a source of reactive oxygen species, were associated with prevalent DSPN {fully adjusted odds ratio (OR) 1.38 [95% confidence interval (CI) 1.10; 1.72] per doubling of MPO}, whereas the association with incident DSPN was less pronounced [OR 1.20 (95% CI 0.92; 1.56)] [49]. Higher levels of methylglyoxal, a reactive dicarbonyl metabolite, were also associated with incident DSPN in younger patients with screen-detected T2D in the ADDITION-Denmark study [OR 1.45 (95% CI 1.12; 1.89)] adjusted for age, sex, and randomization group) [16]. Data for reactive oxygen species as risk factors for DSPN are available from only one prospective study showing that higher superoxide levels were associated with a faster decline in NCV in patients with longstanding T2D [50].

Serum levels of SOD3, the major antioxidant enzyme detoxifying the superoxide anion, were positively associated with incident DSPN in the KORA F4/FF4 cohort [OR 2.14 (95% CI 1.02; 4.48) adjusted for age and sex], but the association was attenuated after further adjustment for cardiometabolic covariables [49]. By contrast, serum levels of SOD3 were lower in individuals with DSPN than in those without DSPN among patients with recent-onset T1D and T2D from the GDS (known diabetes duration of <1 year). Lower SOD3 levels were also correlated with impaired NCV [51].

Increased superoxide generation was found associated with a more pronounced decline in HRV over 6 years in patients with longstanding T2D [50], but data from earlier stages in diabetes progression are not available. In patients with recent-onset T2D from the GDS, subepidermal manganese SOD2 expression was overexpressed and associated with impaired sympathovagal balance [21] (Table 1).

The notion that oxidative stress contributes to DSPN in patients with diabetes is indirectly supported by meta-analyses showing that blood levels of vitamins with antioxidant properties, such as vitamin B12, vitamin D, and folate, are lower in patients with than in those without DSPN [52–55]. However, effect estimates differed between studies, were not based on longitudinal designs of these studies, and often were not adjusted for important confounders, so the degree of evidence with respect to the relationship between antioxidant vitamins and DSPN remains unsatisfactory.

Obesity, a major determinant of oxidative stress, affects the balance between sympathetic and vagal activity of the autonomic nervous system and thus also cardiac autonomic function [15]. Detailed studies on biomarkers of oxidative stress and inflammation as potential risk factors for CAN are scarce (Box 3). However, the available data suggest that cardiac autonomic dysfunction may be associated with cutaneous antioxidative defense [21,50].

In conclusion, both pro- and antioxidant biomarkers have been implicated in the development of DSPN in the early stages of diabetes development, whereas the evidence for CAN is weaker due to the paucity of prospective studies. This may in part be due to analytical challenges in the assessment of systemic oxidative stress, such as the instability of reactive oxygen species and other biomarkers of oxidative stress [56]. Given the biological plausibility of oxidative stress as a pathomechanism for DSPN and CAN, a future focus on clinical and prospective studies would be important, because a better understanding of this association is required for any prevention or intervention approach targeting oxidative stress.

Subclinical Inflammation

DSPN and CAN have been traditionally classified as 'noninflammatory' neuropathies in contrast to acute and chronic neuropathies such as Guillain–Barré syndrome or chronic inflammatory demyelinating polyradiculoneuropathy [57]. Chronic inflammatory demyelinating polyradiculoneuropathy is the most common chronic inflammatory neuropathy, with a complex pathophysiology involving cells (e.g., macrophages, T cells, B cells) and cytokines [tumor necrosis factor alpha (TNFα), interferon-gamma (IFNγ), interleukin (IL)-6, IL-4] from both innate and

adaptive immunity. Studies addressing inflammatory biomarkers as risk factors for DSPN and CAN are scarce, and data from population-based studies or individuals with recent-onset diabetes are mainly limited to the aforementioned KORA F4/FF4 cohort and the GDS, respectively.

Most data on inflammation and DSPN are cross-sectional [10], but two reports from the KORA F4/FF4 cohort comprising approximately 80 biomarkers of inflammation demonstrated that a complex upregulation of multiple pro- and anti-inflammatory cytokines, chemokines, and other biomarkers at baseline preceded the onset of DSPN (Box 4) [58,59]. There was no evidence for differences in these associations depending on prediabetes or T2D status [59]. Biomarkers associated with incident DSPN clustered in pathways reflecting the **innate** and **adaptive immune systems** and involved processes such as antigen presentation and chemotaxis with some evidence also for autoimmune reactivities [59]. Thus, pathway analyses pointed toward complex crosstalk between innate and adaptive immunity in the pathophysiology of DSPN, which represents an interesting hypothesis to be tested in future studies [59].

