Biomarkers in acute coronary syndromes and their role in diabetic patients

Christopher Heeschen

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CHRISTOPHER HEESCHEN

Abstract

Diabetic patients with acute coronary syndromes are at high risk for cardiovascular complications but risk stratification in these patients remains challenging. Regularly, diabetic patients have a less typical clinical presentation, which could lead to delayed diagnosis and subsequent delayed initiation of treatment. Since diabetic patients derive particular benefit from aggressive anti-platelet therapy, early diagnostic and therapeutic risk stratification of these patients is of critical importance to improve their adverse outcome.

Although the electrocardiogram remains a pivotal diagnostic tool in the evaluation of patients suspected of having an acute coronary syndrome, only significant ST-segment changes provide reasonable prognostic information. Therefore, repeated assessment of circulating protein biomarkers represents a valuable diagnostic tool for improving efficacy and safety of decision-making in these patients. The combined use of biomarkers reflecting distinct pathophysiological aspects, such as myocardial necrosis, vascular inflammation, oxidative stress and neurohumoral activation, may significantly improve triage of patients with chest pain. These tools may identify those patients that are at particularly high risk for short-term and/or long-term cardiovascular events. Eventually, tailored medical and interventional treatment of diabetic patients should help to prevent these cardiac events in a cost-effective manner.

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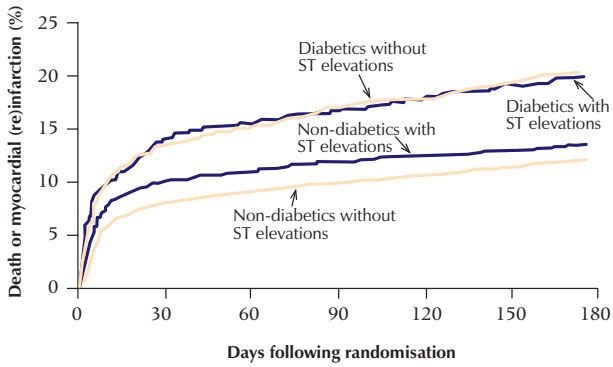
Key words: acute coronary syndrome, unstable angina, biomarker, troponin, risk stratification.

Introduction

Acute coronary syndromes in diabetic patients present a major challenge for risk stratification. Diabetes mellitus is a major risk factor for cardiovascular morbidity and mortality. The burden of cardiovascular disease associated with this condition seems certain to increase dramatically because it is estimated that the prevalence of diabetes will double by the year 2025.[1] Even when other risk factors are controlled, individuals with diabetes have a three- to five-fold greater risk of developing coronary artery disease than non-diabetics.[2] Diabetic patients with no history of heart disease have the same risk for cardiovascular death as non-diabetics with a history of myocardial infarction (MI).[3] As many as 75% of individuals with diabetes will die of coronary artery disease,[2] and up to 33% of patients with insulin-dependent disease will die by the age of 50 years.[4] Most importantly, however, coronary artery disease associated with diabetes has a more aggressive clinical course. Diabetic patients suffer increased mortality from both ST-segment elevation MI[5,6] and non-ST-segment elevation acute coronary syndromes (figure 1).[7,8]

Diabetics with acute coronary syndromes present more commonly with clinical characteristics that are associated with an adverse prognosis. Diabetics are more frequently female, older, suffer from diffuse coronary heart disease, more frequently have heart failure and have a less favourable risk profile.[7,8] Moreover, diabetic patients present more often with less typical symptoms of an acute coronary syndrome, and this may lead to delayed hospitalisation, prolonged diagnostic workup and postponed initiation of appropriate treatment.[9] Even after comprehensive statistical adjustment for other prognostic factors, diabetics show an increased mortality after onset of acute coronary syndromes.[7] These data clearly indicate that thorough risk stratification in patients with diabetes is of critical importance for their clinical outcome.

