Differential Association Between Biomarkers of Subclinical Inflammation and Painful Polyneuropathy: Results From the KORA F4 Study

DOI: 10.2337/dc14-1403

OBJECTIVE
Inflammatory processes have been implicated in the pathogenesis of painful neuropathy in rodents, but the relationship between inflammatory biomarkers and painful distal sensorimotor polyneuropathy (DSPN) has not been assessed in population-based studies. Therefore, we investigated whether circulating levels of seven pro- and anti-inflammatory immune mediators were associated with painful DSPN in older individuals in a large population-based study.

RESEARCH DESIGN AND METHODS
The study population consisted of individuals with painless \( (n = 337) \) and painful DSPN \( (n = 54) \) from a source population \( (n = 1,047) \) of men and women aged 61–82 years who participated in the German KORA F4 survey (2006–2008). We measured circulating levels of seven immune mediators and assessed their associations with the presence of painful DSPN using multiple logistic regression models.

RESULTS
After adjustment for age and sex, we found positive associations between serum concentrations of the cytokine interleukin (IL)-6 and the soluble intercellular adhesion molecule (sICAM)-1 and painful DSPN \( (P = 0.004 \) and \( P = 0.005 \), respectively), whereas no associations were observed for C-reactive protein, IL-18, tumor necrosis factor-\( \alpha \), adiponectin, and IL-1 receptor antagonist (IL-1RA, \( P = 0.07–0.38 \)). Associations between IL-6 and sICAM-1 and painful DSPN remained significant after additional adjustment for waist circumference, height, hypertension, cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction and/or stroke, presence of other neurological conditions, and use of nonsteroidal anti-inflammatory drugs \( (P = 0.005 \) and \( P = 0.016 \), respectively).

CONCLUSIONS
Painful DSPN is linked to systemic subclinical and vascular inflammation in the older population independent of anthropometric, lifestyle, and metabolic confounders.
Distal sensorimotor polyneuropathy (DSPN) represents the most common diabetic microvascular complication, affecting at least one-third of patients with type 2 diabetes (1,2). We previously showed in individuals aged 61–82 years from the population-based KORA F4 study (Augsburg, Germany) that DSPN is also more prevalent in individuals with prediabetes than in those with normal glucose tolerance (NGT) (3). These data indicate that studies in samples from the general population are required to better understand risk factors of DSPN in the older population (2).

Painful DSPN is encountered in 10–26% of diabetic patients and leads to substantial impairment in quality of life, partly due to limited treatment options (4,5). Factors contributing to painful DSPN are incompletely understood and may include obesity, peripheral arterial disease, and dyslipidemia (4,6). Also, functional and structural biomarkers to predict the development of neuropathic pain are still lacking (7). Interestingly, studies using rodent models of painful neuropathy suggest that inflammatory processes contribute to its pathogenesis (5,8,9) because neuropathic pain can be alleviated by genetic knockout of proinflammatory cytokines and by over-expression of anti-inflammatory immune mediators (9). However, it is unclear to what extent pathomechanisms in short-lived animal models of painful neuropathy reflect processes in painful DSPN that develops over the course of years in humans (5). One study of 77 patients with type 1 or type 2 diabetes and DSPN found higher systemic concentrations of C-reactive protein (CRP) and soluble intercellular adhesion molecule (sICAM)-1 in individuals with painful compared with painless DSPN. However, the selection of the study participants and the lack of adjustment for potential confounders precluded inferences to the general population (10).

To investigate the potential link between subclinical inflammation and painful DSPN in the older general population, we measured circulating levels of seven pro- and anti-inflammatory immune mediators in participants of the German KORA F4 study, examined their association with painful DSPN, and assessed whether these associations could be explained by anthropometric, metabolic or lifestyle factors, comorbidities, and medication as potential confounders.

