# Immune Mediators in Patients With Acute Diabetic Foot Syndrome

CHRISTIAN WEIGELT, MD[1]
BETTINA ROSE, MD[1]
ULRIKE POSCHEN, DIPL, OEC, TROPH[1]
DAN ZIEGLER, MD, FRCPE[1,2]
GERD FRIESE, MD[1,3]
KERSTIN KEMPF, PHD[1,4]
WOLFGANG KOENIG, MD[5]
STEPHAN MARTIN, MD[1,4]
CHRISTIAN HERDER, PHD, MSC[1]

**OBJECTIVE** — Subclinical inflammation is an important risk factor for type 2 diabetes and diabetes complications. However, data on the association between inflammation and acute diabetic foot syndrome are scarce. The aim of this study was to compare systemic immune mediators in diabetic patients with and without an ulcer and to identify modulating factors.

**RESEARCH DESIGN AND METHODS** — Circulating levels of acute-phase proteins, cytokines, and chemokines were measured in diabetic patients with an ulcer ($n = 170$) and without an ulcer ($n = 140$). Of the patients, 88% had type 2 diabetes.

**RESULTS** — Patients with an acute foot ulcer had higher levels of C-reactive protein (CRP), fibrinogen, interleukin (IL)-6, macrophage migration inhibitory factor, macrophage inflammatory protein-1α, and interferon-γ–inducible protein-10 as well as lower levels of RANTES (regulated on activation normal T-cell expressed and secreted) (all $P < 0.01$). No differences were found for IL-8, IL-18, and monocyte chemoattractant protein-1. Most of these associations persisted after adjustment for demographic and anthropometric data, metabolic confounders, and diabetes complications. In multivariate models, size of ulcer according to the University of Texas classification but not the grade of infection was independently associated with three markers of subclinical inflammation (CRP, IL-6, and fibrinogen).

**CONCLUSIONS** — We demonstrate in our cross-sectional study that acute foot ulcers and their severity are associated with a marked upregulation of acute-phase proteins, cytokines, and chemokines independently of the concomitant infection. Further studies should investigate whether an activation of the immune system precedes the development of foot ulcer and whether anti-inflammatory therapies might be effective.

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Because the worldwide incidence of diabetes is increasing rapidly (1), the diabetic foot syndrome becomes more and more important as a major diabetes complication. The lifetime risk of a diabetic patient for development of a chronic foot wound has been estimated to reach 15–25% (2), and, despite considerable international efforts, foot ulcers continue to be responsible for a high number of lower-limb amputations that are associated with a substantial decrease in quality of life and increased risk of mortality (3).

The major risk factors for foot ulcer are diabetic polyneuropathy and peripheral arterial disease (4). Interestingly, data on the relevance of systemic inflammation are very scarce in this context, although low-grade immune activation represents an important risk factor not only for the development of type 2 diabetes (5) but also for several macrovascular (myocardial infarction and stroke) and microvascular complications (neuropathy and nephropathy) (6–8). The status of the immune system may be relevant at several stages in the development of chronic wounds. Immune activation may precede the incidence of a diabetic foot ulcer in the same way that it precedes the manifestation of type 2 diabetes and coronary heart disease (5,6). Because pro- and anti-inflammatory processes are crucial in the different phases of wound healing, it is conceivable that disturbances of the immune system interfere with tissue homeostasis and wound healing after the manifestation of ulcers and lead to the chronic, nonhealing wounds that are characteristic of diabetic foot syndrome.

Given the surprising paucity of data on the role of systemic inflammation in diabetic foot ulcers, we evaluated the association between foot ulcers and immune status in a cross-sectional study in diabetic patients with and without foot ulcers by measuring a range of immune mediators (acute-phase proteins, cytokines, and chemokines) representing different aspects of the immune system. The main aims of the study were *1*) to compare circulating levels of these immune mediators between both groups, *2*) to use multivariate regression models to identify potential confounders of these associations, and *3*) to investigate whether systemic immune activation was associated with the severity of the foot ulcer.

