Preoperative plasma fibrinogen levels predict mortality after coronary artery bypass grafting

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Summary
This study was designed to investigate whether plasma fibrinogen levels as well as the β-fibrinogen –455 G/A genotype are associated with outcome after coronary artery bypass graft (CABG) operation. We enrolled 249 consecutive CAD patients one day before they underwent a CABG operation. Data from 220 patients with available plasma fibrinogen levels were analyzed. The primary end-point was total mortality, the secondary end-point mortality from cardiac causes or the need for myocardial revascularization. The 2-year total mortality was 9.1% in the entire cohort. Multivariable analysis revealed an independent relationship between the primary end-point and preoperative plasma fibrinogen levels but not the β-fibrinogen –455 G/A genotype. Neither preoperative plasma fibrinogen levels nor the β-fibrinogen –455 G/A genotype could predict the secondary end-point.

We conclude, that elevated preoperative plasma fibrinogen levels, but not the β-fibrinogen -455 G/A genotype predict the total mortality after CABG operation.

Keywords
Fibrinogen, β-fibrinogen-455 G/A gene polymorphism, coronary artery disease, coronary artery bypass graft operation

Introduction
Impaired haemostasis could be an important risk factor for the outcome after coronary artery bypass graft (CABG) surgery. Up to 25% of grafts are thrombotically occluded within 3 to 4 months after the operation (1). Fibrinogen is an important mediator of thrombus formation. Plasma fibrinogen levels significantly rise during the first five to eight days after CABG surgery (1, 2) and it has been shown that elevated postoperative plasma fibrinogen concentrations indicate early graft occlusion (1). Furthermore, patients who suffered from myocardial infarction after CABG had significantly higher preoperative plasma fibrinogen concentrations compared to CABG patients with a good postoperative course (3).

There is multiple evidence from epidemiological studies that elevated plasma fibrinogen concentrations are an independent risk factor for coronary artery disease (CAD) and acute myocardial infarction (4-7). Elevated plasma fibrinogen levels are also assumed to be an inheritable risk factor of atherosclerosis. Plasma fibrinogen levels are increased in the offspring of men who suffer from premature CAD (8).
Non-genetic characteristics such as age, diet and smoking habits as well as genetic factors influence plasma fibrinogen levels (9, 10). Fibrinogen is a dimeric plasma glycoprotein consisting of three pairs of polypeptide chains (α-, β- and γ-chains) each being encoded by a separate gene (11). The 5'-flanking region of the β-fibrinogen gene contains a G-A substitution 455 base pairs upstream from the start of transcription (4). The presence of the rare -455 A allele has been reported to be associated with elevated plasma fibrinogen levels (8, 12-14), an increased risk of CAD (15), the severity (16) and progression of CAD (17), as well as with ischaemic stroke involving large vessel disease (18). On the other hand, several groups failed to find associations between the β-fibrinogen -455 G/A genotype and the risk of CAD (19) or myocardial infarction (13, 14).

The present analysis was performed to test whether there is an association between plasma fibrinogen levels as well as the β-fibrinogen -455 G/A genotype and the outcome after CABG operation.

Methods

Two hundred and forty-nine consecutive patients were enrolled on the day before they underwent CABG surgery between May 1996 and August 1997. None of the patients had previous cardiac surgery. The study was approved by the institutional ethics committee of the University of Greifswald. Prior to inclusion in the study, all subjects had given informed consent.

Operation details are given elsewhere (20). Survival status, the date of death, recurrent percutaneous transluminal angioplasties (PTCA) or recurrent CABG procedures were obtained from phone calls with the family doctors. Family doctors were also asked to provide information on the cause of deaths. Deaths from cardiac cause were defined as being those due to myocardial infarction, heart failure or sudden death. Myocardial infarction was defined as a rise in cardiac enzymes (total creatinine phosphokinase > two times over the upper limit normal value or troponin I ≥ the value considered diagnostic of a myocardial infarction by the laboratory performing the assay) and evolving ECG changes (progressive changes in the ST segment and T wave compatible with myocardial infarction or without the presence of Q waves). Heart failure was defined according to clinical criteria of systolic left ventricular insufficiency. A sudden cardiac death was assumed if death occurred within 24 h after onset of cardiac symptoms. A death which occurred without evidence for myocardial infarction, heart failure or sudden death was classified as non-cardiac. Whenever necessary, the patient, his or her relatives and local hospitals were contacted for further information. The current status remained unknown in 2 patients (0.8%). Clinical, laboratory and angiographic data were obtained from medical records. Preoperative plasma fibrinogen concentrations were determined according to Clauss (21). Twenty-seven patients had missing data on plasma fibrinogen levels and were excluded from further analyses. This resulted in a total study population of 220 CABG patients. The primary end-point was total mortality. The secondary end-point was major cardiac events, defined as fatal myocardial infarction, fatal heart failure, sudden cardiac death, percutaneous coronary intervention or recurrent CABG during follow-up.

