Comparison of Platelet Glass Bead Retention Techniques in Patients with Clinical Bleeding Disorders

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ABSTRACT

Platelet glass bead retention by the Salzman and infusion pump techniques were compared in 36 control individuals, 10 patients with von Willebrand's syndrome and four patients with thrombocytopathies. No significant differences between the results of the two assays were observed.

Eleven percent or less of the control individuals had diminished platelet glass bead retention. The mean percentage platelet retention of the von Willebrand's group and the thrombocytopathy group was less than the mean platelet retention of the control group. However, at least 50 percent of the patients with von Willebrand's syndrome had normal platelet retention by both methods. In the thrombocytopathy group, glass bead retention was more consistently diminished.

Although an inverse correlation existed between the duration of bleeding time and the platelet retention assays, consistently diminished platelet retention results were observed only when the bleeding time was 18 minutes or longer. It has been concluded by us that while the glass bead retention assays may be helpful in characterizing certain types of platelet disorders, they are not useful screening assays in the diagnosis of von Willebrand's syndrome.

Introduction

Platelet glass bead retention is reduced in von Willebrand's syndrome and in a variety of thrombocytopathies. Hellem was the first to standardize a method of pumping citrated whole blood or platelet-rich plasma through a column of glass beads at a constant rate by means of an electrically driven device. He noted that platelet retention was zero when platelet-rich plasma was used and was diminished in anemic patients as compared to normal individuals. In addition, Hellem detected decreased platelet retention in three patients with thrombasthenia. Because of the slow rate of blood flow through the beads, the method did not...
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distinguish clearly von Willebrand’s syndrome from normal individuals.

Salzman introduced an in vitro technique for platelet glass bead retention in which the patient’s blood passed directly through a glass bead column into a partially evacuated tube containing disodium ethylene diaminetetraacetate (Na₂ EDTA). In his initial report, ten of 11 patients with von Willebrand’s syndrome and one patient with thrombasthenia had a platelet retention assay below the normal range.

Bowie et al modified the Hellem assay so that heparinized blood samples were pumped through a glass bead column at constant velocity. In their report, platelet retention was decreased when flow rates were greater than 3 ml per min for a column containing 2.6 g of glass beads. They reported that six patients with von Willebrand’s syndrome had platelet retention of 17.6 percent ± 19.3 (18 determinations), whereas in 34 determinations in normal subjects, platelet retention was 89.9 percent ± 8.8 (mean ± standard deviation).

It was shown by Bowie et al that blood flow rates through glass bead columns are critical in demonstrating abnormalities of platelet retention in patients with von Willebrand’s syndrome. In addition, it was demonstrated that the type of plastic used in construction of the column, the diameter of the column, quantity of glass beads and type of anticoagulant are variables that can alter the reproducibility of the platelet retention assay.

Platelet glass bead retention also may be reduced in thrombasthenia, storage pool disease, release disorders and other thrombocytopenias. Since numerous sources of technical variation exist in both methods, the purpose of this report is to describe our experience in the use of the Salzman platelet retention (SPR) and infusion pump platelet retention (IPPR) assays in 36 normal individuals and 14 patients with bleeding abnormalities.

**Materials and Methods**

**Patient Population**

From a group of 144 consecutive individuals, studied for the first time in our coagulation laboratory between the years 1972 and 1976, 36 normal individuals, ten individuals with von Willebrand’s syndrome, and four patients with thrombocytopenias were selected. The other 94 patients had a variety of congenital or miscellaneous coagulation abnormalities.

Individuals were placed in the control group when their medical history contained trivial or insignificant bleeding episodes and coagulation studies disclosed a normal platelet count, bleeding time, platelet aggregation, prothrombin time, partial thromboplastin time, thrombin time, factor VIII-procoagulant (VIII : C), factor VIII related antigen (VIII R : Ag), and factor VIII ristocetin co-factor (VIII R : VWF) assays.

Ten individuals were considered to have von Willebrand’s syndrome on the basis of a clinically significant bleeding history, prolonged bleeding time, normal platelet count, decreased factor VIII : C, decreased factor VIII R : Ag and, in some cases, diminished factor VIII R : VWF. All other coagulation assays were normal, including platelet aggregation with ADP, epinephrine and collagen.

