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Association of high-sensitive C-reactive protein with advanced stage β-cell dysfunction and insulin resistance in patients with type 2 diabetes mellitus

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Abstract

Background: Type 2 diabetes mellitus is associated with increased cardiovascular risk. One laboratory marker for cardiovascular risk assessment is high-sensitivity C-reactive protein (hsCRP).

Methods: This cross-sectional study attempted to analyze the association of hsCRP levels with insulin resistance, β-cell dysfunction and macrovascular disease in 4270 non-insulin-treated patients with type 2 diabetes [2146 male, 2124 female; mean age ±SD, 63.9±11.1 years; body mass index (BMI) 30.1±5.5 kg/m^2; disease duration 5.4±5.6 years; hemoglobin A_{1c} (HbA_{1c}) 6.8±1.3%]. It consisted of a single morning visit with collection of a fasting blood sample. Observational parameters included several clinical scores and laboratory biomarkers.

Results: Stratification into cardiovascular risk groups according to hsCRP levels revealed that 934 patients had low risk (hsCRP <1 mg/L), 1369 patients had intermediate risk (hsCRP 1–3 mg/L), 1352 patients had high risk (hsCRP >3–10 mg/L), and 610 patients had unspecific hsCRP elevation (>10 mg/L). Increased hsCRP levels were associated with other indicators of diabetes-related cardiovascular risk (homeostatic model assessment, intact proinsulin, insulin, BMI, β-cell dysfunction, all $p<0.001$), but showed no correlation with disease duration or glucose control. The majority of the patients were treated with diet (34.1%; hsCRP levels 2.85±2.39 mg/L) or metformin monotherapy (21.1%; 2.95±2.50 mg/L hsCRP). The highest hsCRP levels were observed in patients treated with sulfonylurea (17.0%; 3.00±2.43 mg/L).

Conclusions: Our results indicate that hsCRP may be used as a cardiovascular risk marker in patients with type 2 diabetes mellitus and should be evaluated in further prospective studies.

Keywords: high-sensitivity C-reactive protein (hsCRP); homeostatic model assessment (HOMA); insulin resistance; intact proinsulin; type 2 diabetes.

Introduction

Patients with type 2 diabetes suffer from high cardiovascular morbidity and mortality based on extensive and accelerated arteriosclerosis (1). Coronary arteriosclerosis is increasingly recognized as a major complication and the leading cause of death in subjects with diabetes (2). It has been shown by laboratory and experimental studies that arteriosclerosis, as well as being a disease of lipid accumulation, represents a chronic inflammatory process (3). Thus, inflammatory markers have been investigated to identify potential adjunctive predictors for global assessment of cardiovascular risk (4–6). Several large-scale prospective epidemiological studies have demonstrated that high-sensitivity C-reactive protein (hsCRP) is a strong independent predictor for risk of future myocardial infarction and other consequences of arteriosclerosis, even in patients without known macrovascular disease (7–10). With the recognition that inflammation is a critical component in the determination of plaque stability, CRP levels in the low–normal range were found to have predictive value for patients with acute coronary ischemia (11, 12). Levels of hsCRP <1 mg/L, 1–3 mg/L and >3 mg/L have been suggested to define low-, moderate-, and high-risk groups, while levels >10 mg/L may indicate unspecific elevation consequent to a general inflammatory process (9).

In this analysis of the IRIS-II study population (13–15), we investigated the prevalence of hsCRP in a large population of 4270 orally treated type 2 diabetes mellitus patients, and its correlation with the prevalence of insulin resistance, β-cell dysfunction and cardiovascular events.

Patients and methods

The epidemiological cross-sectional study was performed in German patients with orally treated type 2 diabetes mellitus

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in accordance with Good Clinical Practice and the Declaration of Helsinki. The participants completed a standardized questionnaire, and blood samples were drawn in the fasting state. Actual therapy and the prevalence of microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (stroke, coronary and peripheral artery disease, myocardial infarction) disease was assessed by an examination and collection of a detailed medical history. All laboratory data were measured in a central laboratory. Hemoglobin A_{1c} (HbA_{1c}) was measured by HPLC (Menarini, Neuss, Germany; reference range 4.4–6.0%). Glucose was determined using a glucose oxidase method (SuperGL; Ruhrtal Labortechnik, Delecke-Möhnesee, Germany). Insulin and intact proinsulin were determined by means of specific immunoassays (Sciema, Mainz, Germany) as previously published (16). HsCRP was assessed by means of a turbidimetric method (Olympus, Hamburg, Germany).

