

Editorial Focus

The hidden sepsis marker: aPTT waveform analysis

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Downey et al. published a paper in 1997 entitled 'Novel and diagnostically applicable information from optical waveform analysis of blood coagulation in disseminated intravascular coagulation' (1). Using a new automated coagulation analyzer, the MDA-180, the authors observed that during measurement of the aPTT, some samples became turbid immediately after addition of the calcium chloride reagent, well before clot formation. These plasma samples were mainly from intensive care unit (ICU) patients, which also fulfilled the diagnostic criteria for disseminated intravascular coagulation (DIC). The 'biphasic waveform' (BPW) recorded during optical measurement of the aPTT was thus introduced as a diagnostic marker for DIC (2). The authors found BPW also in some patients without DIC, and speculated that the phenomenon was related to coagulation activation or presence of pre-formed fibrin complexes. At that time, our laboratory was involved in an analysis of the effects of ancrod, a thrombin-like snake venom enzyme used for treatment of ischemic stroke (3). Ancrod induces massive intravascular fibrin formation, but none of our probands treated with ancrod developed BPW, indicating that BPW was not related to intravascular fibrin formation. Finally, Toh et al. were able to show that BPW was due to calcium-dependent complex formation between C-reactive protein (CRP) and very low density lipoprotein (VLDL) (4).

Actually, this phenomenon had been described 20 years earlier. Cabana et al. showed that VLDL formed precipitating complexes with CRP, and these complexes were not observed in the presence of EDTA (5). In the same year, Hulman et al. reported that the serum of acutely ill patients agglutinated fat emulsions prepared from soybean oil designated for intravenous infusions, resulting in 'creaming' of the sera (6). Free calcium ions were necessary for 'creaming', and no 'creaming' was observed if citrate was added to the sera. The authors concluded that 'creaming' was a property of acute phase sera, and that this phenomenon is the explanation for adverse reactions to intravenous lipid emulsions, related to lipid microembolism, such as fever, shivering, precordial pains, nausea and vomiting. A 'fat emulsion agglutination test' was developed for measurement of CRP (7). It was later shown that the 'creaming' phenomenon in response to intravenous lipids was only observed in patients with high VLDL

levels (8). Rowe et al. further characterized the interaction between CRP and VLDL and concluded that CRP may be related in some way to lipoprotein metabolism (9). It was also shown in experiments on rabbits that the CRP-VLDL complexes formed in acute phase serum contained predominantly β -VLDL, an abnormal apo-B containing lipoprotein low in apo-E content (10, 11).

Inflammatory stimuli such as endotoxin induce the upregulation of VLDL secretion in the liver (12). Bennett et al. reported that endotoxin has a distinct effect on structural properties of VLDL, resulting in improved utilization by the heart as energy substrate (13). The authors did not consider the possibility that complex formation of CRP and VLDL might be involved.

BPW is an indicator of a systemic inflammatory response, as observed in sepsis. Chopin et al. studied 187 patients with 217 episodes of systemic inflammatory response syndrome, of which 34 were classified as sepsis, 26 as severe sepsis, and 50 as septic shock. The diagnostic sensitivity and specificity of BPW for the combined group of severe sepsis and septic shock was 92% and 67%, respectively. Both CRP and procalcitonin displayed a lower diagnostic sensitivity (14). Another recent study showed a sensitivity of BPW for sepsis of 81%, with a specificity of 76% in ICU patients (15). Combination of aPTT waveform analysis with procalcitonin measurement did not increase sensitivity but raised specificity to 94%. In our own study on ICU patients, the diagnostic sensitivity of BPW for sepsis was 74%, with a specificity of 81% (16).

Within the group of ICU patients with systemic inflammatory response syndrome, BPW identifies a group with increased mortality rate. In the study of Toh et al., the mortality rate of patients with BPW was 44%, compared to 26% in the patients without BPW (17). In the study of Bakhtiari et al., mortality of patients with and without BPW was 43% and 32%, respectively (18). In our study, the mortality rate of ICU patients with and without BPW was 36.8% and 13.1%, respectively (16).

