Subclinical Inflammation and Diabetic Polyneuropathy
MONICA/KORA Survey F3 (Augsburg, Germany)

OBJECTIVE — Subclinical inflammation represents a risk factor of type 2 diabetes and several diabetes complications, but data on diabetic polyneuropathy are scarce. Therefore, we investigated whether circulating concentrations of acute-phase proteins, cytokines, and chemokines differ among diabetic patients with or without diabetic polyneuropathy.

RESEARCH DESIGN AND METHODS — We measured 10 markers of subclinical inflammation in 227 type 2 diabetic patients with diabetic polyneuropathy who participated in the population-based MONICA/KORA Survey F3 (2004–2005; Augsburg, Germany). Diabetic polyneuropathy was diagnosed using the Michigan Neuropathy Screening Instrument (MNSI).

RESULTS — After adjustment for multiple confounders, high levels of C-reactive protein and interleukin (IL)-6 were most consistently associated with diabetic polyneuropathy, high MNSI score, and specific neuropathic deficits, whereas some inverse associations were seen for IL-18.

CONCLUSIONS — This study shows that subclinical inflammation is associated with diabetic polyneuropathy and neuropathic impairments. This association appears rather specific because only certain immune mediators and impairments are involved.

Subclinical inflammation is a risk factor not only for type 2 diabetes (1) but also for diabetes complications such as cardiovascular disease, stroke, diabetic nephropathy, and diabetic retinopathy (2–4). However, the association between subclinical inflammation and diabetic polyneuropathy has only been investigated in small studies without definitive results (5,6). Therefore, the aim of this study was to investigate systematically whether patients with type 2 diabetes with or without diabetic polyneuropathy exhibit a different immune profile and whether associations between subclinical inflammation and diabetic polyneuropathy as well as the individual components of diabetic polyneuropathy are independent of anthropometric and metabolic factors.

RESULTS — The prevalence of diabetic polyneuropathy as defined by an MNSI score >2 was 48.9% (95% CI 42.4–55.4) in our study population (supplementary Figure A1 and Table A1 in the online appendix). Patients with diabetic polyneuropathy had higher levels of C-reactive protein (CRP) (P = 0.013), interleukin (IL)-6 (P = 0.0091), and interferon-γ-inducible protein-10 (P = 0.039) compared with those in patients without diabetic polyneuropathy, whereas leukocyte count and levels of serum amyloid A, IL-18, tumor necrosis factor-α, adiponectin, IL-8, and monocyte chemoattractant protein-1 did not differ significantly (Table A1).

Both CRP (r = 0.23; P = 0.0006) and IL-6 (r = 0.25; P = 0.0001) were highly significantly associated with the continuous MNSI score in univariate analyses and in multiple linear regression models that adjusted for anthropometric, metabolic, and lifestyle factors; anti-inflammatory medication; and recent respiratory infec-
CONCLUSIONS — The present study demonstrates for the first time at the population level that several immune mediators are associated with diabetic polyneuropathy and that many of these associations persist when adjusting for multiple potential confounders. CRP and IL-6 were most consistently associated with diabetic polyneuropathy and some neuropathic deficits. Although the strength of the correlations with the MNSI score was moderate ($r \leq 0.25$), it is important that these associations remained statistically significant after adjustment for multiple founders, including duration of diabetes and A1C, so that immune activation in diabetic polyneuropathy cannot be explained solely as a consequence of hyperglycemia or other metabolic disturbances. Associations of CRP and IL-6 with cardiac autonomic neuropathy have been previously reported in two small studies in patients with type 1 diabetes (11,12). Furthermore, we describe significant associations of subclinical inflammation with some of the individual neuropathic impairments. Interestingly, impaired temperature perception and pain or discomfort in the lower limbs were not associated with any of the measured immune mediators, indicating that the association between subclinical inflammation and diabetic polyneuropathy may only affect certain components of diabetic polyneuropathy, whereas others may be independent of immune activation.

The association between subclinical inflammation and diabetic polyneuropathy appears relatively complex because higher CRP and IL-6 levels were associated with diabetic polyneuropathy, whereas for IL-18, an inverse association was found. CRP is an acute-phase protein that is produced in the liver, and its main inducer is IL-6. This relationship is reflected by the high degree of correlation between these two mediators in the present study ($r = 0.56; P < 0.001$) (supplementary Table A2). Because IL-18 is not elevated to the same degree as IL-6, it is possible that IL-18 is a downstream mediator of IL-6, rather than an independent predictor of polyneuropathy.

The most consistent associations with individual neuropathic deficits were observed for CRP and IL-6, and some associations between IL-18 and neuropathic deficits were found (Table 1). High levels of CRP or IL-6 were associated with impaired ankle reflex, high vibration perception threshold, abnormal appearance of feet, and impaired pain perception (pinprick). In the case of IL-18, low levels of this cytokine were associated with impaired ankle reflex and impaired pain perception (pinprick). Associations for the other immune markers were less pronounced or absent (data not shown).

