THE BLEEDING TIME AS A SCREENING TEST FOR EVALUATION OF PLATELET FUNCTION

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Abstract

The value of the standardized template bleeding time was studied in 100 normal subjects and 136 patients with various disorders. With normal platelets the bleeding time in this test is 4.5 ± 1.5 minutes (± 1 S.D.) when the circulating platelet concentration exceeds 100,000 per microliter, and is 30.5 - (platelet count per μl/3850) minutes at platelet counts between 100,000 and 10,000 per microliter. More prolonged bleeding times reflect impaired platelet function, as associated with acetylsalicylic acid ingestion, uremia, or von Willebrand's disease. In contrast, bleeding times shorter than predicted with normal platelets are due to increased hemostatic competence of young platelets, associated either with idiopathic thrombocytopenic purpura or with bone-marrow recovery after chemotherapy. The standardized bleeding time measures the overall hemostatic role of platelets in vivo, and is thus suitable for systematic screening.

THE concept of measuring the bleeding time to evaluate the hemostatic effectiveness of platelets was introduced by Duke, although his original method was not sufficiently sensitive or reliable for that purpose. Ivy subsequently increased bleeding-time sensitivity by using a sphygmomanometer to elevate capillary pressure and, thus, to increase the hemostatic requirement. Reliability of the test was improved by substitution of a superficial incision of standard depth and length for Ivy's puncture wound. We have found this improved method useful as a bedside screening test of platelet hemostatic function.

METHODS

Subjects Investigated

Measurements of bleeding time and platelet count were performed on 100 normal, young adult males, who denied taking any medication during the preceding two weeks. Ten of these subjects were subsequently given 0.6 g of acetylsalicylic acid (ASA) daily for two days, and the studies were repeated. The same 10 subjects were later given 3 g of ASA daily for two days, and studied over the following eight days.

The bleeding time, platelet count, platelet size and in vitro platelet function were determined in 136 patients assigned to eight different categories: 70 patients with stable thrombocytopenia due to acquired impairment of platelet production (drug-induced or idiopathic) who were receiving no medications during the preceding week (five of these patients who had platelet counts below 10,000 per microliter and bleeding times greater than 60 minutes were studied after freshly prepared platelet concentrates were transfused from normal donors); 11 patients with modest thrombocytopenia due to splenomegaly (cirrhosis of the liver in four patients, myeloid metaplasia in two, Gaucher's disease in two, and lymphoma in three); 10 patients from four different kindreds with autosomal dominant hereditary thrombocytopenia; 12 patients with a diagnosis of idiopathic thrombocytopenic purpura (ITP) (criteria included thrombocytopenia for at least six months in association with increased megakaryocyte number and size, shortened platelet survival with a documented response to steroid therapy, and no other known associated pathologic process); five patients with cancer and normal platelet counts who received experimentally 60 mg per kilogram of intravenous cyclophosphamide on two consecutive days; nine patients with von Willebrand's disease (diagnosis based on low circulating factor VIII levels that responded to cryoprecipitate maximally 18 to 20 hours after infusion, and decreased platelet retention by glass-bead columns); 15 patients with chronic uremia (three without dialysis, four receiving peritoneal dialysis, and eight maintained with hemodialysis); and four patients with Wiscott-Aldrich syndrome (congenital thrombocytopenia, dermatitis, recurrent infections and defective immune mechanism).

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Laboratory Studies

Performance of the bleeding time involved maintenance of venous pressure at 40 mm of mercury with a sphygmomanometer, triplicate template standardized incisions of 1-mm depth and 9-mm length in previously cleansed volar skin of the forearm. The time required for cessation of bleeding was then measured by blotting with filter paper every 30 seconds without disturbing the wound. To avoid visible scarring, incisions were made in a longitudinal direction, and the edges of the wound were held together for 48 hours with butterfly tapes on completion of the test. The template gauge and blade holder were machined from stainless steel to facilitate proper sterilizing for reuse. Placement and adjustment of a disposable blade were simplified by incorporation of the hub of a surgical knife handle and a positioning screw in the blade holder.

Platelet counts were measured with an electronic particle counter on peripheral blood collected in EDTA by means of a previously described method adapted from Bull, Schneiderman and Brecher.

Platelet volume was estimated by means of an electronic particle-sizing apparatus (Coulter Counter) after the method of Bull and Zucker, which involves testing platelet-rich plasma prepared by sedimentation of ACD-drawn blood at 37°C and diluted in Eagle’s solution. Monosized latex particles (volume 3.86 μ) were used for standardization. We calculated an index of platelet volume by dividing the patient’s mean platelet volume by the mean normal platelet volume.

