Prothrombinase enhancement through quantitative and qualitative changes affecting very low density lipoprotein in complex with C-reactive protein

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Summary
The biphasic waveform that can predict for disseminated intravascular coagulation (DIC) is due to the formation of a calcium-dependent complex between C reactive protein (CRP) and very low density lipoprotein (VLDL). As thrombin generation is pivotal to DIC, this aspect has been specifically investigated and the VLDL component has been found to increase prothrombinase activity via both quantitative and qualitative changes. The specific prothrombinase activity of VLDL from patients manifesting the biphasic waveform was 2.5 times that of normal individuals or critically ill patients without the biphasic waveform. This activity was due to an increase in anionic phospholipid surfaces that could be inhibited with excess annexin V and which was dependent on structurally intact apolipoprotein B. The qualitative change appeared to be due to a deficiency of phosphatidylethanolamine in VLDL from patients with the biphasic waveform. The functional consequence of this enhanced prothrombinase activity was an increased procoagulant effect in plasma. Using a modified activated partial thromboplastin time assay, the mean normal clot time decreased significantly when VLDL from patients with biphasic waveforms was substituted. These results indicate that VLDL derived from patients with the biphasic waveform can enhance thrombin procoagulant activity. As the CRP-VLDL complex exists in vivo, it could have a pathogenic role in disseminating the process of intravascular coagulation.

Keywords
DIC, lipoproteins, phospholipids, thrombin, sepsis

Introduction
We have recently identified that a calcium-dependent complex between C reactive protein (CRP) and very low density lipo-protein (VLDL) is the molecular mechanism underlying an atypical optical profile in a simple test of coagulation and exists in vivo (1). The description of this atypical biphasic waveform, primarily seen in critically ill patients with disseminated intravascular coagulation (DIC), followed serendipitous observations during routine performance of the activated partial thromboplastin time (aPTT) using the MDA-180® automated light transmittance analyser (2). The immediate, progressive decrease in light transmittance upon plasma recalcification and prior to formal clot formation causes this biphasic pattern,
which can be quantified via the light transmittance level at 18 seconds (TL18) into the aPTT reaction (3). A normal waveform has a TL18 of 100% (99.4-100.6) (1).

More recently, we have been able to show that waveform analysis can predict clinical outcome. Complex detection by way of the biphasic waveform, within the first hour of admission into the Intensive Therapy Unit (ITU), can significantly predict for the diagnosis of sepsis, DIC development and the risk of mortality (1, 4). The prediction for DIC was strongest at approximately 70% for maximal levels of complex formation and superior to that by D-dimer, a conventional measurement in DIC. Of added significance was that the CRP-VLDL complex better predicted all 3 risk outcomes than CRP or VLDL alone. This suggests that detecting and quantifying the complex of CRP with VLDL provides additional information to that obtained by measuring either component alone, and may relate more directly to the functional activity of either the complex and/or each of its components.

The extent of complex formation directly correlates with the degree of biphasic waveform abnormality. The $K_d$ is 340 nM with a stoichiometry involving 1 mM VLDL binding 178 micrograms/ml CRP. The IC$_{50}$ for calcium is 5.0 mM (1). Although higher than physiological circulating calcium concentrations, Rowe et al have suggested that this complex can form in the circulation following experiments whereby serum from patients with Type III, IV or V hyperlipoproteinaemia were incubated with acute phase serum containing high CRP levels (5). However, no clear physiological function was ascribed to the CRP-VLDL complex in their studies. Our work, through discovering this complex following clinical observations, suggests that it might have a role in the biology of critical illness. For both DIC and sepsis, there is increasing recognition of common and overlapping pathophysiological pathways that link inflammation and coagulation (6).

Thrombin generation in vivo is considered to be pivotal in DIC and its markers increase during disease progression (7, 8). For the enzymatic conversion of prothrombin to thrombin to occur at physiologically relevant rates, the components must be localised to appropriate surfaces (9). In vivo, this is presumed to be supplied by activated platelets, mononuclear and perturbed endothelial cells (10, 11). VLDL, at physiological levels, can also support relevant rates of thrombin generation and this is thought to be of relevance to the significant prediction of triglycerides to cardiovascular events (12, 13). These findings, coupled with the known responses of both CRP and VLDL in the acute stress situation (14, 15), have led us to investigate whether we can attribute enhanced thrombin generation by the CRP-VLDL complex as a reason for its prediction and association with DIC. Preliminary evidence in a few intensive care patients suggested that VLDL-dependent thrombin generation could be increased. In this paper, we present clear evidence that it is only VLDL from patients with the biphasic waveform that significantly increases procoagulant function. The CRP-VLDL complex may therefore have a pathogenic role in DIC.