Four individuals were judged to have a thrombocytopenia because of a significant bleeding problem, prolonged bleeding time with a normal platelet count, and an abnormal platelet aggregation. The latter included the presence of primary aggregation although the percent of maximum platelet aggregation was less than 50 percent of normal with at least two of three reagents including collagen, epinephrine and ADP. Three of these individuals were tested more than once and demonstrated persistent abnormalities of platelet function.

All control subjects and patients had not received any medications within ten
days of the performance of the coagulation and platelet studies.

Thirty-nine SPR and IPPR assays were performed on the control group; 15 SPR and IPPR tests were done on the patients with von Willebrand's syndrome; and 14 SPR and IPPR examinations were carried out on blood from the patients with thrombocytopenias.

**Saltzman Platelet Retention Assay**

One tube of whole blood was collected with a 20 gauge siliconized needle directly into a glass vacuum tube containing Na₂EDTA. A second tube of whole blood was collected with the same needle through polyvinyl tubing,* inner diameter 0.113 inch containing 1.3 g of sodium-lime-silica glass beads with an average diameter of 0.0185 inch (Super-brite, type 070)† into a glass vacuum tube containing Na₂EDTA. Blood filled the second tube in approximately 45 seconds. At each end of the polyvinyl tubing, blood passed through siliconized nylon mesh with openings of 0.002 inch and siliconized adaptors.‡ Platelet counts were then performed on an Autocounter§ on each specimen, and the percent of platelets retained in the glass bead column was calculated.

**Infusion Pump—Platelet Retention Assay**

Ten ml of whole blood were collected with a 20 gauge siliconized needle into a plastic syringe and were transferred into a plastic tube that contained 40 units of heparin and gently mixed. Polyvinyl tubing (see Salzman) was packed with 2.6 g of the glass beads previously described. The heparinized blood was transferred to a 20 ml plastic syringe which was then attached to a pump (model 341).* The blood was pumped through the glass bead-packed polyvinyl column at 6.4 ml per min and collected in 1 ml samples in glass tubes containing Na₂EDTA. Since the greatest number of platelets retained by glass beads was observed by the fourth tube, the platelet count from this sample was consistently used in the calculation of platelet retention. The blood passed through siliconized nylon mesh and adaptors as described under the Salzman technique.

The following platelet and coagulation measurements were performed according to standard methods: platelet count,* bleeding time,† platelet aggregation,* prothrombin time,‡ partial thromboplastin time,§ thrombin time,¶ factor VIII:C assay¶ and factor VIII R:VWF.‖ The factor VIII R:Ag was determined utilizing a heterologous rabbit antiserum in a radioimmunoassay.

**Statistical Test**

Where appropriate, the data were statistically analyzed by a chi-square and Dunnett's T test.

**Results**

Previously established 95 percent reference values for this laboratory were standardized bleeding time, less than 7½ minutes; SPR, 20 to 80 percent; and IPPR, 70 to 100 percent. Platelet response to aggregating agents was judged abnormal when the patient’s maximal platelet aggregation was less than 50 percent of that of the control. The reference values for factor VIII:C were 50 to 150 percent of pooled normal plasma. Those for factor VIII R:Ag were 60 to 185 percent and for factor VIII R:VWF, 50 to 194 percent.

Results from the control group indicated that 2.5 percent (1/39) of the SPR
assays and 7.6 percent (3/39) of the IPPR assays were less than the previously established reference values (figure 1).

The mean percentage glass bead retention of the patients with von Willebrand's syndrome was significantly less than the mean percentage glass bead retention of the control population by the SPR method (p < 0.05) and the IPPR technique (p < 0.01) (figures 2 and 3). However, in 15 assays performed in 10 patients, the SPR assay was less than 20 percent retention in two and the IPPR assay was less than 70 percent in six. Chi square analysis indicated no significant difference between the results of these two measurements. Using both assays together, only 50 percent of patients with von Willebrand's syndrome showed a platelet retention below the previously established reference values.