## RESEARCH DESIGN AND METHODS

— Between August 2003 and November 2005, we recruited 282 consecutive diabetic patients with an acute foot ulcer who were hospitalized at the German Diabetes Clinic and 175 diabetic patients without a history of foot ulcer who were admitted to our outpatient unit. Fasting blood samples were drawn on the first day after admission for the patients with an ulcer and on the day of their medical examination in the outpatient unit for the patients without an ulcer. Plasma and serum samples were stored at −80°C until analysis. The study was approved by the Ethics Committee of Heinrich Heine University Düsseldorf. Written informed consent was obtained

From the [1]Institute for Clinical Diabetology, German Diabetes Center, Leibniz Institute for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany; the [2]Department of Internal Medicine/Metabolic Diseases, Heinrich Heine University Düsseldorf, Düsseldorf, Germany; [3]Medicenter Plettenberg, Plettenberg, Germany; the [4]Westdeutsches Diabetes- und Gesundheitszentrum, Sana Krankenhaus Gerresheim, Sana Kliniken Düsseldorf, Düsseldorf, Germany; and the [5]Department of Internal Medicine II–Cardiology, University of Ulm Medical Center, Ulm, Germany.
Corresponding author: Bettina Rose, bettina.rose@ddz.uni-duesseldorf.de.
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C.W. and B.R. contributed equally to this study.

from all study participants, and the study was performed according to the Declaration of Helsinki.

Of all patients recruited, those with missing essential clinical data, current immunomodulating treatment (e.g., steroids, azathioprine, or ciclosporin), a history of neoplastic disease, surgical or angioplastic intervention before the study, or acute respiratory or genitourinary infections and subjects who had been recruited more than once (only the first obtained biosamples were used) were excluded (supplementary Figs. A1 and A2, available in an online appendix at http://care.diabetesjournals.org/cgi/content/full/dc08-2318/DC1). Thus, the following analyses are based on 170 patients with and 140 patients without a foot ulcer.

Demographic, anthropometric, and metabolic data as well as information on comorbidities and diabetes complications were extracted from the patients' medical records. Levels of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and A1C were determined using standard laboratory methods. The classification of diabetes was based on the guidelines of the German Diabetes Association (9). Diabetic neuropathy was defined as the presence of symptomatic or asymptomatic neuropathy using the neuropathy symptom score and the neuropathy deficit score, respectively. Peripheral arterial disease (PAD) was staged according to Fontaine with reduction of walking distance of <200 m as the minimal criterion for clinically relevant PAD (Fontaine stage IIb) (10).

The diagnosis of retinopathy was based on one-field funduscopy; diabetic nephropathy was defined as urinary excretion of albumin >200 μg/min at two different time points, macroalbuminuria, or renal failure. The size and depth of the foot ulcer was graded according to the University of Texas classification with stratification into grades 0–3 as reported elsewhere (11). The grade of wound infection was subclassified into four different categories: 1, no sign of infection; 2, slight redness; 3, explicit redness and/or erysipelas; and 4, purulent or nonpurulent phlegmon. Hypertension was defined as systolic/diastolic blood pressure >140/90 mmHg or use of any antihypertensive medication. Hyperlipidemia was defined as total cholesterol >200 mg/dl, LDL cholesterol >120 mg/dl, triglycerides >150 mg/dl, or use of any lipid-lowering medication.

### Immunological measurements

C-reactive protein (CRP) and fibrinogen were measured in plasma samples with a high-sensitivity latex-enhanced nephelometric assay on a BN II analyzer (Dade Behring, Marburg, Germany) using immmunonephelometry, respectively (12). Serum levels of the cytokines interleukin (IL)-6, macrophage migration inhibitory factor (MIF), and regulated on activation, normal T-cell expressed and secreted (RANTES) were determined using ELISAs (for IL-6 from Sanquin [Amsterdam, the Netherlands] and for MIF and RANTES from R&D Systems [Wiesbaden, Germany]) as described previously (12–14). Serum levels of IL-8, IL-18, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1α (MIP-1α), and interferon-γ–inducible protein-10 (IP-10) were quantified using a bead-based multiplex assay on a Luminex 100 analyzer (Luminex, Austin, TX) as described earlier (15). The intra- and interassay coefficients of variation of quality control test sera were <10 and <20%, respectively.