Figure 1. Event rates (death, myocardial infarction) in patients with acute coronary syndromes (with/without ST-segment elevations) during six months of follow-up



Results from the GUSTO IIb study[8]

Experimental Surgery, Department of Surgery, Ludwig-Maximilians-University, Marchioninistrasse 15, 81377 Munich, Germany.
Christopher Heeschen, Professor for Oncology and Transplantation Medicine

Correspondence to: Dr Christopher Heeschen
Experimental Surgery, Department of Surgery, Ludwig-Maximilians-University, Marchioninistrasse 15, 81377 Munich, Germany.
Tel: +49 89 7095 3438; Fax: +49 89 7095 6433
E-mail: christopher.heeschen@med.uni.muenchen.de

For several decades, circulating biomarkers have been used to assess myocardial injury in patients with suspected non-ST-segment elevation acute coronary syndromes (NSTE-acute coronary syndromes). However, biochemical markers are now used not only to detect myocardial injury but also for risk stratification and guidance of treatment in patients with acute coronary syndromes.[10-12] A substantial number of studies support the use of cardiac troponins for the detection of minor myocardial injury. Data for concurrent measurement of markers of general inflammation (e.g. high-sensitivity C-reactive protein), markers of vascular inflammation (e.g. placental growth factor), markers of platelet activation (e.g. soluble CD40 ligand) and of neurohumoral activation (e.g. brain natriuretic peptide) are currently emerging and it may be that a multimarker approach will provide additional information. Although markers that are not specific for the coronary arteries and/or myocardium have their limitations, they may be important tools for diagnostic and therapeutic stratification of patients with acute coronary syndromes.

Cardiac troponins as specific markers of cardiac necrosis (and thrombosis)

Numerous studies provide convincing evidence that the blood troponin level is a powerful indicator of risk for patients with NSTE acute coronary syndromes[13-23] whereas creatine kinase-myocardial band (CK-MB) and myoglobin are much less powerful predictors of the patient's risk. A meta-analysis of 14 trials showed that troponins are highly predictive for the risk of death or acute MI. During 30-day follow-up, the relative risk was 2.7 (95% confidence intervals [CI] 2.1–3.4) in patients with unstable angina and elevated troponin T. For troponin I the relative risk was 4.2 (95% CI 2.7–6.4).[24] This predictive capacity of the troponins is independent of important clinical risk factors, including age, ST-segment deviations and presence of heart failure. Another meta-analysis calculated a more than nine-fold increase in the risk for death or MI in patients with elevated troponins.[25]

Angioscopic studies have revealed that the thrombus responsible for the clinical manifestation of unstable angina is more commonly white (platelet-rich) and less commonly red (fibrin-rich), whereas red thrombus tends to be more prominent in acute MI.[26] Pathological studies in patients with unstable angina who have died suddenly demonstrate that the fatal event is often preceded by repetitive embolisation of thrombi from an unstable atheroma.[27,28] This results in focal myocardial necrosis that is too small to be detected by an increase in creatine kinase or CK-MB but that can be detected by troponin measurements. Indeed, it has been shown that troponin T elevation in patients with acute coronary syndromes is significantly linked to visible thrombus, even after prolonged treatment with heparin and in morphologically complex target lesions.[29] Failed resolution of thrombus and complex lesion characteristics provided important prognostic information. In a multivariable analysis, however, troponin T represented the only independent and the most powerful marker for the prediction of cardiac risk in patients with acute coronary syndromes.

The angiographic findings of this study are in full agreement with the concept that troponin T release is related to coronary thrombosis and consecutive embolisation, leading to minor myocardial damage. Thus, even a small increase in troponin levels in patients with acute coronary syndromes is related to 'minor myocardial injury' and should be interpreted as an indicator for microembolisation from thrombus. The increased cardiac risk of troponin T-positive patients may be related less to minor myocardial damage and more to complex coronary morphology and thrombus formation in the culprit lesion.

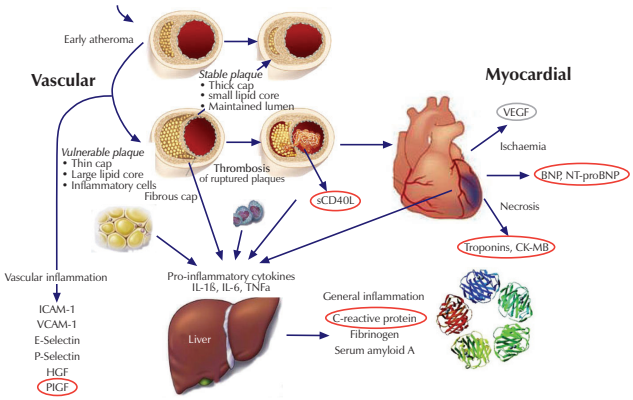
The acute phase reactant C-reactive protein as a marker of general inflammation

