OFFICE OF NAVAL RESEARCH

CONTRACT N00014-88-C-0118
CONTRACT N00014-94-C-0149

TECHNICAL REPORT 95-01

BLEEDING TIME, VOLUME OF SHED BLOOD COLLECTED AT THE BLEEDING TIME SITE, AND THE PERIPHERAL VENOUS HEMATOCRIT

BY

J.P. CROWLEY, J.B. METZGER, AND C.R. VALERI

NAVAL BLOOD RESEARCH LABORATORY
BOSTON UNIVERSITY SCHOOL OF MEDICINE
615 ALBANY STREET
BOSTON, MA 02118

10 JANUARY 1995

ABSTRACT

BACKGROUND: The relation between the bleeding time, the peripheral veous hematocirt and the amount of blood shed at the template bleeding time site has not been previously defined.

METHODS: We studied this relation in 227 individuals: 26 were performed on patients with ITP, 137 on patients with a variety of other bleeding disorders, and 64 in normal subjects. Bleeding times were performed by a modified Ivy technique with a commonly used commercially disposable bleeding time device. Hemoglobin was eluted with sodium hydroxide from the filter paper to collect the shed blood from the skin incision, and the hemoglobin was quantitated using a standardized spectrophotometric method. Complete blood counts were peformed on all individuals.

RESULTS: The bleeding time for the normal group was 7.1 $\pm$ 1.2 minutes, and the amount of shed blood was 136.4 $\pm$ 47.2 µl. In patients with ITP the bleeding time was 14.0 $\pm$ 4.1 minutes and the shed blood was 508.1 $\pm$ 387 µl. There was a significant correlation between the bleeding time and the platelet count ($r = 0.43$, $p<0.05$), in patients with ITP. In the group of individuals with other miscellaneous bleeding disorders, the mean bleeding time was 9.0 + 3.5 minutes and the amount of shed blood was 224.7 $\pm$ 184 µl. Bleeding times from all of the normal and abnormal individuals showed a correlation of 0.75, $p<0.001$ with respect to the amount of shed blood on the filter paper and correlation of 0.28, $p<.001$ with respect to the peripheral venous hematocrit.

CONCLUSIONS: This study demonstrates that the volume of blood shed at the bleeding time site correlates with the peripheral venous hematocrit, and emphasizes the contribution of the hematocrit to primary hemostasis in normal subjects and patients with bleeding disorders.

INTRODUCTION

The bleeding time is a controversial test used to assess hemostasis. Its primary application has been in the evaluation of platelet function and the platelet related bleeding disorders(1,2). A recent review of the bleeding time tests by Rodgers and Levin (3) reported that the bleeding time test has little or no predictive value regarding surgical bleeding. While various modifications of the bleeding time test have been introduced to provide more standardization (3), the test still retains a high degree of unexplained variability and the preoperative bleeding time does not generally correlate with total surgical bleeding (2-6). One source of variability in the bleeding time test may result from the hematocrit (3,5). A recent study in rabbits reported a significant correlation between bleeding in thrombocytopenic rabbits and the hematocrit (7). Previous clinical studies (7-16) have correlated the volume of shed blood collected at the template bleeding time site to the bleeding time. The effect of peripheral venous hematocrit which has been demonstrated to have a high correlation with bleeding time (15) has not been assessed with respect to blood lost during the bleeding time determination.

Willoughby and Allington(10) and Zeigler (16) described the quantitation of blood loss by weighing the filter paper to be used in performing the modified Ivy bleeding time and subtracting the weight of the filter paper from the weight of the filter paper plus dried blood. A comprehensive study of the relation between the preoperative bleeding time and the dried shed blood on the filter paper in patients undergoing coronary bypass surgery has been reported (6). However, the weight of the filter paper may change according to differences in the degree of humidity in office or laboratory, a factor which is difficult to control (17). We modified the method of Zeigler (16) according to the technique of Hallberg and Nilsson (18) which was a method for eluting and quantitating the blood lost during menstruation from tampons and sanitary pads(19). Using this method to quantitate the blood collected on the filter paper during

the measurement of the template bleeding time, we have assessed the relations between bleeding time, the volume of shed blood collected at the template bleeding time site, and the peripheral venous hematocrit in normal subjects and those with bleeding disorders.