### Statistical analyses

Data are given as proportions (percentage) for categorical variables, as means ± SD for continuous variables with Gaussian distribution, and as median (25th percentile; 75th percentile) for continuous variables with non-Gaussian distribution. Categorical variables with two or more than two classes were compared using Fisher's exact test and a $\chi^2$ test, respectively. Continuous variables with Gaussian distribution were compared using Student's *t* test. A Mann-Whitney test or Kruskal-Wallis test (with Dunn's multiple comparison test as a posttest) was used to compare continuous variables without Gaussian distribution. Univariate associations between markers of inflammation were described with Spearman correlation coefficients (*r*). Associations between circulating concentrations of immune mediators and the presence of a foot ulcer or indicators of its severity (University of Texas grade, grade of infection) were analyzed using multiple linear regression models with concentrations of immune markers (ln-transformed in case of non-normality) as dependent variables and existence of a foot ulcer and potentially confounding variables as independent variables. For all statistical analyses, $P < 0.05$ was considered to be statistically significant. Analyses were conducted using SAS (version 9.1; SAS Institute, Cary, NC).

**RESULTS** — Patients with and without a foot ulcer were mostly patients with type 2 diabetes. Those with an ulcer were older, had lower systolic and diastolic blood pressure, lower total and HDL cholesterol levels, lower A1C, more frequent PAD, and other diabetes complications (i.e., neuropathy, retinopathy, nephropathy, and coronary heart disease) and were more often treated with insulin (Table 1).

### Immune activation in diabetic patients with a foot ulcer

In patients with a foot ulcer, median levels of both acute-phase proteins, high-sensitivity (hs)-CRP and fibrinogen, were significantly elevated (4.9- and 1.4-fold, respectively) compared with those in patients without a history of foot ulcer ($P < 0.0001$). Similarly, median levels of the cytokines and chemokines IL-6, MIF, IP-10 (all $P < 0.0001$), and MIP-1α ($P = 0.008$) were elevated 3.3-, 1.8-, 1.4-, and 1.3-fold, respectively, whereas no significant differences were found for IL-18, IL-8, and MCP-1. In contrast, serum levels of RANTES were 1.3-fold lower ($P < 0.0001$) in patients with an ulcer compared with those without an ulcer (Table 2).

To account for imbalances between both groups, the association of immune mediators with foot ulcer was assessed in multiple linear regression models (Table 3). Notably, all associations that were found in unadjusted comparisons persisted after adjustment for age, sex, diabetes type, metabolic factors (BMI, A1C, hypertension, and hyperlipidemia), and comorbidities (PAD, neuropathy, and coronary heart disease) (all $P < 0.003$).

As a sensitivity analysis, all analyses were repeated after exclusion of patients with type 1 diabetes, which led to virtually unaltered results for type 2 diabetes alone (data not shown). Inclusion of other diabetes complications (retinopathy and nephropathy) did not change the observed associations (data not shown).

The analysis of correlations between immune mediators revealed stronger associations in patients with a foot ulcer compared with those without a history of foot ulcer (supplementary Table A1, available in an online appendix). Almost all correlations were positive, and the strongest correlations were found between IL-6, CRP, and fibrinogen (Spearman's *r* 0.59–0.78 in patients with an

