von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death

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Abstract

***Objective*—To evaluate as predictors of reinfarction and mortality tissue plasminogen activator antigen and activity before and after venous occlusion, plasminogen activator inhibitor, von Willebrand factor, and established risk factors.**

***Design*—Prospective study with a mean observation time of 4·9 years.**

***Setting*—Secondary referral centre, the Department of Internal Medicine, University Hospital of Umeå.**

***Patients*—123 consecutive survivors of myocardial infarction under the age of 70 years.**

***Main outcome measures*—Reinfarction and deaths from all causes.**

***Results*—23 patients died and 36 patients had at least one reinfarction. High concentrations of von Willebrand factor were independently associated with both reinfarction and mortality. A history of angina at entry into the study was also independently associated with reinfarction and mortality. Hypertension was independently associated with mortality but not with reinfarction. None of the fibrinolytic or lipid variables was associated with reinfarction or death.**

***Conclusion*—A high concentration of von Willebrand factor was a novel index of increased risk for reinfarction and mortality in survivors of myocardial infarction.**

Fibrinolysis was compromised in survivors of myocardial infarction by an increase in basal plasminogen activator inhibitor activity and reduced tissue plasminogen activator activity after the venous occlusion test.[1 2] Similar findings were reported in patients with angina.[3–5] In one report increased plasma plasminogen activator inhibitor activity was found to be an independent risk factor for reinfarction and cardiac death among young survivors of myocardial infarction,[6] and in another study tissue plasminogen activator antigen was significantly associated with cardiovascular events in patients with severe angina pectoris.[7] Low fibrinolytic activity has also been implicated as risk factor for restenosis after aortocoronary bypass surgery[8] and percutaneous transluminal coronary angioplasty.[9] The key fibrinolytic components—tissue plasminogen activator and plasminogen activator inhibitor—can be regarded as novel cardiovascular risk factors.

Plasma concentrations of von Willebrand factor are increased in disorders that affect the vascular system, such as systemic lupus erythematosus,[10] lupus anticoagulans,[11] thrombotic thrombocytopenic purpura,[12] pregnancy induced hypertension,[13] diabetes mellitus,[14] cerebrovascular disease,[15 16] and deep vein thrombosis.[15] Thus increased concentrations of von Willebrand factor may be important in the pathogenesis of cardiovascular disease.

In the present study, a long term follow up of our earlier cross sectional study[2] of survivors of myocardial infarction, we have compared tissue plasminogen activator, plasminogen activator inhibitor, and von Willebrand factor with previously established risk factors as predictors of reinfarction and mortality from all causes.

Patients and methods

PATIENTS

This study is based on 131 consecutive survivors of myocardial infarction aged less than 70 years who were followed up at the outpatient clinic of the Department of Internal Medicine, University Hospital of Umeå between 20 November 1982 and 28 November 1983.[2] Blood samples were collected three months after discharge from hospital. One patient died before sampling. We also excluded four patients with uncertain diagnosis, one patient in whom blood sampling failed, and two patients in whom clinical data were unaccessible. The remaining 123 patients, 95 men and 28 women, were included in the present study.

At infarction the mean (SD) age of the patients was 59·0 (8·0). Thirty six were smokers and 15 had diabetes mellitus. Table 1 shows the clinical and metabolic data.

FOLLOW UP STUDY PROTOCOL

In November 1988 the patients' records were searched for deaths and myocardial infarctions.

Table 1 Clinical characteristics of 123 survivors of myocardial infarction

Variable	*Number or mean (SD)*
Age (years) (mean (SD))	59·0 (8·0)
Hypertension (n)	37
Diabetes mellitus (n)	15
Smokers (n)	36
Previous angina pectoris (n)	66
Triglyceride (mmol/l) (mean (SD))	2·12 (1·01)
Cholesterol (mmol/l) (mean (SD))	7·00 (1·39)

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Death certificates were obtained for all patients who had died. No patient was lost at follow-up. Myocardial infarction was diagnosed according to the World Health Organisation criteria.[17] The cause of death was taken from the death certificates. The term "any event" used in the statistical analyses indicates reinfarction or death.

BLOOD SAMPLING AND LABORATORY PROCEDURE

Three months after discharge from hospital blood samples were collected at rest and after 10 minutes venous occlusion (100 mm Hg for 10 minutes) and assayed for tissue plasminogen activator activity, tissue plasminogen activator antigen, and von Willebrand factor. Plasminogen activator inhibitor activity was measured only at rest. Assays of tissue plasminogen activator activity and plasminogen activator inhibitor activity were performed by a chromogenic substrate assay. Tissue plasminogen activator antigen and von Willebrand factor were measured by an ELISA. For further details see Nilsson and Johnson.[2]

STATISTICAL ANALYSIS

We used the Statistical Analysis System for statistics and analyses.[18] Standard summary statistics were used to summarise and illustrate the features of the data of interest.

