Waveform analysis of clotting test optical profiles in the diagnosis and management of disseminated intravascular coagulation (DIC)

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Summary

Transmittance waveform charts the changes in light transmittance on standard coagulation assays, such as the prothrombin time (PT) and activated partial thromboplastin time (APTT). Analysis and characterization of these data on photo-optical coagulation analysers provides additional qualitative and quantitative information to that obtained using the clotting time alone. The most thoroughly evaluated clinical application is that of the biphasic APTT waveform with disseminated intravascular coagulation (DIC). The degree of waveform abnormality correlates directly with the severity of haemostatic dysfunction and allows for both the prediction and monitoring from non-overt to overt DIC. As its performance is simple and rapid, this provides the means for targeting therapeutic intervention to an earlier stage of DIC. The recent identification that the mechanism underlying the biphasic waveform is a complex that exists in vivo between C reactive protein with very low density lipoprotein, provides potentially important insights into the molecular pathogenesis of DIC. Thus, in addition to the immediate clinical utility in diagnostic practice, it has important applications as a research tool. Preliminary experience in the application of this technology to the diagnosis and management of the haemophilias and the lupus anticoagulant syndrome has also provided evidence of the power and utility of waveform analysis in essentially simple clotting assays.

Keywords
Clot waveform analysis, DIC, diagnosis, coagulation test

Introduction

Recent advances in the analysis of optical profiles obtained during the routine performance of the prothrombin time (PT) and activated partial thromboplastin time (APTT) have led to the realization that more information can be obtained from a clotting assay than the clotting time alone. Originally, these assays were conducted manually by adding plasma, an activating agent and calcium chloride to reverse the anticoagulant effect of the citrate added during blood collection into a warmed test-tube. The mixture was visually monitored to provide only a single item of information, i.e. the clotting time. However, in the hands of an experienced observer, a qualitative, albeit subjective, assessment of fibrin polymerization could also be made. Thus, important additional information could be obtained. As these tests were automated, to facilitate and standardize their performance, this advantage was lost. Automated devices based on mechanical principles to measure the clotting time clearly could not accommodate the visual attributes of the manual method. While photo-optical systems had this potential, first generation systems were designed to report only clotting times. However, advances in instruments such as the MDA® series of coagulation analysers (bioMérieux, Marcy, France), in both photometer design and the software
capable of processing the optical signal on-line, have led to the recovery of information that can be graphically represented as an optical profile or 'clot waveform' constructed by plotting changes in light transmittance as a function of time (Figure 1). This provides access to qualitative information that was lost when the manual method was replaced. Moreover, graphical display of the information more readily discriminates typical waveform abnormalities or 'signatures' associated with specific disorders of haemostasis (Figure 2). In addition, the data available are now both objective and quantifiable. Coagulation is a dynamic process and complete analysis of the optical profile can yield quantitative information on the time, acceleration, rate and magnitude of change at defined time intervals during the process leading to fibrin polymerization.

Qualitative and quantitative clot waveform analysis on the MDA® coagulation analysers

The ability to perform these procedures has been incorporated exclusively in the MDA® series of coagulation analysers (bioMérieux). The photometer in these analysers is designed to measure relative changes in transmittance instead of absorbance and gather the signal over the entire course of clot formation. Measurement of light absorbance is used frequently in instruments of this type in an attempt to reduce the noise to signal ratio and avoid false trips during the period of recording used to determine the endpoint, i.e. the clotting time. Unfortunately, this comes at a cost to the sensitivity for observing subtle changes in the waveform during this critical phase of the initiation of fibrinogen to fibrin.
conversion. Due to the low noise to signal ratio of the MDA’s photo-optical system, light transmission can be measured directly resulting in an enhanced qualitative ability to detect minor changes in the clot waveform (Figure 3). The instrument also employs kinetic algorithms for clot detection and its software incorporates analytical routines that define and characterize, on-line, the optical data obtained during the performance of the APTT and PT assays (Braun et al., 1997). A set of 10 parameters (Table 1) is defined by dividing the waveform and the data derived from it, the first and second derivatives, into three sectors: the precoagulation, coagulation and postcoagulation phases (Figure 4). Events, with respect to time, acceleration, rate and magnitude, may then be calculated for each. The software has also been designed to facilitate the export and analysis of waveform data for both routine and research settings including the ability to make comparisons between waveforms obtained either by sequential sampling in an individual patient over time or between individual patients. For such comparisons to be meaningful it is obviously essential that each observation be normalized with regard to light levels on a per test basis in order to adjust for the known variations in the optical density of

