Proinflammatory Cytokines Predict the Incidence and Progression of Distal Sensorimotor Polyneuropathy: KORA F4/FF4 Study

Christian Herder,[1,2] Julia M. Kannenberg,[1,2] Cornelia Huth,[2,3] Maren Carstensen-Kirberg,[1,2] Wolfgang Rathmann,[2,4] Wolfgang Koenig,[5,6] Margit Heier,[3] Sonja Püttgen,[1,2] Barbara Thorand,[2,3] Annette Peters,[2,3] Michael Roden,[1,2,7] Christa Meisinger,[2,3] and Dan Ziegler[1,2,7]

Diabetes Care 2017;40:569–576 | DOI: 10.2337/dc16-2259

OBJECTIVE

Experimental and epidemiological studies have implicated inflammatory processes in the pathogenesis of distal sensorimotor polyneuropathy (DSPN), but prospective studies are lacking. We hypothesized that biomarkers of inflammation predict the development and progression of DSPN in a population-based cohort.

RESEARCH DESIGN AND METHODS

This study was based on participants aged 62–81 years from the Cooperative Health Research in the Region of Augsburg (KORA) F4/FF4 cohort, with a mean follow-up of 6.5 years. The predictive value of systemic levels of eight biomarkers of inflammation was assessed for incident DSPN in 133 incident case subjects and 397 individuals without incident DSPN, and for DSPN progression in 57 patients with prevalent DSPN at both time points.

RESULTS

Higher hs-CRP, interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist (IL-1RA), and soluble intercellular adhesion molecule (sICAM-1) and lower adiponectin levels were associated with incident DSPN in age- and sex-adjusted analysis; IL-18 and omentin were not. IL-6 (odds ratio 1.31 [95% CI 1.00–1.71]) and TNF-α (odds ratio 1.31 [95% CI 1.03–1.67]) remained associated with incident DSPN after adjusting for known DSPN risk factors. The addition of both cytokines to a clinical risk model improved model fit and reclassification. sICAM-1 and IL-1RA were positively associated with progression of DSPN.

CONCLUSIONS

Systemic subclinical and vascular inflammation predicted both the onset and progression of DSPN over 6.5 years in an older general population. Thus modulation of inflammatory processes may be relevant to prevent and/or treat diabetic neuropathy.

Distal sensorimotor polyneuropathy (DSPN) is the most frequent neurological complication of type 2 diabetes (1). The pathomechanisms leading to the onset and progression of DSPN are not completely understood, which profoundly limits current prevention and treatment. Advanced age and diabetes duration represent the most important known risk factors, but there is increasing evidence that height,

[1]*Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[2]*German Center for Diabetes Research (DZD), München-Neuherberg, Germany*
[3]*Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany*
[4]*Institute for Biometrics and Epidemiology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University Düsseldorf, Düsseldorf, Germany*
[5]*Deutsches Herzzentrum München, Technische Universität München, Munich, Germany*
[6]*German Center for Cardiovascular Research (DZHK), Partner site Munich Heart Alliance, Munich, Germany*
[7]*Department of Endocrinology and Diabetology, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany*

Corresponding author: Christian Herder, christian.herder@ddz.uni-duesseldorf.de.

Received 21 October 2016 and accepted 5 January 2017.

This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc16-2259/-/DC1.

C.M. and D.Z. contributed equally to this work.

prediabetes, obesity, dyslipidemia, hypertension, and smoking also promote the development of DSPN (2).

Subclinical inflammation has been proposed as one mechanism contributing to the risk of DSPN in animal models and humans (3). However, the cross-sectional design of almost all clinical and epidemiological studies (4–17) so far precludes inferences regarding the temporal relationship between inflammation and DSPN, which are essential for any conclusions about causality. One small prospective study assessed plasma levels of soluble adhesion molecules in 28 individuals with diabetes during a 5-year study period and suggested that endothelial activation is associated with nerve conduction slowing (18). Although that study supports the concept of vascular inflammation as a risk factor for DSPN, larger and especially population-based studies are required to assess the relevance of biomarkers of inflammation as predictors of the development and progression of DSPN.

It is noteworthy that apart from overt diabetes, older individuals with prediabetes show a higher prevalence of DSPN than people with normal glucose tolerance (19), so studies to explore risk factors of DSPN should also focus on the older age ranges of population-based cohorts (4,8).

Therefore, our study used a prospective design and aimed to test the hypotheses that *1*) higher levels of proinflammatory biomarkers and lower levels of anti-inflammatory biomarkers promote the risk of DSPN in the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4/FF4 cohort, *2*) such associations are only partially explained by known risk factors, *3*) associated biomarkers improve the prediction of DSPN risk, and *4*) biomarkers of subclinical inflammation are also associated with the progression of DSPN.

RESEARCH DESIGN AND METHODS

Study Design and Participants

This study is based on data from the KORA F4 (2006–2008) and the KORA FF4 studies (2013–2014), both follow-up examinations of the population-based KORA S4 study (1999–2001); all three were conducted in Augsburg (Germany) and two adjacent counties. The design of the KORA studies has been described before (20). The three examinations were carried out in accordance with the Declaration of Helsinki, including obtaining written informed consent from all participants. The study was approved by the ethics board of the Bavarian Chamber of Physicians (Munich, Germany).