**Table 1—*Characteristics of the study population***

| | *n* | Patients with ulcer | Patients without ulcer | *P* |
|---|---|---|---|---|
| *n* | 310 | 170 | 140 | — |
| Type 1/type 2 diabetes (*n*) | 310 | 13/157 | 23/117 | 0.02 |
| Age (years) | 310 | 67 (59; 75) | 62 (52; 69) | <0.0001 |
| Sex (male/female) | 310 | 100/70 | 76/64 | NS |
| BMI (kg/m$^2$) | 294 | 29.3 ± 6.6 | 29.9 ± 5.4 | NS |
| Systolic blood pressure (mmHg) | 264 | 134 ± 19 | 142 ± 22 | 0.003 |
| Diastolic blood pressure (mmHg) | 264 | 73 ± 10 | 78 ± 10 | 0.0005 |
| Hypertension (%) | 308 | 91 | 75 | <0.0001 |
| Cholesterol (mmol/l) | 289 | 4.86 ± 1.29 | 5.38 ± 1.09 | 0.0007 |
| HDL cholesterol (mmol/l) | 278 | 1.19 ± 0.39 | 1.29 ± 0.41 | 0.024 |
| LDL cholesterol (mmol/l) | 278 | 2.95 ± 1.09 | 3.18 ± 0.93 | NS |
| Triglycerides (mmol/l) | 290 | 1.67 (1.19; 2.17) | 1.79 (1.09; 2.56) | NS |
| Hyperlipidemia (%) | 305 | 78 | 80 | NS |
| A1C (%) | 301 | 7.64 ± 1.58 | 8.27 ± 1.58 | 0.0006 |
| History of diabetes complications | | | | |
| Neuropathy (%) | 307 | 88 | 54 | <0.0001 |
| PAD (%) | 307 | 66 | 9 | <0.0001 |
| Retinopathy (%) | 308 | 74 | 16 | <0.0001 |
| Nephropathy (%) | 305 | 67 | 33 | <0.0001 |
| Coronary heart disease (%) | 306 | 39 | 19 | <0.0001 |
| Diabetes therapy | 303 | | | <0.0001 |
| Diet only (%) | | 5 | 9 | |
| OAD (%) | | 20 | 48 | |
| OAD + insulin (%) | | 8 | 4 | |
| Insulin (%) | | 67 | 39 | |

Data are absolute numbers, proportions, means ± SD, or medians (25th percentile; 75th percentile). OAD, oral antidiabetes drug.

ulcer and 0.43–0.51 in patients without an ulcer; all $P < 0.0001$), whereas correlations between IL-6, MIF, IL-18, IL-8, MCP-1, MIP-1α, and IP-10 were less pronounced or absent.

### Association of proinflammatory mediators with severity of foot ulcer

Foot ulcer comprised all University of Texas grades (0, 1.8%; 1, 18.8%; 2, 35.9%; and 3, 43.5%) and all four grades of infection (1, 7.1%; 2, 14.1%; 3, 27.1%; and 4, 51.8%). The frequency of osteomyelitis differed significantly between University of Texas grades (1, 6%; 2, 18%; and 3, 45%; $P < 0.001$). In unadjusted analyses, patients with foot wounds of University of Texas grade 3 had significantly higher levels of hs-CRP, fibrinogen, and IL-6 compared with patients with foot wounds of University of Texas grade 1, whereas no differences were found for the other immune mediators (Table 4). Again, these associations persisted after adjustment for age, sex, diabetes type, metabolic factors (BMI, A1C, hypertension, and hyperlipidemia), comorbidities (PAD, neuropathy, and coronary heart disease), and grade of infection (CRP $P = 0.010$; fibrinogen $P = 0.004$; IL-6 $P = 0.003$) (supplementary Table A2, available in an online appendix). Additional adjustment for the presence of osteomyelitis virtually did not alter the results (data not shown). When the association between systemic inflammation and grade of infection was analyzed, patients with an ulcer and infection grade 4 had significantly higher circulating concentrations of hs-CRP and IL-6 than patients with infection grades 1 and/or 2 (supplementary Figure A3, available in an online appendix), whereas no differences between grades of infection were found for the other immune mediators (data not shown). However, these associations disappeared after adjustment for University of Texas grade of ulcer and metabolic covariables ($P > 0.1$) (supplementary Table A3, available in an online appendix), so that University of Texas grade but not grade of infection was an independent determinant of markers of systemic inflammation in patients with a foot ulcer.

