Shortened bleeding time in acute myocardial infarction and its relation to platelet mass

P C MILNER, J F MARTIN

Abstract

The bleeding time, using the Simplate method, horizontal incision, and venostasis, was measured in a study of 51 patients admitted to a coronary care unit within 12 hours of the onset of chest pain. The bleeding time was significantly shorter in the 28 patients who were found to have definite myocardial infarction compared with the 23 others with chest pain but no definite infarction ($p<0.0005$). A bleeding time of less than 212 seconds correctly classified 84% of patients (sensitivity for definite myocardial infarction 89%) presenting to the coronary care unit with chest pain. Multiple regression analysis showed the bleeding time in all patients to be determined independently (and with high significance) by the following variables in order of importance: diagnostic group, platelet mass (platelet count $\times$ mean volume), and age. Packed cell volume was not a significant determinant. In the group with definite myocardial infarction considered alone the same order of variables was observed in predicting bleeding time, but none of them was significant. A major variable reducing bleeding time in acute myocardial infarction remains to be determined. There was no association between bleeding time and creatine phosphokinase activity or infarct size in the group with definite myocardial infarction.

Introduction

There is evidence that platelets play a part both in atherogenesis and in arterial occlusion.\(^1\)\(^2\) In myocardial infarction the average mean platelet volume is increased.\(^3\)\(^4\) Studies in man and in animal models show that larger platelets are more reactive than smaller ones;\(^3\)\(^4\) hence their presence in myocardial infarction may be related to the occurrence of the arterial occlusion. Bleeding time estimated with venostasis is probably a sensitive and physiological measurement of platelet reactivity.\(^7\) Bleeding time has already been shown to be shortened three months to five years after myocardial infarction.\(^8\) It was therefore hypothesised that bleeding time would be shorter in the acute phase of myocardial infarction and that the shortening may be related to changes in platelet characteristics. Platelet count may determine bleeding time,\(^9\) and changes in platelet volume have been observed in vascular disease.\(^3\)\(^4\) Platelet count and volume are inversely related.\(^10\)

We have assessed platelet mass, the product of platelet volume and count, as a determinant of bleeding time in myocardial infarction.

Patients and methods

Sixty nine patients admitted to the coronary care unit with chest pain were considered for the study. They were entered sequentially over a three month period during April to July 1984 on the basis of a researcher being free to assess them.

The following groups of patients were excluded: (a) those who had taken aspirin or other non-steroidal anti-inflammatory drugs within the previous 10 days;\(^11\)\(^12\); (b) those with renal impairment, as judged from the serum creatinine concentration; (c) those with a history of bleeding disorders; (d) those whose duration of chest pain was greater than 24 hours.

All patients gave written consent. Full history and examination were undertaken by one investigator. Three serial electrocardiograms, three serial measurements of creatine phosphokinase activity, and serum creatinine concentration, haemoglobin value, and packed cell volume were obtained for each patient.

Patients were classified by World Health Organisation diagnostic criteria as having definite, probable, possible, or no myocardial infarction.\(^13\)\(^14\) All patients with definite myocardial infarction had a creatine phosphokinase activity greater than three times normal.

The study was limited to about 50 patients, since we considered that 44 patients divided equally between definite myocardial infarction and others, with a difference in mean bleeding time of one minute and the same variances, would be significant at the 5% level. A
difference in mean bleeding time of one minute was thought to be a reasonable figure, since local experience had shown the bleeding time of the normal population, as measured by the Mielke method, to range from 2 to 10 minutes. Such a difference in mean bleeding times could be easily contained within this range.