**Table 1.** MDA® analyser PT and APTT clot waveform parameters. Points A-E refer to waveform reference points shown in Figure 4.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-coagulation (A-B)</td>
<td>Slope_1</td>
<td>Initial slope of line fit to data from point A to B.</td>
</tr>
<tr>
<td></td>
<td>Delta_1</td>
<td>Amplitude of signal change from point A to B.</td>
</tr>
<tr>
<td>Coagulation (B-D)</td>
<td>Index_min_2</td>
<td>Time at point B (onset of coagulation, clot time).</td>
</tr>
<tr>
<td></td>
<td>Min_2</td>
<td>Minimum value of second derivative (coagulation acceleration at point B).</td>
</tr>
<tr>
<td></td>
<td>Index_min_1</td>
<td>Time at point C (coagulation mid-point).</td>
</tr>
<tr>
<td></td>
<td>Min_1</td>
<td>Minimum value of first derivative (rate of change at point C; coagulation velocity or slope at coagulation mid-point).</td>
</tr>
<tr>
<td></td>
<td>Index_max_2</td>
<td>Time at point D (end of coagulation phase).</td>
</tr>
<tr>
<td></td>
<td>Max_2</td>
<td>Maximum value of the second derivative (coagulation deceleration at point D).</td>
</tr>
<tr>
<td>Post-coagulation (D-E)</td>
<td>Slope_3</td>
<td>Post-coagulation slope, slope of line fit from point D to E.</td>
</tr>
<tr>
<td></td>
<td>Delta</td>
<td>Amplitude of total signal change between point A and E.</td>
</tr>
</tbody>
</table>

individual plasma specimens. The MDA® series of coagulation analysers automatically make such an adjustment as part of the routine processing of all tests performed on this instrument platform.

The clinical utility of clot waveform analysis

Preliminary studies performed in house by the manufacturer of the MDA® demonstrated that it was possible to predict the presence of heparin or factor deficiencies and provide an estimate of fibrinogen concentration from both PT and APTT assays (Givens et al., 1996; Givens & Braun, 1997). Subsequently, independent investigators have confirmed the utility of waveform analysis in a number of different clinical settings. The first and most developed application is in the diagnosis and management of disseminated intravascular coagulation (DIC).

Waveform analysis in disseminated intravascular coagulation (DIC)

The research in this area was propelled by chance observations by one of us that samples from patients in the Intensive Care Unit at the Royal Liverpool Hospital often showed a characteristically atypical waveform (WF) pattern on the APTT assay (Downey et al., 1997). In contrast to the normal sigmoid shaped APTT WF, the initial plateau prior to the commencement of clotting was replaced by a slope that resulted in an atypical biphasic WF (BPW). This is due to an immediate and increasing fall in light transmittance following activation and re-calcification of the plasma sample prior to formal clot formation (Figure 5). In the initial report, from a cross-sectional study, the BPW was detected in samples from patients who had DIC only. This was regardless of the underlying precipitating cause of the DIC although sepsis was the most common aetiology in this cohort of patients. By comparison, patients with congenital factor deficiencies, acquired coagulation antibodies and those on anticoagulant therapies did not manifest the BPW pattern.

Of importance was the demonstration that the changes were independent of the APTT clotting time and the APTT reagent used with both ellagic acid or silica-based reagents showing the BPW changes. There was also no evidence of interference by preanalytical variables such as time from venepuncture, freeze-thawing or analysis of the platelet-poor plasma on or off cells. Neither was it associated with the use of specific medication such as plasma expanders, heparin or parenteral nutrition. While it could also be seen in the thrombin time assay, its detection in the PT assay was subsequently shown to be reagent-dependent (Toh et al., 2000).

To consolidate these initial observations, a prospective study was undertaken on all consecutive samples processed by the routine hospital coagulation laboratory over a 2-week period (Downey et al., 1998). The APTT WF analysis was performed on 1470 samples and the sensitivity and specificity of the BPW for DIC was 97.6% and 98%, respectively. The positive predictive value for DIC was 74%, which increased with increasing steepness of the BPW slope. These values compare extremely favourably with other tests routinely used in the diagnosis of DIC, such as the PT, APTT, thrombin time, fibrinogen concentration, platelet count, and d-dimer level which, either individually or in combination, have substantially lower sensitivity, specificity and predictive value (Spero et al., 1980; Raimondi et al., 1993). Although the BPW does not always indicate the presence of overt DIC, its presence appeared to be invariably associated with coagulation activation or conditions of haemostatic stress. Milder degrees of the BPW were mainly seen in patients with haemostatic dysfunction of a less overt nature than that of DIC, such as in chronic liver disease, atrial fibrillation and the systemic inflammatory response syndrome. As this suggested that the gradient of the slope might be correlated to the intensity of activation of coagulation, it was important to be able to objectively quantify the magnitude of change in the slope or gradient. The light transmittance level at 25 s (TL25) was used initially on the basis that a normal APTT WF would be