**Table 2—*Systemic immune mediator concentrations in patients with and without diabetic foot ulcer***

| Immune mediator | Patients with ulcer | Patients without ulcer | *P* |
|---|---|---|---|
| CRP (mg/l) | 11.7 (3.3; 38.2) | 2.4 (1.0; 5.0) | <0.0001 |
| Fibrinogen (g/l) | 5.5 ± 1.6 | 3.8 ± 0.9 | <0.0001 |
| IL-6 (pg/ml) | 12.4 (6.9; 30.3) | 3.8 (2.2; 5.2) | <0.0001 |
| MIF (ng/ml) | 7.7 (5.6; 11.9) | 4.3 (3.1; 6.6) | <0.0001 |
| IL-18 (pg/ml) | 118.3 (84.2; 179.0) | 122.7 (85.0; 172.0) | NS |
| IL-8 (pg/ml) | 11.4 (7.1; 18.3) | 10.0 (6.8; 15.9) | NS |
| MIP-1α (pg/ml) | 82.6 (54.6; 132.7) | 61.2 (44.1; 107.4) | 0.008 |
| MCP-1 (pg/ml) | 291.0 (202.2; 381.9) | 303.3 (213.8; 415.6) | NS |
| IP-10 (pg/ml) | 419.8 (309.9; 543.4) | 299.4 (216.9; 401.6) | <0.0001 |
| RANTES (ng/ml) | 76.9 ± 38.2 | 100.3 ± 44.7 | <0.0001 |

Data are means ± SD or medians (25th percentile; 75th percentile).

**CONCLUSIONS** — Our study has three major findings. *1*) Diabetic patients with foot ulceration showed an upregulation of circulating levels of a range of acute-phase proteins, cytokines, and chemokines and lower levels of the chemokine RANTES compared with diabetic patients without a history of foot ulcer. *2*) These associations persisted when age, sex, diabetes type, metabolic factors, and comorbidities were taken into account in multiple regression models. *3*) Severity of foot ulcer based on the University of Texas classification was associated with levels of CRP, IL-6, and fibrinogen in unadjusted comparisons and in multiple linear regression models.

The finding of an altered immune status in patients with foot ulceration is interesting in several ways. First, only some markers of inflammation were upregulated (CRP, fibrinogen, IL-6, MIF, MIP-1α, and IP-10) and others were not (IL-8, IL-18, and MCP-1), whereas RANTES levels were even downregulated. These results indicate a specific and nonrandom alteration of the immune status in patients

**Table 3—*Association between immune mediators and acute foot ulcer: multivariable regression models***

| Immune mediator | Model 1 | | Model 2 | | Model 3a | | Model 3b | | Model 4 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | β | *P* | β | *P* | β | *P* | β | *P* | β | *P* |
| CRP (mg/l) | 1.59 | <0.0001 | 1.59 | <0.0001 | 1.66 | <0.0001 | 1.70 | <0.0001 | 1.74 | <0.0001 |
| Fibrinogen (g/l) | 0.34 | <0.0001 | 0.33 | <0.0001 | 0.35 | <0.0001 | 0.32 | <0.0001 | 0.35 | <0.0001 |
| IL-6 (pg/ml) | 1.58 | <0.0001 | 1.50 | <0.0001 | 1.48 | <0.0001 | 1.51 | <0.0001 | 1.47 | <0.0001 |
| MIF (ng/ml) | 0.70 | <0.0001 | 0.69 | <0.0001 | 0.70 | <0.0001 | 0.76 | <0.0001 | 0.75 | <0.0001 |
| IL-18 (pg/ml) | 0.13 | 0.17 | 0.14 | 0.16 | 0.15 | 0.15 | 0.20 | 0.075 | 0.22 | 0.077 |
| IL-8 (pg/ml) | 0.08 | 0.45 | 0.00 | 1.00 | −0.01 | 0.94 | −0.05 | 0.70 | −0.04 | 0.79 |
| MIP-1α (pg/ml) | 0.17 | 0.072 | 0.22 | 0.023 | 0.23 | 0.028 | 0.33 | 0.0027 | 0.35 | 0.0029 |
| MCP-1 (pg/ml) | 0.06 | 0.49 | 0.03 | 0.74 | 0.03 | 0.73 | 0.10 | 0.31 | 0.06 | 0.53 |
| IP-10 (pg/ml) | 0.61 | <0.0001 | 0.53 | <0.0001 | 0.57 | <0.0001 | 0.49 | <0.0001 | 0.51 | <0.0001 |
| RANTES (ng/ml) | −0.31 | <0.0001 | −0.31 | <0.0001 | −0.29 | <0.0001 | −0.28 | <0.0001 | −0.28 | 0.0004 |