Elevated levels of circulating cardiac troponin are found in about one third of patients with acute coronary syndromes. Although the absolute short-term risk of troponin-negative patients is significantly lower as compared to troponin-positive patients, the large number of patients without troponin elevation means that risk assessment and therapeutic management is clinically challenging. The six-month risk of death or non-fatal MI in troponin-negative patients was 8.4% in the c7E3 Fab Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) trial.[30] Therefore, the availability of a sensitive marker of plaque instability, whose levels become elevated before or even in the absence of myocardial necrosis, should improve diagnostic and therapeutic decision-making. Over the past decade, C-reactive protein (CRP), as a prototypic but non-specific acute-phase reactant, has emerged as a powerful predictor of cardiovascular events. There is compelling epidemiological evidence that CRP is a sensitive marker of inflammation and/or metabolic processes associated with atherogenesis and cardiovascular events such as death, MI and stroke.

Although CRP has been shown to be useful for risk assessment in different populations,[31,33] including patients with acute coronary syndromes,[34-36] debate about whether CRP is a clinically useful biomarker continues. Danesh *et al.* found in the Reykjavik prospective cohort study,[33] which included 2,459 patients with stable coronary heart disease and 3,969 selected controls, that the predictive value of a single baseline measurement of CRP for the 20-year incidence of cardiovascular events was much less impressive than previously reported values.[37,38] Surprisingly, they found that CRP adds little to the predictive value provided by the assessment of traditional risk factors, including low-density lipoprotein (LDL) cholesterol, whereas Ridker and co-workers had previously reported that CRP had greater predictive value than LDL.[39] Since the Reykjavik study included by far the largest number of events (albeit during a follow-up of 20 years) that have been studied in such analyses, these new findings emphasise the need for more research to clarify the use of CRP as a marker of cardiovascular risk in clinical practice.

Two recent studies, one by Nissen *et al.*[40] and one by Ridker *et al.*,[41] confirm that reducing the inflammatory component of cardiovascular disease through the use of statin therapy improves the clinical outcome independently of the reduction in serum cholesterol levels. In the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, which included 502 patients with angiographically doc-

Figure 2. Earlier markers of vascular inflammatory and thrombo-inflammatory activation, respectively, may precede the development of myocardial injury (troponin release) or neurohumoral activation (BNP or NT-proBNP release) in patients with acute coronary syndromes



Key: CK-MB = creatine kinase myocardial band; NT = N terminal; VEGF = vascular endothelial growth factor; PlGF = placental growth factor; IL = interleukin; TNF = tumour necrosis factor; BNP = brain natriuretic peptide; ICAM = intercellular adhesion molecule; VCAM = vascular cellular adhesion molecule

From Fichtlscherer S *et al*. *Curr Opin Pharmacol* 2004;**4**:124-31

umented stable coronary disease, the reduced rate of progression of atherosclerosis associated with intensive statin treatment (80 mg atorvastatin), as compared with moderate statin treatment (40 mg pravastatin), was significantly related to greater reductions in the levels of both atherogenic lipoproteins and CRP.[40] In the Pravastatin or Atorvastatin Evaluation and Infection Therapy-TIMI 22 (PROVE IT-TIMI 22) study, which included 3,745 patients with acute coronary syndromes, atorvastatin 80 mg was more likely than pravastatin 40 mg to result in low levels of LDL cholesterol and CRP.[41] However, meeting these targets was more important in determining the patients' outcome than was the specific choice of therapy. Patients who had low CRP levels after statin therapy had better clinical outcomes than those with higher CRP levels, regardless of the level of LDL cholesterol.