**Methods**

**Clinical samples**

Samples were obtained from patients on the ITU at the Royal Liverpool University Hospital with the approval of the Liverpool Research Ethics Committee. The presence of DIC in these patients was defined by the International Society of Thrombosis and Haemostasis Standardization Sub-Committee cumulative score of 5 or above, derived from changes in the platelet count, prothrombin time (PT), fibrinogen and D-dimer levels (16). These assays along with triglyceride (TG) measurements were performed as previously described (1). aPTT waveform analysis, on the MDA 180® at 580 nm, using Platelin LS reagent on fresh citrated plasma has been well described (1-4). Quantitation of the degree of biphasic waveform abnormality was by the light transmission level at 18 seconds (TL18) into the aPTT reaction and considered biphasic if the TL18 was < 99% (1).

**Materials**

Phosphatidylethanolamine/ phosphatidylserine (PCPS) vesicles, human factors X (FXa), Va (FVα) and prothrombin (FII) were prepared as previously described (17). S-2238 from Chromogenix (Milan, Italy), human recombinant CRP from Calbiochem (Nottingham, UK), annexin V from BD Biosciences (San Diego, CA) with its flourescein labelled form from Boehringer Mannheim (Werk Penzberg, Germany), rabbit anti-human apolipoprotein (apo) B-100 and apo E plus monoclonal mouse anti-human glycoprotein IB from Dako (Glostrup, Denmark), mouse anti-αβ3 integrin complex and mouse anti-lgG1/G2a from BD Pharmingen (San Diego, CA), HRP-conjugated goat-anti-rabbit IgG from Santa Cruz Biotechnology (California, US) and Protein G sepharose from Zymed laboratories Inc. (San Francisco, CA) were used. Apo B and E standards, Infinity cholesterol/ triglyceride reagents, phospholipase A$_2$ (PLA$_2$) from Naja mossambica mossambica and Silica, fumed, were from Sigma (St Louis, MO). All other reagents were of analytical grade.

**VLDL isolation and quantitation**

This was as previously described after removal of chylomicrons through centrifugation of test plasma at 14000 rpm for 10 min at 10°C (1). The plasma was from patients not receiving total parenteral nutrition. The VLDL fraction (density < 1.019 g/mL) was stored at 4°C and used fresh within 4 days of isolation.

**Prothrombinase supporting activity**

Isolated VLDL (100, 300, and 500 µM TG) or PC/PS vesicles (75:25) at 50 µmol/L/phosphate were first diluted in TBS...
(pH 7.4), 5 mM CaCl₂ and incubated with 15 nM FVa. 1 µM FII. The reaction was initiated with the addition of 0.1 nM FXa. At timed intervals, 10 µl was aliquoted into 90 µl TBS, 2 mM EDTA. 10 µl was then aliquoted into a 96 well plate, to which 190 µl (400 µmol/L final conc.) of S-2238 was added. Chromogenic liberation at 405 nm was determined on the Spectramax plate reader. Rates of thrombin generation were established by comparison to a calibration curve constructed from a known human thrombin standard. To assess the contribution of CRP, 500 µM TG VLDL was incubated with 100 µg/ml CRP for 15 min at RT prior to the introduction of the coagulation proteins.

To delineate the contribution of phospholipid surfaces, 500 µM TG VLDL from patients were pre-treated with 50 units PLAs for 15 min at 37°C, prior to the prothrombinase reaction. FACS was performed with fluorescein labelled annexin V incubated with VLDL (final concentration 500 µg TG) in HEPES binding buffer, 50 mM calcium on a Becton Dickinson flow cytometer using Cellquest software. The effect of annexin V addition on prothrombinase generation was assessed by prior incubation of increasing annexin V concentrations (0-100 µg/ml at RT for 15 min) with 300 µM TG VLDL in TBS/5 mM CaCl₂.

The possibility of microparticle contamination in the isolated VLDL was determined by FACS using established platelet and microparticle gates. VLDL (final concentration of 500 µg TG) was incubated with 100 µg/ml fluorescein-conjugated monoclonal antibodies specific for platelet glycoprotein 1b and endothelial cell-αβ₃ integrin complex (10 µl) with the respective isotype controls. Assessment for bound coagulation proteins in the VLDL used specific reaction mixes deficient in factors Va, II or Xa for the standard chromogenic prothrombinase assays. The absence of thrombin generation indicated deficiency of the coagulation factor assessed.

