ABSTRACT

C-reactive protein (CRP) is a liver-derived pattern recognition molecule that is increased in inflammatory states. It rapidly increases within hours after tissue injury, and it is suggested that it is part of the innate immune system and contributes to host defense. Since cardiovascular disease is at least in part an inflammatory process, CRP has been investigated in the context of arteriosclerosis and subsequent vascular disorders. Based on multiple epidemiological and intervention studies, minor CRP elevation [high-sensitivity CRP (hsCRP)] has been shown to be associated with future major cardiovascular risk (hsCRP: <1 mg/L = low risk; 1–3 mg/L = intermediate risk; 3–10 mg/L = high risk; >10 mg/L = unspecific elevation). It is recommended by the American Heart Association that patients at intermediate or high risk of coronary heart disease may benefit from measurement of hsCRP with regard to their individual risk prediction. Elevation of hsCRP is associated with increased risk of type 2 diabetes development in patients with all levels of metabolic syndrome. In type 1 and type 2 diabetes mellitus, hemoglobin A1c significantly correlates with hsCRP levels and future cardiovascular risk. Also, hsCRP levels increase with the stage of β-cell dysfunction and insulin resistance. Non-diabetes drugs that have been shown to reduce hsCRP concentrations include aspirin, statins, cyclooxygenase-2 inhibitors, and fibrates. Recent intervention studies have also demonstrated the distinct efficacy of different anti-diabetes treatments on a variety of cardiovascular risk markers. Intensive insulin therapy may reduce inflammation, but this effect may be influenced by the degree of weight gain. Treatment with peroxisome proliferator-activated receptor γ has lead to substantial reduction of hsCRP and other cardiovascular risk markers in several comparator studies. Since this effect was shown to be independent of the degree of glycemic improvement, it can be regarded as a class-specific effect. Whether these findings translate into a reduction of overall cardiovascular mortality will soon be shown by the currently running thiazolidinedione outcome studies. Positive results in these trials will further strengthen the value and acceptance of hsCRP, which is recommended as a predictive laboratory marker for cardiovascular disease risk also in patients with diabetes mellitus.

INTRODUCTION

TYPE 2 DIABETES is a leading cause of morbidity and mortality, and the number of cases is currently approaching pandemic proportions. Patients with both types of diabetes mellitus have an increased risk of fatal cardiovascular events. While about 75% of patients with type 2 diabetes die from macrovascular complications, this is the case in 35% of the pa-
tients with type 1 diabetes. This significant difference is probably linked to insulin resistance and β-cell dysfunction, the underlying disorders in type 2 diabetes. Atherosclerosis is considered to result from a chronic inflammatory response in arterial walls, and pro-inflammatory cytokines including chemokines (e.g., monocytic chemoattractant protein-1, matrix metalloproteinase-9), adhesion molecules (e.g., intercellular adhesion molecule, vascular cell adhesion molecule), and acute-phase reactants [e.g., C-reactive protein (CRP), fibrinogen] have been suggested as circulating markers for arteriosclerosis and inflammation. Population-based studies show significant correlations between these pro-inflammatory markers and deterioration of glycemic control, obesity, and arteriosclerosis. The currently most accepted laboratory marker for cardiovascular risk is the determination of CRP at low levels [high-sensitivity CRP (hsCRP)], a protein that is normally elevated in human plasma in bacterial infections, in cancer, or after surgical procedures.

BIOLOGY OF HSCRP

CRP was detected 75 years ago in studies with Streptococcus pneumoniae. During inflammation, it is predominantly expressed in hepatocytes and interacts with various ligands (such as phosphocholine), activates the classical complement pathway, stimulates phagocytosis, and binds to immunoglobulin receptors (FcγR). CRP may rise rapidly up to 1,000-fold during acute inflammation and is part of the acute-phase response, in which synthesis of many plasma proteins (like clotting proteins, complement factors, anti-proteases, and transport proteins) is increased after an inflammatory stimulus. Expression of CRP is regulated at the transcriptional level by interleukin (IL)-6 and IL-1β. CRP consists of five identical symmetrically arranged 23-kDa protomers, which are folded into two antiparallel β-sheets, similar to the structure of lectins. CRP can bind to a variety of ligands, including phospholipids, chromatin, histones, fibronectin, small nuclear ribonucleoproteins, laminin, and polycations.

CRP-mediated complement activation is limited to the initial phase of complement activation involving the C1–C4 components and seems to decrease the amount of later-stage aggressive complement activation by up-regulation of endothelial cell expression of complement inhibitory factors. In consequence, CRP participates in host defense while limiting the potentially damaging effects of later-stage complement components, which finally leads to a chronic inflammatory situation.