**RESEARCH DESIGN AND METHODS**

**Study Population**

Data are based on the Cooperative Health Research in the Region of Augsburg (KORA) F4 study (2006–2008), the follow-up examination of the population-based KORA S4 study (1999–2001). The design of both surveys has been described in detail before (11,12). Briefly, the current study is based on 1,047 subjects aged 61 to 82 years who participated in KORA F4 and for whom complete information on glucose tolerance status, clinical DSPN, and immune mediators were available as reported (13). All participants gave written informed consent to the study, which was approved by the Bavarian Medical Association Ethics Committee.

**Assessment of Painful DSPN**

To identify individuals at high risk of DSPN, we used the examination part of the Michigan Neuropathy Screening Instrument (MNSI) (14). The physical examination of the MNSI scores the appearance of feet (normal or deformities, dry skin, callus, infection, fissure, or other irregularities), foot ulceration, ankle reflexes, and vibration perception at the great toes. The total score can range from 0 (all aspects normal) to a maximum of 8 points. We defined DSPN using a cutoff of >2 points, as previously suggested (14,15). This definition of DSPN satisfies the minimal diagnostic criteria for possible DSPN according to the Toronto Diabetic Neuropathy Expert Group (1).

The level of pain in the feet during the 24 h preceding the examination was assessed using an 11-point numeric rating scale from 0 (no pain) to 10 (worst possible pain). Painful DSPN was defined as the combination of DSPN based on MNSI >2 and the presence of pain in the feet (pain intensity >0).

**Assessment of Anthropometric, Lifestyle, and Metabolic Variables**

Height, weight, waist circumference, and blood pressure were measured according to standardized protocols (11,12). Trained medical interviewers collected information on medical history, physical activity, smoking behavior, and alcohol consumption (11,12).

Diabetes was assessed based on validated self-reported diagnosis of type 2 diabetes. Oral glucose tolerance tests, using the World Health Organization diagnostic criteria from 1999 (16) were performed in all individuals without known type 2 diabetes after an overnight fast of 8 h (11,13). Hemoglobin A1c (HbA1c) was determined with a reverse-phase cation-exchange high-performance liquid chromatography method (Analyzer HA 8160; Menarini, Florence, Italy). Total cholesterol was measured by an enzymatic method (Dade Behring, Marburg, Germany).

**Assessment of Biomarkers of Subclinical Inflammation**

The study was based on seven biomarkers reflecting different aspects of subclinical inflammation: CRP as an acute-phase protein, interleukin (IL)-6 and tumor necrosis factor (TNF)-α as general proinflammatory cytokines, IL-18 and IL-1 receptor antagonist (IL-1RA) as pro- and anti-inflammatory cytokines from the IL-1 family, total adiponectin as an anti-inflammatory adipokine, and sICAM-1 as a marker of vascular inflammation. These seven biomarkers have been implicated as potential determinants of painful DSPN in preclinical studies (5,8,9), whereas data from epidemiological studies are scarce or absent.

High-sensitivity CRP (hsCRP) was determined in plasma using a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring) (13). Plasma levels of IL-18 and serum levels of IL-6, TNF-α, IL-1RA, sICAM-1, and total adiponectin were determined with commercially available ELISA kits (13).

**Statistical Analysis**

Participant characteristics were stratified by presence of painful DSPN and are presented as mean ± SD for normally distributed variables and as median (25th; 75th percentiles) for variables without a normal distribution. ANOVA was used to evaluate age- and sex-adjusted differences in continuous variables for subjects with and without painful DSPN. For log-normal variables, ANOVA was performed on a log-scale. Differences in dichotomous variables were examined using logistic regression.

Logistic regression models were fitted to study associations among immune markers as dependent variables (log-transformed continuous variables) and the presence of painful DSPN (dichotomous variable) adjusting for potential confounders as independent variables. As described (13), the multivariable model included age (years), sex, height (cm), waist circumference (cm), hypertension (yes/no), total cholesterol (mg/dL), smoking (never/former/current), alcohol
consumption (abstainer/moderate/high), leisure-time physical activity (low/high), history of acute myocardial infarction and/or stroke (yes/no), the presence of neurological conditions that might cause nerve damage (yes/no), and use of non-steroidal anti-inflammatory drugs (NSAIDs; yes/no). Covariates were based on our previous studies and the published evidence. In particular, we chose these models to ensure comparability of our results with data on subclinical inflammation and DSPN in the same study sample, as previously reported (13).

