THE VOLUME OF BLOOD SHED DURING THE BLEEDING TIME CORRELATES WITH THE PERIPHERAL VENOUS HEMATOCRIT

BY

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ABSTRACT

The relation between the bleeding time, the peripheral venous hematocrit and the amount of blood shed at the template bleeding time site has not been previously defined. We studied this relation in 227 individuals: 26 were patients with ITP, 137 were patients with a variety of other bleeding disorders, and 64 were normal subjects. The bleeding time for the normal group was 7.1 ± 1.2 minutes, and the amount of shed blood was 136.4 ± 47.2 µl. In patients with ITP the bleeding time was 14.0 ± 4.1 minutes and the shed blood was 508.1 ± 387 µl. 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 ± 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 a correlation of 0.28, p<0.001 with respect to the peripheral venous hematocrit. There was also a significant correlation between the bleeding time and the platelet count (r = 0.43, p<0.05), in patients with ITP. 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.

Key Words: Bleeding time, hematocrit, thrombocytopenia, hemostasis
INTRODUCTION

The bleeding time is a commonly used test for assessing hemostasis. Its primary application has been in the evaluation of platelet function and the platelet related bleeding disorders (1,2). However, there has been much controversy regarding the utility of the test. Factors usually viewed as causing variability in the bleeding time test include small variations in technique between operators, direction and placement of the incision on the arm, skin temperature, and peripheral venous hematocrit (3,4). 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). A recent study in rabbits reported a significant correlation between bleeding in thrombocytopenic rabbits and the hematocrit (7). In addition, some 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 the blood shed from the incision during the modified Ivy bleeding time by subtracting the weight of the filter paper prior to the bleeding time from the weight of the filter paper plus dried blood collected during the bleeding time. 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 dried filter paper does not take the effects of the hematocrit into account as it only measures the weight of the
dried hemoglobin. Nor does it consider changes in the weight of the filter paper due to
the
degree of humidity in the office or laboratory, a factor which is difficult to control(17).

Therefore, we modified the method of Zeigler (16) according to the technique of Hallberg and
Nilsson (18,19) of eluting the hemoglobin on the filter paper and measuring it
spectrophotometrically. 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. To further
eliminate variables, all bleeding time tests were performed by a single experienced operator in a
room with a constant temperature.
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 (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°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 cylinder 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 of well mixed blood were 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 ±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 (Na2 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, 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. 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 and the volume of shed blood was 508.1 ± 387 μl (Table 1). In patients with ITP the mean platelet count was 62.3 ± 27.5 x 10^9/L (Table 1). Regression analysis results of bleeding time, hematocrit (Hct) and platelet count (Plt) vs. total μl WB shed and average WB 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. 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. In addition, 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 significantly with the bleeding time in normal individuals 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


Figure 1: The relation of bleeding time to shed blood for all patients

$r=0.75, p<0.001, N=227$
Figure 2: The relation of bleeding time to shed blood in patients with bleeding disorders

$r=0.73, p<0.001, N=137$
Figure 3: The relation between hematocrit and shed blood in normal individuals

$r = 0.32, p < 0.01, N = 64$
Figure 4: The relation between bleeding time and platelet count in patients with ITP.

$r=0.43$, $p<0.05$, $N=26$
Table 1

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

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

+mean±SD
*p<0.05 compared to Normal
Table 2

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

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