Of note, data from this cohort also showed that different measures of obesity were positively associated with incident DSPN and that results were not influenced by prediabetes or diabetes status [65]. Two chemokines [C-C motif chemokine ligand 7 (CCL7) and C-X-C motif chemokine ligand 10 (CXCL10)] and one neuron-specific marker [Δ/Notch-like epidermal growth factor related receptor (DNER)] partly mediated these associations [65]. The fact that there was no evidence for differences in the relationship of both obesity and inflammation with DSPN between participants without and with prediabetes/T2D points toward an overlap in the pathophysiology of DSPN in these groups.

Cross-sectional associations between biomarkers of inflammation and DSPN in recent-onset diabetes were investigated in the GDS. Higher levels of IL-6 and total adiponectin, high-molecular-weight adiponectin, and their ratio were associated with DSPN in patients with T2D, but not in T1D. Higher IL-6 and total and high-molecular-weight adiponectin levels were associated with reduced motor and/or sensory nerve conduction velocity (NCV) in T2D,

Box 4. Biomarkers of Inflammation and Incident DSPN in the Population-Based KORA F4/FF4 Cohort

The first report from this cohort identified IL-6 and TNFα as risk factors for DSPN after comprehensive adjustment for age, sex, anthropometric, metabolic, and lifestyle factors and for comorbidities and medication [58], whereas the second study using a multimarker approach found another 26 biomarkers of inflammation associated with higher risk of DSPN (all $P < 0.05$) [59]. Interaction analyses found no evidence for differences in these associations between individuals without and with prediabetes or T2D [59], so inflammation-related risk factors may largely overlap between nondiabetic and diabetic individuals in the older population.

Adjustment for multiple testing reduced the number of DSPN-associated biomarkers to six (CXCL9, CXCL10, CCL7, DNER, CD40, TNFRSF9), which improved the prediction of DSPN when added to a clinical risk model. The chemokines CXCL9, CXCL10, and CCL7 had neurotoxic effects in an *in vitro* model, which suggests that the association may be physiologically relevant. Of note, CXCL9 and CXCL10 are mainly regulated by the proinflammatory cytokine IFNγ, which has also been identified as a potential upstream regulator of the DSPN-associated biomarkers [59]. IFNγ contributes to insulin resistance, cardiovascular disease, and neurological conditions [60–63], so an involvement of this cytokine produced by natural killer cells and T cells in the development of DSPN merits further studies.

A substudy within the KORA F4/FF4 cohort revealed that circulating levels of sICAM-1, a marker of endothelial dysfunction and vascular inflammation, and of the anti-inflammatory cytokine IL-1RA were positively associated with the progression of DSPN [58]. Neither biomarker was independently linked with the incidence of DSPN, so predictive biomarkers and pathomechanisms may differ between different stages of DSPN. In this context, it should be noted that higher IL-6 and sICAM-1 levels were also associated with painful DSPN in the KORA F4 cohort [64].

whereas in T1D higher total and high-molecular-weight adiponectin were related to faster motor NCV [66]. This study suggests that inflammation-related risk factors for DSPN may differ between T1D and T2D and is thus in line with other observations that point toward partly different risk factors for DSPN between the two diabetes types [67].

Data from clinical studies on the impact of inflammatory processes on cardiac autonomic function are scarce. Acute inflammation triggered by low-dose administration of endotoxin (lipopolysaccharide) decreased HRV and changed the balance between sympathetic and vagal activity [68,69]. Importantly, CAN differs from DSPN, since inflammation and CAN are clearly connected by a bidirectional relationship [70], which is relevant for the interpretation of cross-sectional studies. The effect of the autonomic nervous system on inflammatory processes and the immune system has been described before [71].

Despite the clinical relevance of CAN for morbidity and mortality [1], there is a surprising lack of studies aimed at the identification of systemic biomarkers linked to its prevalence and incidence in patients with diabetes. The majority of studies on systemic levels of inflammation-related biomarkers and cardiac autonomic dysfunction were cross-sectional and performed in samples from the general population or in patients with cardiovascular conditions, but without subgroup comparisons with respect to (pre)diabetes status. These studies reported inverse associations between C-reactive protein (CRP) and IL-6 on the one hand and various indices of HRV on the other hand, thus pointing toward a link between inflammation and cardiac autonomic dysfunction [72].