This study initially included all participants aged 62–81 years in KORA F4 (n = 1,161). Anthropometric and metabolic variables and lifestyle factors were assessed as described previously (20,21). Hypertension was defined as blood pressure 140/90 mmHg or higher or use of antihypertensive medication, given that the subjects were aware of being hypertensive. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation. Alcohol intake was classified as none (0 g/day), moderate ($\geq$0 to $<$20 g/day for women, $\geq$0 to $<$40 g/day for men), or high ($\geq$20 g/day for women, $\geq$40 g/day for men). Study participants were classified as physically active if they participated in sports in summer and in winter and if they reported $\geq$1 h of sports/week in at least one of the seasons.

All participants without known diabetes were assigned to receive a standard 75-g oral glucose tolerance test in both KORA F4 and KORA FF4. Glucose tolerance categories were defined using fasting and 2-h glucose levels according to the 2003 American Diabetes Association criteria (22). Known type 2 diabetes in KORA F4 was defined as self-report that was validated by the responsible physician, or as current use of antidiabetes agents. Newly diagnosed type 2 diabetes in KORA F4 was defined on the basis of fasting and/or 2-h glucose levels in the oral glucose tolerance test using the aforementioned criteria.

Supplementary Fig. 1 describes the study population in detail. Of 1,161 participants in the KORA F4 study (2006–2008) aged 62–81 years, we excluded 28 because of prevalent type 1 diabetes, diabetes forms other than type 1 or type 2, or unclear glucose tolerance status; 8 because blood samples were taken during a nonfasting state; and 36 because of missing baseline values for statistical analysis (age, sex, waist circumference, height, hypertension, total cholesterol, HbA_{1c}, alcohol intake, smoking, physical activity, Michigan Neuropathy Screening Instrument [MNSI] score). Another 454 participants were excluded because of nonparticipation in KORA FF4 (2013–2014) for various reasons (death, moved out of the study area, refused, too ill, not interested, too busy to participate, could not be contacted), which left 635 individuals who participated in both KORA F4 and FF4. From these, we had to exclude 20 because of missing data for MNSI in KORA FF4. This left the following samples for further analysis in this study: *1*) 530 individuals without DSPN in F4 (incidence analysis: 133 incident case subjects, 397 healthy subjects), *2*) 57 individuals with DSPN in F4 and FF4 (progression analysis), and *3*) 28 individuals with DSPN in F4 but not in FF4. Mean duration of follow-up $\pm$ SD was 6.46 $\pm$ 0.23 years.

Assessment of DSPN

Prevalent and incident DSPN were estimated using the examination part of the MNSI (23), including the following components: appearance of feet (normal or deformities, dry skin, callus, infection, fissure, or other irregularities), foot ulceration, ankle reflexes, and vibration perception threshold at the great toes; these resulted in a score from 0 (all aspects normal) to a maximum of 8 points. The age-dependent limits of a normal vibration perception threshold were computed according to the method described by Martina et al. (24). We extended the neuropathy assessment to include a bilateral examination of sensory perception using a 10-g monofilament (Neuropen), as previously described (25). Responses were assigned scores of 1 (absent), 0.5 (decreased), or 0 (normal) for each side. Thus the total MNSI score ranged from 0 (all aspects normal) to a maximum of 10 points. Considering the advanced age of the study population and the addition of the monofilament examination, we defined DSPN using a cutoff at $>$3 points. This definition of DSPN satisfies the minimal diagnostic criteria for possible DSPN according to the Toronto Diabetic Neuropathy Expert Group (1).

Measurement of Biomarkers of Subclinical Inflammation

Plasma levels of hs-CRP and interleukin (IL)-18, and serum levels of IL-6, tumor necrosis factor (TNF)-α, soluble intercellular adhesion molecule (sICAM)-1, IL-1 receptor antagonist (IL-1RA), and total adiponectin and omentin were measured as described before (8,21,26). These biomarkers were selected for

this study based on previous reports describing their associations with DSPN in cross-sectional analyses (5–13,16–18).

Statistical Analysis

Baseline characteristics of the study sample are given as mean ± SD, median and 25th, 75th percentiles, or percentages. Groups were compared pairwise with logistic regression analysis (likelihood ratio tests comparing models with the respective variable, and age and sex as independent variables with models with age and sex only). Biomarkers of subclinical inflammation were $\log_2$-transformed before correlation analysis and logistic regression. Correlations between biomarkers of subclinical inflammation were estimated using the Pearson correlation coefficient (*r*) and corresponding *P* values.

Logistic regression models were fitted to study associations between biomarkers of subclinical inflammation as dependent variables ($\log_2$-transformed, continuous) and the presence of DSPN (yes/no), adjusting for potential confounders as the independent variables. Models of increasing complexity included age (years), sex, height (cm), waist circumference (cm), hypertension (yes/no), total cholesterol (mg/dL), HbA_{1c} (%), smoking (never/former/current), alcohol consumption (none/moderate/high), physical activity (active/inactive), history of myocardial infarction and/or stroke (yes/no), eGFR (mL/min/1.73 m^2), the presence of neurological conditions that might cause nerve damage (yes/no), and the use of nonsteroidal anti-inflammatory drugs (yes/no). Covariables were based on our previous studies and the published evidence. In particular, we chose these models to ensure the comparability of our results with cross-sectional data on subclinical inflammation and DSPN in the KORA F4 study (8,12,26).