In a first sensitivity analysis, we also calculated a third model that additionally included \( \text{HbA}_{1c} \), glucose tolerance status (NGT, impaired fasting glucose [IFG] and/or impaired glucose tolerance [IGT], type 2 diabetes), and use of insulin (yes/no). In a second sensitivity analysis, we fitted linear regression models to study associations among immune markers as dependent variables (log-transformed continuous variables) and pain intensity as a continuous variable instead of using the presence of painful DSPN (yes/no) as a dichotomous variable.

\( P \) values < 0.05 were considered statistically significant. All analyses were performed with STATA 11 statistical software (StataCorp LP, College Station, TX).

RESULTS

Study Population
Among 1,047 participants aged 61–82 years, 391 were affected by DSPN, and 54 of these individuals had painful DSPN (Table 1). Compared with individuals with painless DSPN, those with painful DSPN were more likely to be women and to use NSAIDs and had larger waist circumferences and higher \( \text{HbA}_{1c} \) levels. The groups did not differ with respect to age, glucose tolerance status, blood pressure, cholesterol, lifestyle factors, and history of myocardial infarction, stroke, and neurological diseases (Table 1). Among the pro- and anti-inflammatory immune mediators, circulating levels of IL-6 and sICAM-1 were higher in subjects with painful DSPN (\( P = 0.002 \) and \( P = 0.004 \), respectively, in age- and sex-adjusted analyses), whereas no differences between the groups were seen for hsCRP, IL-18, TNF-\( \alpha \), IL-1RA, and adiponectin (\( P = 0.068-0.373 \)).

Associations Among Immune Mediators and Painful DSPN
After additional adjustment for waist circumference, height, hypertension, total cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction and/or stroke, neurological conditions that might cause nerve damage, and use of NSAIDs, the regression coefficients for the associations between IL-6 and sICAM and painful DSPN remained virtually unchanged (\( P = 0.005 \) and \( P = 0.016 \), respectively, in fully adjusted analyses). Furthermore, no significant associations with painful DSPN were noted for hsCRP, IL-18, TNF-\( \alpha \), IL-1RA, and adiponectin (Table 2).

In a first sensitivity analysis, we assessed the effect of \( \text{HbA}_{1c} \), glucose tolerance status (NGT, IFG and/or IGT, type 2 diabetes) and use of insulin as potential confounders. Results were not altered.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Painless DSPN</th>
<th>Painful DSPN</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.8 ± 5.2</td>
<td>72.1 ± 5.3</td>
<td>0.673</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>57</td>
<td>41</td>
<td>0.025</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167 ± 9</td>
<td>164 ± 9</td>
<td>0.142</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>29.4 ± 4.9</td>
<td>30.9 ± 4.8</td>
<td>0.052</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>101 ± 13</td>
<td>103 ± 12</td>
<td>0.041</td>
</tr>
<tr>
<td>( \text{HbA}_{1c} ) (%)</td>
<td>5.9 ± 0.7</td>
<td>6.1 ± 0.9</td>
<td>0.013</td>
</tr>
<tr>
<td>( \text{HbA}_{1c} ) (mmol/mol)</td>
<td>40.5 ± 8.2</td>
<td>43.4 ± 10.1</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Table 1—Description of the KORA F4 study population with DSPN stratified by the presence of painful and painless DSPN
We previously used the source population of the current study (i.e., all individuals aged 61–82 years in KORA F4) to investigate biomarkers of subclinical inflammation that are related to any DSPN, irrespective of pain, and observed positive associations for IL-6 and IL-1RA (13).

Our results are in line with data from patients with peripheral diabetic neuropathy that assessed differences in immune mediator levels between individuals without and with pain (10). In that study, patients with painful neuropathy had higher circulating levels of CRP and sICAM-1, whereas no differences were found for TNF-α, and IL-6 was not measured (10). The difference compared with our study regarding CRP is interesting given that we previously found associations between CRP and the MNSI score in a sample of diabetic patients (17), but not in the general older population (13), which suggests differences in risk factors of DSPN with respect to diabetic status. One study reported an association between TNF-α plasma levels and neuropathic pain in patients with type 2 diabetes, but the analysis was not adjusted for any confounding variables (18).