In each case we recorded age, sex, and number of previous myocardial infarctions. Site and extent of infarction were recorded in definite cases; site was either inferior or anterior, and extent either subendocardial or transmural, both defined from serial electrocardiograms. Cigarette years was recorded as the average number of cigarettes reported as smoked per day times the number of years smoked. Attenuated cigarette years indicated the possible diminishing effect of smoking with increasing time since giving up smoking (cigarette years in compound fashion by 10% a year for each year since stopping smoking). Patients were said to be shocked if the systolic blood pressure was less than 100 mm Hg and they had nail bed cyanosis. The time from onset of chest pain to venesection for measurement of platelet volume was noted. Patients were either discharged to home or death in hospital (never having been discharged). One new variable was created—namely, platelet mass, which equals platelet count times mean platelet volume.

The bleeding time was measured by the method described by Mielke.14 Two separate horizontal incisions 5 cm apart longitudinally on the lateral aspect of the shaved forearm 5 cm from the antecubital crease were made using two Sipal 1 bleeding time devices. Venostasis was maintained at 40 mm Hg from 30 seconds before the first incision to the arrest of bleeding. All measurements of bleeding time and venostasis were done as soon as possible after admission and all within 24 hours from the onset of chest pain. Bleeding time was measured in all patients by the same investigator.

Blood samples were taken from the cubital vein just after measurement of the bleeding time. A 4.5 ml sample of blood was mixed with 0.5 ml sodium citrate, 38.0 g/l, and 0.01 ml prostaglandin E3, 0.4 mg/ml (citrated blood).

A truly representative platelet population was obtained by centrifuging buffered citrated blood on a Percoll velocity gradient, as described.8 The platelet volume distribution was measured from this sample using a Coulter ZB particle counter coupled to an Apple II microcomputer via an analogue to digital converter. All measurements were made as soon as possible after venesection (mean time 40 minutes). Platelet counts were made at the same time using the Coulter ZB counter on the platelets from the velocity gradient and from diluted blood sedimented for one hour according to the method of Bull et al.9

The ratio of the platelet counts derived from the two different methods—velocity gradient count to sediment count (recovery)—was calculated to ensure that a mean platelet volume was being measured on a representative platelet population. Phase contrast microscopy of platelets harvested by this method showed no platelet aggregates before sizing.

The statistical package for the social sciences was used to calculate the means, standard deviations, standard errors, and correlation matrices of the variables for the different groupings (patients with definite myocardial infarction, others, total).17 The statistical package for the social sciences was also used to consider (a) the difference in means of certain variables between patients with definite myocardial infarction and the others by the unpaired t test; (b) the variation of bleeding time between diagnostic groups by a one way analysis of variance with a linear contrast and an analysis of covariance to examine the compounding effect of age on bleeding time; (c) the simple regression and scattergrams of bleeding time against other variables; (d) the interrelationship of bleeding time with the rest of the variables in the different groups using the new multiple regression program in the statistical package;14 and (e) the possibility of identifying patients with definite myocardial infarction from the rest at presentation by discriminant analysis. All probabilities are based on two tailed tests, and the t tests performed do not assume equal variances.

### Results

Of the 69 patients considered for the study, 18 were excluded. Ten had taken aspirin within 24 hours of admission and after the onset of chest pain (eight cases of myocardial infarction); two had taken non-steroidal anti-inflammatory drugs (one case of myocardial infarction); three refused to give consent (two cases of myocardial infarction); one was comatose (myocardial infarction); one had had a heart transplant (no myocardial infarction); and one was taking warfarin (no myocardial infarction). No patient had renal impairment or a known bleeding disorder. Fifty one of the original 69 patients were studied.

There were 28 patients with definite myocardial infarction and 23 others (six with probable, nine with possible, and eight with no myocardial infarction). Mean ages of the two groups were 60 and 53 years respectively. This difference was significant (p<0.008).

There were four deaths, all occurring in the group classified as having definite myocardial infarction.