Data are regression coefficients (β) and *P* values from linear regression analyses. Concentrations of immune markers were ln-transformed in case of nonnormality (i.e., all except fibrinogen and RANTES). Model 1: adjusted for diabetes type; model 2: model 1 + adjustment for age and sex; model 3a: model 2 + adjustment for BMI, A1C, hypertension, and hyperlipidemia; model 3b: model 2 + adjustment for PAD, neuropathy, and coronary heart disease; and model 4: full model (adjustment for all covariables from models 1, 2, 3a, and 3b).



with foot ulcers rather than a general immune activation.

A parallel upregulation of IL-6, CRP, and fibrinogen is biologically plausible because IL-6 is known to increase the release of both acute-phase proteins, which is reflected in the high correlations between the systemic levels of these markers. Increased concentrations of CRP and fibrinogen in diabetic patients with foot ulcers compared with age- and sex-matched diabetic patients without foot ulcers have been described previously (16), but this study did not control for potential confounders and severity of ulceration.

We also included several chemokines (IL-8, MCP-1, MIP-1α, IP-10, RANTES, and MIF), which are known as crucial mediators in wound healing. The levels of most chemokines in our study were not correlated with levels of IL-6, CRP, and fibrinogen, therefore representing a different compartment of the immune system. Thus, their measurement is not redundant and yields important additional information beyond that for traditional markers of inflammation. Chemokines recruit leukocytes to sites of injury and orchestrate the migration of other cells such as keratinocytes, endothelial cells, and fibroblasts during the different stages of wound healing. Notably, this process is disturbed in patients with diabetes, and a role of altered chemokine expression in this context has been postulated (17). Although the local presence of MCP-1, MIP-1α, and RANTES seems to be crucial for the normal healing of the wound in mice and humans, elevated levels of MIF and IP-10 impaired this process (18–21). Our study demonstrates that patients with foot ulcer display elevated systemic levels of MIP-1α, MIF, and IP-10, decreased RANTES levels, and unchanged concentrations of MCP-1 and IL-8. Although the putative local expression of chemokines does not necessarily translate into systemic concentrations, our results may reflect a combination of normal and abnormal wound repair mechanisms, combined with an activation of the acute-phase reaction by the acute wound.

Although the association between an acute-phase response and diabetic foot ulcer has been described before (16), our study considerably extends previous work. First, it comprises a comprehensive measurement of multiple immune mediators regulated independently from each other. Second, it takes into account the effect of confounding factors on the association between systemic inflammation and foot ulcer to ensure that the associations reported here are not affected by age, sex, obesity, metabolic markers, and other comorbidities. Finally, we describe an association between IL-6, CRP, and fibrinogen with the severity of the ulceration and grades of infection. Importantly, the association with University of Texas grades persisted after adjustment for multiple confounders including infection, whereas the association with grades of infection was abolished by adjusting for University of Texas grades and metabolic confounders. An association of fibrinogen and CRP with severity and infection of foot ulcers has recently been described (22,23). However, our data reveal that the elevated levels of markers of an acute-phase response are mainly associated with the severity of ulceration, whereas the