To illustrate the relation between a possible prognostic factor and the incidence of reinfarction or mortality we divided baseline variables into quartiles, and calculated the incidence of events in each quartile per 100 patient years. These quartiles were *not* used to test relations. To test for a relation between a baseline variable and the incidence of reinfarction or mortality we used a Cox regression analysis.[19] Variables having a statistically significant relation with the incidence of events in a univariate analysis were further studied in a multivariate analysis. For each model we calculated a statistic (R) that measures the predictive ability of the model. R^2 = (model χ^2 − 2p)/− 2L(0)) where p is the number of variables in the model and L(0) is the log likelihood with no variables in the model—that is all regression coefficients set to 0. Individual R statistics, partial Rs, were also calculated for each variable in a model. These are defined as R = (MLE χ^2 − 2)/(− 2L(0))$^{\frac{1}{2}}$ and the sign of the corresponding regression coefficient is attached to R. R values are between − 1 and + 1 and provide a measure of the contribution of variables independent of the sample size. We used the Mann-Whitney test to compare groups with and without events. Two tailed tests were performed and p values below 0·05 were regarded as statistically significant.

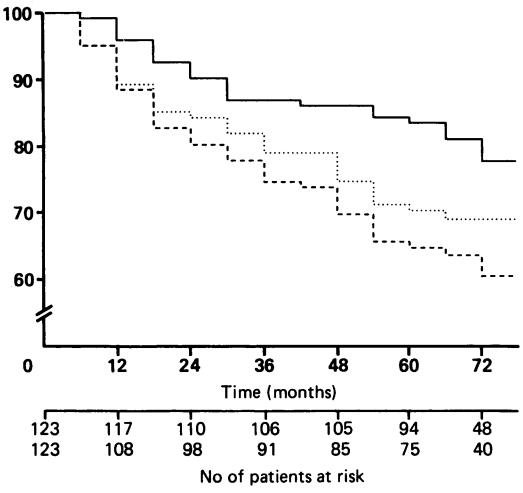

Figure 1 Life table showing the proportion of patients alive (solid line), remaining free of reinfarction (dotted line), or remaining free from any event (broken line). The numbers given underneath the graph show the number of patients at risk (uper row alive; lower row, free of reinfarction).

Results

The mean follow up time was 4·9 years. In the course of follow up 23 patients died (including four patients with fatal myocardial infarction) and 36 patients had at least one myocardial infarction (four fatal and 32 non-fatal). Figure 1 shows the life tables for the proportion remaining free of reinfarction, death, and any event. A 34 year old drug addict died from an overdose of methadone and one patient died from pulmonary thromboembolism. All the other 21 deaths were due to coronary heart disease.

The concentration of von Willebrand factor was significantly higher in the group with reinfarction and in the group of patients that died during follow up than in the groups without these events (table 2). There were no significant differences in the fibrinolytic vari-

Table 2 von Willebrand factor, fibrinolytic variables, and lipid variables according to the different outcome variables.

