Blood Platelet Count and Function Are Related to Total and Cardiovascular Death in Apparently Healthy Men

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Background. Experimental animal and clinical studies indicate that blood platelets have an important role in atherosclerosis and formation of thrombi. Prospective studies presenting evidence of an association between blood platelet count and cardiovascular mortality have not been performed.

Methods and Results. From 1973 to 1975, blood platelets were counted, and their responsiveness to aggregating agents was studied in healthy middle-aged men. The aim was to assess the possible association between these variables and coronary heart disease. At 13.5 years of follow up, a significantly higher coronary heart disease mortality was observed among the 25% of subjects with the highest platelet counts. Platelet aggregation performed in a random subsample (150 of the 487 men), moreover, revealed that the 50% with the most rapid aggregation response after ADP stimulation had significantly increased coronary heart disease mortality compared with the others. These associations could not be explained by differences in age, lipids, blood pressure, or smoking habits.

Conclusions. The present study is the first to present conclusive, prospective evidence of an association between platelet concentration and aggregability and long-term incidence of fatal coronary heart disease in a population of apparently healthy middle-aged men. (*Circulation* 1991;84:613–617)

Experimental animal studies indicate that blood platelets both initiate atherogenesis and trigger its complications.[1,2] Furthermore, angiographic and angioscopic studies in patients with acute myocardial infarction or unstable angina pectoris demonstrate a central role of blood platelets in the formation of thrombi and embolism.[3] The latter has earlier been observed in subjects dying suddenly and suggests an important role of blood platelet emboli for the development of myocardial electrical instability and subsequent lethal arrhythmias.[4]

Although the Northwick Park Heart Study demonstrated prospectively a strong association between high plasma levels of factor VII activity and plasma fibrinogen and coronary heart disease (CHD) mor-

tality,[5] we are not aware of prospective studies presenting evidence for a possible association between blood platelet count and in vitro platelet responsiveness with mortality due to cardiovascular causes. The present study was designed to test this possibility.

Methods

In a cardiovascular survey aimed at detecting previously unknown and unsuspected CHD, 2,014 men aged 40–59 years were studied. All were healthy according to detailed information from health files obtained from the five companies for whom they were working. Details about the selection procedures, examination program, and exclusion criteria have been presented elsewhere.[6,7] In the baseline study conducted between 1972 and 1975, a random 50% of the second half of the study population had undergone platelet function tests and platelet counts. After careful exclusion of all who had ingested drugs known to influence platelet function during the 2 weeks before the study (aspirin and nonsteroidal anti-inflammatory drugs), data from 487 randomly chosen men remained for analysis of platelet counts. All platelet tests were performed at 8:30 AM in the fasting and nonsmoking state. In a subsample, 150 of

the 487 men were randomly chosen to undergo examination for platelet aggregation (one fourth of the subjects were examined daily). Platelet counts and function were assessed in each subject at the baseline visit only.

The clinical examination was performed with standardized technique.[8] Platelet aggregation was examined in a dual-channel aggregometer (Payton Scientific, Inc., Buffalo, N.Y.). Platelet-rich plasma (PRP) was prepared by centrifuging blood that was anticoagulated in 0.013 M/l sodium citrate (final concentration) at 280g for 15 minutes at room temperature. Platelets in PRP were counted, but adjustment to a fixed number was not performed for practical reasons and because dilution of PRP can cause indeterminate changes in platelet responsiveness. Aggregation was started 45 minutes after PRP preparation and was finished within 90 minutes. The following were used as agonists: 2.1 μM ADP; 3.6 μM adrenaline, and 2.1 μg/ml collagen (Sigma Chemical Co., St. Louis, Mo.) final concentrations. Evaluation was by lag time (time from addition of agonist to incipient deflection) and by maximal rate (decrease in optical density per unit time at steepest slope).

The patients included in the survey were reinvestigated with an exercise test 7 years after the primary investigation to assess the incidence of angina pectoris, myocardial infarction, and a positive exercise electrocardiographic response de novo as well as total and CHD death. Data for the latter two are available for a 13.5-year follow-up period also.[9] Angina pectoris was diagnosed by a positive score on a World Health Organization questionnaire, on a personal interview, or on development of typical angina during the exercise test.[7] Myocardial infarction was diagnosed by electrocardiographic changes according to Minnesota code 1.1, by a 13.5-year questionnaire follow-up, and by a positive history of hospitalization for confirmed myocardial infarction. The diagnosis of fatal and nonfatal cardiovascular end points was made by personnel without knowledge of the results of platelet count and platelet aggregation studies.

Ninety-seven percent of the men responded to the questionnaire, and thus, this high percentage allowed estimation of the minimum incidence of nonfatal myocardial infarctions during the 13.5 years after the baseline survey.