METHODS

I. SUBJECTS

Twenty-six determinations were made in patients with ITP, and 137 determinations in patients received for evaluation of a bleeding disorder other than ITP. There were more women (97) than men (40) in the bleeding disorder group reflecting a sex difference noted in other studies (ref 15,20). The patients with bleeding disorders other than ITP fell into the following diagnostic categories: von Willebrand's disease (33), idiopathic prolonged bleeding time (14), suspected drug sensitivity (16), myeloproliferative disease(18), lymphoproliferative disease(10), collagen vascular diseases (12), and storage pool disorders (3). Thirty-one determinations were performed on individuals for whom eventually no specific diagnosis was found to explain their bleeding tendency. Their test results were compared to the results of 64 determinations in hematologically normal individuals. A total of 227 bleeding times were performed in which the amount of shed blood was measured. Informed consent was obtained prior to all studies.

II. BLEEDING TIMES

Bleeding times were modified (21) from the method of Buchanan and Holtkamp(22) using the Simplate II device (Organon Teknika, Jessop, MD). All determinations were performed by placing the device firmly against the forearm in the perpendicular (vertical) position relative to the elbow crease(21-24). The length of the incision was 5 mm. The drops of blood flowing from the wound were gently collected by touching the edge of the Whatman #1 filter paper to the blood every thirty seconds until bleeding ceased. All tests were performed by a single experienced person (JPC) in a room with a constant temperature of 70^{o}F (24).

III. HEMOGLOBIN ELUTION

After completing the bleeding time, the filter paper was allowed to air dry overnight and then was rolled into a small cyclinder and placed into a test tube containing 10 ml of 5% NaOH(18). The tube was gently rocked for five to six hours to elute the hemoglobin. Standards were prepared using known amounts of normal fresh blood diluted in 10 ml of 5% NaOH. The absorbance of each solution was read against 5% NaOH at 546A in a Gilford spectrophotometer, and, using linear regression, the slope of the absorbance of the standards versus their values was determined. As an assay control, one day prior to the assay a blood sample was selected and 100 µl pipetted onto a filter paper in spots to mimic the pattern of a bleeding time. Duplicates were prepared. These were dried overnight and eluted in the same manner as the filter papers used to perform the bleeding time determinations. An identical quantity of the same blood was placed directly into 10 ml 5% NaOH. The amount of blood placed on the filter paper and in the test tube was calculated and these controls were always determined to be within $\pm$ 1 mg of each other. The results which were expressed as mg of hemoglobin were converted to µl whole blood using each patient's peripheral blood hemoglobin level measured on the day of the bleeding time study.

IV. HEMATOLOGIC MEASUREMENTS

Hemoglobin levels and platelet counts were measured from blood collected in lavender topped vacutainer tubes (Na_2 EDTA) on a Coulter Counter, Model T540 (Coulter Electronics, Hialeah, FL).

V. STATISTICAL ANALYSIS

The mean and standard deviation (SD) was calculated for each parameter measured in each group studied. The means of the groups were compared using factorial ANOVA

analysis. Linear regression, correlation coefficients (r), and multiple regression analyses were used to measure the relation between the parameters studied. Since all measurements were performed on all of the individuals studied, the number of samples (N) in each group remains consistent (see Methods I. Subjects). A significance limit (p) of <0.05 was considered significant for all tests.

RESULTS

Hematological values (mean ± SD) bleeding times (BT), whole blood shed (WB shed) and the significance (p) of differences in these parameters for each of the groups is shown in Table 1. Women had a more prolonged bleeding time than men ($p<0.01$) in the bleeding subgroup (9.8 ± 3.4 vs 8.3 ± 3.1). Regression analysis results of bleeding time, hematocrit (Hct) and platelet count (Plt) vs. total µl blood shed and average volume of blood shed per minute are shown in Table 2. The relation between bleeding time and shed blood for all patients is shown in Fig 1, and for patients with bleeding disorders in Fig 2. The relation between hematocrit and shed blood is shown in Fig 3 for normal subjects and is summarized in Table 2 for bleeding disorders. The mean bleeding time in the normal group was 7.1 ± 1.2 minutes (Table 1) which was significantly lower than for the other groups. In patients without ITP, but in whom a diagnosis of some other bleeding disorder was established, the mean bleeding time was 9.0 ± 3.5 minutes with 224.7 ± 184 ml of shed blood for a mean blood loss rate of 23.3 ± 14.4 µl/min (Table 1). In patients with ITP the mean bleeding time was 14.0 ± 4.1 minutes (Table 1). The volume of shed blood was 508.1 ± 387 µl. In patients with ITP the mean platelet count was 62.3 ± 27.5 x 10^9/L (Table 1). There was a significant correlation between bleeding time and platelet count in the ITP group ($r = 0.43$, $p<.05$) (Figure 4). There was an overall correlation between hematocrit and bleeding time in the entire population studied with an r of 0.28 ($p<0.001$) (Table 2). A significant correlation was observed in the normal group with the µl of shed blood correlating with peripheral hematocrit, ($r = 0.32$, $p<0.01$) (Table 2) and in the group with bleeding disorders ($r=0.26$, $p<0.01$) (Table 2).