Genetic analysis was performed by laboratory personnel who were blinded from the clinical data. Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (High Pure PCR Template Preparation Kit, Roche Diagnostics GmbH, Mannheim, Germany). Genotyping of the β-fibrinogen –455 G/A genotype was performed using a PCR/restriction fragment length polymorphism (RFLP)-based technique. A fragment spanning the 5'-flanking region and the first exon of the β-fibrinogen gene was amplified using PCR conditions and primers as described by Thomas et al. (12). The presence of the –455 G nucleotide created an additional HaeIII-site which is lost when the A nucleotide is in this position. The digestion of the PCR products with the Hae III isoschizomer BsuRI (MBI Fermentas GmbH, St. Leon-Rot, Germany) was performed according to the manufacturer’s instructions. This resulted in three different bands in GG homozygotes (575 bp, 383 bp and 343 bp), in two bands in AA homozygotes (958 bp and 343 bp) and in all four bands in GA heterozygotes (958 bp, 575 bp, 383 bp and 343 bp). All digested samples were separated by electrophoresis on 4% agarose gels and visualized by ethidium bromide staining.

Statistics

Data on quantitative characteristics are expressed as median and range. Data on qualitative characteristics are expressed as percentage values or absolute numbers as indicated. The median plasma fibrinogen level was used to divide patients into two groups of similar size (fibrinogen < 3.5 g/l 107 patients; fibrinogen ≥ 3.5 g/l 113 patients). Patients were further divided into three genotype groups according to the β-fibrinogen –455 G/A genotype (GG, GA, AA). Comparisons between groups were made using the χ²-test (nominal data) or the Mann-Whitney-U- and the Kruskal-Wallis-H-test (interval data). The cumulative survival and the cumulative event-free survival were calculated by Kaplan-Meier analysis. The comparison of survival curves was done by the log rank-test. The analysis of independent predictors for the primary and secondary end-points was performed with multivariable Cox regression analysis with estimation of significance by Wald statistics. In addition, e^β [exp(β)] as an estimation of the relative risk was calculated, values being given with its 95%-confidence interval (CI). Partial correlation was used to evaluate the contribution of individual characteristics on the variability of end-points. All statistical assumptions were met and no multi-collinearity problems were found. A value of p <0.05 was considered statistically significant.
Results

The genotype frequencies of the β-fibrinogen –455 G/A gene polymorphism were GG 53.2% (n = 117), GA 41.8% (n = 92) and AA 5.0% (n = 11) patients. The total frequency of the A allele was 0.259. The genotype distribution was compatible with the Hardy-Weinberg equilibrium. The genotype groups did not differ with respect to baseline clinical characteristics (Table 1), operation details and most angiographical characteristics (Table 2). Patients with the β-fibrinogen –455 A allele had more often a stenosis > 50% of the right coronary artery than patients with the β-fibrinogen –455 GG genotype (Table 2). Furthermore, patients with the –455 A allele had elevated plasma fibrinogen concentrations compared to those with the –455 GG genotype (Fig. 1), but data did not obtain statistical significance.

Patients with plasma fibrinogen levels ≥ 3.5 g/l were older, more often female and had less often disturbances in left ventricular wall motion than patients with fibrinogen levels < 3.5 g/l (Tables 1 and 2). All other clinical and angiographical characteristics, and operation details were similarly distributed among both plasma fibrinogen level groups (Tables 1 and 2).

The 2-year total mortality was 9.1%. Of the 20 deceased patients seven died from myocardial infarction, two from heart failure and one from sudden cardiac death (cardiac causes). Other causes of death were: postoperative sepsis (n = 1), ischemic cerebral infarction (n = 1), intracerebral bleeding (n = 1), suicide (n = 2), pneumonia (n = 2) and malignant disease (n = 3). The mortality was 11.1% in patients with the β-fibrinogen –455 GG genotype, 6.5% in patients with the GA genotype and 9.1% in patients with the AA genotype (p = 0.52). The mortality of patients with preoperative plasma fibrinogen levels of < 3.5 g/l was 4.7% compared to the mortality of 13.3% in patients with plasma fibrinogen levels ≥ 3.5 g/l (p < 0.05) (Fig. 2). The statistical significance of this association vanished after excluding the 30-day postoperative mortality. Multi-variable Cox regression analyses identified the plasma fibrinogen levels (exp (β) 1.59, 95%-CI 1.08 – 2.33) but not the β-fibrinogen genotype as an independent risk factor of 2-year-mortality.