The mean bleeding time in the group with definite myocardial infarction (170 s) was significantly shorter (p<0.0005) than in the others (258 s) (table). This result was still significant when an analysis of covariance was used to correct for the observed difference in mean ages between the groups (F(1,46)=10.8; p<0.002). Most of the variation of bleeding time with age in the group who had not had a myocardial infarction came from the four youngest patients. When they were omitted the difference in mean ages between the two groups became non-significant (60 v 57.5 years), while the difference in bleeding time (170 v 238 s) remained significant (p<0.05). There was no significant difference in platelet mass and packed cell volume between the two groups. The table shows the measured differences between the two groups. Values are given either as the mean and standard deviation (in square brackets) or as number and percentage, whichever is appropriate.

One way analysis of variance—The differences in mean bleeding times among the diagnostic groups were examined by one way analysis of variance using a linear contrast. The differences were found to be highly significant (F(3,46)=17.3; p<0.0005) (fig 1).

![Comparison of bleeding times for patients with definite myocardial infarction and others.](image_url)

**Comparison of bleeding times for patients with definite myocardial infarction and the others.** Mean values expressed with standard deviations in square brackets.

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Correlation analysis—The bleeding time was found to be strongly correlated with platelet mass (p < 0.008; fig 2) and age (p < 0.003) when all cases were considered. Platelet count was also significantly correlated with bleeding time (p < 0.019) but not as significantly as platelet mass. There were slight negative correlations between bleeding time and both packed cell volume and haemoglobin concentration but no significant associations were shown. Age and platelet mass were not significantly correlated.

Multiple linear regression was undertaken to assess the conditional effects of diagnosis, platelet mass, and age on bleeding time. All the models were developed using a stepwise procedure with a probability for inclusion of any variable of p < 0.05. The multiple regression equation developed for the group who had suffered a definite myocardial infarction was:

$$\text{bleeding time} = 548 - 77 \times \text{platelet mass} - 3.2 \times \text{age}.$$  

None of the diagnostic variables—for example, diagnosis, maximum creatine phosphokinase activity—were considered for this model. The assumptions of multiple regression were not violated. Platelet mass and age (in that order) were the best predictors of bleeding time in this group, and were both highly significant: for platelet mass t = -2.695, p < 0.01; for age t = -2.72, p < 0.01. In the group with definite myocardial infarction all variables excluding the diagnostic ones were considered. No variables entered the regression equation at a probability for inclusion of p < 0.05. When no constraints were placed on the probability for inclusion, however, platelet mass and age (in that order) were the first and second variables predicting bleeding time (as in the other group). The correlation between bleeding time and platelet mass was t = -2.29, p < 0.05; and between bleeding time and age was even more significant than in the group who had not suffered a definite myocardial infarction.

A bleeding time of less than 212 seconds on admission to the coronary care unit was a good discriminator of definite myocardial infarction—sensitivity (25/28) 89%; specificity (18/23) 78%; proportion of cases correctly classified (43/51) 84%; \( r^2 = 23.62, p < 0.001 \). The discriminating value of 212 seconds was chosen as the one that maximised the total number of cases correctly classified into the categories of definite myocardial infarction and others while balancing the need for a high sensitivity against a not too low specificity. Figure 1 shows the effect on these parameters of choosing different discriminant cut off values. The ability to detect the cases of definite myocardial infarction at presentation was assessed by the discriminant analysis program of the statistical package for the social sciences. A total of 18 variables available at presentation such as age, sex, platelet count, packed cell volume, bleeding time, etc were included in the analysis and no model was developed which discriminated better than by using the bleeding time alone.

There was no correlation in the group with definite myocardial infarction between bleeding time and shock (r = -0.03; p = 0.88). Platelet count in this group was strongly correlated with both the mean (r = -0.41; p = 0.003) and standard deviation (r = -0.50; p = 0.000) of the platelet volume frequency distribution curve. The considerably lower association was less pronounced in the others—count vs mean: r = -0.28; p = 0.19; count vs standard deviation: r = -0.46, p = 0.02. The packed cell volume was inversely related to mean platelet volume (r = -0.52; p < 0.01) and age (r = -0.41; p < 0.05) but directly related to platelet count (r = 0.42; p < 0.05).