The exact source of elevated CRP levels among patients with acute coronary syndromes remains unclear. Given that myocardial damage is also a major inflammatory stimulus, it is important to note that, in a recent combined analysis of the Fragmin and fast Revascularisation during InStability in Coronary artery disease II (FRISC-II) trial and Global Utilisation of Strategies to Open Occluded arteries (GUSTO)-IV, CRP elevation over a period of up to 120 hours after onset of symptoms was found only in patients with elevated troponin levels.[42] In CAPTURE patients, CRP levels were consistently significantly higher in troponin-positive patients,[36] suggesting that an acute inflammatory process induced by myocardial damage is superimposed on a chronic inflammatory condition. Thus, current research activities have shifted to the identification of more upstream markers of the inflammatory cascade which may be more representative of vascular inflammation (figure 2).

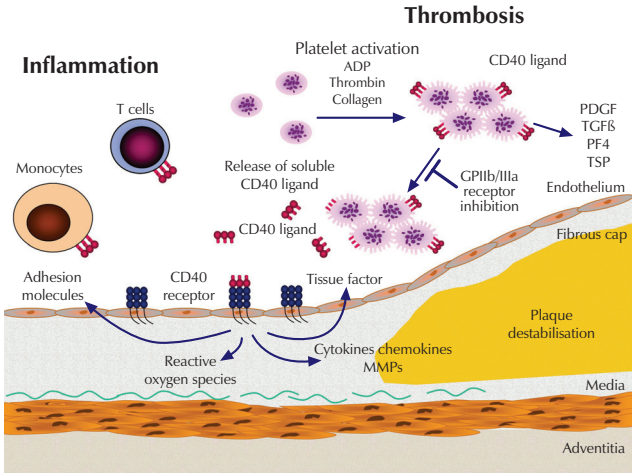
Placental growth factor as a primary vascular inflammatory instigator

Placental growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, was recently shown to be profoundly up-regulated in early and advanced atherosclerotic lesions.[43] PlGF, originally identified in the placenta,[44] stimulates vascular smooth muscle growth, recruits macrophages into atherosclerotic lesions, up-regulates tumour necrosis factor (TNF)α and monocyte chemoattractant protein (MCP)-1 production by macrophages, enhances production of tissue factor and stimulates pathological angiogenesis.[43,45] All these processes are known contributors to plaque progression and destabilisation. Most importantly, inhibition of the PlGF effects by blocking its receptor Flt-1 was shown experimentally to suppress both atherosclerotic plaque growth and vulnerability via inhibition of inflammatory cell infiltration.[46] These data suggest that PlGF may act as a primary inflammatory instigator of atherosclerotic plaque instability.

PlGF blood levels are markedly up-regulated in patients with acute coronary syndromes, independent of the presence of myocardial injury.[47] Moreover, data from the CAPTURE trial established PlGF blood levels as a novel, powerful and independent prognostic determinant of clinical outcome in patients with acute coronary syndromes.[47] Measuring PlGF levels significantly extends the predictive and prognostic information gained from traditional inflammatory markers in acute coronary syndromes. The predictive value of PlGF levels was found to be independent of myocardial necrosis as evidenced by elevated troponin levels,[29] and of platelet activation as evidenced by elevation of soluble CD40 ligand.[48] Intriguingly, elevated PlGF levels identified not only those patients with acute chest pain who developed acute coronary syndromes, but also those patients who suffered from an increased risk of recurrent instability after discharge after an initial acute coronary syndrome. PlGF levels may represent a reliable and powerful clinical tool for identifying both patients with high-risk lesions and those with ongoing vascular inflammation of the coronary circulation.

The role of PlGF as a primary inflammatory instigator of atherosclerotic lesion instability makes sense given its well documented pro-inflammatory effects in animal models of atherosclerosis and arthritis.[43] Although PlGF belongs to the VEGF family, its role in causation of disease appears to be related more to vascular inflammation than to angiogenesis.[46] Whereas VEGF is activated by hypoxia and elevation of VEGF levels is regarded as an early adaptation of the myocardium to deprivation of blood flow,[49] PlGF is not affected, and may even be down-regulated, by hypoxia.[50,51] In line with these data, analysis of CAPTURE did not reveal any correlation between PlGF levels and VEGF levels as a marker of myocardial ischaemia or between PlGF levels and troponin levels as a marker of myocardial necrosis. PlGF levels do not appear to be confounded by myocardial necrosis, whereas VEGF levels are linked to troponin elevation,