Platelet aggregation was estimated from changes in optical transmission† of 0.02 M sodium citrated plasma at 37°C with a concentration of 300,000 platelets per microliter within 70 minutes of drawing. Aggregation was observed in 1-ml aliquots after the addition of ADP (0.25, 0.50, 0.75, 1.00 and 5.00 μg), thrombin (0.2 NIH units), collagen and epinephrine (25 μg). Plastic equipment was used throughout, and plasma, freshly drawn, was kept at room temperature before testing. Platelet adhesion to glass beads was measured by the modified Salzman method. In this procedure 7 ml of blood is drawn into a plastic syringe without anticoagulant and immediately passed through the standard column of beads with a constant infusion pump at the rate of 2 ml per minute. The delay between blood drawing and initiation of flow through the beads was less than 10 seconds. Platelet counts in four serial 1-ml samples of effluent blood were compared with the circulating platelet count. In 35 normal subjects, 40 ± 9 per cent (± 1 S.D.) of the platelets in the first sample and 79 ± 12 per cent in the fourth sample remained adherent in the column of glass beads. Platelet adhesion to collagen was evaluated by direct counts and by optical-density measurement. Platelet phospholipid availability (platelet factor 3) was estimated by comparison of kaolin recalcification times of platelet-poor plasma with a plasma suspension of platelets standardized at a count of 50,000 platelets per microliter. Clot retraction was measured as the amount of serum extruded one hour after incubation at 37°C of 0.5 ml of recalcified 0.02 M citrated plasma containing 50,000 platelets per microliter.

RESULTS

Effect of Platelet Count on Bleeding Time

The bleeding time† in 100 normal subjects with a mean platelet count of 250,000 ± 40,000 platelets per microliter was 4.5 ± 1.5 minutes. In patients with thrombocytopenia due to impaired production, bleeding times remained in the normal range until the concentration of platelets fell below 100,000 per microliter. At counts below 10,000 platelets per microliter, bleeding times exceeded 30 minutes. Between these platelet levels the bleeding time had a direct inverse relation with the concentration of circulating platelets (Fig. 1). The correlation coefficient was 0.971 (p less than 0.001), and the regression line was represented by y = -3850x + 117,500 (standard error of the estimate was 7220, and the standard error of the slope was 141).

Figure 1. Inverse Relation of Bleeding Time to Circulating Platelet Count in Patients with Thrombocytopenia on the Basis of Impaired Production when the Concentration of Platelets Is between 10,000 and 100,000 per Microliter.

The regression line is shown by the solid line, and 95 per cent confidence limits are indicated by the shaded area.

Normal platelets transfused into five unimmunized recipients with aplastic thrombocytopenia (platelet counts less than 10,000 per microliter) predictably shortened the bleeding time in with the elevation achieved in the platelet (Fig. 2). Furthermore, the expected relation between platelet count and bleeding time remained as the platelet count progressively fell over the next four or five days.

Bleeding times also correlated with platelet concentration in 11 patients whose thrombocytopenia was related to massive splenomegaly and the associated increased platelet sequestration (Fig. 3). Similarly, the normal relation between bleeding time and platelet count applied to 10 patients with hereditary thrombocytopenia (Fig. 3).

Effect of Young Platelets on Bleeding Time

In 12 patients with ITP and a mean platelet count...
Figure 2. Predictable Shortening in Bleeding Time when the Platelet Count Is Increased in Unimmunized Aplastic Recipients (Platelet Count Less than 10,000 per Microliter) by Transfusion of Fresh Platelet Concentrates.

As the platelet count subsequently falls, the appropriate prolongation in the bleeding time follows. The shaded area indicates the normal values with 95 per cent confidence limits.

Figure 3. Expected Relation between Platelet Count and Bleeding Time in Patients with Thrombocytopenia Due to Splenic Pooling (○) or to a Heritable Defect of the Autosomal-Dominant Type (●).

Figure 4. More than Predictable Shortening of the Bleeding Time for Normal Platelets by Young Platelets, as Found in Patients with ITP (●) or Rapidly Returning Bone-Marrow Function after Chemotherapy (▼).

Bleeding times remain short even after adjustment for the increase in platelet volume (○). Impaired platelet plug formation is reflected as bleeding times longer than predicted by the platelet count as shown by 3 g of ASA daily (□) and von Willebrand's disease (○), which corrects with cryoprecipitate infusion (●). In uremia platelets have marked dysfunction in the untreated state (▼), which improves after hemodialysis (▼) and reverses to normal with peritoneal dialysis (▼). The modestly disparate bleeding times in Wiscott–Aldrich syndrome (WAS) (▲) become appropriate after adjustment for the small platelet volume (●).

Five patients undergoing transient bone-marrow suppression from high-dose cyclophosphamide showed a progressive fall in the platelet count and predictable prolongation of bleeding times. Heralding the return of marrow function, the bleeding time shortened to normal values before any rise in the platelet count was observed (Fig. 4). Based on concomitant measurements of platelet survival and platelet counts, the bleeding time shortened when the newly released platelets approximated a concentration of 25,000 per microliter of circulating blood. Platelet volume was not measurably increased at this time.