DISCUSSION

Our data show that normal individuals and those with bleeding disorders who have higher hematocrits have shorter bleeding times and less blood shed at the site of bleeding times than do subjects with lower hematocrits. There has been interest in the role of the hematocrit on bleeding going back to the original demonstration by Duke in 1910(25) that bleeding times improved after correction of anemia by transfusion of packed red blood cells, a finding confirmed in more recent studies of patients with anemia due to renal failure(5,26-29). In the study of Gerrard et al(15), the correlation between hematocrit and bleeding time was greater than any other abnormality, including the platelet count, platelet aggregations to collagen, epinephrine, ADP and arachadonic acid, von Willebrand's factor antigen, patient age, platelet adhesion to glass beads and prothrombin consumption. The effect of hematocrit in reducing bleeding has been attributed to the release of a platelet aggregating factor from the red blood cells(2-6), a factor later identified to be ADP(28). Another line of investigation has demonstrated that a rheological effect of hematocrit was also involved: Turrito and Weiss(30) demonstrated that platelet deposition on the subendothelium was proportional to the hematocrit of the flowing blood. The present study supports the positive relation between hematocrit and bleeding time as well as actual blood loss in a group of subjects who were either normal or who had bleeding disorders other than ITP.

In patients with ITP the stronger correlation of the platelet count in predicting the bleeding time probably obscured the role of hematocrit in this setting. The relatively normal hematocrits in virtually all of the ITP patients probably did not provide enough variation in hematocrit to see the strong effect demonstrated between bleeding, thrombocytopenia and hematocrit by Blajchman et al(7) in rabbits and by Gerrard et al in patients with bleeding disorders(15). Moreover, the younger age and size of the platelets in ITP(31) may have altered the relationship between the bleeding time and hematocrit observed in normals and patients with

bleeding disorders. Experimental studies have generally supported negative correlation between hematocrit and bleeding. Hopkins et al (32) measured the rate of bleeding from standardized wounds in rats with varying levels of hematocrit. In that study packed red blood cells were administered to animal pairs selected at random to increase hematocrit. Their results showed a significant inverse correlation of the rate of bleeding with hematocrit ($r = -0.44$, $p<0.01$ $n = 75$). As mentioned earlier, Blajchman et al (7) showed a strong effect of decreasing hematocrit on enhanced bleeding in thrombocytopenic rabbits. In contrast, Cadroy and Hanson (33) demonstrated a prolongation of bleeding time in normal baboons with low as compared to high or normal hematocrits, but they did not consider the prolongation significant. Species differences may explain the variations in the relation between bleeding time, blood loss and hematocrit in these studies.

The present study confirms the finding of Sutor, Bowie and co-authors (11-14) that the intensity of bleeding is greater in patients with bleeding disorders and platelet dysfunction than normal. The magnitude of bleeding that we observed was greater than that of Sutor et al(11), both in normal subjects and in patients with bleeding disorders. This difference is explained by the difference in their technique, which involved a light superficial puncture(0.8 - 1.2 mm) versus a firmly applied 5 mm incision, which results in wound gaping and a consequent release of a greater amount of blood. Bleeding times in this study were of similar magnitude to those reported by Gerrard et al (15). They found a mean bleeding time of 9.48 ± 3.3 minutes similar to our finding of 9.0 ± 3.5 minutes in bleeding patients. Differences in the performance of bleeding times and collection of shed blood emphasize the importance of standardized technique and the difficulty that arises when studies done by different techniques are compared (3,4).

