Assessment of Neonatal Platelet Function
Using a Viscoelastic Technique*

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ABSTRACT

The Sonoclot®, a simple viscoelastometer, measures changes in the viscoelastic properties of plasma as it is recalcified. Platelet rich plasma (PRP) and platelet poor plasma (PPP) each have characteristic Sonoclot® tracings. This technique was applied to evaluate the coagulability and platelet function, specifically, in PRP prepared from 53 normal term newborn cord bloods and whole blood from 17 very low birth weight premature infants. These values were compared to each other and to those previously reported adult control values obtained by one of the authors (C.B.). The cord PRP samples had shorter time related parameters than adult controls. The values from premature infants were suggestive of diminished platelet function when compared to cord values but were similar to adult values. This technique allows the use of small sample volumes, the minimization of the introduction of infection risks, and avoids manipulation of fragile, very premature infants.

Introduction

Assessment of platelet function has presented a challenge to clinical investigators. Neonatal platelet function, specifically, has been assessed in both quantitative and qualitative studies. Quantitative studies have demonstrated that preterm and term newborn infants have platelet counts that fall within the normal adult range. Qualitative tests have shown conflicting results and, thus, what remains controversial and largely unknown is the relative ability of the neonatal platelet to function in vivo.

Qualitative studies include in vitro and in vivo methods. When compared in vitro with adult and/or children's platelets, neonatal platelets show the following relative properties: Aggregation of platelets in platelet rich plasma (PRP) is decreased when low molar adenosine diphosphate (ADP), collagen, epinephrine, or thrombin are used as the aggregating agent. Platelet factor 3 (PF3) release and content are defective and clot retraction is decreased. Platelet adhesiveness to glass surfaces has been
shown to be normal. Another in vitro system, the thromboelastograph, has been used to assess platelet function in adults and newborns; however, it requires relatively large blood volumes, and data collection and interpretation are difficult. All of these in vitro studies may be difficult to interpret in light of ongoing coagulation events in vivo.

The accepted in vivo test of platelet function is the bleeding time. This test reflects true platelet-capillary interaction. Fuesner used a modified Mielke technique to demonstrate that bleeding times of healthy term and preterm infants did not differ from those of older children and adults. In spite of its usefulness, the bleeding time in neonates may be a difficult procedure to perform and standardize. The variables of what cuff pressure to use, what surface to use, variation in incision depth with variation in subcutaneous tissue depth, and the interference of bleeding from venules rather than capillaries remain difficult to control. It may further represent an infection inducing risk. Owing to patient manipulation, it may present an adverse stimulus in fragile premature infants since one of the proposed etiologies for intraventricular hemorrhage is rapid changes in blood pressure, cardiac output, and central venous pressure in the face of an immature cerebral circulation.

In addition to the previously mentioned tests of platelet function, single step coagulation tests are prolonged in newborns as compared to adults. This reflects relative deficiencies of vitamin K dependent and early contact factors. Nevertheless, healthy neonates do not demonstrate a bleeding diathesis and, in fact, have shorter whole blood clotting times than do adults. This contradiction has been termed the “paradoxical hypercoagulability” of the neonate.

Recently, another in vitro method has been described by Saleem et al which measures overall coagulability and platelet function, specifically. This method, the Sonoclot®, uses a simple viscoelastometer to measure changes in viscoelasticity of plasma as it is recalcified. In the description and application of this technique, Saleem et al showed that one Sonoclot® parameter, the shoulder to peak (S-P) interval, is a sensitive, reproducible indicator of platelet function. In that study, mean bleeding time was prolonged in patients with abnormal Sonoclot® tracings who demonstrated clinical platelet dysfunction (i.e., bleeding). Sonoclot® shoulder to peak intervals allowed a better separation of the groups into normal versus abnormal platelet function than did bleeding time.

Since this technique requires minimal blood volumes, minimal stimulus to the patient, and is a specific index of platelet function, Sonoclot® technique was used to study 53 normal newborn cord bloods and samples obtained within the first 24 hours of life in 17 ill, very low birth weight (VLBW) premature infants. These two populations have been compared to each other and to the previously reported normal adult controls.

Materials and Methods

Patient Population

All patients were born at the Jefferson Davis Hospital, Houston, Texas. The experimental protocol used in the study was approved by the institutional review committee of Baylor College of Medicine. Enrollment of infants occurred after informed consent was obtained from the parents after the nature of the procedure had been fully explained. The assent of the child was waived because of age.

As shown in table I, the mean gestational age, birth weight, and Apgar

scores of the infants from whom cord blood was obtained were 39.2 (0.2) weeks, 3275 (83) grams and Apgar Scores of 8/9, respectively. [Values are mean (S.E.M.)] Likewise, the premature infants had gestational age 29 (0.5) weeks, birth weight 1133 (49) grams, and Apgar scores of 5/7, respectively.