To assess the additional predictive value of biomarkers of subclinical inflammation, we compared a risk model including all covariables from the fully adjusted logistic regression model with a risk model including those covariables and the biomarkers of subclinical inflammation that showed significant associations in the logistic regression models. To compare these models, we report the likelihood ratio test result, the C-statistic, and the 95% CI for the difference in C-statistics using a nonparameteric approach, as implemented in SAS (27). To test for differences in C-statistics, we used the bootstrap test implemented in the pROC package in R software (28). In addition, we calculated the category-free net reclassification improvement (NRI) and the integrated discrimination improvement (IDI) (29). We tested NRI and IDI significance, as previously described (29), using the predictABEL package in R software (30).

The association between biomarkers of subclinical inflammation and progression of DSPN (defined as change in MNSI: $MNSI_{FF4} - MNSI_{F4}$) was assessed in all individuals with DSPN in KORA F4 and FF4 using linear regression analysis including covariables identical to those used in the logistic regression analysis.

All statistical analyses were performed with R version 3.2.4 (https://www.r-project.org/) and SAS version 9.4 (SAS Institute Inc., Cary, NC). A *P* value <0.05 was considered to indicate statistical significance.

RESULTS

Study Population

The study population comprised 615 individuals who participated in both KORA F4 and FF4 and for whom all relevant data for the analysis were available (Supplementary Fig. 1).

Supplementary Table 1 shows that hs-CRP, IL-6, IL-18, TNF-α, IL-1RA, and sICAM-1 were positively correlated with one another (*r* between 0.13 and 0.41). Adiponectin and omentin were also positively correlated with each other ($r = 0.36$), whereas their correlations with the aforementioned biomarkers were weaker and in some cases inverse.

Supplementary Table 2 presents a dropout analysis and shows that individuals who participated in KORA F4 but not in FF4 ($n = 546$) were older and in general less healthy; for example, they had higher BMI and HbA_{1c}, lower eGFR, higher MNSI score, and a more extensive proinflammatory state than participants in both KORA F4 and FF4.

Biomarkers of Subclinical Inflammation and Incidence of DSPN

Table 1 shows the baseline characteristics of the KORA F4 study population stratified by incidence of DSPN in KORA FF4. Individuals who developed DSPN were older, taller, and had both a higher BMI and larger waist circumference than individuals who remained free of DSPN. They also had a higher HbA_{1c}, were more likely to smoke and less likely to be physically active, and had a higher MNSI score. No differences were seen between both groups with respect to sex, glucose tolerance status, hypertension, serum lipids, antihypertensive medication use, eGFR, alcohol intake, history of myocardial infarction, presence of neurological conditions that might cause nerve damage, and use of nonsteroidal anti-inflammatory drugs.

Systemic levels of inflammation-related biomarkers differed for six of eight measured biomarkers. Patients with incident DSPN had higher systemic levels of hs-CRP, IL-6, TNF-α, IL-1RA, and sICAM, as well as lower levels of adiponectin (all $P < 0.05$, adjusted for age and sex), than those without incident DSPN, whereas systemic levels of IL-18 and omentin did not differ (Tables 1 and 2).

Further adjustment for anthropometric variables, hypertension, cholesterol, HbA_{1c}, lifestyle factors (all added in model 2), eGFR, medication use, and cardiovascular and neurological comorbidities (all added in model 3) partially explained these associations, but the positive associations of IL-6 (odds ratio 1.31 [95% CI 1.00–1.71]; $P = 0.048$) and TNF-α (odds ratio 1.31 [95% CI 1.03–1.67]; $P = 0.031$) with incident DSPN remained statistically significant (Table 2).

When comparing the accuracy of a clinical risk prediction model for DSPN that contained all variables from model 3 with an extended model that also contained IL-6 and TNF-α, we found a better fit of the extended model ($P = 0.031$ for the likelihood ratio test), but no significant improvement in the C-statistic (Table 3). However, the extended biomarker model showed both a significant category-free NRI (0.243 [95% CI 0.046–0.439]; $P = 0.015$) and IDI (0.014 [95% CI 0.003–0.025]; $P = 0.016$) (Table 3).

Biomarkers of Subclinical Inflammation and Progression of DSPN

The population in which the associations between biomarkers of subclinical inflammation and progression of DSPN were analyzed comprised 57 individuals with DSPN from both KORA F4 and FF4. MNSI scores (mean ± SD) were 4.24 ± 0.61 in KORA F4 and 5.00 ± 1.03 in KORA FF4, with a mean change in MNSI of

Table 1—Baseline characteristics of the KORA F4 study population stratified by incidence of DSPN