Statistical Analysis

The association between time until death and the blood platelet variable as well as the covariables of age, cholesterol level, systolic blood pressure, and smoking were investigated by means of proportional-hazard models.[10] The main assumption in these models is that there is a proportional relation between the change in the value of a variable and the change in the associated hazard.

The initial model that was investigated included blood platelet counts and all the potential covariables mentioned above as the independent variables. Because all of these covariables were significantly re-

TABLE 1. Relation Between Total Platelet Counts and Annual Coronary Heart Disease and Total Mortality in 487 Apparently Healthy Middle-Aged Men Followed Up for 13.5 Years

	Quartile			
	1	2	3	4
CHD deaths (%)	0.36	0.41	0.44	1.04
Total deaths (%)	0.73	0.94	0.89	1.53
Median platelet Count (10^9/l)	176	211	239	298
n	123	126	117	121

lated to CHD mortality, the final model was equal to the initial one.

The proportional-hazards assumption for the model was checked and found to be fulfilled. The model was computed with the use of the proportional-hazards general linear model procedure in the SAS computer package (SAS, Cary, N.C.). When comparing frequencies, we applied the χ^2 test. When comparing two groups of persons according to a continuous variable, we used a two-sided Wilcoxon's ranked-sum test.[11] The correlation between two continuous variables was assessed by Spearman's correlation method.[11]

Continuous variables are presented as mean$\pm$SD. The material was subdivided in quartiles of platelet counts to study possible interquartile differences in total and CHD mortality. We studied platelet aggregation in only 150 men; therefore, before analysis, we decided to compare mortality in subjects with aggregation responses above and below the median. The decision to use quartiles for platelet count and the median for platelet aggregation studies was established a priori and was not selected on the basis of subgroup analysis.

Results

Of the 487 men who had platelets counted, 67 died within the first 13.5 years of follow-up, giving an annual death rate of 1.02% (0.56% CHD mortality per year and 0.46% non-CHD mortality per year). As expected, the mortality rate appeared to increase exponentially with time.

Table 1 shows that the annual total and CHD mortality increased with increasing platelet concentration. Mortality among those in the highest quartile of platelet counts was 1.53% and was 0.87% among the others ($p=0.017$). An increased death rate was only observed among subjects in the fourth quartile and was entirely confined to an increased CHD mortality. CHD mortality was in fact 2.5 times higher in the fourth quartile (1.04%) than in the other quartiles (0.41%) ($p=0.0025$), whereas deaths from other causes were evenly distributed among the quartiles. A close scrutiny of the 25% of the patients with the highest platelet counts showed that the 10% of the 487 men with the highest platelet counts (mean platelet count, 305×10^9/l) had an annual CHD mortality of 1.27%, and the remaining subjects of the

TABLE 2. Relation Between the Platelet Aggregation Response Induced by ADP and Annual CHD and Total Mortality in 150 Apparently Healthy Middle-Aged Men Followed Up for 13.5 Years

	CHD mortality (%)	Total mortality (%)
Aggregation rate		
50% with the slowest	0.20	0.50
50% with the fastest	0.98	1.27
Aggregation log time		
50% with the longest	0.25	0.58
50% with the shortest	1.06	1.30

TABLE 4. Relative Risk of Death From Cardiovascular Disease in Apparently Healthy Middle-Aged Men Followed Up for 13.5 Years

	RR	95% CI	*p*
Age (2 SD, 11 years)	2.4	1.2–4.8	0.010
Current smoking	2.0	1.1–3.6	0.022
Platelet counts in quartile 4	2.6	1.4–4.9	0.002
Systolic BP (2 SD, 37 mm Hg)	1.9	1.1–3.3	0.025
Cholesterol (2 SD, 2.64 mmol/l)	1.7	1.0–2.9	0.045

RR, relative risk; CI, confidence interval; BP, blood pressure.

fourth quartile had an annual CHD mortality of 0.93%. Only six of all 487 men had platelet counts higher than $400 \times 10^9/l$ (405, 410, 410, 420, 595, and $705 \times 10^9/l$). During the observation time, one of these six men died (from a non-CHD cause).

During the baseline study, coronary artery disease was suspected in 51 of the 487 studied according to the exercise electrocardiographic response. Positive responses were distributed equally in all four platelet count quartiles (1, 10.6%; 2, 6.4%; 3, 12.8%; 4, 12.4%). A test was positive when the electrocardiogram showed at least a 1.5-mm ST segment depression in precordial leads or a 1.0-mm ST segment depression in standard leads 0.08 second from the J point.