Table 1 — Association among MNSI, individual components of diabetic polyneuropathy, and immunological variables (multivariable-adjusted analysis)

| Dependent variable                        | Model 1 | Model 2 | Model 3 | Model 4 |
|-------------------------------------------|---------|---------|---------|---------|
| MNSI score                                | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | 0.17    | 0.0005  | 0.14    | 0.0043  | 0.14    | 0.0055  | 0.14    | 0.0067  |
| IL-6 (pg/ml)                              | 0.09    | 0.0015  | 0.08    | 0.0082  | 0.08    | 0.0090  | 0.08    | 0.0076  |
| IL-18 (pg/ml)                             | -0.03   | 0.13    | -0.03   | 0.15    | -0.03   | 0.094   | -0.03   | 0.11    |
| Ankle reflex score                        | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | 0.14    | 0.0037  | 0.10    | 0.060   | 0.09    | 0.076   | 0.09    | 0.086   |
| IL-6 (pg/ml)                              | 0.08    | 0.0058  | 0.05    | 0.080   | 0.05    | 0.087   | 0.06    | 0.079   |
| IL-18 (pg/ml)                             | -0.05   | 0.012   | -0.04   | 0.023   | -0.05   | 0.014   | -0.05   | 0.013   |
| Vibration perception score                | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | -0.01   | 0.075   | -0.008  | 0.23    | -0.008  | 0.23    | -0.007  | 0.28    |
| IL-6 (pg/ml)                              | -0.01   | 0.0036  | -0.008  | 0.034   | -0.008  | 0.031   | -0.008  | 0.035   |
| IL-18 (pg/ml)                             | 0.003   | 0.22    | 0.002   | 0.34    | 0.002   | 0.31    | 0.003   | 0.26    |
| Temperature perception score              | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | -0.04   | 0.39    | -0.05   | 0.23    | -0.06   | 0.17    | -0.06   | 0.21    |
| IL-6 (pg/ml)                              | -0.04   | 0.18    | -0.04   | 0.17    | -0.04   | 0.15    | -0.04   | 0.13    |
| IL-18 (pg/ml)                             | 0.01    | 0.35    | 0.02    | 0.22    | 0.02    | 0.25    | 0.02    | 0.31    |
| Appearance of feet                        | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | 0.39    | 0.010   | 0.36    | 0.019   | 0.34    | 0.026   | 0.36    | 0.024   |
| IL-6 (pg/ml)                              | 0.20    | 0.037   | 0.19    | 0.041   | 0.19    | 0.047   | 0.20    | 0.040   |
| IL-18 (pg/ml)                             | 0.03    | 0.65    | 0.01    | 0.85    | -0.004  | 0.95    | 0.004   | 0.94    |
| Pain perception (pinprick)                | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | 0.11    | 0.55    | 0.003   | 0.99    | 0.01    | 0.94    | -0.03   | 0.88    |
| IL-6 (pg/ml)                              | 0.25    | 0.023   | 0.20    | 0.082   | 0.20    | 0.076   | 0.20    | 0.085   |
| IL-18 (pg/ml)                             | -0.15   | 0.021   | -0.14   | 0.045   | -0.13   | 0.059   | -0.15   | 0.035   |
| Pain or discomfort in the lower limbs     | β       | P       | β       | P       | β       | P       | β       | P       |
| CRP (mg/l)                                | -0.06   | 0.74    | -0.04   | 0.79    | -0.05   | 0.76    | -0.11   | 0.52    |
| IL-6 (pg/ml)                              | -0.06   | 0.59    | -0.06   | 0.53    | -0.06   | 0.55    | -0.07   | 0.53    |
| IL-18 (pg/ml)                             | 0.10    | 0.095   | 0.11    | 0.087   | 0.10    | 0.10    | 0.10    | 0.10    |

Regression coefficients $\beta$ and $P$ values for MNSI score and individual impairments are from multiple linear regression models with ln-transformed concentrations of immune mediators as dependent variables. Details on the assessment of neuropathic deficits are given in the online appendix. Model 1: adjusted for age and sex; model 2: adjustment for model 1 variables plus waist circumference, duration of diabetes, A1C, hypertension, and total cholesterol; model 3: adjustment for model 2 variables plus smoking, high alcohol intake, and physical activity; model 4: adjustment for model 3 variables plus lipid-lowering medication, nonsteroidal anti-inflammatory drugs, and recent respiratory infections.

Tions ($P < 0.01$ in all models; Table 1).
also widely considered a proinflammatory cytokine (13), the hypothesis of IL-18 being protective against at least some neurological symptoms (14,15) needs to be corroborated by further studies.

The population-based design of the MONICA/KORA Survey F3, the extensive immunological phenotyping, and the multiple regression analyses represent strengths of this study. This study also has limitations. First, MNSI as a diagnostic tool only allows identification of patients with high risk of diabetic polyneuropathy. Although most patients will have diabetic polyneuropathy, in some cases neuropathy may not have been due to diabetes, but an exclusion of other potential causes of neuropathy for this relatively large study sample was not feasible. However, MNSI has been validated in other diabetic populations (7–10). In addition, we did not conduct more objective electrophysiological tests to assess neuropathic deficits, which could have had an impact on our findings. Second, this study is cross-sectional, so one cannot distinguish between true risk factors and associations that could be due to reverse causation or simply coincidental. Third, the study lacked a nondiabetic control group, which would have been necessary to demonstrate unequivocally that the diabetic study participants had increased levels of proinflammatory markers compared with those in healthy individuals without type 2 diabetes. Fourth, the study relied on measurements of immune mediators at a single time point only and at different stages of disease. Fifth, the study was not designed to assess neuropathic pain in detail; thus, further studies are necessary to clarify the association between subclinical inflammation and this specific neuropathic symptom. Sixth, we performed multiple tests in this study, so type I errors cannot be ruled out.

Taken together, the data indicate that diabetic polyneuropathy and some of its individual impairments are significantly associated with subclinical inflammation (in particular with CRP, IL-6, and, inversely, IL-18). Prospective studies will be required to assess the time course and causal relevance of subclinical inflammation in the development of diabetic polyneuropathy in order to test whether immunomodulation could become a treatment option for patients with diabetic polyneuropathy.

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