Variable	No incident DSPN (n = 397)	Incident DSPN (n = 133)	P value
Age (years)	67.9 ± 4.6	70.2 ± 5.0	<0.001
Sex (%)			0.171
Male	49.1	55.6	
Female	50.9	44.4	
BMI (kg/m^2)	27.6 ± 3.8	29.1 ± 4.0	<0.001
Waist circumference (cm)	94.7 ± 11.3	99.8 ± 11.4	<0.001
Height (cm)	166 ± 9	167 ± 9	0.031
HbA_{1c} (% [mmol/mol])	5.68 ± 0.50 [39 ± 6]	5.82 ± 0.68 [40 ± 7]	0.028
Glucose tolerance status (%)			0.689
NGT	45.1	37.6	
IFG	21.4	20.3	
IGT	9.1	10.5	
IFG and IGT	9.6	11.3	
Newly diagnosed T2D	5.5	5.3	
Known T2D	9.3	15.0	
Hypertension (%)*	56.2	66.2	0.316
Total cholesterol (mmol/L)†	5.16 ± 0.85	4.83 ± 0.61	0.203
Fasting triglycerides (mmol/L)†	1.31 (0.96, 1.75)	1.45 (1.03, 2.05)	0.462
Use of lipid-lowering drugs (% yes)	23.2	25.6	0.866
eGFR (mL/min/1.73 m^2)	80.3 ± 13.1	77.0 ± 14.1	0.458
Smoking (%)			0.003
Never	51.9	55.6	
Former	42.3	33.1	
Current	5.8	11.3	
Alcohol intake (%)			0.070
None	30.5	30.1	
Moderate	60.5	55.6	
High	9.1	14.3	
Physically active (%)	62.2	42.9	0.001
Myocardial infarction (%)	5.0	6.8	0.880
Neurological conditions that might cause nerve damage (%)	14.7	21.1	0.041
Use of nonsteroidal anti-inflammatory drugs (%)‡	1.0	2.3	0.385
MNSI	2.0 (0.5, 2.0)	2.0 (2.0, 2.5)	<0.001
hs-CRP (mg/L)	1.31 (0.70, 2.52)	1.60 (0.76, 3.42)	0.023
IL-6 (pg/mL)	1.33 (0.94, 1.96)	1.71 (1.23, 2.50)	<0.001
IL-18 (pg/mL)	306 (250, 402)	325 (254, 416)	0.992
TNF-α (pg/mL)	1.92 (1.39, 2.74)	2.11 (1.60, 3.52)	0.013
IL-1RA (pg/mL)	224 ± 47	240 ± 58	0.008
sICAM-1 (ng/mL)	306 ± 128	349 ± 175	0.015
Adiponectin (μg/mL)	10.15 (6.57, 15.44)	9.44 (5.41, 13.58)	0.046
Omentin (ng/mL)	496 ± 150	506 ± 155	0.706

Data are given as mean ± SD, median (25th, 75th percentiles), or percentages. The P values are derived from logistic regression analysis (likelihood ratio tests comparing models with the respective variable, age, and sex as independent variables with models with age and sex only). All analyses were adjusted for age and sex except associations with age (sex-adjusted only) or sex (age-adjusted only). Biomarkers of subclinical inflammation were $\log_2$-transformed before logistic regression. IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T2D, type 2 diabetes. *Blood pressure ≥140/90 mmHg or use of antihypertensive medication, given that the subjects were aware of being hypertensive. †Individuals using lipid-lowering drugs were excluded (n = 123). ‡Except acetylsalicylic acid when used as platelet aggregation inhibitor.

0.76 ± 0.97 (P < 0.001). In addition, this group was on average older, more likely to be male, taller, more obese, and had higher IL-6 and IL-1RA levels than the group without DSPN at baseline (n = 530) (Supplementary Table 3).

A small group (n = 28) had DSPN at baseline (MNSI 4.18 ± 0.66), but not at the follow-up examination (MNSI 2.38 ± 0.60; mean change −1.80 ± 0.90; P < 0.001). This group was older and had higher HbA_{1c} than the group without DSPN at baseline, but lower cholesterol levels than the group with DSPN at both time points (Supplementary Table 3).

In the initial analysis adjusting for age and sex, higher sICAM-1 and higher IL-1RA were associated with an increase in MNSI (Supplementary Table 4). These associations were strengthened by further adjustment and reached a β value of 1.075 (95% CI 0.218–1.932) for sICAM-1 and 0.792 (0.292–1.293) for IL-1RA in the fully adjusted model 3 (Fig. 1). In addition, we observed a trend for an association between higher hs-CRP and increase in MNSI (P = 0.052, model 3).

CONCLUSIONS

Higher systemic levels of hs-CRP, IL-6, TNF-α, IL-1RA, and sICAM-1, and lower adiponectin levels, were associated with a higher risk of DSPN over 6.5 years in the population-based KORA F4/FF4 cohort study. Adjustment for known risk factors attenuated some of these associations, but higher IL-6 and TNF-α levels remained associated with incident DSPN after extensive adjustment. The addition of both proinflammatory cytokines to a clinical risk model improved model fit, category-free NRI, and IDI. Higher systemic levels of sICAM-1 and IL-1RA were associated with progression of DSPN.

IL-6, TNF-α, and Incident DSPN

This is the first prospective, population-based study to investigate whether biomarkers of subclinical inflammation are associated with incident DSPN. The first main finding of our study is that systemic levels of six of eight biomarkers differed between individuals with incident DSPN and those without 6 years before DSPN diagnosis, which indicates that a proinflammatory state and lower adiponectin levels precede the onset of DSPN and suggests a role for inflammation in its pathogenesis.