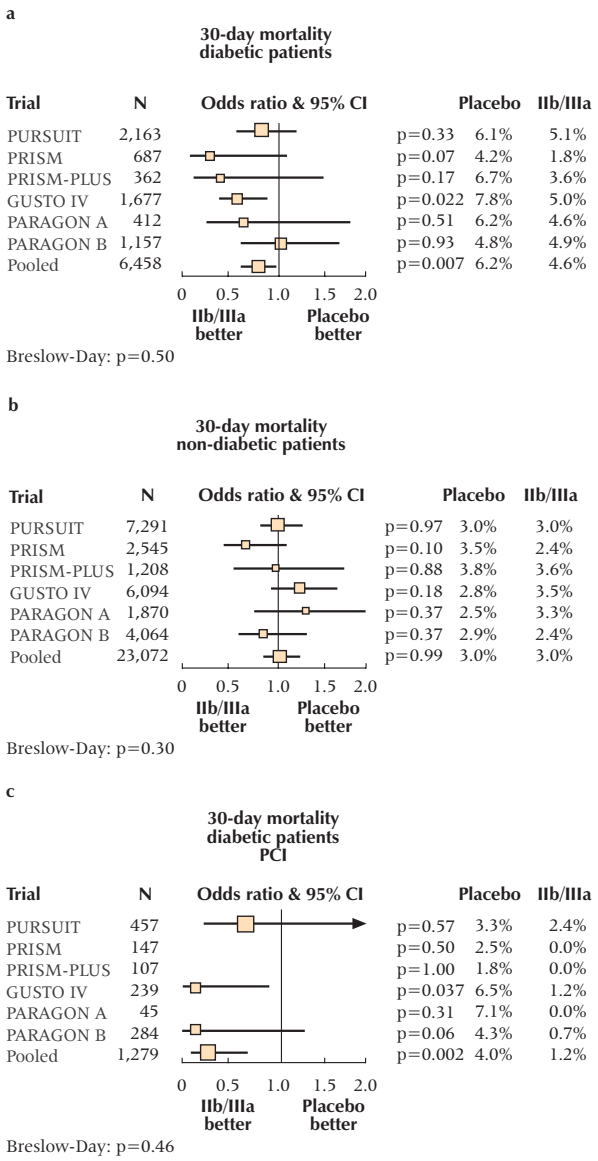
Figure 3. The pathophysiological role of soluble CD40 ligand in plaque destabilisation and thrombosis of patients with acute coronary syndromes



Key: PDGF = platelet-derived growth factor; MMP = matrix metalloproteinase

From Fichtlscherer S *et al*. *Curr Opin Pharmacol* 2004;**4**: 124-31

Figure 4. Therapeutic benefit of treatment with glycoprotein IIb/IIIa receptor antagonists in patients (a) with and (b) without diabetes. Therapeutic benefit of treatment with glycoprotein IIb/IIIa receptor antagonists in diabetic patients treated by percutaneous coronary intervention (c)



From Roffi M *et al*. *Circulation* 2001;**104**:2767-71

impaired TIMI flow and clinical evidence of myocardial ischaemia.[48] Most notably, since the pro-inflammatory effects of PlGF can be specifically inhibited by blocking its receptor Flt-1, these findings may also provide a rationale for a novel anti-inflammatory therapeutic target in patients with coronary artery disease.[43]

Soluble CD40 ligand as a marker for platelet activation

Troponins are surrogate markers for unstable thrombus formation[30] but more immediate biochemical markers of platelet activation may prove to be helpful for identifying patients who are in a prothrombotic state, even before myocardial necrosis occurs. In this respect, increasing evidence suggests that CD40 ligand is associated with platelet activation.[52] Soluble CD40 ligand is actively released following platelet stimulation (figure 3).[53,54] Circulating soluble CD40 ligand can activate CD40 on endothelial cells and thereby induce a pro-inflammatory cascade in the vessel wall. Soluble CD40 ligand can also activate CD40 which is expressed on inflammatory cells such as monocytes and T cells. The subsequent activation of these inflammatory cells and their invasion into the ruptured or eroded plaque results in further inflammatory perturbation of the vessel wall. In patients with coronary heart disease, soluble CD40 ligand is released primarily from activated platelets; elevated levels have been reported for patients with acute coronary syndromes.[55,56] Moreover, it has been shown that soluble CD40 ligand is a powerful biochemical marker of thrombotic inflammatory activation in patients with acute coronary syndromes, supporting the close relationship between inflammation, thrombotic activation and acute coronary syndromes.[48,57] The latter two studies have clearly demonstrated that combining this new marker with classical markers of necrosis (troponins) can help us to identify those patients at highest risk for subsequent cardiovascular events.