Table 1: Clinical characteristics of β-fibrinogen G–455A genotype and plasma fibrinogen level groups at baseline

<table>
<thead>
<tr>
<th></th>
<th>β-fibrinogen GG –455 genotype N= 117</th>
<th>β-fibrinogen GA –455 genotype N= 92</th>
<th>β-fibrinogen AA –455 genotype N= 11</th>
<th>p*</th>
<th>Plasma fibrinogen level &lt; 3.5 g/l N= 107</th>
<th>Plasma fibrinogen level ≥ 3.5 g/l N= 113</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>62.7 [40.9 – 86.2]</td>
<td>65.2 [41.8 – 81.9]</td>
<td>63.2 [39.8 – 75.5]</td>
<td>0.36</td>
<td>62.6 [40.9 – 81.9]</td>
<td>65.2 [39.8 – 86.2]</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Sex (male)</td>
<td>93 (79.5%)</td>
<td>75 (81.5%)</td>
<td>9 (81.8%)</td>
<td>0.93</td>
<td>94 (87.9%)</td>
<td>83 (73.5%)</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Triglycerides [mmol/l]</td>
<td>2.1 [0.3 – 7.6]</td>
<td>2.2 [0.7 – 9.9]</td>
<td>1.9 [1.6 – 3.2]</td>
<td>0.32</td>
<td>2.1 [0.3 – 9.9]</td>
<td>2.1 [0.8 – 8.0]</td>
<td>0.82</td>
</tr>
<tr>
<td>LDL-Cholesterol [mmol/l]</td>
<td>3.8 [1.4 – 6.4]</td>
<td>3.8 [1.4 – 12.5]</td>
<td>4.5 [2.4 – 5.5]</td>
<td>0.58</td>
<td>3.8 [1.4 – 7.0]</td>
<td>3.8 [1.4 – 12.5]</td>
<td>0.22</td>
</tr>
<tr>
<td>HDL-Cholesterol [mmol/l]</td>
<td>1.0 [0.4 – 2.1]</td>
<td>1.0 [0.5 – 1.9]</td>
<td>1.2 [0.6 – 1.7]</td>
<td>0.22</td>
<td>1.0 [0.4 – 2.1]</td>
<td>1.0 [0.5 – 2.0]</td>
<td>0.59</td>
</tr>
<tr>
<td>Systolic blood pressure [mmHg]</td>
<td>125 [90 - 180]</td>
<td>130 [95 - 170]</td>
<td>120 [100 - 160]</td>
<td>0.27</td>
<td>125 [100 - 180]</td>
<td>130 [90 - 180]</td>
<td>0.73</td>
</tr>
<tr>
<td>Diastolic blood pressure [mmHg]</td>
<td>80 [50 - 100]</td>
<td>80 [60 - 110]</td>
<td>80 [60 - 90]</td>
<td>0.97</td>
<td>80 [55 - 100]</td>
<td>80 [50 - 110]</td>
<td>0.31</td>
</tr>
<tr>
<td>Hypertension</td>
<td>70 (59.8%)</td>
<td>57 (62.0%)</td>
<td>6 (54.5%)</td>
<td>0.88</td>
<td>66 (61.7%)</td>
<td>67 (59.3%)</td>
<td>0.72</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>34 (29.1%)</td>
<td>29 (31.5%)</td>
<td>3 (27.3%)</td>
<td>0.91</td>
<td>31 (29.0%)</td>
<td>35 (31.0%)</td>
<td>0.75</td>
</tr>
<tr>
<td>Smoking (current)</td>
<td>24 (20.9%)</td>
<td>22 (24.2%)</td>
<td>4 (36.4%)</td>
<td>0.48</td>
<td>25 (23.8%)</td>
<td>25 (22.3%)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

* χ²-test (nominal data) or Kruskal-Wallis-H-test/ Mann-Whitney-U-test (interval data)
Other independent risk factors were the lack of use of the internal mammary artery graft (exp (β) 23.12, 95%-CI 5.59 – 95.7), a decreased left ventricular ejection fraction (exp (β) 0.95, 95%-CI 0.9 – 0.99), hypertension (exp (β) 3.74, 95%-CI 1.04 – 13.38) and increased diastolic blood pressure (exp (β) 1.10, 95%-CI 1.02 – 1.19). Together these factors explained 77.2% of the variability of total mortality. Plasma fibrinogen levels alone explained 13.0% of the variability of total mortality.