We previously reported that higher levels of IL-6, IL-18, IL-1RA, and sICAM-1 were associated with higher MNSI scores in older individuals (8), and that omentin levels were inversely associated with DSPN in individuals with type 2 diabetes (12). Thus, cross-sectional and prospective associations only partially overlapped, emphasizing

Table 2—Odds ratios (OR) and 95% CI for associations between biomarkers of subclinical inflammation and incident DSPN

	Model 1		Model 2		Model 3	
Variable	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
hs-CRP	**1.17 (1.02–1.35)**	**0.024**	1.09 (0.94–1.28)	0.255	1.09 (0.93–1.27)	0.306
IL-6	**1.53 (1.21–1.95)**	**<0.001**	**1.33 (1.02–1.72)**	**0.034**	**1.31 (1.00–1.71)**	**0.048**
IL-18	1.00 (0.70–1.42)	0.992	0.83 (0.57–1.22)	0.348	0.82 (0.56–1.21)	0.321
TNF-α	**1.32 (1.06–1.65)**	**0.013**	**1.29 (1.01–1.63)**	**0.036**	**1.31 (1.03–1.67)**	**0.031**
IL-1RA	**1.53 (1.08–2.18)**	**0.017**	1.09 (0.74–1.61)	0.666	1.09 (0.73–1.61)	0.681
sICAM-1	**2.44 (1.25–4.73)**	**0.009**	1.63 (0.80–3.35)	0.182	1.59 (0.77–3.27)	0.209
Adiponectin	**0.76 (0.58–1.00)**	**0.046**	0.95 (0.70–1.27)	0.710	0.92 (0.68–1.25)	0.595
Omentin	1.10 (0.67–1.82)	0.706	1.43 (0.84–2.45)	0.193	1.36 (0.79–2.34)	0.267

ORs, 95% CIs, and corresponding *P* values for incident DSPN are given for a doubling in circulating levels of biomarkers. Model 1 was adjusted for age and sex. Model 2 was adjusted for the factors in model 1 plus waist circumference, height, hypertension, total cholesterol, HbA_{1c}, alcohol intake, smoking, and physical activity. Model 3 included variables in model 2 plus the use of lipid-lowering drugs, use of nonsteroidal anti-inflammatory drugs, eGFR, prevalent myocardial infarction, and neurological conditions that might cause nerve damage. Boldface type indicates statistical significance ($P < 0.05$).

the need for prospective studies to assess genuine risk relationships between biomarkers and DSPN.

The second main finding of this study is the identification of higher serum levels of the proinflammatory cytokines IL-6 and TNF-α in individuals with incident DSPN compared with those without incident DSPN after adjustment for anthropometric or metabolic risk factors for DSPN. The finding for IL-6 not only corroborates the report from the KORA F4 study (8) but also is in line with other cross-sectional studies investigating DSPN or impaired nerve conduction (5,17). By contrast, associations between higher TNF-α and DSPN or its components have so far been limited to small studies that did not adjust for confounding factors (6,7,9,15,31), whereas no associations were found in studies that considered confounders (5,8). From a mechanistic point of view, it is important to note that IL-6 and TNF-α are functionally related: TNF-α activates the transcription factor nuclear factor-κB and thereby induces IL-6 expression.

It is noteworthy that increased systemic levels of IL-6 and TNF-α are unlikely to be specific for incident DSPN. Higher levels of both cytokines are associated with a higher risk of coronary heart disease (32) and have been implicated in the development of impaired kidney function (33,34) and diabetic retinopathy (35,36). Therefore, further analyses are planned to investigate whether IL-6 and TNF-α also predict these micro- and macrovascular complications in the KORA cohort and whether it will be possible to identify distinct biomarker profiles for the incidence of specific comorbidities and for their combined risk.

It is currently not known to what extent our findings may be specific to the old age group in this study or to DSPN related to type 2 diabetes. Cross-sectional analyses indicated associations between IL-6, TNF-α, and DSPN in younger individuals and those with type 1 diabetes (6,9,17,37), but prospective data for these populations are not available.

The inverse association between adiponectin and DSPN in the age- and sex-adjusted model in our study is also interesting because cross-sectional studies reported associations between *higher* adiponectin and DSPN and/or impaired nerve conduction (10,11,17). This discrepancy suggests some degree of residual confounding or reverse causality in the latter analyses, which seems to be no longer relevant in studies using a prospective design.

Table 3—Improvement of a clinical risk model for the prediction of DSPN by biomarkers of subclinical inflammation

	Clinical risk model*	Clinical risk model plus IL-6 and TNF-α
Log likelihood	−251.78	−255.24
P value	Reference	0.031
C-statistic (95% CI)	0.746 (0.696–0.797)	0.756 (0.706–0.805)
Δ C-statistic (95% CI)†	N/A	0.009 (−0.007–0.025)
P value	N/A	0.268
Category-free NRI (95% CI)	Reference	0.243 (0.046–0.439)
P value	N/A	0.015
IDI (95% CI)	Reference	0.014 (0.003–0.025)
P value	N/A	0.016

*The clinical risk model includes all variables from the fully adjusted logistic regression model (see Table 2, model 3; i.e., age, sex, waist circumference, height, hypertension, total cholesterol, HbA_{1c}, alcohol intake, smoking, physical activity, use of lipid-lowering drugs, use of nonsteroidal anti-inflammatory drugs, eGFR, prevalent myocardial infarction, and neurological conditions that might cause nerve damage). †Δ C-statistic indicates the difference between models with biomarkers compared with the model without biomarkers. N/A, not applicable.

sICAM-1, IL-1RA, and Progression of DSPN

The third main finding of this study is the identification of sICAM-1 and IL-1RA as biomarkers for the progression of manifest DSPN. These results suggest that different biomarkers and mechanisms may be relevant for incidence as opposed to progression of DSPN, which deserves further study.