All infants whose cord blood was assayed were ready for discharge 72 hours after delivery and none developed bleeding or thrombotic disorders. One infant had a positive direct Coomb’s test without evidence of hemolysis; three infants required screening for sepsis owing to prolonged rupture of membranes; one infant had extra postaxial digits bilaterally; and one infant required an intramuscular injection of benzathine penicillin for a positive RPR.

All 17 VLBW infants were intubated and mechanically ventilated. All received 0.5 mg vitamin K₁ oxide (Aquamephyton®) intramuscularly shortly after delivery. No infant received indomethacin. There was no maternal history of recent salicylate ingestion nor did cord blood or infant blood used in the study contain significant amounts of salicylate as measured by a high pressure liquid chromatography technique. This method would detect levels as low as 0.5 mg per dl. No mothers received large doses of penicillin prior to delivery. One cord blood and two additional premature infants were eliminated from the study on the basis of detectable levels of salicylate.

Blood Sample Collection

Immediately after delivery of the infant and before delivery of the placenta, the initial 30 cm segment of double clamped umbilical cord was given to one of the investigators. As recommended by Hathaway, the umbilical vein was cannulated immediately with an 18 gauge needle, and the blood was placed in ethylenediamine tetraacetic acid (EDTA) for hematocrit and platelet count determination and in sodium citrate for Sonoclot® studies. Initial spun hematocrits were obtained. The cord bloods were all anticoagulated in 3.8 percent sodium citrate with rigid correction to the spun hematocrit to maintain a plasma:citrate ratio of 5:1. An aliquot of cord blood was also used for salicylate determinations.

Blood was collected from VLBW infants via indwelling umbilical artery catheters. As these catheters were routinely infused with solutions containing 1.0 unit of heparin per ml of infusate, sample collections were preceded by clearance of 5 ml of blood from the catheter. Samples were aliquoted similarly to the cord samples with rigid correction according to hematocrit to maintain a plasma:citrate ratio of 5:1.

Preparation of PRP and PPP

Platelet rich plasma (PRP) was obtained by centrifugation of citrated whole blood at 100 × g for ten minutes at room temperature. Platelet poor plasma (PPP) was obtained by further centrifugation of the remaining contents at 1000 × g for 15 minutes at room temperature. Sonoclot® measurements were performed within two hours of sample collection.
PLATELET COUNTS

Platelet counts were performed manually by the method of the Unopette® microcollection system and phase microscopy.

HEMATOCRITS

Hematocrits were obtained by centrifugation of blood in heparinized capillary tubes.

SONOCLOT® TRACING

Sonoclot® tests were performed by the method of Saleem et al.10 Four hundred microliter of PRP were placed in a cuvette containing a stir bar and were equilibrated to 37°C for three minutes. Twenty microliter of 0.5 molar CaCl₂ were added to the plasma. The mixture was stirred for 15 seconds; the probe was then lowered carefully into the cuvette. The recorder then was activated.

STATISTICAL ANALYSIS

Comparison between three groups was made by analyzing the data with the unpaired Student's t test corrected for multiple comparisons by the Bonferroni method. Multiple regression analyses were performed to assess the relationship between platelet count and Sonoclot® parameters.

RESULTS

A typical Sonoclot® tracing is shown in figure 1. Following recalcification of platelet rich plasma, there is a lag period (A) followed by an upward deflection (primary wave, B). Further, a shoulder (C) is produced, and this is followed by another upward deflection (secondary wave, D). The secondary wave peaks (E) and is followed by a downward deflection. Platelet poor plasma shows no deflection after the primary wave reaches a plateau.

Mechanical correlates of the different phases of the Sonoclot® curve can be described.10 Initially, the vibrating probe is inserted in a medium of uniform low viscoelasticity. As CaCl₂ is added, a lag time is observed and the primary wave signals the beginning of fibrin monomer formation with constantly increasing impedance to probe vibration in a medium of increasing viscoelasticity. Electron microscopic studies of the clot show that at the time of production of the secondary wave, platelets have become an integral part of the clot and fibrin threads have become attached to the platelet surface. At about the same time, visual inspection shows the clot
retracting from the sides of the cuvette. This phenomenon appears to correspond to the stimulation of platelet contractile protein and the contraction of cytoplasm toward the center of the platelet. These events increase clot density, which is reflected in the production of the secondary wave. At about the time the secondary wave peaks (E), the clot completely retracts onto the probe and this new probe-clot complex vibrates in expressed serum, a medium of low viscoelasticity. This results in the production of the final downward deflection of the Sonoclot® tracing.¹⁰

The results of the present study are summarized in table II. Mean Sonoclot® parameters of term cord blood showed significantly shorter time intervals and steeper slopes than did adult mean values. Comparison of 17 VLBW preterm infants’ Sonoclot® values to previously obtained adult values showed that, within the first day of life, all parameters approached adult normal values in contrast to the fast, steep slopes of the term infant cord samples. When term and preterm infants are compared, term infants have a shorter lag time and S-P interval and steeper primary and secondary wave slopes (p values for all parameters compared were <0.01). Platelet counts did not differ significantly among the three populations.