	Alive			*Dead*			*No reinfarction*			*Reinfarction*			*No event*			*Any event*		
Variable	*n*	*Mean*	*CI*	*n*	*Mean*	*CI*	*n*	*Mean*	*CI*	*n*	*Mean*	*CI*	*n*	*Mean*	*CI*	*n*	*Mean*	*CI*
vWf (% of normal)	100	143	131 to 155	23	177	157 to 196*	87	138	128 to 149	36	176	152 to 200*	78	134	123 to 144	45	176	156 to 196†
vWF occlusion (% of normal)	98	188	175 to 202	23	228	204 to 253*	86	185	172 to 199	35	222	196 to 247*	77	182	168 to 195	44	221	199 to 243*
tPA activity (U/ml)	100	0·10	0·08 to 0·12	23	0·13	0·09 to 0·17	87	0·10	0·08 to 0·12	36	0·11	0·09 to 0·13	78	0·10	0·08 to 0·12	45	0·12	0·08 to 0·16
tPA activity occlusion (U/ml)	98	2·0	1·4 to 2·6	23	2·0	1·2 to 2·8	86	2·0	1·4 to 2·6	35	2·0	1·2 to 2·8	77	2·0	1·4 to 2·6	44	2·1	1·4 to 2·8
tPA antigen (μg/l)	100	11·5	10·6 to 12·4	23	13·2	10·3 to 16·1	87	11·9	10·8 to 13·0	36	11·6	10·0 to 13·2	78	11·7	10·7 to 12·7	45	12·1	10·4 to 13·8
tPA antigen occlusion (μg/l)	98	36·4	31·5 to 41·3	23	40·6	27·7 to 53·5	86	37·6	32·3 to 42·9	35	36·3	26·7 to 45·9	77	36·8	31·4 to 42·2	44	38·0	29·3 to 46·7
PAI (U/ml)	100	10·0	8·7 to 11·3	23	9·7	6·8 to 12·6	87	10·5	9·1 to 11·9	36	8·8	6·8 to 10·8	78	10·4	9·0 to 11·8	45	9·1	7·1 to 11·1
Triglycerides (mmol/l)	87	2·0	1·8 to 2·2	18	2·5	1·8 to 3·2	77	2·1	1·9 to 2·3	28	2·1	1·8 to 2·5	70	2·1	1·9 to 2·3	35	2·2	1·8 to 2·6
Cholesterol (mmol/l)	89	7·0	6·7 to 7·3	18	7·3	6·7 to 7·9	78	7·0	6·7 to 7·3	29	6·9	6·4 to 7·4	71	7·0	6·7 to 7·3	36	6·9	6·5 to 7·3

* $p < 0·01$, † $p < 0·001$.
CI, 95% confidence interval; vWF, von Willebrand factor; tPA, tissue plasminogen activator; PAI, plasminogen activator inhibitor.

Table 3 Relation between risk factors and cardiovascular events (univariate Cox regression analysis)

	Mortality		*Reinfarction*		*Any event*	
Variable	*R*	*p*	*R*	*p*	*R*	*p*
Age		> 0·40		> 0·40		> 0·40
Sex		0·23		0·20		0·091
Smoking		> 0·40		> 0·40		> 0·40
Hypertension	0·13	0·018		> 0·40		0·18
Diabetes		> 0·40		> 0·40		> 0·40
Angina	0·17	0·005	0·15	0·0048	0·15	< 0·001
Triglyceride		0·090		> 0·40		> 0·40
Cholesterol		0·37		> 0·40		> 0·40
ASAT		> 0·40		> 0·40		> 0·40
vWF	0·13	0·016	0·19	0·0014	0·20	< 0·001
vWF occlusion	0·14	0·011	0·14	0·0038	0·14	0·0017
tPA activity		0·27		> 0·40		> 0·40
tPA activity occlusion		> 0·40		> 0·40		> 0·40
tPA-s		> 0·40		> 0·40		> 0·40
tPA-s occlusion		> 0·40		> 0·40		> 0·40
tPA antigen		0·10		> 0·40		> 0·40
tPA antigen occlusion		> 0·40		> 0·40		> 0·40
PAI		> 0·40		> 0·40		0·39

R, partial R statistic, see statistical methods; ASAT, aspartate aminotransferase; vWF, von Willebrand factor; tPA, tissue plasminogen activator; tPA-s, measurements in acidified blood; tPA activity, measurements in acidified plasma; PAI, plasminogen activator inhibitor.

ables between the group with events and the group without.

PROGNOSTIC FACTORS FOR REINFARCTION

Univariate Cox regression analyses showed that three of the variables studied were associated with reinfarction (table 3). Reinfarctions were more common in patients with previous angina and were positively associated with the concentration of von Willebrand factor both at rest and after venous occlusion (fig 2). The increase in the number of reinfarctions with increasing concentrations of von Willebrand, from the second to the fourth quartile accords with the close correlation between these two variables.

PROGNOSTIC FACTORS FOR MORTALITY

In univariate Cox regression analyses we found four of the studied variables to be associated with mortality (table 3). Of the primary risk factors for coronary heart disease, only hypertension was significantly associated with mortality; whereas age, sex, smoking, diabetes mellitus, serum cholesterol and triglyceride did not show a significant association. Those patients who had had angina at entry to the study were more likely to die. Figure 3 shows the number of deaths through quartiles one to four of the von Willebrand factor at rest and after venous occlusion. For both variables the number of deaths increased with increasing concentrations.

