



Elevated levels of plasma prekallikrein, high molecular weight kininogen and factor XI in coronary heart disease

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Abstract

Increased levels of hemostatic factors may play a role in the pathogenesis of myocardial infarction by triggering thrombin formation. We measured factor XII (FXII), factor XI (FXI), plasma prekallikrein (PK) and high-molecular-weight kininogen (HK) in 200 patients having survived myocardial infarction for at least 2 months, and in 100 healthy controls. We found significantly elevated levels of FXI clotting activity (FXI:C), HK:C and of the amidolytic activity of PK (PK:Am) among the patients as compared to the controls. Plasma levels of FXI:C, HK:C and PK:Am in the highest quartile were associated with an odds ratio of 1.9 (95% CI: 1.0–3.8), 2.0 (95% CI: 1.0–4.0) and 5.4 (95% CI: 2.6–11.2), respectively, compared to the respective plasma levels in the lowest quartile. After correction for established clinical and laboratory risk factors, the association between PK:Am plasma levels and myocardial infarction remained significant ($P = 0.0007$). Combination of high PK:Am plasma levels and smoking or arterial hypertension, respectively, resulted in a more than additive relative risk for myocardial infarction. © 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Myocardial infarction; Contact phase activation; Prekallikrein; High molecular weight kininogen; Factor XI

1. Introduction

The contact activation system of the intrinsic pathway of coagulation consists of four plasma proteins: factor XII (FXII), factor XI (FXI), prekallikrein (PK) and high-molecular-weight kininogen (HK). FXII and PK reciprocally activate each other upon contact with negatively charged surfaces [1,2]. Activated FXII (FXIIa) in turn activates FXI [3] resulting in FXIa, which then activates factor IX. HK serves as a nonenzymatic cofactor in these reactions [4]. FXI and PK circulate in plasma noncovalently complexed with HK [5–7].

Coronary artery thrombosis superimposed on a disrupted atherosclerotic plaque is considered to be the

pivotal pathophysiologic event in acute coronary syndromes, such as unstable angina, myocardial infarction, and sudden death. The clinical manifestations depend on the extent and duration of thrombus deposition, which are determined by local and systemic risk factors. The thrombotic response to plaque disruption involves both platelet activation and thrombin generation [8,9]. Thrombin formation may occur via activation of the extrinsic or the intrinsic pathway of coagulation. Elevated plasma levels of fibrinogen are associated with an increased risk for ischemic heart disease, whereas the data for elevated plasma levels of factor VII are controversial [10–12]. The second Northwick Park prospective cardiovascular survey has revealed evidence of activation of factor VII and factor IX in men at high risk of fatal coronary heart disease [13]. Activation of the contact system has been found to contribute to activation of factor VII [14] in vitro and factor IX may be enhanced by contact system activation as well. There

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is indeed evidence for the activation of the contact system in patients with increased risk for coronary heart disease [15–17]. Several studies reported decreased levels of FXII, PK, HK and FXI in acute and subacute coronary artery disease [18–21]. Low FXII levels, however, may be explained by a recent report showing FXII to be a negative acute-phase protein [22] and do not necessarily support activation of FXII. In survivors of myocardial infarction, increased plasma levels of FXII and HK were observed [23], whereas other authors reported a high prevalence (10.3%) of decreased FXII plasma levels in patients with coronary heart disease awaiting cardiac surgery [24]. Therefore, the role of the contact activation system in the pathogenesis of ischemic heart disease needs further clarification, particularly regarding an estimation of the risk for coronary artery disease associated with plasma levels of the contact activation proteins.

In the present study, we provide evidence that plasma levels of FXI, PK and HK are elevated in a group of 200 survivors of myocardial infarction compared to the respective plasma levels in a group of 100 healthy controls. Furthermore, we demonstrate a high PK level to be an independent risk factor for myocardial infarction.