The only study that compared associations of biomarkers of inflammation with cardiac autonomic function between the two diabetes types was the GDS, which found intriguing differences between participants with recent-onset T1D and T2D. Whereas no consistent associations were found in T1D, in T2D higher IL-18 levels were related to lower vagal activity, and higher levels of total and high-molecular-weight adiponectin showed associations with reduced sympathetic activity. Higher concentrations of soluble intercellular adhesion molecule-1 (sICAM-1) were associated with both lower vagal and sympathetic activity and higher sE-selectin with lower vagal activity only. CRP and IL-6 were also inversely related to cardiac autonomic function, but adjustment for cardiometabolic risk factors attenuated these associations [73].

The temporal relationship between biomarkers of inflammation at baseline and 5-year changes in cardiac autonomic function has been analyzed in the large Whitehall II cohort. Higher levels of IL-1 receptor antagonist (IL-1RA) were associated with greater increases in heart rate. Higher hsCRP, IL-6, and IL-1RA levels were linked with reductions in HRV, but these associations were explained by BMI and other confounders. Higher adiponectin levels were associated with more pronounced decreases in heart rate and increases in three indices of HRV reflecting both sympathetic and vagal activity in individuals with T2D, whereas no such associations were found in participants without diabetes [74].

Taking these findings together, there is emerging evidence that subclinical inflammation precedes the onset of DSPN in the elderly population and in patients with recent-onset T2D. Comparable studies for T1D are lacking, but cross-sectional data indicate that inflammation-related risk factors may differ between T1D and T2D. Prospective data for inflammation and CAN in individuals with prediabetes or diabetes are again too scarce for solid conclusions, so research in this field should be prioritized given the high impact of CAN on morbidity and mortality. Additionally, the stimuli of inflammatory processes and the tissues throughout the body involved in them need to be defined more precisely.

Implications for Prevention and Treatment

Lifestyle Intervention

Several clinical trials have shown that reducing obesity as a risk factor for both DSPN and CAN by various interventions such as lifestyle intervention may exert favorable effects on nerve function and pathology [10,75]. In the lifestyle arm of the Diabetes Prevention Program, persons with prediabetes who did not develop diabetes had a lower prevalence of DSPN than those who did develop diabetes [76]. Likewise, heart rate and QT indexes decreased and HRV increased following lifestyle intervention [77]. Weight loss induced by caloric restriction appears to exert favorable effects on autonomic nervous system activity via enhancing vagal activity and/or reducing sympathetic modulation. In obese individuals with T2D, low-energy diets differing in fiber, red meat, and coffee intake resulted in comparable weight loss, decline in heart rate, and enhanced vagal tone, which was associated with enhanced oxidative glucose utilization and diminished fat oxidation [78]. Exercise interventions focusing on CAN have also shown that physical activity can increase cardiovagal activity and reduce sympathetic overactivity. In particular, long term and regular, but also supervised endurance and high-intensity and high-volume, exercise improves cardiac autonomic function in patients with T2D. By contrast, the evidence in those with T1D or with prediabetes or metabolic syndrome is weaker [75]. Large-scale controlled studies should ultimately determine whether favorable modulation of autonomic tone toward augmentation of vagal activity accompanied by attenuated insulin resistance can be translated into a reduction of cardiovascular endpoints in people with diabetes.

Bariatric Surgery

Bariatric surgery results in sustained weight loss and is associated with other important health outcomes, among which the effect on remission of T2D is substantial. However, only a few uncontrolled studies have assessed the effects of bariatric surgery on measures of DSPN and CAN. In a small uncontrolled study the Neuropathy Disability Score (NDS) improved after 12 months following Roux-en-Y gastric bypass (RYGB) in relation to improvements in systemic biomarkers of oxidative, nitrosative, and carbonyl stress [79], but in another study motor and sensory NCV did not change after 12 months following RYGB [80]. A meta-analysis of four studies reported that gastric sleeve resection was associated with improvement in vagal tone and sympathovagal balance, while RYGB was, rather, associated with improved insulin sensitivity. However, the increase in vagal tone seems to be independent of the reduction in insulin resistance [81]. Altogether, low-grade evidence indicates that bariatric surgery may improve measures of DSPN and CAN, but RCTs randomized clinical trials (RCTs) are required to confirm these preliminary findings. The possible benefit of bariatric surgery should be weighed against the risk of subacute axonal neuropathy caused by micronutrient deficiencies, particularly low thiamine levels [82] and autonomic dysfunction including orthostatic intolerance [83].