PROGNOSTIC FACTORS FOR ANY EVENT

In univariate Cox regression analyses we found three of the studied variables were associated with the occurrence of "any event" (table 3). The incidence of "any event" was more common in patients with previous angina, and was positively associated with von Willebrand factor at rest and after venous occlusion. Figure 4

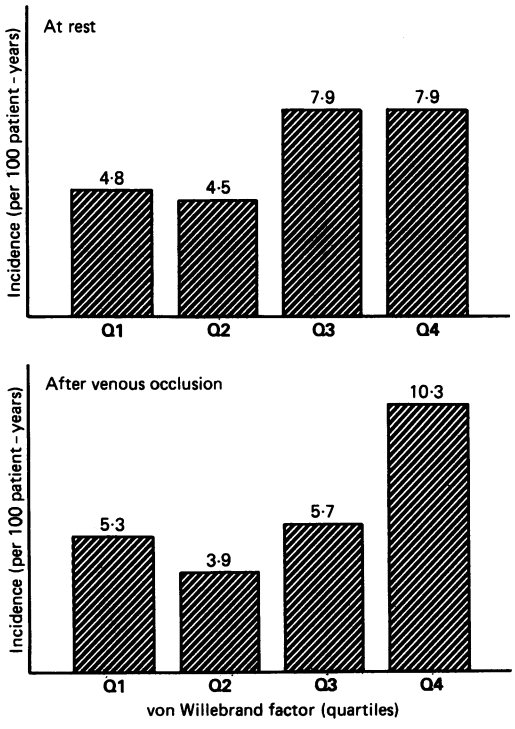

Figure 2 Incidence of reinfarction in relation to von Willebrand factor at rest and after venous occlusion.

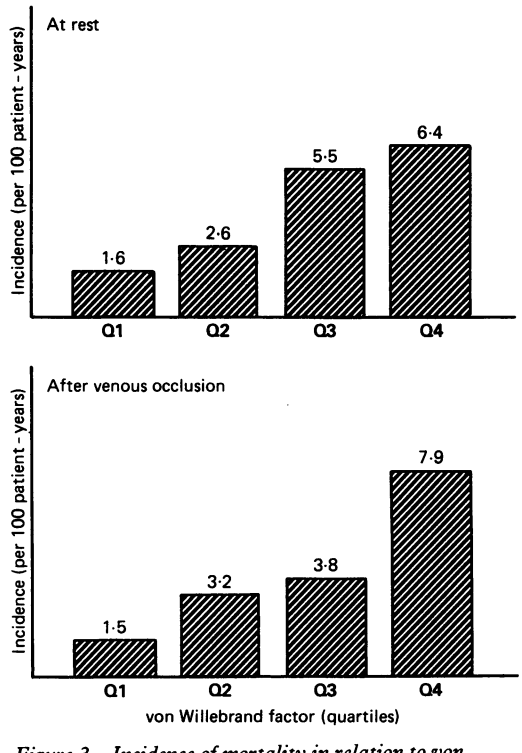

Figure 3 Incidence of mortality in relation to von Willebrand factor at rest and after venous occlusion.

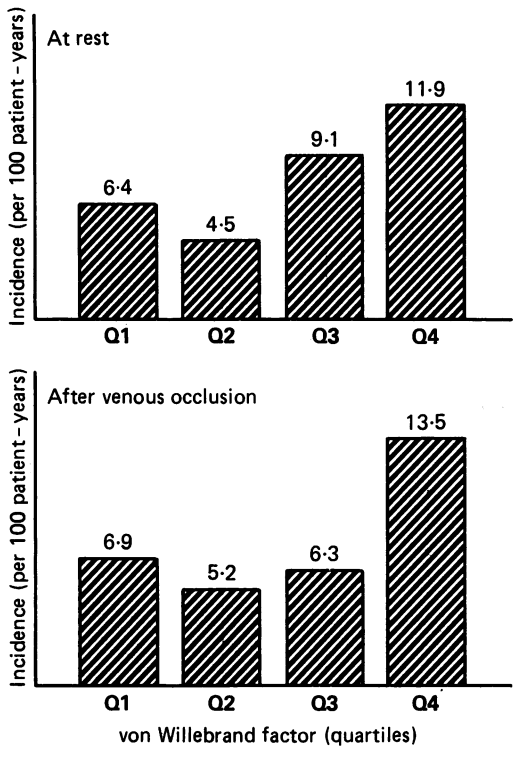

Figure 4 Incidence of any event in relation to von Willebrand factor at rest and after venous occlusion.

shows the number of any events through quartiles one to four of the von Willebrand factor at rest and after venous occlusion. Except for the first quartile both variables show an increasing number of any events with increasing concentrations.

