Changes in platelet size and count in unstable angina compared to stable angina or non-cardiac chest pain

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Aims An increase in platelet aggregability is associated with unstable angina and myocardial infarction. Platelet size and activity correlate and mean platelet volume was found to be increased before acute myocardial infarction. We measured the mean platelet volume and platelet count in patients with stable angina, unstable angina and non-cardiac chest pain.

Methods and results We studied 981 patients (734 men; 247 women) defined clinically as stable angina (n=688), unstable angina (n=108) and unstable angina requiring immediate angioplasty (n=52). After coronary angiography the patients were subdivided into single (n=269), double (n=304) and triple-vessel disease (n=311) and the control group of non-cardiac chest pain (n=97). There was no significant difference in platelet count between the control group and patients with 1, 2, or 3-vessel disease. However, the platelet size in patients with coronary artery disease was significantly larger (single: $8 \cdot 7 \pm 1 \cdot 19$ fl; double: $8 \cdot 7 \pm 1 \cdot 12$ fl; triple-vessel disease: $8 \cdot 8 \pm 1 \cdot 18$ fl) than the control group ($8 \cdot 2 \pm 0 \cdot 95$ fl) ($P<0 \cdot 01$). Patients with stable angina similarly had no significant difference in platelet count compared to the control group but did have a significantly increased mean platelet volume ($8 \cdot 7 \pm 1 \cdot 13$; $P<0 \cdot 01$). In contrast, patients with unstable angina had a decreased platelet count ($245 \pm 56 \times 10$/l) compared to either stable angina ($262 \pm 62 \times 10$/l; $P<0 \cdot 05$) or the control group ($261 \pm 58 \times 10$/l; $P<0 \cdot 05$); furthermore, the mean platelet volume ($9 \cdot 4 \pm 1 \cdot 23$ fl) was significantly greater than for stable angina ($P<0 \cdot 01$). Patients with unstable angina requiring immediate PTCA had an even lower platelet count ($231 \pm 55 \times 10$/l) and higher mean platelet volume ($10 \cdot 4 \pm 1 \cdot 03$ fl) ($P<0 \cdot 01$) than the rest of the population with unstable angina.

Conclusions In stable angina the platelet count is unchanged compared to patients with normal coronary arteries but the platelet size is increased. However, in unstable angina there is a decrease in platelet count and an even larger increase in platelet size. We interpret this as meaning that unstable angina might be associated or preceded by an increase in platelet destruction rate that is not completely compensated for by an increase in platelet production rate. The large, more reactive platelets might be causally related to an ongoing coronary artery obstruction in unstable angina.
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Key Words: Platelets, unstable angina, coronary atherosclerosis.

Introduction

Unstable angina is a syndrome of chest pain and myocardial ischaemia usually in the presence of coronary artery atherosclerosis, whose natural history is either to resolve or to proceed to myocardial infarction. There is evidence that causal occlusion of the coronary artery in unstable angina is mediated by a platelet-rich thrombus. Treatment with aspirin and antibodies to the glycoprotein IIb/IIIa receptor, which inhibit platelet function, improves outcome in high-risk PTCA. In patients with unstable angina, aspirin and antibodies decrease progression to myocardial infarction and relieve pain, respectively[1–4]. Platelet function and size correlate since larger platelets, produced from activated megakaryocytes in the bone marrow, are more reactive than normal platelets in control populations[5]. Larger platelets have been shown to be an independent risk factor for myocardial infarction[6].

Platelet production is governed by various agents. Thrombopoietin (c-mpl ligand) has been shown to be a major agent controlling platelet number, and changes in platelet production from megakaryocytes is probably modulated by a series of cytokines (IL-3, IL-6, IL-11)[7–9] that are also involved in the inflammatory response. Recently it has been shown that the presence of activated megakaryocytes in the bone marrow in atherosclerosis correlates with increased circulating levels of the inflammatory cytokine IL-6 found in

atherosclerosis[10]. Liuzzo *et al.*[11] have demonstrated increases in C-reactive protein in unstable angina, postulating that a systematic inflammatory process may be causally related to thrombus formation in the coronary artery in unstable angina. We therefore measured platelet count and size in patients with stable and unstable angina. Since atherosclerosis itself may influence the bone marrow megakaryocytes before platelet production[12,13], we also assessed the degree of coronary artery atherosclerosis in all patients by coronary angiography.