EPIDEMIOLOGY OF HSCRP IN SUBJECTS WITH AND WITHOUT DIABETES

During the past decades, many efforts have been undertaken to develop risk factors that improve global risk prediction for cardiovascular disease. A very successful approach had been the Framingham Risk Score in the 1980s, which nowadays forms the basis for most coronary risk detection and prevention programs. It has now been demonstrated that hsCRP is a most promising marker to additionally improve the predictive value of these existing programs. To date, more than 22 prospective studies have been presented that demonstrate the positive relation of hsCRP to future cardiovascular risk, including the Physicians Health Study, the Women's Health Study, the Atherosclerosis Risk in Communities Study (ARIC), the Monitoring of Trends and Determinants of Cardiovascular Disease (Monica) study, and the Reykjavik Study. In consequence, the Centers for Disease Control and Prevention and the American Heart Association have published guidelines that endorse the use of hsCRP as the only inflammatory biomarker currently available with adequate standardization and predictive value that is suitable as an adjunct to traditional risk factor screening.

The current recommendations for risk assessment are given in Table 1. Values below 1 mg/dL are considered to be associated with low future cardiovascular risk. Values above 10 mg/dL cannot be clearly correlated to any risk score since the probability of unspecific inflammatory elevation not linked to atheroscle-
rosis, e.g., induced by bacterial infection, is too high at these levels. A recent review has documented the added value of hsCRP assessment also in patients suffering from the metabolic syndrome. The authors concluded that based on the consistency of prognostic data for hsCRP and the practicability of its use in outpatient clinical settings, the time has come to consider the addition of hsCRP as a clinical criterion for metabolic syndrome and for the creation of an hsCRP-modified risk score useful for global risk prediction in both men and women.

HSCRP IN PATIENTS WITH DIABETES MELLITUS

The evaluation of the potential value of hsCRP assessment in patients with diabetes needs a consideration of the potential association of chronic inflammation with autoimmunity, absolute lack of insulin, insulin resistance, and β-cell dysfunction. In patients with type 1 diabetes, intensive insulin therapy can reduce previously elevated hsCRP levels, especially if the subjects do not extensively gain body weight. A recent analysis from the Diabetes Control and Complications Trial study demonstrates that the impact of intensive insulin therapy on inflammation is complex and that the risk of atherosclerosis among patients with diabetes may be influenced by the degree of weight gain while undergoing this therapy. There is a significant association between slightly elevated hsCRP levels and reduced coronary vasoreactivity in young subjects with type 1 diabetes. Also, hemoglobin A1c and overall glycemic control significantly correlate with levels of hsCRP in this patient population. In a number of prospective studies in subjects with type 1 diabetes, especially with early carotid atherosclerosis, and in critically ill subjects, hsCRP has been strongly related to risk of cardiovascular disease.

A number of studies have also addressed the situation in type 2 diabetes. Insulin action at the metabolically active cell increases glucose uptake, but activation of insulin receptors at the inner layer of the arteries induces the production of nitric oxide and mediates a variety of vasoprotective and anti-inflammatory effects at the endothelial level. Therefore, insulin resistance leads not only to hyperglycemia, but also to an impairment of vasoprotection and a substantial endothelial dysfunction, with increased secretion of adhesion molecules and increased monocyte activation and migration into the vascular wall.

At the same time, β-cell dysfunction is followed by increased secretion of the insulin precursor proinsulin, which is considered to be a driver for adipogenesis and a cardiovascular risk factor by way of stimulating plasminogen activator inhibitor-1 and IL-6 expression and inhibition of fibrinolysis. Intact proinsulin secretion forced by increasing insulin resistance can be regarded as the independent contribution of β-cell dysfunction to the overall increased cardiovascular risk in patients with type 2 diabetes, and an elevated level of fasting intact proinsulin is a very specific indirect indicator for insulin resistance.

It is likely to be the combination of postprandial glucotoxicity and hyperlipidemia with the impaired endothelial protection and overall pro-inflammatory macrophage activation (induced by adipogenesis) that provides the background for the systemically occurring severe arteriosclerosis in many patients with type 2 diabetes. Based on this background, it is to be expected that inflammatory surrogate markers for cardiovascular risk should be prevalent and elevated in patients with type 2 diabetes. Kang et al. showed that hsCRP concentrations in patients with type 2 diabetes are associated with carotid intima-media thickness, an important clinical surrogate marker for macrovascular disease. It was also shown that hsCRP is associated with insulin resistance and cardiovascular autonomic dysfunction.