The present study supports the generalization that when a highly standardized bleeding time test is performed the amount of shed blood correlates signficantly with the bleeding time in normals as well as patients with bleeding disorders. This suggests that the bleeding time test

should predict excessive bleeding during and following surgery. Why this prediction differs from observations that indicate the bleeding time test does not correlate with total surgical bleeding(2-4,6) deserves further consideration. Most blood lost during surgery comes from the severing of relatively large blood vessels, and is due to incomplete ligation or cautery of surgically divided blood vessels, or failure of ligation or cautery of previously divided blood vessels (surgical bleeding). The remainder is related to microcapillary oozing (nonsurgical bleeding). The poor predictive value of the bleeding time probably stems from the difficulty encountered in designing studies that separate surgical from nonsurgical bleeding. Our data suggest that the bleeding time test should correlate with nonsurgical bleeding since the amount of blood shed at the site of the incision is strongly correlated with the duration of the bleeding time. Clinical studies are indicated to assess the clinical impact of variation in hematocrit on bleeding in the perioperative period. A major challenge in the design of these studies will be to separate surgical from nonsurgical blood loss since the volume of blood shed during the bleeding time test should have predictive value only for that portion of the total blood loss that was nonsurgical.

REFERENCES

1. Bowie EJ, Owen CA. The bleeding time. Prog in Hemostasis and Thrombosis, NY, Grune-Stratton 2:249-271, 1974.

2. The bleeding time. The Lancet 1991;337:1447-1448.

3. Rodgers RPC, Levin J. A critical reappraisal of the bleeding time. Sem Throm Hemost 1990;16:1-20.

4. Lind SE. The bleeding time does not predict surgical bleeding. Blood 1991;77:2547-2552.

5. Boneu B, Fernandez F. The role of the hematocrit in bleeding. Transfusion Medicine Reviews 1987;1:182-185.

6. De Caterina R, Lanza M, Manca G, Strat GB, Maffei S, Salvatore L. Bleeding time and bleeding: An analysis of the relationship of the bleeding time test with parameters of surgical bleeding. Blood 1994;84:3363-3370.

7. Blajchman MA, Bordin JO, Bardossy L, Heddle NM. The contribution of hte haematocrit to thrombocytopenic bleeding in experimental animals. British J Haematology 1994;86:347-350.

8. DeNicola P. Critical evaluation of methods for the study of blood coagulation. Thromb Diam Haemorrh 1962;7:325-335.

9. Adelson E, Crosby W. A new method to measure bleeding time: The "inversion" method. ACTA Haematol 1957;18:281-286.

10. Willoughby MLN, Allington MJ. The rate of blood loss from skin punctures during the Ivy bleeding time test. J Clin Path 1961;14:381-384.

11. Sutor A, Bowie EJW, Owens CJ. Quantitative bleeding time (hemorrhagometry): A Review Mayo Clinic Proc 1977;52:238-243.

12. Sutor, AH, Bowie EJW and Owens CJ. Effective aspirin sodium salycylate and acetaminophen on bleeding. Mayo Clinic Proc 1971;46:178-184.

13. Bowie EJW, Owen C, Hansen RJ, Isaacson J. Electronic method for quantitation of bleeding time. AM J Clin Path 1972,58:255-299.

14. Sutor AH, Bowie EJW, Thompson JH, Didisheim P, Mertens BF, Owens CA. Bleeding from standardized skin punctures: Automated technic for recording time, intensity, and pattern of bleeding. Am J Clin Path 1971,55:541-550.

15. Gerrard JM, Docherty JC, Israels SJ, Cheang MS, Bishop AJ, Kobrinsky NL, Schroeder ML, Israels ED. A reassessment of the bleeding time: Association of age, hematocrit, platelet function, von Willebrand factor, and bleeding time thromboxane B_2 with the length of the bleeding time. Clin Inves Med 1989,12:165-171.

16. Zeigler ZR. Non-occlusive bleeding times may improve the value of Ivy bleeding times. Thrombosis and Haemostasis 1990;63:371-374.

17. Blaedel WJ, Meloche VW, eds. Elementary Quantitative Analysis Theory and Practice Harper & Row, 1963.

18. Hallberg L, Nilsson L. Determination of menstrual blood loss. Scand J Clin Lab Invest 1964;16:244-348.

19. Rees MCP. Role of menstrual blood loss measurements in management of complaints of excessive menstrual bleeding. British Journal of Obstetrics and Gynaecology 1991;98:327-328.