Subjects with an ADP-induced platelet aggregation rate above the median level had a significantly higher CHD mortality than those with an aggregation rate below the median level ($p < 0.01$) (Table 2). The same finding was observed in relation to lag time ($p < 0.01$). Thus, the 50% with the fastest ADP-induced aggregation rate had significantly higher

CHD mortality than did the others. No difference in mortality from any cause was observed when platelet aggregation responses to adrenaline or collagen were analyzed. Platelet counts in PRP and aggregation responses induced by ADP are shown in four quartiles of platelet counts of the 150 subjects that had undergone testing for platelet aggregation (Table 3).

Platelet counts in PRP, (mean, $393 \pm 91 \times 10^9/l$) used for the aggregation tests, correlated significantly with total platelet counts in whole blood (mean, $231 \pm 57 \times 10^9/l$) ($r = 0.71$, $p < 0.01$), indicating that the preparation of PRP did not lead to loss of platelets to any measurable extent. The platelet counts in PRP were higher in the subjects with the shortest lag time after ADP application ($430 \pm 75 \times 10^9/l$) than in the subjects with the longest lag time ($353 \pm 75 \times 10^9/l$) ($p < 0.001$). There were no differences in PRP platelet counts in the 50% with the fastest or slowest rate of ADP-induced aggregation.

Smokers had significantly higher platelet counts ($241 \pm 63 \times 10^9/l$) than did nonsmokers ($223 \pm 51 \times 10^9/l$) ($p < 0.01$). However, although the Cox regression analysis identified smoking as an important independent predictor of CHD mortality, platelet counts remained a

TABLE 3. Distribution of Platelet Counts in Platelet-Rich Plasma and Aggregation Responsiveness in Four Quartiles of Platelet Counts in 150 Men in Whom Platelet Aggregation Was Assessed at the Baseline Survey

	Quartile			
	1	2	3	4
Platelet counts				
Distribution	104–193	195–222	225–260	264–705
Mean	161	209	243	342
PRP				
Distribution	160–448	225–552	303–480	270–650
Mean	320	364	401	486
($r = 0.71$, $p < 0.0001$)				
ADP aggregation				
Lag time				
Distribution	4–128	3–132	10–132	5–140
Mean	51	51	64	68
($r = 0.26$, $p < 0.0014$)				
Max rate				
Distribution	6–26	7–27	2–22	2–35
Mean	12	13	12	14
($r = 0.07$, $p < 0.37$)				

PRP, platelet-rich plasma.
Correlation (r) between platelet counts and the variables given.

TABLE 5. Risk Factors in 487 Apparently Healthy Middle-Aged Men Distributed According to Blood Platelet Count Quartiles

	Quartile			
	1	2	3	4
Age (yr)	51.4±5.5	51.8±5.3	52.5±5.6	51.3±5.4
Cholesterol (mmol/l)	6.8±1.3	6.7±1.4	6.8±1.2	6.7±1.3
Triglycerides (mmol/l)	1.5±1.2	1.4±0.8	1.3±0.7	1.4±0.9
Systolic BP (mm Hg)	134±18	131±17	137±19	133±19
Diastolic BP (mm Hg)	89±9	88±10	90±10	88±12
Current smoking (%)	33.3	35.9	50.0	54.0

BP, blood pressure.

strong and independent predictor of CHD mortality after adjusting for smoking (Table 4).

The hematocrit values were the same in the four platelet count quartiles (1, 46.8±3.0%; 2, 47.2±3.0%; 3, 47.4±2.9%; 4, 47.3±2.5%), demonstrating that differences in platelet counts were not secondary to differences in plasma volume.

Platelet counts and aggregability (ADP, maximal rate response) were not significantly correlated to age, plasma cholesterol level, or plasma triglyceride level.

The unadjusted cumulative mortality was much higher in the quartile with the highest level of blood platelet counts than in the other three quartiles. The rate of death from CHD was 2.6 times higher in this quartile after adjusting for age, smoking, cholesterol level, and systolic blood pressure (Table 4). All five independent variables were significantly related to CHD mortality; the relative risk of death associated with an increase in age by 2 SD (11 years) was 2.4, and that associated with an increase in systolic blood pressure by 2 SD (37 mm Hg) was 1.9 (Table 3). For current smoking, the relative risk was 2.0.

The distribution of conventional risk factors in the four quartiles is presented in Table 5; only smoking was unevenly distributed. Age, systolic and diastolic blood pressures, cholesterol levels, or triglyceride plasma levels did not differ among the four quartiles.

The incidence of nonfatal cardiovascular events (angina pectoris, myocardial infarction, or the development of a positive exercise electrocardiographic response de novo) were distributed equally in the four platelet count quartiles (Tables 6 and 7).