Patients and methods

We studied 981 consecutive patients (734 men; 247 women; mean age 58 ± 7.2 years) referred to one centre (Department of Cardiology, University of Bonn) for diagnostic coronary angiography over a period of 9 months. Patients on oral anticoagulation, intravenous antianginal medication or with thrombocytopaenia or myocardial infarction within the last 8 weeks were excluded. The vast majority of patients with suspected or known coronary artery disease was on aspirin (824 patients: usually $100 \, mg \, . \, day^{-1}$; however, aspirin has no effect on platelet size[14]). Additional medication included calcium channel antagonists, beta-blockers, nitrates, angiotensin converting enzyme (ACE) inhibitors, digitalis and diuretic agents; the medication was evenly distributed within the study groups, except for patients with a past history of myocardial infarction, who were more often treated with nitrates and ACE inhibitors (but both with no effect on platelet size) and patients with unstable angina, who were more often treated with heparin subcutaneously (which also has no effect on platelet volume)[15].

On the day of the scheduled diagnostic coronary angiography, a fasting blood sample was obtained from all patients in a sitting position between 0800 and 0900h for the measurement of platelet count and mean platelet volume, which is a good indicator of platelet size[16]. Every precaution was taken to ensure good flow. Blood was taken into 20 gauge syringes containing dipotassium edetic acid (EDTA $1.3 \, mg \, . \, ml^{-1}$). All measurements were performed within 2–3 h of collection of blood because of the known effect of EDTA on platelet volume[17]. All blood samples were analysed on a Technicon-H system (Bayer Diagnostics, Munich, Germany). Platelet size was determined by measuring laser light scattered at a single scatter angular interval. The light source of the optical system is a red HeNe laser with a wavelength of 632 nm. Red cells and platelets are counted from the signals from a common detector with two different gain settings. Coincidence correction is made to each of the counts so that accurate counts are made over a wide range of each cell type. The range of expected values for mean platelet volume in our laboratory is 7·1 to 11·1 fl, for a platelet count of 130 to 400 ($\times 10/l$). The Technicon-H system underwent quality control once a week for within-run precision (mean

platelet volume nominal value 8·0 fl; standard deviation 0·20; intra-coefficient of variation 2·5%) using 20 aspirations of a single sample. In addition, daily quality control was performed by calibration to predetermined reference values with a maximum allowable deviation between the average of five repeated determinations of 5% for platelet count and 2% for mean platelet volume.

Patients were defined in two ways. Firstly on clinical grounds, into stable angina (group A; n=688) and unstable angina (group B; n=108). Stable angina was defined as chest pain on effort relieved by rest or sublingual nitrates (stable situation for the last 3 months). Most of these patients had a positive treadmill test and/or thallium scintigraphy and more than half of them had a history of myocardial infarction for more than 8 weeks (n=392). Unstable angina was defined as chest pain at rest, or marked progression of known angina within the last 2 weeks or recent and progressive onset of angina with evidence of ischaemia on the ECG. A third group of patients was identified: those that had unstable angina with symptoms at rest during the hospital stay despite antianginal medication (<24 h before the angiography). They had undergone immediate primary angioplasty during the diagnostic angiographic procedure (group C; n=52). In 36 patients it was not possible to distinguish between stable or unstable angina and thus they were excluded.

All patients were defined angiographically since they all underwent coronary artery angiography to assess the degree of atherosclerosis. Coronary obstruction was assessed visually by experienced investigators and a luminal narrowing of >50% in a major vessel (left anterior descending, circumflex artery and right coronary artery) or major side branch (first or second marginal or diagonal) was assumed to be a significant narrowing. Patients were therefore classified as having a normal coronary angiogram (n=97; group 0), single-vessel (n=269; group 1), double-vessel (n=304; group 2) or triple-vessel disease (n=311; group 3). The patients with a normal coronary angiogram and chest pain were classified as having non-cardiac chest pain and were defined as a control group for either stable or unstable angina patients (with proven coronary atherosclerosis) and patients with single-, double- and triple-vessel coronary artery disease, respectively.

Statistical analysis

Comparisons between groups were made with the unpaired Student's t-test. Probability (*P*) values <0·05 were considered significant. Results are expressed as mean ± standard deviation.