Use of the aPTT waveform analysis is not limited to ICU patients. Smith et al. have shown that also non-ICU patients with BPW are more likely to have positive blood cultures (19). Similarly, the study now published by Hussain et al. (20) shows that BPW can identify patients with sepsis within a specific high-risk patient group. Myelosuppressive therapy results in neutropenia

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and imposes a considerable risk of developing sepsis. Often, patients are treated with non-steroidal anti-inflammatory drugs as part of analgesia, because of which the patients do not develop fever. Early identification of septic patients is essential, and the use of an assay, which is frequently performed as part of routine diagnostics (aPTT) and generates a reliable and specific warning signal for sepsis may have considerable impact on survival rates. The aPTT is performed daily in the majority of these patients, and the BPW result is generated at no extra cost. The authors show that BPW precedes positive blood culture results by up to three days.

BPW was first introduced as a marker for DIC, and the concordance between BPW and DIC in Liverpool was striking (2). In the study of Bakhtiari et al. (18), BPW had a sensitivity of 88% and a specificity of 97% for DIC. In contrast, Matsumoto et al. (21) found that BPW had a sensitivity of only 59.2% for DIC, although specificity was 95.4%. BPW might simply be an indicator of infection, and the concordance between BPW and DIC is a result of the high rate of DIC among patients with severe sepsis. Alternatively, it is possible that the mechanisms underlying the BPW phenomenon are involved in coagulation activation processes leading to DIC. Lipoproteins, and especially VLDL may support prothrombinase and other procoagulant enzyme complexes and thus may complement activated platelets (22). VLDL particles contain negatively charged phospholipids, which are essential for the binding of the coagulation factor complexes. These phospholipids render the VLDL particles procoagulant, but may also be transferred to platelets in the course of platelet activation (23). The procoagulant effect of VLDL is already observed at

physiological levels and is suspected to become even more relevant if VLDL particles are present at higher concentration. It has been observed that postprandial VLDL increase is indeed associated with coagulation activation (24). Apart from enhancing prothrombinase activity, VLDL also appears to induce tissue factor-independent factor VII activation (25) and to stimulate the contact system of coagulation (26). Oxidation of phospholipids may further enhance the procoagulant effect (27).

The aPTT waveform result is generated at no additional cost as part of routine aPTT measurement. The aPTT is especially suited for this type of additional analysis, since samples are first stabilized with citrate solution (which prevents 'creaming' of the VLDL-CRP complexes), and calcium is added at a fixed concentration, which reduces variability induced by differences in calcium concentration. Thus, complex formation and generation of the turbidity signal occurs under standardized conditions, which greatly increases the reliability. The phase between addition of calcium and onset of coagulation is sufficient for observing the BPW phenomenon in most cases. The majority of coagulation analyzers used in hospital laboratories are based on optical clot detection and could easily accommodate aPTT waveform analysis. Astonishingly, the hard- and software needed for the detection of BPW is not a general feature of coagulation analyzers. One reason might be the confusion caused by claiming that BPW is a marker primarily for DIC. According to the present results, BPW is a reliable marker for infection and complements other assays, such as procalcitonin, for detection and monitoring of patients with sepsis.