One can first speculate from our findings that IL-6 and IL-6 receptor (IL-6R)–mediated signaling may be involved in the development of painless and also painful DSPN. Interestingly, there is evidence from a mouse model that the inhibition of IL-6 activity with a monoclonal antibody against IL-6R reduces neuropathic pain (19). On one hand, it remains to be elucidated whether intracellular signaling downstream of the IL-6R/gp130 complex (e.g., by activation of the transcription factors Janus kinase and signal transducer and activator of transcription (20)) contribute to the development of neuropathic pain. On the other hand, IL-6–mediated activation of macrophages, microglia, and other cell types and the subsequent release of other immune mediators could also be relevant (21).

Second, our data suggest that IL-1β–related processes, which are reflected by increased IL-1RA levels, may be more important at the early stages of DSPN before progression to painful DSPN. However, inhibition of IL-1β has been found effective to block neuropathic pain in several rodent models (9), pointing also toward an interaction between IL-1β and nociception. Sensory neurons express the IL-1 receptor (22), and IL-1β increases their excitability (23,24). Therefore, a direct effect of IL-1β on the development of neuropathic pain is conceivable (25). Moreover, IL-1β activates macrophages, Schwann cells, and neurons, which in turn release cytokines, prostaglandins, and other mediators, so that indirect effects to the generation of neuropathic pain are also possible (26,27). Thus, IL-1β–mediated mechanisms represent potentially relevant contributors to neuropathic pain despite our null finding for IL-1RA. In this context, it is important to note that measurement of IL-1β would have been interesting in our study, but currently available assays are not sensitive enough to capture the low levels of this potent proinflammatory cytokine in the circulation.

Third, it can be hypothesized that vascular inflammation, reflected by elevated sICAM-1 levels, may contribute to the development of pain in DSPN. Increasing plasma levels of soluble cell adhesion molecules (sICAM-1, E-selectin) were associated with decreasing peroneal nerve conduction velocity during a 5-year period in a small group of diabetic patients, which indeed indicated a vascular involvement in the development and progression of DSPN (28). Elevated concentrations of cell adhesion molecules such as sICAM-1 in the circulation result from increased expression and/or shedding of these proteins from the cell surface in response to endothelial activation. They most likely represent markers of vascular inflammation rather than genuine cardiovascular risk factors, but this does not preclude a potential prognostic value

Table 2—Association between circulating levels of immune mediators and painful DSPN

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P</td>
<td>β</td>
<td>P</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>0.156</td>
<td>0.269</td>
<td>−0.004</td>
<td>0.980</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>0.542</td>
<td>0.004</td>
<td>0.621</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>−0.314</td>
<td>0.380</td>
<td>−0.498</td>
<td>0.198</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>0.280</td>
<td>0.229</td>
<td>0.283</td>
<td>0.259</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>1.750</td>
<td>0.005</td>
<td>1.638</td>
<td>0.016</td>
</tr>
<tr>
<td>IL-1RA (pg/mL)</td>
<td>0.411</td>
<td>0.202</td>
<td>−0.012</td>
<td>0.975</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>−0.514</td>
<td>0.069</td>
<td>−0.325</td>
<td>0.296</td>
</tr>
</tbody>
</table>

β Coefficients and P values are shown from linear regression analyses with log-transformed immune mediator levels as dependent variables. Significant associations are indicated by bold print. Model 1: adjusted for age and sex. Model 2: model 1 + waist circumference, height, hypertension, total cholesterol, smoking, alcohol intake, physical activity, history of myocardial infarction and/or stroke, neurological conditions that might cause nerve damage, and use of NSAIDs.
of these proteins for the development of painful DSPN, which needs to be explored in future prospective studies. It is also conceivable that microvascular inflammation contributes to the association of DSPN with increased risk for a first cardiovascular event in type 2 diabetic patients (29).