2. Material and methods

2.1. Patients and controls

Two hundred Caucasian patients (174 males, 26 females) were consecutively selected from the data files of the Division of Cardiology of the University Hospital of Bern. The inclusion criteria were: (i) history of acute myocardial infarction having occurred at least 2 months before investigation, (ii) stable coronary artery disease and (iii) no concomitant diseases with a life expectancy of less than 6 months. The time period between myocardial infarction and study inclusion was 9 months (median, range 2–228). All except two patients had undergone coronary angiography. Three-, two- or one-vessel disease was present in 53, 72 and 67 patients, respectively, whereas six patients had normal coronary angiography. Seventeen percent (34/200) of the patients had suffered more than one myocardial infarction and 59% had a family history of coronary artery disease. Sixty-seven patients were treated with oral anticoagulation and 126 patients with platelet aggregation inhibitors. Angiotensin-converting-enzyme inhibitors, β -blockers, nitrates, diuretics or calcium antagonists were taken by 68, 98, 58, 33, 47 patients, respectively. None of the patients had a debilitating systemic or malignant disease or overt hepatopathy. Female patients did not take either oral contraceptives or postmenopausal estrogen substitution, and were not

pregnant. Coronary artery disease was in a stable phase in all patients at the time of investigation. Healthy Caucasian control subject, of the same age (± 5 years) and sex and without any history of thromboembolic event or bleeding tendency were selected from the hospital staff or their relatives. Thus, the control group consisted of 87 men and 13 women not taking oral contraceptives or postmenopausal estrogen replacement. Height and weight were measured in the 200 patients, and in 88 controls, and the body mass index (BMI) was calculated (weight (in kg)/height² (in m²)). Patients and controls were asked for a history of hypertension and diabetes mellitus. Additionally, we assessed medication for these or other conditions. We classified as smoker any individual who reported smoking > 5 pack-years, and all others as nonsmokers. Informed consent was obtained from all subjects investigated, and the study was approved by the Ethics Committee of the University of Bern.

2.2. Blood sampling

Blood was collected in the fasting state in the morning from an antecubital vein with a 19 gauge butterfly needle and after obtaining 5 ml of EDTA–blood and 5 ml of native blood, 2 \times 9 ml were collected into two plastic syringes (Monovette[®], Sarstedt, Nümbrecht, Germany), each containing 1 ml 0.106 M trisodium citrate. Plasma was prepared by centrifuging twice at 1500 $\times g$ for 10 min each at 15–18 °C and was then stored in polypropylene tubes at –70 °C.

2.3. Assays

Dilutions of a normal human plasma pool (NHP) of 42 healthy men were used to obtain calibration curves. All plasma samples from patients and controls were assayed at two different dilutions as previously described [25]. Clotting activity of FXII (FXII:C), FXI (FXI:C), and HK (HK:C) were each measured by an activated partial thromboplastin time (aPTT)-based coagulation assay using Neothromtin[®] (ellagic acid–phospholipid mixture, Behring, Germany). We used immunodepleted FXII- and FXI-deficient plasmas (Behring) and congenitally kininogen-deficient plasma (George King Biomedical Inc., Overland Park, KS), respectively, as substrate plasmas for the determination of the respective coagulation factors. Plasma levels of prekallikrein were assessed by measuring the amidolytic activity (PK:Am) in a chromogenic substrate assay as described [26]. The activator of this assay is composed of ellagic acid, cephalin and a plasma fraction containing FXII and HK ensuring full PK activation even in the complete absence of FXII and/or HK in the test plasma. FXII antigen levels (FXII:Ag) were measured by a dot immunobinding assay as reported [27]. Fibrin-

Table 1
Cardiovascular risk factors in patients and controls

Parameter	Patients (<i>n</i> = 200; 174 men, 26 women)	Controls (<i>n</i> = 100; 87 men, 13 women)	<i>P</i> value ^a
Age (years)	57 (32–72) ^b	56 (32–74)	0.35
Hypertension (%)	48	11	<0.001
Diabetes mellitus (%)	21	0	–
Smoker (%)	74	36	<0.001
Body mass index (kg/m ²)	26.1 (20.6–43.3)	24.7 (18.6–39.1) ^c	0.002
Total cholesterol (mmol/l)	5.97 (3.09–9.75)	5.76 (3.72–8.65)	0.01
Fibrinogen (g/l)	2.8 (1.8–4.8)	2.6 (1.7–4.5)	<0.0001
White blood cells ($\times 10^9/l$)	7.2 (3.9–15.9)	6.5 (3.8–13.5)	0.003
Serum albumin (g/l)	39.5 (33.0–46.1)	39.7 (32.6–44.8)	0.5

^a Mann–Whitney *U*-test or chi-square test where appropriate.