Adding annual incidence of nonfatal myocardial infarction and CHD death according to data obtained after 13.5 years of follow-up revealed a significantly higher incidence of CHD events in the fourth platelet count quartile (1.47%) compared with the other three quartiles (1, 0.96%; 2, 0.94%; 3, 0.70%) (p=0.04).

Discussion

It is well documented that subjects with pathologically increased platelet counts, that is, thrombocyto-

TABLE 6. Annual Incidence of Angina Pectoris, Myocardial Infarction, or a Positive Exercise Electrocardiographic Response After 7 Years of Follow-up in Apparently Healthy Middle-Aged Men in Four Platelet Count Quartiles

	Quartile			
	1	2	3	4
AP (%)	1.74	2.27	1.96	2.13
MI (%)	0.59	0.46	0.61	0.24
Exercise ECG (%)	3.49	2.73	3.79	3.43

AP, angina pectoris; MI, myocardial infarction; ECG, electrocardiogram.

sis, have an enhanced risk of thrombotic complications.[12] However, the present study is the first to demonstrate that in apparently healthy middle-aged men there is an association between high platelet counts within the normal range and a risk of CHD mortality. Moreover, the present study suggests that subjects with the most reactive platelets (identified by ADP-induced aggregation) have a higher risk of developing fatal cardiovascular events. Thus, not only increased number of circulating blood platelets, but also an enhanced reactivity seems to increase the risk of cardiovascular death.

In contrast to reports of platelet hyper-reactivity in patients with hyperlipidemia,[13] the present study found no relation between platelet counts or aggregability and plasma cholesterol levels, triglyceride levels, and age. Even though smokers, on average, had higher platelet counts than did nonsmokers, multivariate analysis proved blood platelet counts to be an independent risk factor of CHD mortality.

Collagen or adrenalin-induced aggregation showed no relation to CHD mortality. With ADP, however, a highly significant correlation to CHD mortality was evident, whether the evaluation was by lag time or maximal rate (Table 3). The observed effect on ADP aggregation is probably not caused by an increase in the number of platelets in PRP because this increased aggregation effect was not seen with collagen or adrenaline. Aggregation induced by ADP seems to indicate that this platelet property is related to CHD mortality. The suggestion of a specific qualitative platelet involvement is strengthened by the fact that neither collagen nor adrenaline showed such a relation, albeit the procedure was performed on the same PRP samples. The reason why collagen and adrenalin-induced aggregation appeared to be unrelated to CHD death is obscure.

As far as we know, this is the first study to demonstrate that both blood platelet concentration

TABLE 7. Annual Incidence of Myocardial Infarction After 13.5 Years According to Questionnaire Follow-up in Apparently Healthy Middle-Aged Men Presented in Four Quartiles of Platelet Counts at the Baseline Study

	Quartile			
	1	2	3	4
Myocardial infarction (%)	0.60	0.53	0.25	0.43

and blood platelet responsiveness in apparently healthy subjects are associated with the subsequent development of fatal CHD. In the present study, there was no association between platelet counts or the development of angina pectoris or a de novo positive exercise electrocardiographic response. Thus, the data suggest a role of blood platelets in precipitating complications to stenotic CHD but not of the progression of the atherosclerotic disease itself.

In contrast to CHD death in this study, blood platelet counts could not be related to nonfatal myocardial infarction. This puzzling discrepancy may be due to a chance phenomenon or may relate to differences in blood platelet involvement in fatal and nonfatal complications to coronary artery disease. Acute myocardial infarction is usually caused by an occlusive coronary artery thrombus, whereas the mechanisms of sudden cardiac death is uncertain and frequently not associated with an occlusive thrombus. Increased platelet concentration may be a factor that propagates the conversion of a relatively mild thrombotic process into an aggressive and more extensive one. Another possible explanation is that sudden death is to some extent precipitated by electrical instability in minor infarcts induced by platelet aggregate emboli.[4]

Holter monitoring in other studies has documented the presence of silent myocardial ischemia in patients with asymptomatic and symptomatic CHD. Continuous electrocardiographic monitoring has further identified a circadian distribution of ischemic attacks with a peak in the early morning hours that coincides with the circadian distribution of sudden death and acute myocardial infarction.[14,15] Examination of blood platelet aggregability likewise has identified early morning hours as a period of enhanced blood platelet reactivity.[16] These studies suggest that there is a relation between increased ischemic activity, enhanced blood platelet responsiveness, and risk of developing complications to atherosclerotic heart disease. They seem to substantiate our assumptions of an association between blood platelets and complications to stenotic coronary artery disease. An increased blood platelet concentration and an enhanced blood platelet aggregation responsiveness induced by ADP seem to rep-

resent an elevated risk of developing fatal complications to ischemic heart disease.

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