Natriuretic peptides as markers of neurohumoral activation of the heart

Since the description of elevated levels of the neurohormone B-type natriuretic peptide (BNP) and the N-terminal fragment of the BNP prohormone, NT-proBNP, in patients with congestive heart failure, several investigations have focused on the prognostic value of neurohumoral activation in the setting of acute MI. More recently, the prognostic implications of BNP and NT-proBNP, have also been extend-

ed to patients with unstable angina and non-ST-elevation MI. In a first small case-control study of patients with non-ST-elevation acute coronary syndromes, NT-proBNP levels were higher among patients who died than those who survived.[58] This pilot study was followed by a *post hoc* analysis of the orbofiban in patients with unstable coronary syndromes (OPUS)-TIMI 16 trial: this included 2,525 patients in whom BNP was measured approximately 40 hours after the onset of symptoms.[59] Rates of death and heart failure during 10 months of follow-up increased with higher baseline levels of BNP, and this finding was consistent across the spectrum of acute coronary syndromes.

More recently, in a consecutive series of patients with chest pain and no ST-segment elevation, Jernberg and colleagues also found that NT-proBNP levels measured at the time of arrival in the emergency room were strongly associated with long-term mortality, again independent of the index diagnosis (MI or unstable angina).[60] A more recent study in patients with stable coronary heart disease demonstrated that elevated levels of BNP are independently associated with inducible ischaemia. This provides the rationale for the hypothesis that NT-proBNP levels in patients with acute coronary syndromes may also reflect ischaemia-induced left ventricular dysfunction, even in the absence of myocardial necrosis.[61]

In a heterogeneous population of patients with non-ST elevation acute coronary syndromes enrolled in the Platelet Receptor Inhibition in Ischemic Syndrome Management (PRISM) trial, it was demonstrated that serial measurements of NT-proBNP significantly enhance the predictive value as compared to a single baseline NT-proBNP measurement.[62] A second blood sample, drawn 72 hours after the onset of symptoms, provided important information about the further clinical course of the patients. A rapid fall in NT-proBNP levels may indicate responsiveness to the therapeutic regimen chosen for the individual patient and, thus, may explain the reduced event rates observed in patients with declining NT-proBNP levels during the first days after onset of symptoms. Intriguingly, none of the other investigated biomarkers (troponin T and CRP) demonstrated a similar pattern during clinical stabilisation of these patients. These data suggest for the first time that serial measurements of NT-proBNP in patients with acute coronary syndromes can be used for dynamic risk assessment and may be helpful for rapid identification of patients who are suitable for early discharge or who may need more intensive therapy.

Summary

Diabetic patients represent a high-risk population of patients with acute coronary syndromes. Unfortunately, clinical presentation in these patients is frequently not typical, which can delay the diagnostic workup and initiation of appropriate treatment. Since diabetic patients derive particular benefit from aggressive antiplatelet therapy (figure 4),[63] early risk stratification of these patients is of great importance for their short- and long-term prognosis. The electrocardiogram still remains the most useful and cost-effective first-line tool in the evaluation of patients with chest pain. After exclusion of the presence of ST-segment elevation, repeat quantitative or qualitative troponin measurements provide valuable diagnostic tools for improving efficacious and safe decision-making in patients suspected of having an acute coronary syndrome.

Increasing evidence suggests that the combined use of biomarkers reflecting distinct pathophysiological features such as myocardial necrosis, vascular inflammation, oxidative stress and neurohumoral activation may add significantly to our ability to identify correctly patients who are at high risk for short-term and long-term cardiovascular events and subsequently to tailor medical treatment to improve their adverse outcome.

Conflict of interest

Dr Heeschen has received research support from Dade Behring Inc., Roche Diagnostics, and Abbott.

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