All patients who died from a non-cardiac cause (n = 10) were excluded from further analyses. The secondary end-point was observed in 12.4% of all patients. Ten patients died from a...
cardiac cause, 16 had a PTCA and one a recurrent CABG operation. The incidence was 10%, 13.5% and 27.3% in patients with the β-fibrinogen –455 GG, GA and AA genotype, respectively (p = 0.19). The cumulative cardiac event incidence was 8.6% in patients with plasma fibrinogen levels < 3.5 g/l versus 16.2% in patients with plasma fibrinogen levels ≥ 3.5 g/l (p = 0.11) (Fig. 3). Multivariate statistical analyses did not reveal independently acting predictors for the secondary end-point.

**Discussion**

Our study shows that elevated preoperative plasma fibrinogen levels are associated with an increased 2-year mortality after CABG operation. This relationship was mainly due to an increased 30-day postoperative mortality in patients with elevated preoperative plasma fibrinogen levels. Increased plasma fibrinogen concentrations measured prior to the operation could have been caused by an activated inflammatory system, which may make patients more susceptible to postoperative sepsis and early vascular complications (22). Several studies (23-25) show that the risk profile of complications and deaths following CABG changes after about 30 days from risk factors predominantly related to inflammation and sepsis to other risk factors more related to atherosclerosis. While increased plasma fibrinogen levels may have contributed to atherosclerosis in the present study our investigation period of two years may have been too short to detect atherogenic effects of fibrinogen and to find an association with the secondary end-point.

Patients with the -455 A allele had higher plasma fibrinogen levels compared to patients with the -455 GG genotype, but this difference was not statistically significant. This finding is in
agreement with some studies (15, 26), although other studies (7, 9, 12-14) have found statistically significant associations between the \( \beta \)-fibrinogen –455 genotype and plasma fibrinogen levels. Furthermore, there is evidence for a functional role of the \( \beta \)-fibrinogen –455 G/A gene polymorphism beyond an association with basal plasma fibrinogen levels (2, 26, 27). Thus, the extent of the rise of plasma fibrinogen levels after CABG (2), exercise (27) or surgical trauma (26) have been reported to be associated with the presence of the A allele. Together these data indicate that there may be a weak association between the \( \beta \)-fibrinogen –455 G/A gene polymorphism and plasma fibrinogen levels.

We did not find an association between the \( \beta \)-fibrinogen –455 G/A gene polymorphism and the outcome after CABG. While our data are consistent with several studies that failed to find an association between the \( \beta \)-fibrinogen –455 G/A gene polymorphism and the risk of CAD (19) or myocardial infarction (13, 14) others reported that the rare \( \beta \)-fibrinogen –455 A allele is associated with an increased risk of CAD (15) and ischaemic stroke (18) as well as with the severity (16) and progression of CAD (17). This discrepancy may in part be due to the widely acknowledged weaknesses of genetic association studies of vascular phenotypes (28), which are prone to both the widely acknowledged weaknesses of genetic association and ischaemic stroke (18) as well as with the severity (16) and progression of CAD (17). This discrepancy may in part be due to the widely acknowledged weaknesses of genetic association studies of vascular phenotypes (28), which are prone to both false positive (type 1 error) and false negative results (type 2 error). Thus, while our data do not support an association between the \( \beta \)-fibrinogen –455 G/A gene polymorphism and the outcome after CABG, the number of patients enrolled in our study was too small to ultimately exclude a role for the \( \beta \)-fibrinogen gene in the clinical course after CABG.

The comparison of baseline characteristics revealed that patients with elevated plasma fibrinogen levels had less often left ventricular wall motion disturbances (Table 1) and patients with the \( \beta \)-fibrinogen –455 A allele had more often a stenosis > 50% of the right coronary artery than patients with the \( \beta \)-fibrinogen –455 GG genotype (Table 2). There is no biologically plausible explanation for these associations. We assume these effects to be artifacts due to multiple testing. Possible confounding influences of these associations were excluded by multivariable statistical analyses.

The lack of use of the internal mammary artery graft was the main predictor of 2-year post CABG total mortality. This finding is in good agreement with results from other studies (25, 29, 30). Furthermore, in keeping with our results, a decreased left ventricular ejection fraction (23, 25, 31, 32) and hypertension (25, 33) have been shown to shorten the mid- and long-term survival after CABG surgery.

We conclude, that elevated preoperative plasma fibrinogen levels, but not the \( \beta \)-fibrinogen –455 G/A genotype predict the 2-year mortality after CABG operation.

References


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