^b Values are presented as median (range) or percentage.

^c Body mass index available in 88 controls.

nogen was determined according to Claus [28]. White blood cell count, serum albumin and total cholesterol were measured by conventional methods.

2.4. Statistics

Median and range or proportions for baseline risk factors were calculated for patients and controls. Comparison of continuous variables between patients and controls was performed by Mann–Whitney *U*-test; categorical data were analyzed by chi-square test. Correlation of the association between observations was done using Spearman's rank correlation and expressed as the correlation coefficient and tested for significance using Spearman's test for correlation. *P*-values were two-sided and were considered significant if ≤ 0.05 . Confidence intervals (CI) were calculated at the 95% level. Correction for multiple comparisons was done using the Bonferroni method [29]. Statistical analysis was done using SIGMASTAT (Jandell, Erkrath, Germany). We calculated crude odds ratios by standard methodology as estimates of the relative risk for myocardial infarction for plasma levels of the respective contact activation factors. To find a dose–response relation we stratified the plasma levels of coagulation factors into quartiles and calculated the odds ratios for the three higher quartiles as compared to the lowest one. The quartiles were defined based on the control group. In addition, we calculated odds ratios among smokers and hypertensive individuals separately in order to study possible differences in risk among these subgroups. To simultaneously adjust for the effects of other coronary risk factors, we used logistic regression (SAS System, SAS Institute Inc., Cary, NC) to estimate the relative risk. Adjustment was made for the dichotomized risk factors sex, hypertension (yes/no), diabetes mellitus (yes/no), cigarette smoking including former smokers (yes/no), and for the continuous risk factors age, BMI, cholesterol, white blood cell count and fibrinogen.

3. Results

3.1. Patients and controls

Table 1 shows the baseline characteristics of the patients and controls. As expected, the percentages of individuals with hypertension, diabetes mellitus and smoking habit were significantly higher among the patients, as were BMI and the levels of total cholesterol, fibrinogen and white blood cells.

3.2. Contact activation factors

The plasma levels of the four contact activation factors FXII, FXI, PK, and HK are given in Table 2. We found no difference in the plasma levels of FXII between patients and controls, neither when FXII was measured as clotting activity (FXII:C) nor in the antigen assay (FXII:Ag). However, significantly higher plasma levels among the patients compared to the controls were observed for FXI:C, PK:Am, and HK:C. Moreover, PK:Am levels were significantly higher ($P = 0.05$) among the patients with diabetes mellitus (median 118%, range 82–172%, $n = 42$) compared to the patients without diabetes mellitus (median 112%, range 76–168%, $n = 158$). Apart from this finding, no differences in the plasma levels of the four contact activation

Table 2
Contact activation factors in patients and controls

Parameter	Patients (<i>n</i> = 200)	Controls (<i>n</i> = 100)	<i>P</i> value ^a
FXII:C	97 (41–167) ^b	100 (49–144)	0.12
FXII:Ag	98 (45–201)	97 (50–156)	0.5
FXI:C	111 (56–190)	107 (71–170)	<0.05
PK:Am	114 (76–172)	104 (73–147)	<0.0001
HK:C	107 (74–162)	102 (65–152)	<0.05

^a Mann–Whitney *U*-test.

^b Values in percentage of a normal human plasma pool (NHP) are given as median (range).