Our data extend a previous study that followed 28 individuals with diabetes (13 of whom also had DSPN) for 5 years and observed associations between higher baseline plasma levels of cell adhesion molecules and stronger deterioration of peroneal nerve conduction velocity (18). Our findings for sICAM-1

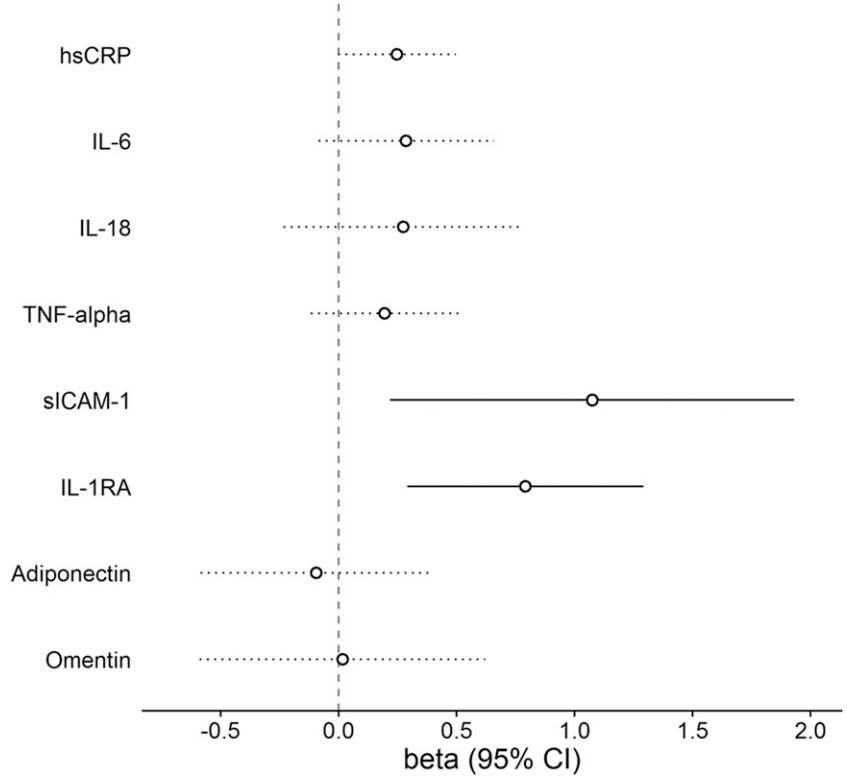

Figure 1—Associations between biomarkers of subclinical inflammation and progression of DSPN assessed by changes in MNSI in individuals with DSPN in KORA F4/FF4. The figure shows regression coefficients (β) and corresponding 95% CIs for changes in MNSI ($MNSI_{FF4} - MNSI_{F4}$) that are given for a doubling of circulating levels of biomarkers. Data are adjusted for age, sex, waist circumference, height, hypertension, total cholesterol, HbA_{1c}, alcohol intake, smoking, physical activity, use of lipid-lowering drugs, use of nonsteroidal anti-inflammatory drugs, eGFR, prevalent myocardial infarction, and neurological conditions that might cause nerve damage (model 3). Solid lines indicate $P < 0.05$; dotted lines, $P > 0.05$.

are in line with these data, although the fact that the study included both individuals with type 1 diabetes and individuals with type 2 diabetes limits the comparability with our results. Cell adhesion molecules such as ICAM-1 are expressed by activated endothelial cells and are shed into the circulation under inflammatory conditions. Therefore, the data collectively suggest that vascular inflammation and endothelial activation promote the progression of DSPN.

Serum IL-1RA has not been assessed as a biomarker of DSPN progression before. Given the anti-inflammatory properties of the protein, it seems likely that IL-1RA represents a risk marker of, rather than a genuine risk factor for, the progression of DSPN.

Our study included a small subgroup with DSPN at baseline, but not at the follow-up examination. Apart from higher HbA_{1c} and lower serum lipid levels at baseline, the remaining clinical characteristics do not point toward obvious factors that could have influenced DSPN regression, which has also been observed following placebo treatment in controlled clinical trials (38). However, the size of this subgroup precluded a precise analysis of predictors of DSPN regression.

Clinical Implications

The fourth main finding of this study is the improvement of model fit, category-free NRI, and IDI by adding IL-6 and TNF-α to a clinical risk model of DSPN. The improvement of model fit and both measures of reclassification means that the estimation of disease risk in DSPN-free individuals is more accurate in our extended risk model that contains both cytokines compared with the clinical model without them. Validation of our data in other cohorts is doubtless necessary, and it is likely that clinically relevant progress in DSPN risk prediction requires that *1*) subclinical inflammation is characterized in more detail, and *2*) the biomarkers used herein are combined with those reflecting other mechanistic pathways involved in the development of DSPN.

From a therapeutic point of view, it should be mentioned that IL-6 and TNF-α are established drug targets. Inhibitors of both cytokines have been used for years against severe proinflammatory conditions. However, this treatment also revealed substantial adverse effects such as immunosuppression and increased risk of severe infections, which preclude their use for the prevention of DSPN. By contrast, clinical approaches targeting other cytokines (e.g., using a monoclonal antibody against IL-1β) or more general anti-inflammatory strategies (e.g., inhibition of nuclear factor-κB) that are currently being tested in ongoing trials to prevent or treat type 2 diabetes and its complications (39,40) seem to have better safety profiles. Further studies need to show whether these approaches can be extended to prevent the incidence and/or progression of DSPN.