Figure 4. A normal transmittance waveform and plots of the first and second derivatives are shown. In order to monitor changes in the waveform the transmittance plot has been divided into 3 phases using the second derivative:
• Pre-coagulation (a-b)
• Coagulation (b-d)
• Post-coagulation (d-e).
expected to have a light transmittance level of 100% at 25 s into the reaction. Conversely, the development of a BPW would demonstrate a corresponding reduction in the level of light transmittance at this time point which could then be measured on the waveform print out as shown in Figure 5. More recently, we have chosen to make this reading at 18 s (TL18) to allow for the fact that occasionally an APTT clot time may be less than 25 s. In addition, on-line quantification of the gradient of the slope (Slope_1 – See Table 1) on the MDA® analyser has now been developed and validated. In the latest edition of the instrument’s software this measurement is automatically flagged (A2 Flag) when a user-definable threshold is exceeded to alert the laboratory and, in turn, the attending physician to the emergence of this important and potentially life threatening medical complication. Using this facility to quantify the BPW slope we have demonstrated a close real-time correlation between WF changes and clinical events. Increasing falls in light transmittance invariably correlated with a worsening clinical state towards decompensation and death (Figure 6). Conversely, resolution towards a normal waveform pattern in serial samples was associated with clinical recovery and an improving prognosis.

Waveform analysis in non-overt DIC

The Subcommittee on DIC of the Scientific and Standards Committee (SSC) of the International Society on Thrombosis & Haemostasis (ISTH) has recently recommended that a non-overt or developing form of DIC should be recognized (Taylor et al., 2001). This reflects the increasing recognition that monitoring these more subtle changes in haemostasis offer a window of opportunity to assess early inflammatory stress of the microvasculature that may become rapidly irreversible. There has been speculation that the frequent failures in trials of new therapeutic modalities in the treatment of sepsis reflects the failure to intervene before this occurs (Astiz & Rackow, 1998). Unfortunately, clinical signs and symptoms alone are frequently unhelpful at this stage and there is an urgent need for a simple, reliable and easy-to-perform screening test.

Waveform analysis of the APTT appears to have the potential to fulfil this need. We have shown that the BPW frequently appears before the consumptive process of DIC prolongs the clotting times (Downey et al., 1997). We have also demonstrated that, in patients who subsequently were confirmed to have overt or fully developed...
DIC, the development of the BPW often preceded the appearance of other established markers such as d-dimer and thrombin-antithrombin (TAT) and plasmin-antiplasmin (PAP) complexes by up to 48 h (Toh & Downey, 1997). Moreover, its performance has substantial practical advantages. The analysis is performed on a frequently requested screening test, i.e. the APTT, and is simple, robust and rapidly performed and thus compares favourably with the more complex and technically demanding molecular marker assays such as d-dimer, TAT and PAP. Thus, it appears to offer a simple means of monitoring patients at risk and optimizing clinical protocols for therapeutic intervention with agents such as recombinant human activated protein C (Bernard et al., 2001). Its clinical utility in this context should now be confirmed by appropriate controlled clinical management studies. Such trials are now being planned.

The molecular mechanism of the biphasic waveform.

As BPW changes correlated well with real-time clinical events and had clinical prognostic application, a biological mechanism underlying this phenomenon was suspected. The finding that the mechanism was thrombin-independent and divalent cation-dependent led to isolation and purification procedures, which identified this to be a calcium-dependent complex of very low density lipoprotein (VLDL) and C-reactive protein (CRP). The Kd of the CRP–VLDL interaction was 340 nm (Toh et al., 2002) and the IC50 for Ca2+ was 5.0 mm. This complex has also been demonstrated to exist in vivo from sera of patients with the BPW and the VLDL component manifests enhanced prothrombinase activity. These findings suggest that the CRP-VLDL complex underlying the APTT BPW plays a pathogenic role in DIC by its ability to fuel increased thrombin generation in vivo. This further underpins the diagnostic utility of WF analysis by directly guiding therapeutic timing of a drug, such as rhAPC with its anticoagulant properties, in sepsis and DIC. Ongoing investigations on the biochemistry of this complex detected by WF analysis holds promise of further improving outcome in this critical area.

**Conclusion**

It is clear that the application of waveform analysis to simple clotting test such as the APTT provides more...
information than clotting time alone. Moreover, this information appears to offer significant advantages in patient management as demonstrated by our findings in patients who may or may not be suspected of having DIC. Other applications of waveform analysis are now emerging. The analysis of the PT shows great promise in the detection of lupus anticoagulants (Su et al., 2002). Finally, complete waveform analysis of the APTT appears to provide additional discriminatory power over clotting time and/or one-stage factor assay in defining the clinical severity of the classical haemophilias. Moreover, it appears to provide a more accurate measurement of Factor VIII below 1.0 μ/dL (Shima et al., 2002). Thus, waveform analysis has the potential to streamline diagnostic test procedures by providing additional information during the performance of a single test. In turn, this provides the possibility for improving patient outcomes, by expediting and optimizing therapeutic interventions, and decreasing overall health care costs.

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