20. Bain B, Forster T. A sex difference in the bleeding time.

21. Smith PS, Baglini R, Meissner GF. The prolonged bleeding time in hemophilia A: comparison of two measuring technics and clinical associations. Am J Clin Path 1985;83:211-215.

22. Buchanan GR, Holtkamp CA. A comparative study of variables affecting the bleeding time using two disposable devices. Am J Clinical Pathology 1989;91(1):45-51.

23. Mielke CH. Aspirin prolongation of the template bleeding time. Influence of venostasis and direction of incision. Blood 1982;60:1139-1142

24. Valeri CR, Khabbaz K, Khuri SF, Marquardt C, Ragno G, Feingold H, Gray AD. Effect of skin temperature on platelet function in patients undergoing extracorporeal bypass. J Thor Cardiovas Surg 1992;104:108-116.

25. Duke, WW. The relation of blood platelets to hemorrhagic disease. Description of a method for determining the bleeding time and coagulation time and report of three cases of hemorrhagic disease relieved by transfusion. JAMA 1910;55:1185-1190.

26. Hellem AJ, Borchgrevink CF, Ames SB. The role of red cells in haemostasis: the relation between haematocrit, bleeding time and platelet adhesiveness. Brit J Haemat 1961;7:42-50.

27. Small M, Lowe GDO, Cameron E, Forbes CD. Contribution of the haematocrit to the bleeding time. Haemostasis 1983;13:379-384.

28. Saniabadi AR, Lowe GDO, Barbenel JC, Forbes CD. Haematocrit, bleeding time, and platelet aggregation. The Lancet 1984;1409-1410.

29. The bleeding-time and the haematocrit. The Lancet May 4; 1984;997-998.

30. Turrito VT,Weiss HJ: Red blood cells. Their dual role in thrombus formation. Science 1980;207:541-543.

31. Harker LA, Slichter SJ. The bleeding time as a screening test for evaluation of platelet function. NEJM 1972;287:155-159.

32. Hopkins RW, Fratianne RB, Rao KV and Damewood CA. Effects of hematocrit and viscosity on continuing hemorrhage. Jour Traum 1975;14:482-493.

33. Cadroy Y, Hanson SR. Effects of red blood cell concentration on hemostasis and thrombus formation in a primate model. Blood 1990;75:2185-2193.

Table 1

Hematologic Parameters, Bleeding Times, and Shed Blood Measurements In The Four Groups Studied+

	Number	Hemoglobin gm/dl	Hematocrit %	Platelet $x10^9/l$	Bleeding Time min	WB shed μl	WB shed/min μl/min
All	227	13±1.8	38.4±4.8	265.3±154	9.1±3.7	232.3±221.2	23.3±14.5
Bleeding Patients	137	13.1±2.1	38.7±5.5	303.4±168	9.0±3.5*	224.7±184*	23.3±14.4*
ITP Patients	26	12.4±1.3	36.5±3.7	62.3±27.5*	14±4.1*	508.1±387*	34.1±22.3*
Normal	64	13.0±1.3	38.4±3.3	266.1±61	7.1±1.2	136.4±47.2	19.1±6.2

+mean±SD
*$p<0.05$ compared to Normal

Table 2

Regression Analysis of Bleeding Times and Shed Blood Measurements with Platelet Counts and Hematocrits

	All	Bleeding Patients	ITP Patients	Normal
	r,p	r,p	r,p	r,p
BT vs μl shed	0.75, 0.001	0.73, 0.001	0.61, 0.001	0.47, 0.001
BT vs μl/min	0.4, 0.001	0.30, 0.001	0.34, 0.05	0.05, NS
Plt vs BT	0.34,0.001	0.19,0.05	0.43, 0.05	0.07, NS
Plt vs μl/min	0.07, NS	0.05, NS	0.2, NS	0.3, 0.05
Plt vs μl shed	0.23,0.001	0.05, NS	0.32, NS	0.24, NS
BT vs Hct	0.28, 0.001	0.27,0.01	0.21, NS	0.34, 0.01
μl shed vs Hct	0.27, 0.001	0.26,0.01	0.35, NS	0.32,0.01
μl/min vs Hct	0.24,0.001	0.22, 0.05	0.32, NS	0.16, NS