MULTIVARIATE COX REGRESSION ANALYSES

All variables with $p < 0.05$ in univariate regression (table 3) were included in multivariate analyses (except that the two von Willebrand factor variables, which are closely interrelated, were not included simultaneously), with reinfarction, mortality, and any event as dependent variables. The analyses were based on the 23 deaths and 99 living patients (35 patients with reinfarction and 87 patients without reinfarction; 45 with any event and 77 without any event) for all of whom data were complete. Previous angina and von Willebrand factor each made an independent contribution to the prediction of reinfarction and of any event (table 4). Hypertension, previous angina, and von Willebrand factor each made an independent contribution to the prediction of death (table 4).

Discussion

We found that high concentrations of von Willebrand factor, either at rest or after venous occlusion, were independently associated with reinfarction and mortality.

von Willebrand factor is synthetised by the vascular endothelium[20] and megakaryocytes.[21] It is an essential factor in primary haemostasis, and a hereditary deficiency of von Willebrand factor is associated with a bleeding disorder, von Willebrand disease. Increased concentrations of von Willebrand factor were associated with previous myocardial infarction in case-control studies,[22 23] and we found a tendency for patients to have higher concentrations than controls, but this difference did not reach statistical significance.[2] In an earlier study on survivors of myocardial infarction the patients who died within a year had significantly higher concentrations of von Willebrand factor.[24] In that study the blood samples were taken within the first week of admission and may thus have been affected by a non-specific acute phase reaction. There is also one study that showed no difference between survivors of myocardial infarction and controls,[1] but the patients in that study were younger, less than 45. In a longitudinal population study, there was a tendency for ischaemic heart disease events to increase with increasing concentrations of factor VIII (which correlates closely with the concentration of von Willebrand factor), but the association was not significant.[25]

Increased concentrations of von Willebrand factor have also been associated with clinical severity of angina,[26] deep venous thrombosis,[15] and ischaemic cerebrovascular disease.[15 16] These case-control studies tend to support the hypothesis that a high concentration of von Willebrand factor is an index of atherosclerosis or of increased risk of thromboembolic complications or both. Moreover, high concentrations of von Willebrand factor seem to be correlated with high concentrations of atherogenic growth factors.[27] In a previous prospective study high concentrations of von Willebrand factor were associated with an increased incidence of thromboembolic events. In that study, high concentrations of von Willebrand factor in preoperative samples were correlated with an increased risk of postoperative deep vein thrombosis after major abdominal surgery.[28]

von Willebrand factor has a role both in adhesion and aggregation of platelets and in coagulation. It is reasonable to assume that increased concentrations of von Willebrand factor may increase the risk of thrombus formation, at least in patients with pre-existing vascular wall disease such as coronary artery disease. On the other hand, endothelial damage in atherosclerotic coronary arteries may result

Table 4 Relation between risk factors, reinfarction, and cardiovascular mortality (multivariate Cox regression analysis)

	Reinfarction		*Mortality*		*Any event*	
Risk factor	*R*	*p*	*R*	*p*	*R*	*p*
Angina	0·13	0·0164	0·14	0·0150	0·10	0·0135
vWF at rest	0·11	0·0090	0·12	0·0280	0·15	< 0·001
Hypertension			0·14	0·0135		

vWF, von Willebrand factor; R, partial R statistic, see statistical methods.
Variables showing a significant ($p < 0.05$) univariate association (table 2 and 3) were entered into the model.

in thrombin generation, causing increased secretion of von Willebrand factor. Thus our prospective data established that increased concentrations of von Willebrand factor are an indicator of increased risk, although the precise mechanism(s) for this are not yet known.

In our study there was no association between plasminogen activator inhibitor activity and reinfarction, death, or any event. This accords with our previous findings when we assessed plasminogen activator inhibitor as an index of risk for cardiovascular events in patients with severe angina pectoris and found that tissue plasminogen activator antigen concentration but not plasminogen activator inhibitor activity, was significantly associated with cardiovascular events.[7] High concentrations of plasminogen activator inhibitor, however, were independently related to reinfarction in young (< 45 years) survivors of myocardial infarction.[6]

Several of the established risk factors for myocardial infarction were not associated with reinfarction, death, or any event in the present study. This is probably because we did not study a large group of patients. This emphasises the potential value of von Willebrand factor measurements but does not mean that the other risk factors are unimportant.

Changes in the concentrations of both fibrinolytic variables and von Willebrand factor can be regarded as markers of endothelial cell dysfunction.[10 11 14 29] It may prove to be important to look for several markers of endothelial cell dysfunction when predicting cardiovascular events in patients with established coronary heart disease; and, moreover, the relative sensitivity of specific variables may vary with the population studied.

We thus regard a high concentration of von Willebrand factor as a novel index of increased risk for reinfarction and all-cause mortality in survivors of myocardial infarction.

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