In a recent study, Bahceci et al. investigated the correlation among hemoglobin A1c, insulin re-

<table>
<thead>
<tr>
<th>hsCRP concentration</th>
<th>Cardiovascular risk assessment</th>
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<tbody>
<tr>
<td>0 to &lt;1 mg/L</td>
<td>Low risk</td>
</tr>
<tr>
<td>1-3 mg/L</td>
<td>Intermediate risk</td>
</tr>
<tr>
<td>&gt;3 to 10 mg/L</td>
<td>High risk</td>
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<tr>
<td>&gt;10 mg/L</td>
<td>Unspecific elevation</td>
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Pfützner and Forst
istance, and hsCRP in 105 male patients with and without coronary disease. Mean hsCRP levels were higher in men with cardiovascular disease, independent of the prevalence of type 2 diabetes, and there was a positive correlation among hsCRP, fasting insulin, and insulin resistance. The authors concluded that inflammation, insulin resistance, and hyperglycemia jointly contribute to the elevated cardiovascular risk in this patient group.

In Germany, the cross-sectional IRIS-II study investigated the prevalence of insulin resistance and β-cell dysfunction in 4,265 patients with orally treated type 2 diabetes mellitus. Insulin resistance was assessed by the insulin resistance homeostasis model assessment score and several other clinical resistance scores, and by measurement of intact proinsulin, an indicator of insulin resistance and later stage β-cell dysfunction. The correlation of hsCRP with the different stages of β-cell dysfunction as described by Forst et al. and prevalence of macrovascular complications are given in Figure 1. All β-cell dysfunction groups showed an increased cardiovascular risk as assessed by hsCRP levels above 3 mg/L. In addition, increased severity of β-cell dysfunction was positively correlated with an increase in hsCRP concentrations and increased prevalence of stroke, cardiovascular disease, myocardial infarctions, and peripheral occlusive artery disease. It is noteworthy that neither progression of β-cell dysfunction nor cardiovascular risk was associated with disease duration in this study.

Next to diagnostic evaluations, interventional studies are required to investigate the impact of currently available therapeutic options on hsCRP levels and cardiovascular risk in patients with type 2 diabetes.

### INSULIN

In addition to the above reported findings from the Diabetes Control and Complications
Trial trial, further studies have investigated the anti-inflammatory effect of insulin substitution. Insulin has been found to inhibit nuclear factor \( \kappa B \) in vascular endothelial cells and mononuclear cells and to have an acute anti-inflammatory effect, which could be demonstrated by a decrease of pro-inflammatory cytokines.\(^{40,41} \) A decrease in hsCRP was observed in Japanese patients with poorly controlled type 2 diabetes shortly after initiation of insulin therapy,\(^4^2 \) and a substantial decrease was also seen during insulin therapy in a relatively long-term observation over 16 weeks.\(^4^3 \)

One can argue that an overall long-term reduction of inflammation by insulin therapy, which is accompanied by a decrease in cardiovascular mortality, has not been seen in the United Kingdom Prospective Diabetes Study. It needs to be stated, however, that this valuable epidemiological study is by its design not able to clarify this question, since patients were not equally randomized to the different treatment moieties at baseline.\(^4^4 \) Next to insulin, many other interventions have been suggested to reduce hsCRP levels and to improve cardiovascular outcome in patients with and without diabetes mellitus. They include, but are not limited to, diet and exercise, aspirin, statins, peroxisome proliferator-activated receptor (PPAR) \( \gamma \) agonists, cyclooxygenase-2 inhibitors, fibrates, gemfibrozil, and absciximab\(^4^5 \) (see Deans and Sattar\(^4^6 \) in this issue).

**STATINS**

The currently most accepted class for primary prevention of coronary artery disease is the 3-hydroxy-3-methylglutaryl Coenzyme A reductase inhibitors, or statins. Next to lowering lipids, the statins exhibit anti-inflammatory effects mediated by inhibition of macrophage function, leading to reduced macrophage activation and foam cell formation. They induce expression of PPAR\( \gamma \), prevent matrix degradation by metalloproteinases, modulate endothelial function, and can up-regulate nitric oxide synthesis \(^4^7,4^8 \).