Table 3
Spearman correlation coefficients (r_s) and P -values (P) for contact activation factors among patients and controls

		FXII:Ag		FXI:C		PK:Am		HK:C	
		Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
FXII:C	r_s	0.88	0.79	0.12	0.25	0.42	0.37	0.16	0.05
	P	<0.001	<0.001	NS	NS	<0.001	<0.001	NS	NS
FXII:Ag	r_s			0.06	0.00	0.37	0.24	0.09	0.02
	P			NS	NS	<0.001	NS	NS	NS
FXI:C	r_s					0.43	0.35	0.44	0.17
	P					<0.001	<0.001	<0.001	NS
PK:Am	r_s							0.52	0.54
	P							<0.001	<0.001

NS: not significant after correction for multiple comparison.

proteins were observed comparing patients with and without diabetes mellitus, hypertension, or smoking habit, respectively.

A normal range of the contact activation factors was defined as the nonparametric 95%-interval in the controls, i.e. 56–137% for FXII:C, 53.5–143.5% for FXII:Ag, 73–146.5% for FXI:C, 73–144% for PK:Am, and 71.5–144% for HK:C. Accordingly, 2.5% of the controls were below the lower limit of this normal range for each parameter. We found among the patients 3.5% (95% CI: 2.2–4.8%), 2% (95% CI: 1–3%) and 6% (95% CI: 4.3–7.7%) with levels of FXII:C, FXII:Ag, and FXI:C, respectively, below this normal range. No patients were found with a PK:Am level or a HK:C level below the lower limit of the normal range.

3.3. Correlation coefficients

Table 3 shows Spearman rank correlation coefficients for the contact activation factors among patients and controls. As expected, FXII:C and FXII:Ag levels showed a strong and significant correlation, both in patients and in controls. PK:Am levels showed an intermediate, however significant, correlation with levels of FXII:C, HK:C and FXI:C, respectively, among patients and controls. FXI:C was significantly correlated with HK:C among patients but not among control subjects.

3.4. Relative risk analysis

Next, we calculated the relative risks associated with the three upper quartiles of the plasma levels of the clotting activities of FXII, FXI and HK and of the amidolytic activity of prekallikrein as compared to the lowest quartile for each parameter (Fig. 1). No association was found between FXII (neither FXII:C nor FXII:Ag) and coronary artery disease, whereas the odds ratios were 1.9 (95% CI: 1.0–3.8) for the fourth quartile of FXI:C and 2.0 (95% CI: 1.0–4.0) for the

fourth quartile of HK:C levels (Table 4). The relative risk associated with PK:Am levels increased steadily from the second to the fourth quartile (Fig. 1) showing an odds ratio of 5.4 (95% CI: 2.6–11.2) for the fourth quartile. The four quartiles for PK:Am levels were ≤ 91 , 92–104, 105–114, and $> 114\%$, respectively. After adjustment for the risk factors age, sex, BMI, smoking, diabetes mellitus, hypertension, cholesterol, fibrinogen, white blood cell count, and, since they were correlated with each other, for FXI:C, HK:C, and FXII:C, PK:Am remained the only factor significantly associated with myocardial infarction (Table 4). The odds ratios calculated in this logistic regression model for an increase in PK:Am of 25 and 50% were 3.1 (95% CI: 1.6–6.0) and 9.5 (95% CI: 2.6–35.3), respectively.

Smoking and arterial hypertension were strong risk factors for myocardial infarction in our study population: smoking was associated with a fivefold increased risk (odds ratio 5.1, 95% CI: 3.0–8.5) and hypertension with a sevenfold increased risk (odds ratio 7.5, 95% CI: 3.8–14.8). We therefore explored whether the risk associated with PK:Am was different for smokers and hypertensive patients compared to nonsmokers and normotensive patients. The calculated odds ratio for PK:Am in the fourth quartile was equal among smokers and nonsmokers (4.9, 95% CI: 1.7–13.7 and 4.9, 95% CI: 1.6–14.9, respectively), and among normotensive individuals (4.6, 95% CI: 1.9–11.0), was higher in the hypertensive group (11.3, 95% CI: 1.8–72.0). Next, we also studied the interaction of PK:Am plasma levels with these two strong clinical risk factors. In this analysis, we calculated odds ratios for myocardial infarction for PK:Am plasma levels in the fourth quartile, together with smoking and/or hypertension in relation to PK:Am in the lowest quartile together with nonsmoking and normal blood pressure (Table 5). The results indicate that high PK:Am levels lead to a substantially higher risk in combination with smoking and/or hypertension. There were 29 patients but no control individual with both clinical risk factors present together with

