Evaluation of the SonoClot Analyzer™ for the Measurement of Platelet Function in Whole Blood*

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ABSTRACT

A viscoelastometer, the SonoClot Coagulation Analyzer™, has been proposed for use in the evaluation of platelet function. The purpose of this study was to evaluate systematically this instrument when used with whole blood. Under laboratory conditions, the coefficient of variation (cv) of determinations of whole blood activated clotting time (ACT) on the instrument was approximately 7.0 percent. In contrast, the cv of measurements on whole blood related to the graphic events associated with clot formation ranged from 9.2 to 41.7 percent. Because of the large and variable cvs associated with these measurements of clotting, the SonoClot Analyzer™ cannot presently be recommended for use in studies designed to examine quantitatively the clotting function in whole blood.

Introduction

Infants may have altered platelet function because of prematurity,10 the use of drugs known to affect platelet function,7 or the use of invasive procedures, such as extracorporeal membrane oxygenation (ECMO).6 Altered hemostasis may occur as a result of either decreased numbers of platelets1 or a decreased function of platelets, or both;5 furthermore, the occurrence of hemorrhage in neonates managed with ECMO and administered heparin is associated with significant increases in morbidity and mortality.9

The SonoClot Coagulation Analyzer™, a viscoelastometer, has been proposed for use in measuring both activated clotting time (ACT) and characteristics of the clot formation process. This instrument might be of value in neonatal ECMO by determining the overall coagulability of plasma and whole blood during the process,2,3,8 by monitoring heparin administration during cardiopulmonary bypass,4,11 or by examining the changing characteristics of platelet function during the ECMO procedure. However, previously published data obtained with the

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instrument using whole blood showed a large variability in some of the clotting parameters reported in groups of patients. Part of this overall variability might have been due to the variability in the instrument measurements. The purpose of this present work, therefore, was to evaluate systematically the SonoClot Analyzer™ when used to monitor platelet function in whole blood.

Methods

MEASUREMENT OF ACT AND SLOPES ON THE SONOCLOT ANALYZER™

The SonoClot Analyzer™ was obtained from the manufacturer. The SonoClot Analyzer ACT and the entire SonoClot "Signature" were obtained on re-calculated, citrated, whole blood. For all experiments, whole blood was collected in Vacutainers™ containing 3.8 percent sodium citrate with a blood/anticoagulant ratio of 9:1 (v/v); aliquots were maintained on ice, warmed to 37°C by incubating in a waterbath for three minutes immediately before assay, and re-calculated with the addition of 25 µl of 0.5 M CaCl₂. Stability of clotting activity in these cooled, re-warmed, and re-calcified whole blood samples over six hours was demonstrated by analyzing the data for consistent changes in clotting activity. No unidirectional changes were detected.

In figure 1 is illustrated an idealized SonoClot "Signature" curve. Activated clotting time and slopes were calculated essentially as described by others. For the purposes of this study, the ACT was defined as the time, in seconds, for the viscosity of the sample to change by one chart division. Then R1 was calculated as the slope of the steepest straight line drawn from the curve of the initial viscosity change, expressed as [change in chart divisions/cm]/60, at a chart speed of one cm per minute. Then R2 and R3 were similarly calculated from the second slope and the downward deflection, respectively.

QUALITY CONTROL PROCEDURES

The SonoClot Analyzer™ quality control protocol consisted of weekly determinations of the viscosity of a standard material (SonoCal Kit™) and determinations of the temperature in the sample chamber and the chart recorder speed. The instrument was judged to be in control if determined values fell within expected ranges.

Results

In table I are listed means, standard deviations, and variability associated with determinations of the SonoClot Analyzer ACT™ and the slopes, R1, R2, and R3, calculated from the "Signature"
curves of re-calcified, citrated whole blood. Technicians for ECMO were used to determine inter-individual variation on the instrument; the personnel for ECMO received standardized training in the use of the instrument prior to determinations. As seen in table I, the cvs associated with ACT determinations averaged approximately seven percent. Also noted in table I is the range of cvs for the slopes which was 9.2 to 41.7 percent. The inconsistent slope cvs, evident when comparing intra-individual and inter-individual results, most likely reflect differences in the properties of the specific whole blood samples used in the studies as well as to the week-to-week variation of the instrument. No whole blood control material is presently available which either contains platelets or simulates platelet function; therefore, no material is available which can be used to assess the accuracy and precision of R1, R2, and R3 measurements on the SonoClot Analyzer™. It has been suggested by others that the concentration of platelets is one factor which may influence the shape of the SonoClot “Signature”™ curve and therefore, the calculation of R1, R2, and R3. SonoCal QC™, a carbohydrate polymer solution of known viscosity, can be used to examine week-to-week SonoClot Analyzer™ variability related to viscosity measurements. In the present study, the cv of the instrument using this non-blood control, was 0.77 percent measured over a four week period.

Discussion

The SonoClot Analyzer™ was originally designed as a bedside screening instrument useful in a qualitative assessment of clotting; recently, the instrument has been used for coagulation measurements after bypass surgery. In the present study, the large and inconsistent cv calculated from the slopes of the SonoClot “Signature”™ curve preclude the use of the instrument for the quantitative measurement of platelet function in whole blood. This study supports the conclusions of others that the instrument is a good viscometer. Under laboratory conditions, the SonoClot Analyzer™ can measure ACT in re-calcified, citrated whole blood similarly to other commercially-available instruments. It can measure the viscosity of pure solutions in an extremely reproducible manner.

The variation in the ability of the instrument to replicate slopes in this study can account for a large part of the variation noted in groups of patients, previously reported. The slopes calculated from the SonoClot “Signature”™ curves cannot be reasonably reproduced by either the same individual or by different individuals using the identical instrument.

An additional problem associated with using the SonoClot Analyzer™ for the

<p>| TABLE I |
| Variability of SonoClot Analyzer™ Activated Clotting Time and “Signature” Slopes of Citrated Whole Blood |</p>
<table>
<thead>
<tr>
<th>ACT</th>
<th>Slope R1</th>
<th>Slope R2</th>
<th>Slope R3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-individual Variation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>129</td>
<td>0.36</td>
<td>0.24</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>sd</td>
<td>10</td>
<td>0.08</td>
<td>0.10</td>
</tr>
<tr>
<td>cv</td>
<td>7.8</td>
<td>22.2</td>
<td>41.7</td>
</tr>
<tr>
<td>Inter-individual Variation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \bar{x} )</td>
<td>201</td>
<td>0.7</td>
<td>0.35</td>
</tr>
<tr>
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<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>sd</td>
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<tr>
<td>cv</td>
<td>6.3</td>
<td>9.2</td>
<td>20.0</td>
</tr>
</tbody>
</table>

ACT = activated clotting time.
\( \bar{x} \) = mean ACTs or slopes expressed as changes in chart units per time.
n = number of determinations.
sd = standard deviation.
cv = coefficient of variation expressed as a percentage.
measurement of platelet function in whole blood is the lack of suitable quality control material for assessing the accuracy and precision of the instrument, particularly between hospitals or laboratories. Finally, the correlation of the SonoClot "Signature"™ slopes, R1, R2, and R3, with the actual biologic events involved in clotting is circumstantial, at best.2,8,11 Few, if any, published studies provide data to support this correlation. In summary, this study does not support the use of the SonoClot Analyzer™, at this time, to examine quantitatively platelet function.

Acknowledgment

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References