Discussion

The method that we used for measuring bleeding time is more sensitive than other methods.\(^8\) In a group of patients with chest pain in a coronary care unit we assessed the relative importance of platelets and packed cell volume in determining bleeding time and for the first time showed that platelet mass (count x mean volume) is a major determinant of bleeding time.

In the group who had not had a definite myocardial infarction but had suffered the same symptoms and same external environment, platelet mass was the main observed determinant of bleeding time, and that relation was highly significant (p < 0.01). In the definite myocardial infarction group platelet mass was still the main observed determinant of bleeding time, though the relation was not significant (p < 0.17). The considerably shortened bleeding time observed in myocardial infarction was therefore due to a variable which was not platelet mass itself. In both cases age was the second determinant of bleeding time and that relation was independent of the more important determinant, platelet mass. The variation of bleeding time with age was similar to that described by Jorgensen et al.\(^8\)

The shortened bleeding time in myocardial infarction could either be related to the cause or be an effect of myocardial infarction; however, there are several arguments that the shortened bleeding time may have predated the myocardial infarction. Firstly, O’Brien et al have shown that the bleeding time is shorter between three months and five years after myocardial infarction,\(^9\) pointing to a chronic change. Secondly, there was no relation in this study between the size of the infarct as measured by creatine phosphokinase activity and a decrease in bleeding time; nor a relation between bleeding time and either the existence of cardiogenic shock or the time from onset of symptoms to measurement of the bleeding time. Thirdly, such a change with acute myocardial infarction was predictable from previous evidence.\(^9\)\(^10\) Plainly these arguments do not rule out entirely the possibility that the decrease in bleeding time is secondary to the myocardial infarction.

Platelets are produced from megakaryocytes. In myocardial infarction megakaryocytes have been shown to be larger than in controls similar to those patients without myocardial infarction studied here.\(^11\) In sudden cardiac death large megakaryocytes are present at the time of death.\(^12\) In an animal model such large megakaryocytes produced platelets which disproportionately decreased the bleeding time and produced more thromboxane A\(_2\) (a platelet aggregator and vascularconstrictor) per unit volume of platelet cytoplasm compared with platelets produced from normal sized megakaryocytes.\(^13\) Furthermore, Thorngren et al measured thromboxane B\(_2\) (the stable metabolite of thromboxane A\(_2\)) in the blood emerging from skin incisions and showed that it was increased in men with a lower bleeding time.\(^11\) A possible explanation, therefore, for the shortened bleeding time observed here is that the platelet mass, determining bleeding time, is composed of cytoplasm from large megakaryocytes with increased thromboxane A\(_2\) producing ability per unit mass. Further indirect evidence that platelet thromboxane A\(_2\) production may be an associated cause in some myocardial infarctions comes from the double blind study of crescendo angina, where aspirin (an inhibitor of thromboxane A\(_2\) production) reduced the occurrence of myocardial infarction by 50%.\(^14\)
The early diagnosis of myocardial infarction in patients with chest pain is difficult. Here bleeding time successfully classified 84% of such patients with a sensitivity of 89% for the diagnosis of changing myocardial infarction. The data were obtained from the onset of symptoms, using a simple test taking about five minutes and performed at the bedside. A discriminant cut off value of less than 212 seconds for the bleeding time for definite myocardial infarction was derived from a retrospective fit of the data. Its use for prospective diagnostic classification, therefore, will provide slightly inferior results than those quoted here. Nevertheless, bleeding time may still be a useful diagnostic tool in definite myocardial infarction.