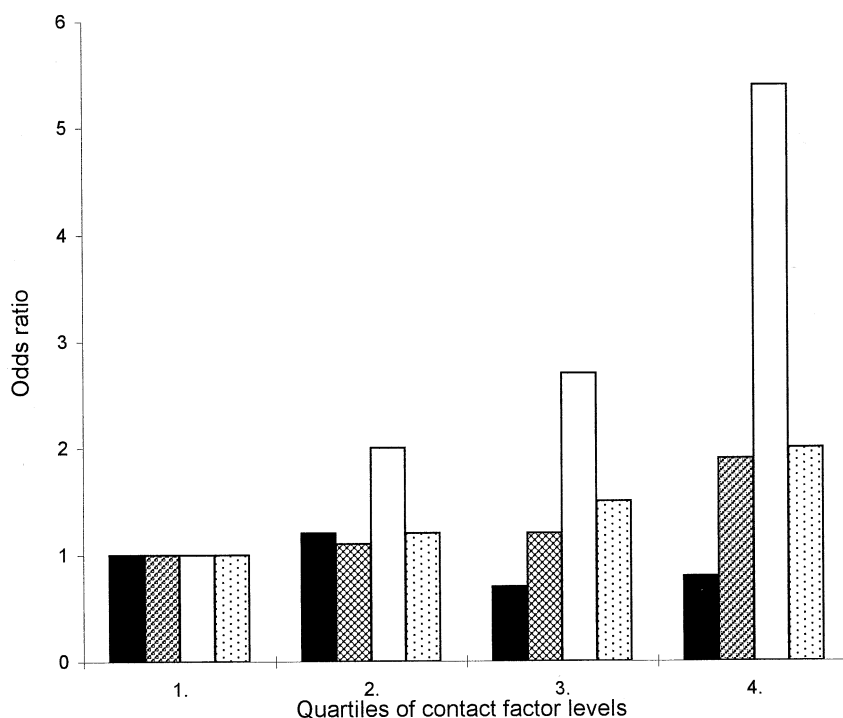


Fig. 1. Relative risk (odds ratio) of myocardial infarction associated with plasma concentrations of contact system factors: factor XII (FXII:C) ■, factor XI (FXI:C) ▨, prekallikrein (PK:Am) □, and high molecular weight kininogen (HK:C) ▤. Odds ratios were calculated for the three higher quartiles (2–4) as compared to the lowest quartile (1) for each parameter.

a PK:Am level in the fourth quartile. The relative risk of this combination could therefore not be quantitated.

4. Discussion

The present data show plasma concentrations of the contact system proteins FXI, HK and PK to be higher in patients with a history of myocardial infarction as compared to controls. The main finding was a strong and independent association between plasma prekallikrein activity and myocardial infarction: high plasma levels of PK:Am were found to be associated with a 5.4-fold (95% CI: 2.6–11.2) risk of myocardial infarction, which remained significant after adjustment for other risk factors such as age, sex, BMI, smoking, diabetes mellitus, hypertension, cholesterol and fibrinogen.

To our knowledge, there have been no previous larger epidemiological studies investigating plasma levels of FXI, PK and HK in survivors of myocardial infarction. Our finding of higher PK:Am levels among diabetic patients as compared to patients without diabetes mellitus corresponds to previous reports [30,31], and this has tentatively been attributed to altered glycosylation of this glycoprotein in diabetic subjects [32].