In the Cholesterol And Recurrent Events (CARE) study, post-myocardial infarction patients were treated with pravastatin, which resulted in a rapid and extensive reduction in hsCRP levels and in a better clinical outcome.\(^4^9 \) Statins reduced CRP levels in a low-density lipoprotein-cholesterol-independent manner in 1,702 patients with no history of cardiovascular disease, who were randomized to statin or placebo in the prospective double-blind Pravastatin Inflammation/CRP Evaluation (PRINCE) study, regardless of age, smoking status, body mass index, presence of diabetes, and use of aspirin.\(^5^0 \) In this study, a reduction of hsCRP by 17% was seen in the statin group, while no change was seen in the placebo group after 6 months \( (P < 0.001) \). In the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS-TexCAPS), a 5-year randomized placebo-controlled prospective study of primary prevention with lovastatin with 5,742 participants, the rates of coronary events increased significantly with an increase in baseline hsCRP concentrations. Statin treatment effectively prevented coronary events in individuals with elevated hsCRP independent from their lipid profile.\(^5^1 \)

**PPAR\( \gamma \) AGONISTS**

PPARs can be found in the cellular nucleus and act as transcription factors that control the expression of target genes and influence multiple cellular functions, such as regulation of lipoprotein metabolism, uptake of oxidized low-density lipoprotein, and processing of cholesterol in the macrophages.\(^4^8 \) The subfamily member PPAR\( \gamma \) is highly expressed by monocytes, macrophages, and foam cells.\(^5^2 \) The pathophysiologically important expression of adhesion molecules in endothelial cells can be inhibited by PPAR\( \gamma \) agonists, like rosiglitazone or pioglitazone.\(^5^3 \) PPAR\( \gamma \) agonists have been shown to improve insulin resistance and long-term blood glucose control in patients with type 2 diabetes, when used as monotherapy, and also in combination with sulfonylurea drugs and metformin.\(^5^4,5^5 \) They also exert short-term anti-inflammatory effects, which can accumulate into anti-atherogenicity.\(^5^6 \) Clinical data suggest that PPAR\( \gamma \) agonists reduce inflammatory biomarkers of arteriosclerosis, like hsCRP, matrix
metalloproteinase-9, or soluble CD40L, in treated patients, thus potentially modulating their cardiovascular risk. Most of these studies were placebo-controlled and as such did not allow a dissection of metabolic from non-metabolic drug effects. However, in a recent randomized parallel study in 173 patients with orally treated type 2 diabetes, we have been able to demonstrate that pioglitazone in comparison with glimepiride significantly reduces biomarkers for cardiovascular risk, such as hsCRP (–28%), matrix metalloproteinase-9 (–14%), or monocyte chemoattractant protein-1 (–7%), independently from glycemic control. These effects were accompanied by a reduction in carotid intima-media thickness, reduction of insulin resistance scores and intact proinsulin concentrations. When given in combination with glimepiride, rosiglitazone could be shown to effectively inhibit impairing effects on the cardiovascular risk profile observed with sulfonylurea monotherapy, and to reduce biomarkers of β-cell dysfunction, insulin resistance, and cardiovascular risk including hsCRP (–42%) in a dose-dependent manner. In the recently terminated double-blinded placebo-controlled PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) Study with 5,238 patients in secondary prevention, pioglitazone given on top of an intensified diabetes regimen significantly decreased the number of composite macrovascular endpoints by 16% after an observation period of only 2.8 years.

An extensive program of outcome studies with hard cardiovascular endpoints is currently under way (DREAM, RECORD, ADOPT, BARI-2D, etc.), which may be able to provide the evidence on whether the observed anti-inflammatory and β-cell protective actions of thiazolidinediones will finally translate into a significant lower cardiovascular risk for patients with impaired glucose tolerance.

**ASPIRIN**

In the Physicians’ Health Study, aspirin has been shown to decrease the risk of first cardiovascular events by 44%. It was demonstrated that the highest risk reduction can be achieved in subjects with high hsCRP levels. This finding suggests that the observed beneficial effects of aspirin on cardiovascular risk may be attributable to the anti-inflammatory effects of this drug. Aspirin has become a readily available over-the-counter drug in most countries in the world and is widely used nowadays as an unspecific pain reliever but also to improve blood rheology. The direct effect on hsCRP, however, seems to be minimal.

**CONCLUSIONS**

In recent years, hsCRP has become an accepted laboratory marker for prediction of cardiovascular risk. Many epidemiological and prospective studies have demonstrated the correlation between the hsCRP risk group and the observed cardiovascular outcome in populations without diabetes, but also now in patients with diabetes mellitus. While diet and exercise are still the cheapest and most effective ways to reduce cardiovascular risk, different drugs, like statins and PPAR agonists, have been demonstrated to effectively reduce hsCRP levels. Given the increased cardiovascular risk associated with both types of diabetes mellitus on one hand, and the convincing overall consistency of the reported findings and the potential value of the additional information provided by determination of hsCRP about the chronic vascular inflammatory state before during and after therapeutic interventions on the other hand, introduction of this marker into routine diagnostic procedures and guidelines for diagnosis and treatment of diabetes mellitus is recommended.

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