Duke in 1910 showed the bleeding time to be a function of platelet count, but others have argued that this was only correct for platelet counts below 100 x 10^3/μL. Using more accurate methods for bleeding time, and measuring platelet volume distribution on a truly representative population, we have shown in patients in a coronary care unit that changes in bleeding time are related to platelet mass. It seems reasonable to assume that this would hold for the normal population. Since platelet volume and count are a function of thrombopoiesis, then for a constant platelet destruction rate the total volume of megakaryocyte cytoplasm producing platelets would be a main determinant of bleeding time, more important than platelet count alone. Since megakaryocytes can change their mean volume rapidly by undergoing polyploid change, this ability may be a major homeostatic mechanism in determining vascular integrity in man.

The role of red cells in shortening bleeding time in conditions where the packed cell volume is abnormal has recently been debated. Our results suggest that their role is secondary to that of platelets when the packed cell volume is in the normal range—that is, in most cases. Their relative importance to each other and to age, however, requires study in normal and pathological populations by the newer methods of measurement and analysis used here.

We thank Mr J Nicholl (department of community medicine, Sheffield University) for his comments on the statistical methods used, and Dr J S Fleming and the consulting physicians of the Royal Hallamshire Hospital for allowing us to study their patients.

The analog-to-digital converter and computer program are available from Dr J F Martin.

100 YEARS AGO

At a meeting of the St Pancras Vestry, held on Thursday, January 8th, the subject of the recent charges of mismanagement and ill-treatment of small-pox patients at Darent Camp was again brought under consideration. Mr. N. Robinson, a member of the St. Pancras Vestry, and also of the Metropolitan Asylums Board, is reported to have said that: "The men had no chance of linen for three weeks, that the food was not properly cooked, and that there were no proper bathing facilities." Sir E. H. Currie, in a letter to Mr. Robinson, utterly denies these charges, and expresses his regret that Mr. Robinson should have made, in public, such allegations of mismanagement against a board of which he himself is a member. In reply to this, Mr. Robinson states that he has been misreported, that what he said at the vestry meeting was prepared to say anywhere; and he was of opinion that what he had said was the best possible means of easing the public mind with regard to allegations which had arisen in other quarters. Whatever may be the truth of these charges of mismanagement at Darent, it is certain that there have been recently a large number of letters in the public prints, from late patients, complaining of their treatment while at the camps, and of the general management of the place; but it is to be hoped that, if any grounds for such complaints did exist, they have now been effectually removed. As a striking instance of the current absurdities which have been uttered, we note the following. It has been asserted that the food of the patients was handled by hands that ought not to handle it, which we found, on inquiry, to mean that the small-pox convalescents cut their own bread by means of a machine. If they may not cut their own bread with their own hands, of course, they may not eat it with their own hands, and, consequently, must be fed like helpless idiots. In all the small-pox hospitals we have known, the convalescents have been utilized in the general work of the hospital, and we fail to see the slightest objection to the practice. The Asylums Board has a very difficult task in the management of such an institution, and it is not if at first, there are some hitchs. The wonder is that it has gone on so well.

To manage a camp containing, at times, upwards of a thousand persons, many of them practically well, and some of them certainly not easily managed, appears to us to be work for some military officer, who could enforce military discipline, rather than for a medical man; and it seems unjust to blame the medical man if, in some instances, the task has proved too much for him. (British Medical Journal 1885;ii:74:96.)

Some interesting experiments are being made at Munich as to the natural object of preserved meats, etc., as a good nutritive substitute for fresh meat, etc., especially as the means are being provided for the army in time of war. A special company of non-commissioned officers and men from all twelve companies of the 13th Bavarian infantry has been told off to march daily for a fortnight, with the exception of an occasional rest-day, for six hours, and to go through field exercises, fully equipped, as in time of war. During this time, they receive no fresh food of any kind, only preserved meats, a kind of biscuit composed of flour, bacon, and chopped-up meat, with salt and spices, etc. While off duty, they are watched, to prevent them from eating or drinking anything else, and they are continually weighed. The object of these experiments is to see how far soldiers can remain healthy and fit for fighting when only living on preserved food, which they can carry themselves. So far, the results have been satisfactory. (British Medical Journal 1885;ii:188.)