Multiple regression analyses of these data show no significant correlation for the lag time, S-P interval, primary or secondary wave slopes with platelet concentration. This analysis was also used to assess these relationships in the 17 VLBW infants and, likewise, no significant correlation was found.

**Discussion**

The present study demonstrates that cord blood PRP samples from 53 healthy newborns coagulate without difficulty; in fact, all parameters correlating with platelet function are increased over normal adult values. This is true in spite of a possible decrease in clotting factor concentrations which would be expected to increase the lag time and decrease the primary and secondary wave slopes. Clot retraction is normal, also, with all samples completing a final downward deflection. None of these infants demonstrated bleeding or thrombotic disorders. None had detectable levels of salicylate. It is impossible to exclude completely effects from contamination of the cord blood specimens with amniotic fluid or Wharton’s jelly, which might result in accelerated time related parameters of the Sonoclot® recording. However, the consistent, marked differences in term cord blood Sonoclot® recordings as compared to the normal adults suggest that at least the cord blood platelet related Sonoclot® findings are comparable to normal adults, and not an artifact of specimen collection.

When 17 ill, VLBW premature infants were examined, they demonstrated Sonoclot® values which approach those of normal adults but are significantly prolonged (p < 0.01) when compared with term, healthy newborn cord bloods.
Several points should be made with regard to these infants. Shortly after birth, all infants received 0.5 mg vitamin K$_1$ oxide via intramuscular injection. As can be seen in Table II, the platelet counts of these samples were slightly lower than those of healthy newborns. This was not significant by unpaired Student's $t$ test ($p = 0.48$). Additionally, multiple linear regression analysis did not reveal a significant correlation between the platelet counts and Sonoclot® parameters. Finally, blood was extracted via indwelling catheters infused with heparin containing solutions. Any heparin remaining in specimens for Sonoclot® determinations after recommended clearance of the catheter would be expected to delay time related parameters of the Sonoclot® recording and not artifactually skew the Sonoclot® findings of the premature infants’ blood toward normal adult values.

Despite reported *in vitro* defects of platelet function in term and preterm infants, *in vivo* measurements of bleeding times in these infants have been reported as normal relative to the adult. Fuesner, using a standardized template bleeding time; found that healthy term and preterm infants had values which did not differ from adults with bleeding times (mean ± S.D.) of 3.4 ± 0.9 and 3.2 ± 1.0 minutes, respectively.$^1$ Setzer, et al, evaluated the role of preexisting coagulation and platelet defects in VLBW premature infants with respect to occurrence of intraventricular hemorrhage and found normal bleeding times in 46 of 58 infants studied. In infants with documented intraventricular hemorrhage, two-thirds had bleeding times less than six minutes.$^{11}$ McDonald et al$^6$ reported bleeding time determinations in 50 VLBW premature infants on the first and second days of life. Infants without intraventricular hemorrhage ($n = 26$) had mean bleeding times of 4.4 and 3.6 minutes while infants with intraventricular hemorrhage ($n = 20$) had bleeding times of 5.1 and 5.6 minutes, respectively. Using a rather liberal definition of normal bleeding time (less than nine minutes), only four of 50 infants studied were felt to have prolonged bleeding times.

Two VLBW premature infants were excluded from the study because of detectable serum salicylate levels (4.3 and 2.8 mg per dl). Interestingly, these two infants had Sonoclot® S-P intervals greater than two standard deviations above the mean for normal adult values. The present authors are aware, of course, that salicylate levels per se cannot rule out lingering effects of cyclooxygenase acetylation from maternal aspirin ingestion several days prior to birth. However, when this study was performed, the methodology for determination of platelet malondialdehyde formation was not available to the investigators. Any undetected salicylate effect, again, would be expected to artifactually impair platelet related Sonoclot® measurements rather than improve those measurements to values equivalent or better than those of normal adults.

In summary, this study demonstrates that the Sonoclot® technique for assessment of platelet function is easily applicable to newborn infants. It is useful because it requires small blood volumes, requires no special manipulation of the infant nor does it introduce infection risks. The method has potential applicability in addressing qualitative platelet function in the neonate and such questions as the relationship of intraventricular hemorrhage and platelet function, diet and platelet function and the problem of drug induced platelet dysfunction.

A range of observed values for term cord blood samples have been presented. These data may reflect the described "paradoxical hypercoagula-
bility” of the newborn in comparison to the adult. The small population of ill, VLBW infants studied suggests that, indeed, even these infants possess overall coagulability and platelet function comparable to those of normal adult controls. Assessment of platelet function in the context of participation in ongoing coagulation may be more meaningful than assessment by conventional in vitro techniques.

References