The mean platelet retention of the patients in the thrombocytopathy group was significantly less than the mean of the normal population by the SPR method (p < 0.01) and the IPPR technique (p < 0.01) methods (figures 2 and 3). The SPR and IPPR assays were each less than the previously established reference values in 12 of 14 evaluations on four patients.

In addition, a rough correlation was observed between platelet glass bead retention and length of bleeding time in 28 assays performed on 10 patients with von Willebrand's syndrome and four patients with thrombocytopathies (figure 4). However, the platelet retention assays were not consistently less than reference values until the bleeding time was greater than 18 minutes.
Discussion

Hellem reported reduced platelet adhesiveness in patients with thrombosthenia but normal adhesion in patients with von Willebrand’s syndrome, idiopathic thrombocytopenia and in patients with a variety of defects in circulating procoagulants. Because of the complexity of the procedure, it was rarely used as a routine laboratory assay.

Salzman devised a method whereby native blood flowed over glass beads into a vacuum tube containing Na2 EDTA. With this method, contact time between blood and foreign surface was short and no anticoagulant was necessary before blood came into contact with glass beads. He noted that platelet retention was normal in patients with bleeding disorders secondary to a procoagulant deficiency but was reduced in 10/11 patients with von Willebrand’s syndrome.

In the Salzman assay, the flow rate varies as the vacutainer fills. O’Brien and Heywood showed that the rate of flow was crucial in demonstrating a difference between platelet retention in patients with von Willebrand’s syndrome and in normal individuals. Bowie and associates modified the Hellem and Salzman tests to control for the variable flow rates. Blood was collected in a plastic syringe containing four units per ml of sodium heparin. One ml of blood was put into a tube containing Na2 EDTA and platelet counts were performed on this control sample. The rest of the sample was pumped, at a constant rate, through a column of beads and the results were expressed as a percentage of platelets retained. Bowie et al found that the difference in platelet retention between von Willebrand’s syndrome and normals was much greater than that demonstrated in the Salzman test. The platelet retention was decreased in von Willebrand’s syndrome without regard to clinical severity. Platelet retention was also decreased in patients with Glanzmann’s thrombosthenia, thrombocytopenies and myeloproliferative disorders. In addition, it was reduced in patients with valvular heart disorders and intravascular coagulation.

In the current study, one of 36 normal individuals had a decreased platelet retention by the SPR method and three of 36 showed diminished retention by the IPPR technique. Although the mean percentage of platelet retention from patients with von Willebrand’s syndrome was significantly less than the mean platelet retention of the control group, most of the results of the platelet assays from patients with von Willebrand’s patients were within the reference values. In addition, platelet retention was less than the previously established reference values in only 20 percent of patients evaluated by the SPR assay and 50 percent of patients tested with the IPPR technique.

Results of platelet retention from patients with thrombocytopenias were usually below the previously established reference values. The values were less in 12 of 14 platelet retention assays performed by both methods on four patients.

A cooperative study indicated that only 15 percent of patients with von Willebrand’s syndrome could be clas-
sified as having had normal platelet retention, but this was determined at a level that misclassified 20 percent of normals. Were we to accept 20 percent false positive rate for normals, then a greater proportion of our patients would have had a decreased platelet retention.

The current study suggests a rough correlation between the standardized bleeding time and decreased platelet retention. In addition, the platelet retention assays were consistently less than normal when the bleeding time was greater than 18 minutes.

These data suggest that the platelet glass bead retention assay by either method was less sensitive than the standardized bleeding time as a screening assay for patients with von Willebrand’s disease and thrombocytopenies.

In summary, the data presented in this report suggest that the mean percentages of platelet retention from patients with von Willebrand’s syndrome and thrombocytopenies were significantly less than the control group. However, in the von Willebrand’s group the results of the platelet retention assays were more frequently in the low normal than abnormal range. In the thrombocytopenies, the results of the platelet retention assays were usually less than the previously established normal range. Thus, neither platelet retention method appeared to be an effective screening assay for von Willebrand’s syndrome. The platelet retention assays may be helpful, however, in characterizing some platelet disorders.

References