Assessment of insulin resistance was performed by analysis of fasting intact proinsulin values or homeostatic model assessment ($HOMA_{IR}$) score calculation. Patients with intact proinsulin values exceeding the normal reference value of 11 pmol/L were considered to be insulin-resistant (14, 15). In the case of normal intact proinsulin values, $HOMA_{IR}$ score calculation was applied, as previously published (17). The estimate of insulin resistance by $HOMA_{IR}$ score was calculated using the following formula: fasting serum insulin (μU/mL)×fasting plasma glucose (mmol/L)/22.5. As described by Hedblad et al. in a non-diabetic population, patients with $HOMA_{IR}$ score values exceeding the 75th percentile (i.e., 2.0) were considered to have insulin resistance (18). The β-cell dysfunction stage was assessed by considering steady state of insulin and intact proinsulin on the fasting morning, as previously reported (19).

Statistical analysis

As hsCRP has been reported to be associated with body mass index (BMI), hsCRP concentrations were adjusted for BMI before further analysis (20). To compare the means of the variables measured, Student's t-test, and the Mann-Whitney U-test were used. All tests were carried out as two-sided. Results with p-values less than 0.05 were considered statistically significant. All calculations were made with the SPSS statistical package (version 9.0; SPSS Inc., Chicago, IL, USA).

Results

The study was completed by 2146 male and 2124 female patients with type 2 diabetes on oral medication and/or diet [age (mean±SD) 63.9±11.1 years (range 21–96); disease duration 5.4±5.6 years (range 0–52); BMI 30.1±5.5 kg/m^2 (range 16.0–67.2); waist/hip ratio 1.0±0.1 (range 0.6–1.4); HbA_{1c} 6.8±1.3% (range 4.2–16.0%)]. No significant differences in demographic data could be observed between male and female patients.

Stratification of the patients into the different β-cell dysfunction stages, as previously defined (19), led to the following results: stage I (no insulin resistance or β-cell secretion disorder), 1042 patients (24.4%); stage II (insulin resistance without secretion disorder), 1658 patients (38.8%); and stage III (insulin resistance and secretion disorder), 1570 patients (36.7%). The clinical characteristics of these groups are given in Table 1. Calculation of the mean hsCRP concentration in all three β-cell dysfunction groups revealed high risk levels for all stages of β-cell dysfunction. A higher cardiovascular risk was associated with more advanced stages of β-cell dysfunction ($p<0.001$), as shown in Figure 1.

Stratification of the patients into the hsCRP risk groups as suggested by Ridker and Cook (9) revealed that 934 patients had low risk (hsCRP <1 mg/L, 21.8%), 1369 patients were in the medium-risk group (hsCRP 1–3 mg/L, 32.0%), and 1352 patients were in the high-risk group (hsCRP >3–10 mg/L, 31.6%). The number of patients with unspecific elevated hsCRP values (>10 mg/L) was 610 (14.3%).

The differences observed for both hsCRP-associated cardiovascular risk and severity of β-cell dysfunc-

Table 1 Clinical and laboratory results for patients in the different β-cell dysfunction stages.

Parameter	β-Cell dysfunction stage		
	I	II	III
n	1042	1658	1570
Gender, female/male	501/538	746/912	899/672
Age, years	65±12	64±11**	65±11*
Disease duration, years	5.5±6.1	5.1±5.5	5.4±5.2
BMI, kg/m^2	27.2±4.4	30.5±5.2***	30.9±5.3***
Macrovascular disease, %	25.0	23.5	31.1
Hypertension, %	12.1	14.8	17.3
Neuropathy, %	12.2	14.8	17.3
Retinopathy, %	5.6	5.7	7.1
Nephropathy, %	16.0	14.9	21.2
HOMA score, mU·mmol/L^2	1.38±0.39	3.66±2.22***	6.61±5.36***
HbA_{1c}, %	6.3±0.9	6.7±1.2***	7.1±1.3***
hsCRP (not BMI-adjusted), mg/L	4.0±7.4	5.5±12.3***	6.8±14.0***
Insulin, μU/mL	6.1±2.0	13.5±7.1***	20.4±13.3***
Glucose, mmol/L	5.3±1.2	6.6±1.7***	7.0±1.7***
Intact proinsulin, pmol/L	4.3±2.0	6.1±2.1***	22.1±15.8***
Triglyerides, mmol/L	1.45±1.65	1.88±1.48***	2.31±1.74***
HDL, mmol/L	1.3±0.35	1.20±0.33***	1.08±0.28***
LDL, mmol/L	3.13±0.83	3.23±0.83	3.18±0.8

*$p<0.05$; **$p<0.01$; ***$p<0.001$ vs. stage I. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