**Table 4—*Systemic immune mediator levels stratified by ulcer grade (University of Texas classification)***

| Immune mediator | UT grade 1 | UT grade 2 | UT grade 3 |
|---|---|---|---|
| *n* | 32 | 61 | 74 |
| hs-CRP (mg/l) | 5.2 (2.3; 19.4) | 11.1 (3.7; 30.7) | 21.0 (7.9; 61.6)* |
| Fibrinogen (g/l) | 4.8 ± 1.3 | 5.3 ± 1.7 | 6.0 ± 1.6* |
| IL-6 (pg/ml) | 7.9 (3.4; 19.1) | 10.5 (6.9; 25.6) | 19.8 (8.9; 48.6)† |
| MIF (ng/ml) | 7.9 (5.8; 10.9) | 7.0 (5.3; 9.9) | 8.6 (6.4; 13.4) |
| IL-18 (pg/ml) | 131.3 (79.9; 193.8) | 122.2 (90.7; 169.5) | 111.3 (85.8; 188.5) |
| IL-8 (pg/ml) | 9.4 (6.7; 20.2) | 11.6 (7.6; 18.5) | 12.9 (7.0; 18.1) |
| MIP-1α (pg/ml) | 83.5 (50.3; 214.2) | 85.1 (56.2; 127.4) | 77.6 (54.2; 136.1) |
| MCP-1 (pg/ml) | 256.6 (158.8; 321.3) | 348.4 (220.0; 471.6)‡ | 284.2 (206.8; 367.9) |
| IP-10 (pg/ml) | 431.6 (320.0; 558.5) | 427.2 (344.9; 517.5) | 353.2 (275.0; 543.6) |
| RANTES (ng/ml) | 75.3 ± 35.7 | 77.7 ± 43.6 | 75.7 ± 34.1 |

Data are means ± SD or medians (25th percentile; 75th percentile). *$P < 0.01$; †$P < 0.001$; ‡$P < 0.05$, compared with University of Texas (UT) classification grade 1.

grade of infection or the presence of osteomyelitis had only a minor impact. Despite the aforementioned association of several chemokines with the presence of an acute ulceration, no association between the chemokines analyzed and severity of ulceration could be observed. This finding again indicates that acute-phase reactants and chemokines reflect different aspects of the immune status.

Our study has several limitations that should be mentioned briefly. First, the study is a cross-sectional study so that cause and effect in the association between systemic inflammation and foot ulcer cannot be investigated. Longitudinal studies are needed to investigate the impact of inflammation on the development of foot ulcerations and their prognostic value. Second, our study population reflected the high percentage of patients with type 2 diabetes in our clinic. The proportion of patients with type 1 diabetes was too small to run separate analyses. Therefore, our results are valid mainly for patients with type 2 diabetes as indicated by sensitivity analyses. Whether the observed results are specific for diabetic foot ulcer or represent a common feature of impaired regeneration of chronic wounds cannot be inferred from our data as no nondiabetic control group with chronic wounds was available, but the scarce data on systemic immune markers in nondiabetic chronic wounds indicate that at least some of the observed results may be characteristic of chronic ulcers in general (24). Third, we cannot assess whether systemic inflammation was associated with long-term response to treatment or reoccurrence of ulceration as follow-up data are not available. Fourth, the two groups of patients studied were not matched for potential confounders such as age, duration, and type of diabetes, A1C, smoking habits, and cardiovascular risk factors. Furthermore, we cannot exclude confounding effects on immune reactions by psychic stress due to hospital admission and bed rest in patients with a foot ulcer, although bed rest seems to impair rather than activate immune function via modulation of cortisol levels (25).

The strengths of our study include a relatively large study population, comprehensive immunological phenotyping comprising different compartments of the immune system, and the availability of additional data on potential confounders so that multiple regression analyses were possible to analyze the impact of metabolic factors or comorbidities on immune activation.

In summary, our study shows that patients with foot ulcers exhibit a specific and nonrandom upregulation of several acute-phase proteins, cytokines, and chemokines and decreased levels of the chemokine RANTES in the circulation. These associations were independent of multiple potential confounders and were mainly associated with severity of ulceration. Further studies are needed to test whether this immune activation precedes the development of foot ulcer. Moreover, the characterization of beneficial and deleterious immune mediators in the process of wound healing in patients with ulcerations would be important to identify potential therapeutic targets and immunomodulating treatment options.

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