Results

Angiography

There was no significant difference in platelet count between the control group (0) and patients with 1, 2, or

Table 1   *Platelet count and mean platelet volume in patients with coronary athero-sclerosis and normal coronary angiogram*

Group	Description	n	Platelet count ($\times 10/l$)	Mean platelet volume (fl)
0	Normal angiogram	97	261 ± 58 ns	$8 \cdot 2 \pm 0 \cdot 95$*
1	Single-vessel	269	263 ± 64 ns	$8 \cdot 7 \pm 1 \cdot 19$ ns
2	Double-vessel	304	264 ± 58 ns	$8 \cdot 7 \pm 1 \cdot 12$ ns
3	Triple-vessel	311	258 ± 64 ns	$8 \cdot 8 \pm 1 \cdot 18$ ns

*$P<0\cdot01$ for mean platelet volume in group 0 vs groups 1, 2 or 3.
ns=no significant difference in mean platelet volume between groups 1, 2 and 3 and all mean platelet counts (0–4).

Table 2   *Platelet count and mean platelet volume in patients with stable and unstable angina and in patients with non-cardiac chest pain*

Group	Description	n	Platelet count ($\times 10/l$)	Mean platelet volume (fl)
0	Non-cardiac chest pain	97	261 ± 58	$8 \cdot 2 \pm 0 \cdot 95$
A	Stable angina	688	262 ± 62	$8 \cdot 7 \pm 1 \cdot 13$
B	Unstable angina	108	245 ± 56	$9 \cdot 4 \pm 1 \cdot 23$
C	Unstable angina+PTCA	52	231 ± 55	$10 \cdot 4 \pm 1 \cdot 03$

Platelet count: 0 vs A: ns; A vs B: $P<0\cdot01$; A vs C: $P<0\cdot01$; C vs O: $P<0\cdot01$; B vs C: ns (t-value: 1·49)
Mean platelet volume: A vs O: $P<0\cdot01$; A vs B: $P<0\cdot01$; A vs C: $P<0\cdot001$; B vs C: $P<0\cdot01$; C vs O: $P<0\cdot001$.

3-vessel atherosclerosis as shown in Table 1. Even if all patients with atherosclerosis (n=884; $262 \pm 63 \times 10/l$) were compared with the control group with the normal angiogram ($261 \pm 58 \times 10/l$) there was no significant difference in platelet count. Thus platelet count failed to distinguish between patients with and without coronary artery obstruction. However, platelet size in patients with either single- ($8 \cdot 7 \pm 1 \cdot 19$ fl), double- ($8 \cdot 7 \pm 1 \cdot 12$ fl) or triple-vessel disease ($8 \cdot 8 \pm 1 \cdot 18$ fl) was significantly larger than the control group ($8 \cdot 2 \pm 0 \cdot 95$ fl; $P<0\cdot01$). However, there was no significant difference in platelet size with regard to the degree of atherosclerosis present (1, 2 or 3-vessel disease).

Clinical classification

Table 2 shows the results of platelet count and mean platelet volume for patients defined by symptoms and compared to the same control group as in Table 1 (0; non-cardiac chest pain). There was no significant difference in platelet count in patients with stable angina compared to the control group but there was a significant increase in mean platelet volume ($8 \cdot 7 \pm 1 \cdot 13$ fl; $P<0\cdot01$) of a similar order of magnitude to the increase in mean platelet volume for groups 1, 2 and 3 in Table 1. Interestingly, patients with unstable angina had a decreased platelet count ($245 \pm 56 \times 10/l$) compared to either stable angina ($262 \pm 62 \times 10/l$; $P<0\cdot01$) or the control group ($261 \pm 58 \times 10/l$; $P<0\cdot05$). Furthermore,

the mean platelet volume ($9 \cdot 4 \pm 1 \cdot 23$ fl) was significantly greater than that for stable angina, regardless of the amount of the underlying coronary obstruction ($P<0\cdot01$). Additionally, those patients with unstable angina requiring immediate PTCA (group C) had an even lower platelet count ($231 \pm 55 \times 10/l$; non-significant) than the rest of the population with unstable angina. They also had an average mean platelet volume ($10 \cdot 4 \pm 1 \cdot 03$ fl) that was considerably higher than any other group (C vs B: $P<0\cdot01$; C vs A and vs 0: $P<0\cdot001$).