Effect of Platelet Dysfunction on Bleeding Time

Bleeding times were prolonged to 9.4 ± 2.3 minutes in the 10 normal subjects ingesting 0.6 g of ASA per day for two days (p less than 0.01). A daily dose of 3.0 g of ASA for two days further lengthened the bleeding time in all these subjects (Fig. 4) to a mean of 14.5 ± 2.6 minutes (p less than 0.01). The effect on the bleeding time diminished gradually, so that base-line values were reached four days after medication was stopped. Inhibitory effects were also noted on ADP-induced and epinephrine-induced secondary platelet aggregation and platelet adhesion to collagen fibers, although these results were less predictable.

In three patients with severe chronic uremia the
versely, peritoneal dialysis management of uremia
bleeding time was greater than 60 minutes. Con-
versely, peritoneal dialysis management of uremia
in four other patients resulted in normalization of
the bleeding times (5.3 ± 1.0 minutes). In eight
uremic patients maintained by hemodialysis the
bleeding time was 20.4 ± 2.6 minutes (Fig. 4); mod-
ification of the dialysis program to a low-flow
dialysis regimen (100 ml per minute instead of 500
ml per minute) shortened the bleeding time to 7.4
± 2.1 minutes. Changes in platelet retention by
glass-bead column and platelet adhesion to collagen
fibers paralleled the alterations in bleeding time,
although with greater variability.

In nine patients with von Willebrand's disease,
the mean bleeding time was 41.4 ± 10.8 minutes
(Fig. 4). In two of these patients transient correction
of the bleeding time to normal followed the infu-
sion of 15 bags of cryoprecipitate (Fig. 4).

Bleeding times in four patients with Wiscott-
Aldrich syndrome averaged 28.3 ± 3.4 minutes,
which was longer than expected for the plate-
let count. However, when their platelet counts were
adjusted for the decrease in platelet size (platelet
index, 0.6), bleeding times appeared to correlate
appropriately (Fig. 4).

**DISCUSSION**

The observations in patients with acquired hypo-
proliferative thrombocytopenia indicate that platelet
plug formation is unimpaired when normal platelets
circulate at levels of 100,000 per microliter or
higher. At lesser concentrations, however, bleeding
is inversely related to the platelet count (Fig. 1).
This relation is valid down to a platelet level of
10,000 per microliter, in the absence of interfering
medications or disease. The precision of this rela-
tion is verified by the predictable shortening of the
bleeding time produced by transfused fresh normal
platelets into aplastic recipients (Fig. 2). The effect
of normal platelets on the bleeding time between
concentrations of 100,000 and 10,000 per microliter
is expressed by the equation:

\[
\text{bleeding time (minutes)} = 30.5 - \frac{\text{platelet count per μl}}{3.850}
\]

The template bleeding time therefore appears to
be a useful measure of platelet hemostatic function
in vivo, and applies to thrombocytopenia due not
only to marrow failure but also to other conditions,
such as splenomegaly (Fig. 3). These data indicate
that the platelets left in circulation by an enlarged
spleen function normally, findings at variance with
observations by Shulman et al.15 Similarly, the
platelets that circulate in patients with autosomal dom-
inant thrombocytopenia function normally (Fig. 3)
although the concentration is reduced as a result of
defective thrombopoiesis.16

Studies with ASA demonstrate the sensitivity of
the bleeding time for detecting platelet dysfunc-
tion.17-19 When normal subjects ingest 0.6 g of ASA,
the bleeding time doubles, whereas a 3-g daily dose
in the same subjects produces a threefold prolonga-
tion (Fig. 4). The usefulness of monitoring platelet

*Derived from the regression line \( y = -3.850x + 117,500 \) (Fig. 1) by
solving for the bleeding time "x".

function with the bleeding time is also illustrated
by the uremic patients, in whom the marked prolon-
gation before treatment improved greatly with
hemodialysis therapy, and was completely corrected
by peritoneal dialysis, apparently reflecting the
difference in efficiency of artificial and physiologic
dialyzing membranes, respectively.20 In accord with
published observations, the bleeding time reliably
detects pathologically impaired platelet plug forma-
tion associated with hereditary disorders of platelet
function involving both the extrinsic disorders (e.g.,
von Willebrand's disease) and intrinsic abnormali-
ties (e.g., thrombasthenia).21-24 Although the bleed-
time in Wiscott-Aldrich syndrome is abnormally
prolonged in terms of platelet number, function
appears normal when related to platelet mass
(Fig. 4).

The unexpected shortening of the bleeding time
in patients with ITP suggests that these platelets
have enhanced hemostatic capacity; these results
are in agreement with animal and in vivo stud-
ies.25-28 Although these young platelets are of
increased size, significant functional enhancement is
evident even after platelet-mass correction (Fig. 4).
The increased functional integrity of young platelets
is further evidenced by the shortening of the bleed-
time to normal with returning marrow function
after cyclophosphamide suppression (Fig. 4).

On the basis of these studies, the standardized
template bleeding time is considered a valuable
measure of the platelet's role in hemostasis with
sufficient sensitivity and reliability to serve as the
best clinical screening test of platelet function. It is
particularly useful in the systematic evaluation of
patients with thrombocytopenia because of its ca-
pacity to discriminate between platelets with nor-
mal and those with decreased or increased function.

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