These three hypotheses need to be corroborated by longitudinal analyses in humans with repeated examinations and measurements in which the development of painless and painful DSPN, as well as changes in inflammation-related biomarkers and other risk factors, are monitored simultaneously. Given that the underlying pathophysiology of pain in DSPN is not well defined, mechanistic studies examining the link between local inflammatory responses, enhanced excitability of sensory neurons, and central sensitization are necessary. Furthermore, the relationship between circulating immune markers and local levels in the central and peripheral nervous system also need to be better understood.

This work has several strengths and limitations. The population-based sample and the adjustment for multiple confounding variables represent strengths of our analysis. The population-based approach is relevant because DSPN without and with pain also affects a considerable proportion of nondiabetic individuals among the older population, as previously reported (3,6,17) and reviewed (2). A detailed analysis of this intriguing finding is beyond the scope of this study. However, it seems noteworthy that age does not appear to be the main determinant because we did not find significant age differences between individuals with painless and painful DSPN among the entire study sample (Table 1) or among the subgroup with NGT (data not shown). The adjustment for confounding factors represents an important part of the current analysis because this was largely neglected in previous clinical or epidemiological studies.

The main limitations are the cross-sectional design and the definition of painful DSPN. The cross-sectional nature does not allow us to draw conclusions with respect to the causality of the relationships described herein, so that prospective studies are required to corroborate and extend our findings. In the absence of a generally accepted definition of neuropathic pain in patients with diabetes, we used a definition for painful DSPN that combines the presence of neuropathic deficits (impairments) based on the MNSI and pain intensity in the feet during the last 24 h. We used this definition in a previous KORA survey (6), which showed a prevalence of painful DSPN of 13% in the diabetic population and only 1% in subjects with NGT. On the basis of these data, we believe that our definition of painful DSPN is conservative. However, we cannot exclude that patients with, for example, chronic inflammatory pain or chronic arthropathy, were included in the painful DSPN group, but the likelihood is low. In addition, it should be noted that we analyzed associations for seven biomarkers of inflammation, of which only the association for IL-6 would remain significant after adjustment for multiple testing according to Bonferroni.

In conclusion, our data indicate a differential association of biomarkers of subclinical and vascular inflammation with the presence of painful DSPN and pain intensity in older individuals from the general population. Prospective studies are required to assess their predictive value for incident painful DSPN. Mechanistic studies exploring the relationship between inflammation and peripheral and central sensitization appear relevant to improve our limited understanding of the underlying pathophysiology and to identify potential novel therapeutic targets.

Acknowledgments. The authors thank Ulrike Poschen, Gabi Gornitzka, and Karin Röhrig (all German Diabetes Center) for excellent technical assistance and are grateful to the field staff in Augsburg who were involved in the conduct of the KORA F4 study.

Funding. This work was supported by the Ministry of Science and Research of the State of North Rhine-Westphalia (MIWF NRW) and by the German Federal Ministry of Health (BMG). The diabetes part of the KORA F4 study was funded by a grant from the German Research Foundation (DFG; RA 459/3-1). This study was partly supported by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD e. V.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The KORA research platform and the KORA Augsburg studies are financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, which is funded by the German Federal Ministry of Science and Research (Berlin, Germany) and by the State of Bavaria.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

Author Contributions. C.H. planned the study, contributed to data analysis and interpretation, and wrote the manuscript. B.W.C.B. performed data analysis and reviewed and edited the manuscript. W.R. and D.Z. planned the study, contributed to data analysis and interpretation, and reviewed and edited the manuscript. M.H. and W.K. contributed data and reviewed and edited the manuscript. B.K., B.T., M.R., and C.M. reviewed and edited the manuscript. W.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prior Presentation. Data from this study were presented at the 49th Annual Meeting of the European Diabetes Epidemiology Group, Cagliari, Italy, 29 March–01 April 2014, and at the 49th Annual Meeting of the German Diabetes Association, Berlin, Germany, 28–31 May 2014.

References