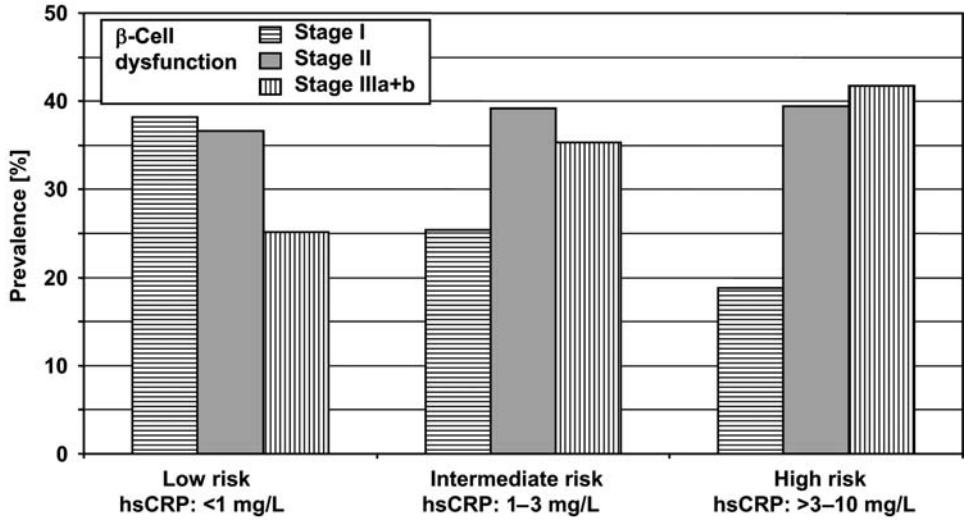

Figure 1 Stratification of patients (% distribution) with high and low cardiovascular risk (according to hsCRP risk group definition) into the β-cell dysfunction stages as defined by Pfützner et al. (19). Higher cardiovascular risk is associated with a higher degree of β-cell dysfunction.

tion were independent of disease duration. A summary of the mean values for HbA_{1c}, $HOMA_{IR}$, hsCRP, intact proinsulin, BMI, disease duration, and the prevalence of macrovascular disease in the hsCRP risk groups is given in Table 2. It is evident that other well-known cardiovascular risk indicators in type 2 diabetes mellitus, such as BMI, insulin resistance and intact proinsulin, are strongly correlated with the hsCRP risk staging according to Ridker and Cook (7, 9), while the correlation with markers of glucose metabolism (HbA_{1c}, glucose) is less pronounced, although still significant because of the high number of patients.

The association of hsCRP values with prescribed therapy is shown in Figure 2. Patients receiving combination therapy of peroxisome proliferator activated receptor γ (PPARγ) agonists with metformin presented the lowest hsCRP mean values ($p < 0.05$).

Discussion

The cross-sectional IRIS study was initially performed to identify diagnostic parameters for insulin resistance in non-insulin-treated patients with type 2 diabetes that are suitable for daily practice (13–15). Analysis of the same patient population presented here was performed to evaluate the correlation between cardiovascular risk, as identified by hsCRP risk assessment and β-cell dysfunction, and insulin resistance. In our non-insulin-treated type 2 diabetes patients with approximately 5 years of disease duration, the prevalence of macrovascular complications was 20–30%. Serum levels of hsCRP were correlated with the severity of insulin resistance and β-cell dysfunction. All analysis groups, however, had hsCRP levels indicating high cardiovascular risk.

Chronic vascular inflammation may be the reason why hsCRP was elevated in our type 2 diabetes population. Strong correlations were evident between cardiovascular risk as assessed by hsCRP stratification and other parameters that are associated with increased risk or increased prevalence of cardiovascular complications in patients with type 2 diabetes, such as BMI, insulin resistance and elevated proinsulin (21–23). No or only weak associations, however, were evident for age, disease duration and the metabolic markers HbA_{1c} and fasting glucose. This result is in line with previous reports demonstrating that

Table 2 Clinical and laboratory results in the hsCRP risk groups.

Parameter	Low risk (hsCRP <1 mg/L)	Intermediate risk (hsCRP 1–3 mg/L)	High risk (hsCRP >3–10 mg/L)	p-Value for correlation
n	934	1369	1352	
Age, years	64±11	65±11	64±11	n.s.
Disease duration, years	6.3±6.5	5.4±5.3	5.1±5.3	n.s.
BMI, kg/m^2	27.3±4.1	29.2±4.2	31.2±5.3	<0.001
HbA_{1c}, %	6.59±1.10	6.73±1.16	6.95±1.32	n.s.
Fasting glucose, mmol/L	5.9±1.6	6.2±1.8	6.4±1.9	<0.05
Fasting insulin, μU/mL	10.9±9.0	13.2±9.6	15.3±11.5	<0.001
$HOMA_{IR}$ score, $mU \cdot mmol/L^2$	3.0±3.3	3.7±3.3	4.5±4.2	<0.001
Fasting intact proinsulin, pmol/L	8.7±8.2	13.2±9.6	15.3±11.5	<0.001
hsCRP (BMI-adjusted), mg/L	0.53±0.28	1.87±0.57	5.50±1.86	<0.001
Prevalence of macrovascular complication	208 (22.3%)	382 (27.9%)	383 (28.3%)	<0.001
Prevalence of microvascular complications	260 (27.8%)	397 (29.0%)	406 (30.0%)	<0.001