Conflicting data have been reported concerning FXII activity in coronary heart disease patients. Whereas FXII levels were found to be lowered during the acute

phase of coronary heart disease [18–21], they were reported to be elevated [23] or normal and comparable to those in controls [33] in patients having survived a myocardial infarction for at least 2 months. Several case reports described the occurrence of myocardial infarction in subjects with FXII deficiency [34–38] or prekallikrein deficiency [39]. The reported high prevalence (10.3%) of decreased FXII:C in 426 coronary heart disease patients awaiting cardiac surgery [24] may even suggest FXII deficiency to be a common risk factor for coronary heart disease, possibly by leading to insufficient intrinsic fibrinolytic activity. Our data do not support this hypothesis: the percentage of patients

Table 4

Relative risk of myocardial infarction associated with the highest quartile of the clotting activity of FXI (FXI:C) and high molecular weight kininogen (HK:C) and of the amidolytic activity of prekallikrein (PK:Am) as compared with the lowest quartile for the respective parameter

Parameter	Crude odds ratio	95% CI	Adjusted ^a odds ratio	95% CI
FXI:C	1.9	1.0–3.8	0.8	0.2–2.7
PK:Am	5.4	2.6–11.2	5.5	1.3–22.5
HK:C	2.0	1.0–4.0	0.3	0.1–1.2

^a Logistic regression was used to adjust for possible confounders (age, sex, BMI, smoking, diabetes mellitus, hypertension, cholesterol, fibrinogen, white blood cell count, and—since they were correlated with each other—FXII:C, FXI:C, HK:C, and PK:Am.

Table 5
Relative risk of myocardial infarction associated with prekallikrein amidolytic activity (PK:Am) in the highest quartile, smoking and hypertension as compared with PK:Am in the lowest quartile, nonsmoking and normal blood pressure

PK:Am ^a	Smoking	Hypertension	Patients	Controls	OR	95% CI
Low	No	No	3	14	1 ^b	
Low	Yes	No	8	8	4.7	0.96–22.8
Low	No	Yes	4	2	9.3	1.1–76.9
Low	Yes	Yes	4	2	9.3	1.1–76.9
High	No	No	13	11	5.5	1.3–24.3
High	Yes	No	40	12	15.6	3.8–63.3
High	No	Yes	16	2	37.3	5.4–257
High	Yes	Yes	29	0	–	

^a Low and high denote PK:Am in the lowest and highest quartile, respectively.

^b All odds ratios are relative to the reference category, i.e. those individuals with PK:Am in the lowest quartile who were normotensive and nonsmokers.

with decreased FXII:C (3.5, 95% CI: 2.3–4.8) was not higher compared to that in controls (2.5% by definition), thus excluding FXII deficiency as a risk factor for myocardial infarction.

Next, we studied the contribution of the contact factors to the relative risk for myocardial infarction. In crude analysis, high levels of FXI:C, PK:Am and HK:C were each associated with a two- to fivefold increased risk of myocardial infarction. Multivariate analysis controlling for potential confounding variables found that the association for FXI:C and HK:C lost statistical significance largely due to the influence of PK:Am, diabetes mellitus and history of hypertension, whereas a significantly increased risk remained for high PK:Am. This finding indicates that high levels of PK:Am may be a risk factor for myocardial infarction. The 'dose-response' relationship supports the existence of a true association. High PK:Am levels lead to a substantially higher risk in combination with smoking or hypertension, suggesting an interaction between high PK:Am levels and smoking or hypertension, respectively.

Our study does not allow us to draw any conclusions regarding the pathophysiological significance of PK:Am in coronary artery disease. The mechanism relating high prekallikrein activity to myocardial infarction is unclear. One hypothesis is that high PK plasma levels may favor contact activation resulting in increased generation of activated FXII and FXI, leading to enhanced activation of the intrinsic pathway of coagulation (FIX) and subsequent thrombin formation. This hypothesis would be compatible with data from the second Northwick Park study showing activation of factor IX in men at high risk of fatal coronary heart disease [13], and, furthermore, with several reports showing evidence for the activation of FXI and FXII in patients with increased risk for coronary heart disease [15–17]. However, it is still a matter of debate whether the contact system indeed contributes to activation of the coagulation pathway in vivo or rather to the activation of the fibrinolytic system [40].

In conclusion, we suggest that measurement of PK:Am plasma levels merits further evaluation and it remains to be confirmed that high PK:Am is a predictor of coronary artery disease in addition to conventional risk factors.

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