**Immunoadsorption analysis**

Rabbit anti-human apo B/E (1mg) were coupled to 150 µl protein G sepharose beads. Control sepharose, without antibody addition, was prepared similarly. 250 µl VLDL standardised for TG was mixed by end over end rotation and incubated overnight at 4°C. Harvested supernatant was analysed for prothrombinase supporting assembly and apo B/E quantitation by ELISA. This involved coating plates overnight at 4°C with 100µl goat-anti-human apo B/E at 5 µg/ml in 50 mM sodium hydrogen carbonate pH 9.5, per well. Following washes with 2% bovine serum albumin (BSA)/HEPES buffered saline (HBS)/ Tween 20, plates were blocked for 1h at RT with phosphate buffered saline (PBS)-2% BSA. 100µl standard (A4183 for apo B and A2673 - A2456 for apo E) /sample fractions were applied and incubated for 2h at RT. Detection was with 100 µl rabbit-α-human apo B/E at 2 µg/ml followed by 100µl goat-anti-rabbit-HRP conjugate at 1:20000 in washing buffer. The signal was generated with 100 µl 2.9% O-phenylenediamine dihydrochloride in 6ml deionised water with 2.5 µl 30% hydrogen peroxide (Sigma-Aldrich Company Ltd., St. Louis, MO, US) and the reaction stopped by 50 µl 0.5M Sulphuric acid. Plates were read on Spectramax Plus (Molecular Devices Corp., Stanford, CA) at 490 nm.

**Chromatography**

Two-dimensional thin layer chromatography (TLC) used plates (Whatman LabSales, Hillsboro, OR) activated for 30 min at 125°C (18). The sample was spotted 15 mm from the edge and run for 140 mm in methyl acetate: n-propanol: chloroform: methanol: 0.25% potassium chloride (25:25:10:9) in a well-saturated tank. The plate was dried under nitrogen before running perpendicularly. They were sprayed with ninhydrin and then 50% aqueous sulphuric acid before heating to 120°C for 15 min. Standards used were phosphatidylcholine (PC), -serine (PS), -ethanolamine (PE), sphingomyelin (SM), and cerebrosides.

![Figure 1: Prothrombinase supporting activity. (A) shows the comparative activity generated by PCPS (●), VLDL from normal individuals (□) and from patients with the biphasic waveform (◆). (B) shows the differences in 81 samples from normal VLDL (□), intensive care patients with (◆) and without (∆) the biphasic waveform.](image-url)
Procoagulant cofactor clotting assay
To determine procoagulant activity of VLDL in the plasma milieu, a modified aPTT was performed on the Spectramax microtitre plate reader. 25 µl platelet-poor plasma from normal, overnight-fasted individuals was incubated with 25 µl of 0.175% silica in imidazole buffer at 37°C. After 180 seconds, 50 µl of a 50:50 mix between VLDL (from normal individuals or patients with biphasic waveform abnormalities with equivalent TG levels) and 25 mM CaCl₂ was added with the time at half-maximum absorbance change recorded as time to clot formation. Each run, with all samples performed in duplicates, compared VLDL from a normal versus VLDL from a patient with a biphasic waveform added to the same normal plasma. To ascertain dose-dependence, normal VLDL was admixed with VLDL from patients with the biphasic waveform in the ratios of 1:0, 2:3, 1:3 and 0:1, respectively.

Statistics
Means were compared using either a univariate ANOVA (for prothrombinase experiments) or the analysis of variance by students t-test. Non-parametric testing was also used to supplement the t-test to make no assumption about data distribution. A value of p<0.05 defined statistical significance.