Glycemic Control and Multifactorial Risk Intervention

Intensive insulin therapy aimed at achieving near-normal glycemia is essential to prevent, albeit not completely, or delay progression of DSPN in T1D [1]. However, there is no convincing evidence in T2D patients to suggest that intensive diabetes therapy has a favorable effect on the development or progression of DSPN [84].

Numerous studies have shown the efficacy of controlling individual cardiovascular risk factors in preventing or slowing the development of cardiovascular disease in people with diabetes, but only a few studies assessed the effect of multifactorial cardiovascular risk intervention (CVRI) on DSPN and CAN [85–87]. In the Steno-2 Study, intensified CVRI had no effect on DSPN whereas the progression of CAN could be delayed, suggesting that at least CAN could be amenable to CVRI [85].

Clinician's Corner

Despite their major clinical impact, diabetic neuropathies remain largely underdiagnosed and undertreated, although multiple tools are available that allow their early detection.

Both DSPN and CAN have been shown to develop to a significant extent in predisposed individuals early in the course of even well-controlled diabetes and in those with prediabetes.

Recent years have witnessed progress in the identification of biomarkers of inflammation and oxidative stress to predict the development of DSPN and CAN, but it remains to be seen whether these will ultimately find their way into clinical practice.

Lifestyle intervention and effective control of modifiable risk factors should constitute the mainstay of prevention and treatment of both DSPN and CAN.

Large-scale controlled studies should determine whether favorable modulation of autonomic tone toward the augmentation of vagal activity accompanied by attenuated insulin resistance can be translated into a reduction of cardiovascular endpoints in people with diabetes.

Interventions Targeting Oxidative Stress and Inflammation

Among the antioxidants assessed for efficacy in diabetes patients with DSPN or CAN, α-lipoic acid has reached the best evidence level from RCTs and meta-analyses, suggesting that treatment with α-lipoic acid improves neuropathic symptoms within 3–5 weeks and neuropathic impairments over 4 years in patients with DSPN and may also improve HRV in those with CAN [88]. By contrast, other antioxidants, such as vitamin E, did not show favorable effects on measures of DSPN [89].

Although recent results from the KORA F4/FF4 study indicated that an increase in multiple biomarkers of inflammation precedes DSPN [58,59], it is not possible to infer a causal link from these observational data. If experimental and clinical studies corroborate the association between inflammation and DSPN, it would be promising to test the efficacy of anti-inflammatory therapies to prevent and/or treat DSPN [90]. In obese individuals, weight loss following lifestyle interventions or bariatric surgery attenuates subclinical inflammation [91], which may be one mechanism for how these interventions may reduce the risk of DSPN, CAN, and other micro- and macrovascular comorbidities of prediabetes and diabetes. Results from the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS) demonstrated that targeting IL-1β, a proinflammatory cytokine that is far upstream in inflammatory cascades, reduces the risk of recurrent cardiovascular events [92]. Future studies will need to test whether targeting of specific cytokines or signaling cascades may be similarly effective for individuals with DSPN or CAN.

Conclusions and Future Perspectives

Recent studies have provided insights into the potential role of novel biomarkers, pathways, and pathomechanisms in the development of DSPN and CAN before and early after the onset of diabetes. Given the limitations of animal models of diabetic neuropathy, further clinical and epidemiological studies are warranted to improve our knowledge of mechanisms including oxidative stress and inflammation leading to DSPN and CAN. Currently, the scarcity of prospective biomarker studies and the lack of hypothesis-free omics studies (e.g., genomics, transcriptomics, proteomics, metabolomics) limit our understanding of the pathomechanisms and lead to a lack of reliable biomarkers for the prediction of DSPN and CAN in individuals without and with diabetes. Finally, there is an urgent need for novel therapeutic approaches addressing new targets and not only diabetic but also prediabetic neuropathy in intervention studies (see Outstanding Questions).

Outstanding Questions

Can the associations found in the GDS baseline cohort between multiple risk factors and diabetic neuropathy be confirmed and the precise temporal sequence of the early alterations be defined by prospective studies?

Can future prospective studies corroborate and extend initial findings on the associations of biomarkers of oxidative stress and subclinical inflammation with DSPN and CAN?

To what extent can hypothesis-free omics approaches identify additional pathways and pathomechanisms contributing to DSPN and CAN?

Are there differences in the processes leading to DSPN and CAN between individuals with T1D, those with T2D, and older persons with NGT or prediabetes?

What are the consequences of recent advances in the identification of novel biomarkers and assessment tools for the design of future clinical trials assessing novel pharmacotherapies?

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