Strengths and Limitations

This study is, to our knowledge, the first adequately large study to assess the association between biomarkers of subclinical inflammation and both the incidence and the progression of DSPN. The prospective design, the population-based sample, the sample size, and the use of different metrics to compare risk models are major strengths. In addition, study participants were well phenotyped, which allowed extensive adjustment of results for known DSPN risk factors. Finally, multivariable regression models were complemented by the assessment of the incremental predictive value of IL-6 and TNF-α for incident DSPN.

The main limitation of this study is the clinical definition of DSPN, which could not be confirmed by nerve conduction studies in this epidemiological setting. Another limitation is the large number of study participants for whom no follow-up data were available. On the basis of their risk factor profiles at baseline, it can be assumed that a relevant proportion of them had developed DSPN before being lost to follow-up, which may have affected our results. This study assessed only systemic levels of biomarkers of subclinical inflammation rather than local levels (e.g., in skin biopsies), which

would be valuable with respect to mechanistic analyses but were not feasible in this epidemiological context. Finally, the data were derived from an elderly population of European descent and with high prevalence of prediabetes and type 2 diabetes, so these data cannot be extrapolated to younger cohorts, individuals with type 1 diabetes, or other ethnic groups.

Conclusion

This population-based study demonstrated that subclinical inflammation precedes both the onset and the progression of DSPN. The combination with novel biomarkers reflecting other pathomechanisms contributing to DSPN may be required to improve risk prediction at a clinically relevant level. The data further support the concept that modulation of inflammatory processes may be relevant to prevent and/or treat DSPN.

Acknowledgments. The authors thank Ulrike Partke, Gabi Gornitzka, and Karin Röhrig (German Diabetes Center, Düsseldorf, Germany) for excellent technical assistance.

Funding. This study was funded by grants from the German Diabetes Association (DDG; to C.He.) and the German Diabetes Foundation (DDS; to C.He. and W.R.). This work was also supported by the Ministry of Science and Research of the State of North Rhine-Westphalia (MIWF NRW) and 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 supported in part by a grant from the German Federal Ministry of Education and Research (BMBF) to the German Center for Diabetes Research (DZD). The KORA study was initiated and financed by the Helmholtz Zentrum München–German Research Center for Environmental Health, which is funded by the BMBF and by the State of Bavaria. KORA research was also supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.

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

Author Contributions. C.He. designed the study, contributed data, drafted the analysis plan, interpreted data, and wrote the manuscript. J.M.K. drafted the analysis plan, performed the statistical analysis, and interpreted data. C.Hu., M.C.-K., W.K., M.H., and S.P. contributed data. W.R., B.T., A.P., M.R., and C.M. contributed and interpreted data. D.Z. designed the study, contributed to the analysis plan, and contributed and interpreted data. All authors reviewed and edited the manuscript and approved its submission. C.He. 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.