Discussion

Platelets play a crucial role in the course of acute coronary syndromes since there is evidence that myocardial infarction, unstable angina and sudden cardiac death are associated with changes in the haemostatic system, particularly with changes in platelet behaviour[18,19]. An increase in platelet aggregability has been shown to be an independent risk factor for a future coronary event and platelet activity and responsiveness to aggregating stimuli are increased in patients who will undergo cardiovascular events[20–22]. The prognostic importance of platelet behaviour in acute coronary syndromes is further demonstrated by the therapeutic efficacy of inhibition of platelet function either with aspirin or with the use of monoclonal antibodies directed against the platelet glycoprotein IIb/IIIa receptor[1–4].

Several studies indicate that platelet volume and platelet function correlate since large platelets are more reactive haemostatically. In experimental animal models the production of large platelets that follows platelet destruction is accompanied by a decrease in bleeding time which is an in vivo indicator of platelet activity[5]. In patients with thrombocytopaenia the frequency of bleeding episodes correlates with the mean platelet volume (the higher the mean platelet volume the lower the number of bleeding episodes)[23] and in patients with myocardial infarction the mean platelet volume was found to be inversely related to the bleeding time[24]. Large platelets are denser, they produce more thromboxane B2 per unit volume of platelet cytoplasm and decrease bleeding time more than control platelets[5]; they contain more alpha and dense granules and preferential aggregation of large platelets is observed after addition of ADP to platelet suspensions[25]. Finally, larger platelets aggregate more rapidly upon collagen challenge, release more serotonin and other granule contents and express more GPIb per unit area[26–29].

In the present study, we demonstrated that there is no change in platelet count between patients with a normal coronary artery angiogram and those with atherosclerotic disease. However, mean platelet volume is significantly increased in coronary atherosclerosis, consistent with our previous findings[10].

However, in unstable angina, in addition to the pronounced increase in platelet volume, compared to either stable angina or patients with normal coronary arteries and non-cardiac chest pain, the platelet count was decreased. Further, in the group of patients where unstable angina was so severe as to require immediate angioplasty, the platelet count was even lower and platelet size on average was increased by more than 25% compared to the control population. This indicates that on top of atherosclerosis unstable angina might be associated with or preceded by a systemic increase in platelet destruction rate that is not completely compensated for by an increase in platelet production rate. In addition, platelet consumption at the site of the coronary atherosclerotic culprit lesion may contribute to coronary artery thrombus formation in unstable angina and thus to a further increase in platelet destruction rate. Disruption of an atherosclerotic plaque may initiate the formation of platelet- and fibrin-rich thrombi by exposure of thrombogenic components of the vessel wall to the circulating platelets[30,31]. This intraluminal process of platelet adhesion and activation is dynamic and repetitive[32–34]. In some patients this may lead to intermittent or transient vessel occlusion and ischaemia by a labile thrombus which may resolve by treatment or even spontaneously; in others the thrombus formation may be progressive and lead to total occlusion with subsequent myocardial infarction. Since larger platelets are more reactive haemostatically, once initiated the thrombus formation which leads to the flow-limiting obstruction may progress in the presence of these larger platelets. The larger the platelets the greater may be the severity of unstable angina, as seen in this study.

The presence of larger platelets in patients with ongoing symptoms may in part be interpreted as a consequence of platelet consumption at the site of the coronary lesion, ultimately leading to the release of larger platelets from the bone marrow. However, since changes in platelet size are determined at thrombopoiesis[16,35] and platelets circulate in man for 10 days it is likely that the large platelets were circulating at the initiation of symptoms. Our data suggest that the increased mean platelet volume contributes to the prethrombotic state in acute ischaemic syndromes. They are consistent with previous findings that the mean platelet volume is increased at time of admission with an acute myocardial infarction[36,37]. Furthermore, large platelets are a risk factor for myocardial infarction and death[6], and activated megakaryocytes are present in the bone marrow at the time of sudden cardiac death[38], compared to controls and in patients with coronary artery atherosclerosis prior to cardiac surgery[39].

In addition to an increase in platelet destruction rate leading to an increase in platelet production (mediated by thrombopoietin), systemic changes independently may affect platelet production. The increase in C-reactive protein in unstable angina shown by Liuzzo and colleagues[11] supports the hypothesis, that a systemic inflammatory process in addition to coronary plaque rupture is involved in unstable angina. Interleukin-6, which is an inflammatory cytokine involved in megakaryocyte maturation in vitro, is increased in patients with atherosclerosis[10]. A better knowledge of the control mechanisms governing the size of platelets produced from megakaryocytes might help in understanding the nature of acute coronary syndromes.

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