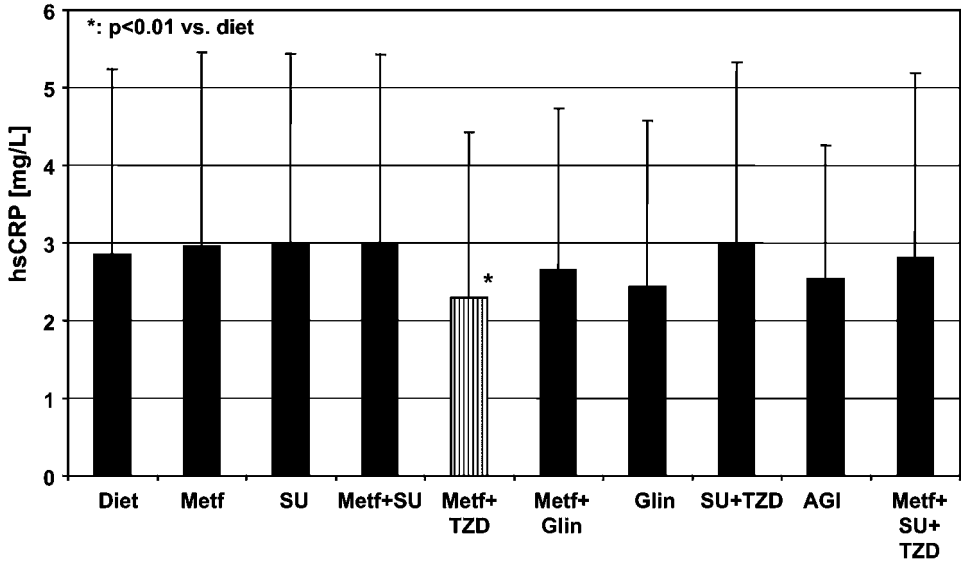

Figure 2 hsCRP values (adjusted for BMI) in patients treated with different oral treatment regimens; SU, sulfonylurea; Metf, metformin; TZD, thiazolidinediones; Glin, glinides; AGI, α-glucosidase inhibitors.

postprandial glucose, rather than fasting glucose and HbA_{1c}, is a strong predictor for cardiovascular mortality in patients with type 2 diabetes, as it may increase adhesion molecule secretion by endothelial cells when glucose concentrations increase above toxic levels (24).

A weakness of our study is the cross-sectional approach, which does not allow conclusions to be drawn about the impact of different therapeutic interventions on the metabolic and macrovascular risk scenario. It has, however, been demonstrated that impairment of β-cell dysfunction, as indicated by increased intact proinsulin levels, is associated with the use of insulinotropic substances, such as sulfonylurea drugs (15). In this analysis the highest hsCRP levels were also observed in patients receiving sulfonylurea therapy. These results support the hypothesis that cardiovascular disease risk may be associated with sulfonylurea treatment, as suspected decades ago (25, 26) and reported again recently (27). Unfortunately, contradicting reports from studies such as the UK Prospective Diabetes Study (UKPDS) did not involve well enough characterized patient populations to allow direct comparison of the results (28). However, prospective studies using the same diagnostic markers as used in our current analysis have provided evidence of a beneficial impact of other therapeutic interventions compared to sulfonylurea therapy. Treatment of insulin resistance, e.g., using PPARγ agonists seems to be an effective measure for decreasing cardiovascular and metabolic risk (29–32).

The importance of determining hsCRP in at-risk populations, such as diabetic patients, is further strengthened by recent reports suggesting that hsCRP is not only a risk indicator, but may also allow monitoring of arteriosclerosis regression during successful therapeutic interventions. Patients who have low CRP levels after statin therapy have better clinical outcomes than those with higher CRP levels, regardless of the resultant level of LDL cholesterol (33). In the ''Reversal of Atherosclerosis with Aggressive Lipid Lowering'' study (REVERSAL), the reduced rate of progression of atherosclerosis associated with intensive statin treatment, as compared with moderate statin treatment, was significantly related to greater reductions in the levels of both atherogenic lipoproteins and CRP in patients with coronary artery disease (34). The same may hold true for patients with type 2 diabetes. Whether determination of hsCRP can provide additional risk information in type 2 diabetes, similar to the analysis of Ridker et al. in the Framingham Risk Score population (35), needs to be explored in prospective epidemiological trials.

The results of our analysis indicate that impaired insulin resistance and impaired β-cell function were associated with higher hsCRP levels and a higher prevalence of macrovascular complications. The clear association between hsCRP and other established or suspect risk markers support the suitability of hsCRP as a cardiovascular risk marker suitable for routine clinical use in type 2 diabetes mellitus.

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