References

1. Tesfaye S, Boulton AJM, Dyck PJ, et al.; Toronto Diabetic Neuropathy Expert Group. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285–2293
2. Ziegler D, Papanas N, Vinik AI, Shaw JE. Epidemiology of polyneuropathy in diabetes and prediabetes. Handb Clin Neurol 2014;126:3–22
3. Pop-Busui R, Ang L, Holmes C, Gallagher K, Feldman EL. Inflammation as a therapeutic target for diabetic neuropathies. Curr Diab Rep 2016;16:29
4. Di Iorio A, Cherubini A, Volpato S, et al. Markers of inflammation, vitamin E and peripheral nervous system function: the InCHIANTI study. Neurobiol Aging 2006;27:1280–1288
5. Herder C, Lankisch M, Ziegler D, et al. Subclinical inflammation and diabetic polyneuropathy: MONICA/KORA Survey F3 (Augsburg, Germany). Diabetes Care 2009;32:680–682
6. Doupis J, Lyons TE, Wu S, Gnardellis C, Dinh T, Veves A. Microvascular reactivity and inflammatory cytokines in painful and painless peripheral diabetic neuropathy. J Clin Endocrinol Metab 2009;94:2157–2163
7. Purwata TE. High TNF-alpha plasma levels and macrophages iNOS and TNF-alpha expression as risk factors for painful diabetic neuropathy. J Pain Res 2011;4:169–175
8. Herder C, Bongaerts BW, Rathmann W, et al. Association of subclinical inflammation with polyneuropathy in the older population: KORA F4 study. Diabetes Care 2013;36:3663–3670
9. Hussain G, Rizvi SA, Singhal S, Zubair M, Ahmad J. Serum levels of TNF-α in peripheral neuropathy patients and its correlation with nerve conduction velocity in type 2 diabetes mellitus. Diabetes Metab Syndr 2013;7:238–242
10. Jung CH, Kim BY, Mok JO, Kang SK, Kim CH. Association between serum adipocytokine levels and microangiopathies in patients with type 2 diabetes mellitus. J Diabetes Investig 2014;5:333–339
11. Pradeepa R, Surendar J, Indulekha K, Chella S, Anjana RM, Mohan V. Association of serum adiponectin with diabetic microvascular complications among south Indian type 2 diabetic subjects - (CURES-133). Clin Biochem 2015;48:33–38
12. Herder C, Bongaerts BW, Ouwens DM, et al.; KORA Study Group. Low serum omentin levels in the elderly population with type 2 diabetes and polyneuropathy. Diabet Med 2015;32:1479–1483
13. Magrinelli F, Briani C, Romano M, et al. The association between serum cytokines and damage to large and small nerve fibers in diabetic peripheral neuropathy. J Diabetes Res 2015; 2015:547834
14. Kallestrup M, Møller HJ, Tankisi H, Andersen H. Soluble CD163 levels are elevated in cerebrospinal fluid and serum in people with type 2 diabetes mellitus and are associated with impaired peripheral nerve function. Diabet Med 2015;32: 54–61
15. Ge S, Xie J, Zheng L, et al. Associations of serum anti-ganglioside antibodies and inflammatory markers in diabetic peripheral neuropathy. Diabetes Res Clin Pract 2016;115:68–75
16. Roustit M, Loader J, Deusenbery C, Baltzis D, Veves A. Endothelial dysfunction as a link between cardiovascular risk factors and peripheral neuropathy in diabetes. J Clin Endocrinol Metab 2016;101:3401–3408
17. Schamarek I, Herder C, Nowotny B, et al.; German Diabetes Study Group. Adiponectin, markers of subclinical inflammation and nerve conduction in individuals with recently diagnosed type 1 and type 2 diabetes. Eur J Endocrinol 2016;174:433–443
18. Jude EB, Abbott CA, Young MJ, Anderson SG, Douglas JT, Boulton AJ. The potential role of cell adhesion molecules in the pathogenesis of diabetic neuropathy. Diabetologia 1998;41: 330–336
19. Bongaerts BW, Rathmann W, Kowall B, et al. Postchallenge hyperglycemia is positively associated with diabetic polyneuropathy: the KORA F4 study. Diabetes Care 2012;35:1891–1893
20. Rathmann W, Strassburger K, Heier M, et al. Incidence of type 2 diabetes in the elderly German population and the effect of clinical and lifestyle risk factors: KORA S4/F4 cohort study. Diabet Med 2009;26:1212–1219
21. Herder C, Ouwens DM, Carstensen M, et al. Adiponectin may mediate the association between omentin, circulating lipids and insulin sensitivity: results from the KORA F4 study. Eur J Endocrinol 2015;172:423–432
22. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2003;26(Suppl. 1):S5–S20
23. Feldman EL, Stevens MJ, Thomas PK, Brown MB, Canal N, Greene DA. A practical two-step quantitative clinical and electrophysiological assessment for the diagnosis and staging of diabetic neuropathy. Diabetes Care 1994;17:1281–1289
24. Martina IS, van Koningsveld R, Schmitz PI, van der Meché FG, van Doorn PA. Measuring vibration threshold with a graduated tuning fork in normal aging and in patients with polyneuropathy. European Inflammatory Neuropathy Cause and Treatment (INCAT) group. J Neurol Neurosurg Psychiatry 1998;65:743–747
25. Paisley A, Abbott C, van Schie C, Boulton A. A comparison of the Neuropen against standard quantitative sensory-threshold measures for assessing peripheral nerve function. Diabet Med 2002;19:400–405
26. Herder C, Bongaerts BW, Rathmann W, et al. Differential association between biomarkers of subclinical inflammation and painful polyneuropathy: results from the KORA F4 study. Diabetes Care 2015;38:91–96
27. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 1988;44: 837–845
28. Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12:77
29. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med 2008;27:157–172; discussion 207–212
30. Kundu S, Aulchenko YS, Janssens ACJW. PredictABEL: assessment of risk prediction models [software online], 2014. Available from https://CRAN.R-project.org/package=PredictABEL. Accessed 23 January 2017

31. Duksal T, Tiftikcioglu BI, Bilgin S, Kose S, Zorlu Y. Role of inflammation in sensory neuropathy in prediabetes or diabetes. Acta Neurol Scand 2016;133:384–390
32. Kaptoge S, Seshasai SR, Gao P, et al. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. Eur Heart J 2014;35:578–589
33. Donate-Correa J, Martín-Núñez E, Muros-de-Fuentes M, Mora-Fernández C, Navarro-González JF. Inflammatory cytokines in diabetic nephropathy. J Diabetes Res 2015;2015:948417
34. Pena MJ, de Zeeuw D, Mischak H, et al. Prognostic clinical and molecular biomarkers of renal disease in type 2 diabetes. Nephrol Dial Transplant 2015;30(Suppl. 4):iv86–iv95
35. Tang J, Kern TS. Inflammation in diabetic retinopathy. Prog Retin Eye Res 2011;30:343–358
36. Simó-Servat O, Simó R, Hernández C. Circulating biomarkers of diabetic retinopathy: an overview based on physiopathology. J Diabetes Res 2016;2016:5263798
37. González-Clemente JM, Mauricio D, Richart C, et al. Diabetic neuropathy is associated with activation of the TNF-alpha system in subjects with type 1 diabetes mellitus. Clin Endocrinol (Oxf) 2005;63:525–529
38. Ruggenenti P, Lauria G, Iliev IP, et al.; DEMAND Study Investigators. Effects of manidipine and delapril in hypertensive patients with type 2 diabetes mellitus: the Delapril and Manidipine for Nephroprotection in Diabetes (DEMAND) randomized clinical trial. Hypertension 2011;58:776–783
39. Donath MY. Targeting inflammation in the treatment of type 2 diabetes: time to start. Nat Rev Drug Discov 2014;13:465–476
40. Tousoulis D, Oikonomou E, Economou EK, Crea F, Kaski JC. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. Eur Heart J 2016;37:1723–1732