References

- Downey C, et al. Novel and diagnostically applicable information from optical waveform analysis of blood coagulation in disseminated intravascular coagulation. *Br J Haematol* 1997; 98: 68–73.
- Downey C, et al. Early identification and prognostic implications in disseminated intravascular coagulation through transmittance waveform analysis. *Thromb Haemost* 1998; 80: 65–69.
- Dempfle CE, et al. Analysis of fibrin formation and proteolysis during intravenous administration of anecrod. *Blood* 2000; 96: 2793–2802.
- Toh CH, et al. Biphasic transmittance waveform in the aPTT coagulation assay is due to the formation of a Ca(++)-dependent complex of C-reactive protein with very-low-density lipoprotein and is a novel marker of impending disseminated intravascular coagulation. *Blood* 2002; 100: 2522–2529.
- Cabana VG, et al. Interaction of very low density lipoproteins (VLDL) with rabbit C-reactive protein. *J Immunol* 1982; 128: 2342–2348.
- Hulman G, et al. Agglutination of intralipid by sera of acutely ill patients. *Lancet* 1982; 2: 1426–1427.
- Richter D, et al. The fat emulsion agglutination test: a reliable and cost effective alternative to the latex agglutination test for rapid bedside CRP measurement. *Clin Chim Acta* 1997; 261: 141–148.
- Zagara G, et al. C-reactive protein and serum agglutination in vivo of intravenous fat emulsions. *Lancet* 1989; 1: 733.
- Rowe IF, et al. Circulating human C-reactive protein binds very low density lipoproteins. *Clin Exp Immunol* 1984; 58: 237–244.
- Rowe IF, et al. Rabbit and rat C-reactive proteins bind apolipoprotein B-containing lipoproteins. *J Exp Med* 1984; 159: 604–616.
- Cabana VG, et al. Effects of the acute phase response on the concentration and density distribution of plasma lipids and apolipoproteins. *J Lipid Res* 1989; 30: 39–49.
- Aspichueta P, et al. Endotoxin promotes preferential periportal upregulation of VLDL secretion in the rat liver. *J Lipid Res* 2005; 46: 1017–1026.
- Bennett MJ, et al. Utilization of very low density lipoprotein by rat heart: the effect of endotoxin. *Am J Physiol Endocrinol Metab* 2000; 278: E802–810.
- Chopin N, et al. Activated partial thromboplastin time waveform analysis: a new tool to detect infection? *Crit Care Med* 2006; 34: 1654–1660.
- Zakariah AN, et al. Combination of biphasic transmittance waveform with blood procalcitonin levels for diagnosis of sepsis in acutely ill patients. *Crit Care Med* 2008; 36: 1507–1512.
- Dempfle CE, et al. Utility of activated partial thromboplastin time waveform analysis for identification of sepsis and overt disseminated intravascular coagulation in patients admitted to a surgical intensive care unit. *Crit Care Med* 2004; 32: 520–524.
- Toh CH, et al. Early identification of sepsis and mortality risks through simple, rapid clot-waveform analysis. Implications of lipoprotein-complexed C reactive protein formation. *Intensive Care Med* 2003; 29: 55–61.
- Bakhtiari K, et al. Prospective validation of the International Society of Thrombosis and Haemostasis scoring system for disseminated intravascular coagulation. *Crit Care Med* 2004; 32: 2416–2421.
- Smith EY, et al. Biphasic activated partial thromboplastin time waveform and adverse events in non-intensive care unit patients. *Am J Clin Pathol* 2004; 121: 138–141.
- Hussain N, et al. The Biphasic Transmittance Waveform: An early maker of sepsis in patients with neutropenia. *Thromb Haemost* 2008; 100: 146–148.
- Matsumoto T, et al. Frequency of abnormal biphasic aPTT clot waveforms in patients with underlying disorders associated with disseminated intravascular coagulation. *Clin Appl Thromb Hemost* 2006; 12: 185–192.
- Moyer MP, et al. Plasma lipoproteins support prothrombinase and other procoagulant enzymatic complexes. *Arterioscler Thromb Vasc Biol* 1998; 18: 458–465.
- Ibrahim S, et al. Transfer of very low density lipoprotein-associated phospholipids to activated human platelets. *J Lipid Res* 2006; 47: 341–348.
- Elmas E, et al. Activation of coagulation during alimentary lipemia under real-life conditions. *Int J Cardiol* 2007; 114: 172–175.
- Kjalke M, et al. Plasma lipoproteins enhance tissue factor-independent factor VII activation. *Arterioscler Thromb Vasc Biol* 2000; 20: 1835–1841.
- Klein S, et al. Phosphatidylethanolamine participates in the stimulation of the contact system of coagulation by very-low-density lipoproteins. *Arterioscler Thromb Vasc Biol* 2001; 21: 1695–1700.
- Rota S, et al. Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: modulation by oxidation and vitamin E. *Blood* 1998; 91: 508–515.