Figure1: The relation of bleeding time to shed blood for all patients

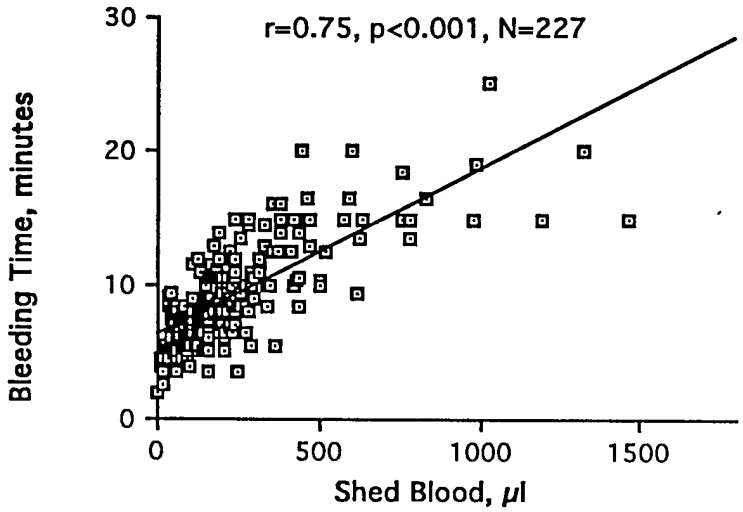

Figure 2: The relation of bleeding time to shed blood in patients with bleeding disorders

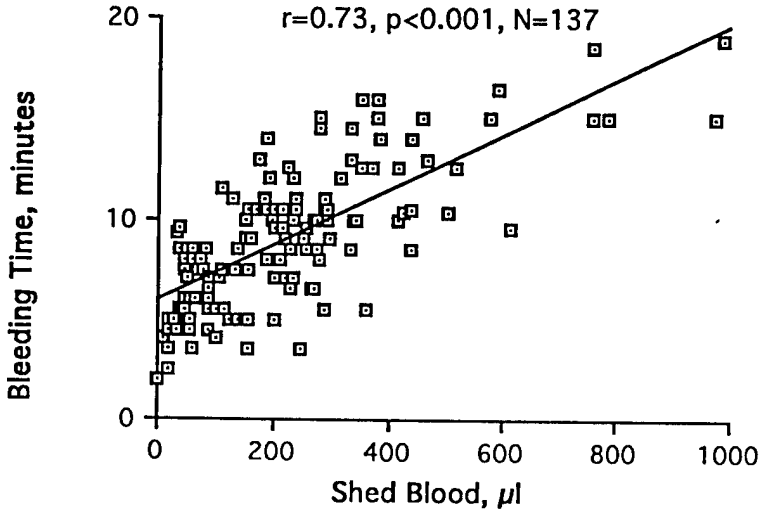

Figure3: The relation between hematocrit and shed blood in normal individuals

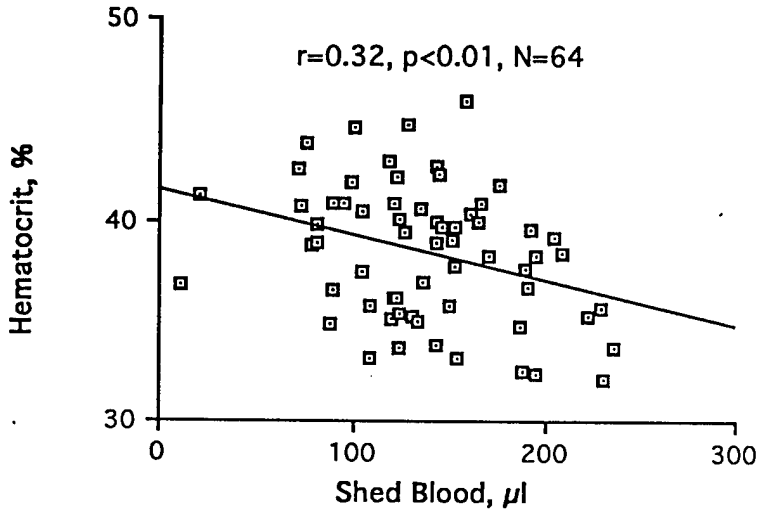

Figure4: The relation between bleeding time and platelet count in patients with ITP