Results
Qualitative and quantitative VLDL changes in patients with the biphasic waveform enhance prothrombinase activity
The kinetic contribution of VLDL to thrombin generation was compared to PCPS vesicles. Figure 1A demonstrates the immediate response with PCPS vesicles with half maximal response within 30 seconds. By comparison, the half maximal response of VLDL in patients with the biphasic waveform was delayed, with half maximal response occurring at 2 minutes. Figure 2A shows plasma triglyceride concentrations between normal individuals, patients with normal waveforms and biphasic waveforms (BPW) presented as box plots representing 25th to 75th percentile interquartile ranges. The median is the thickened line and the extreme upper and lower data points are represented outside the interquartile range. Figure 2B shows the correlation between triglyceride (TG) levels and the aPTT waveform in patients with the biphasic waveform. Figure 2C and 2D show time courses from 2 patients who recover and die from sepsis, respectively, in terms of total prothrombinase activity (N), as calculated by the product of plasma TG and the specific prothrombinase activity/ mM triglyceride, and aPTT-TL18 values (GG). Their respective TG levels and DIC scores are also shown with DIC defined by a score of 5 or greater.
response for biphasic VLDL is 8 min and 25 min for normal VLDL. This was further assessed in a large sample set that examined differences between VLDL in different patient groups. Rates of thrombin formation were determined at numerous concentrations of normal VLDL (n=22) and VLDL from ICU patients with (n=30) and without the biphasic waveform (n=29). Slopes of the relationships depicted in figure 1B indicate that the biphasic waveform patients were 2.5 fold more potent than normal VLDL and VLDL from ITU patients without the waveform abnormality (p<0.0001). Interestingly, there was no difference between VLDL from normal and patients without the biphasic waveform in the rate of thrombin generation (p=0.23). The inclusion of increasing concentrations of CRP did not significantly vary the rates of thrombin generation from the different groups of VLDL tested.

In the same samples, the total triglyceride content was quantified prior to VLDL separation. Figure 2A shows that the total triglyceride level is significantly elevated in patients with the biphasic waveform and there is a positive correlation with increasing waveform abnormality, as reflected by falls in TL18 (Fig. 2B). As both qualitative and quantitative changes in VLDL are relevant in terms of total thrombin generating potential, the product of plasma triglyceride level and the specific prothrombinase activity [IIa (sec-1)/mM TG] was ascertained. This relationship is demonstrated in individual patient series from samples over several days in figures 2C and D, which respectively illustrate examples of severe sepsis treated to resolution and where there is terminal decline. DIC, as defined by a score of 5 or greater, was diagnosed from day 2 in both these cases and increased TG levels (up to 4 mM) contributed to the increased thrombin generating potential at time points of DIC.

**VLDL surface enhancement of thrombin generation in patients with the biphasic waveform patients**

Pre-treatment of patient VLDL with purified PLA₂ for 15 minutes ablated the prothrombinase activity (data not shown), suggesting that intact phospholipid in VLDL is necessary. In further experiments utilising fluorescein-labelled annexin V, a calcium-dependent binding protein specific for coagulant-active phospholipids (19, 20), we compared VLDL from 16 patients with the biphasic waveform to a corresponding number from normal individuals by FACS. The more intense signal from VLDL isolated from the biphasic patients (geo. mean fl 58.02 vs. 19.25) suggests increased coagulant-active phospholipid exposure. Figure 3A illustrates typical experimental findings. This was unaffected by incubation with CRP (data not shown). The ability of annexin V to inhibit prothrombinase activity is demonstrated in figure 3B with data of VLDL from 4 normals and 4 patients with biphasic waveforms. There was significant inhibition of thrombin generation for biphasic VLDL at 100 ng/ml annexin V (p=0.003). The degree of inhibition of prothrombinase activity is far greater on the VLDL derived from patients with the biphasic waveform when compared to normal. This suggests that the increased thrombin generation was due to increased coagulant-active phospholipid exposure in VLDL from biphasic waveform patients.
The possibility of either in vivo and/or ex vivo contamination with platelet or endothelial microparticles was excluded by flow cytometry of lipoprotein microparticles failing to identify fragments specific for each (data not shown). The possible contribution of bound prothrombinase proteins was also excluded as no significant thrombin was generated in the absence of purified factors Va, II or Xa by means of the prothrombinase assay. Furthermore, there was no variation in the degree of oxidation between VLDL from patients as compared to normal (data not shown).

To define the contribution of VLDL proteins, immobilised antibodies against apo B-100 or apo E were tested for their ability to adsorb prothrombinase activity. This was efficiently achieved, as the quantified apo B or E in the adsorbed supernatant was 5% of that from control Sepharose beads. The adsorption process itself led to some loss of prothrombinase potential but specific apo B removal caused significant loss of activity (p=0.0002) from 3 different starting VLDL (Fig. 3C). Further FACS to assess VLDL structural integrity after apo B immunoadsorption showed no identifiable particles. The contribution of apo B is therefore most likely as the main structural component of the particle, especially as blocking apo B with a monoclonal antibody had no effect on VLDL prothrombinase activity (data not shown). As apo E immunoadsorption did not diminish prothrombinase activity (Fig. 3C) it does not appear essential for thrombin enhancement.

VLDL from biphasic waveform patients lack PE but contain PC, SM and Cerebrosides

To investigate the phospholipid moieties involved, TLC was performed. The method, in being sensitive down to detection limits of at least 0.5 µg of amino-containing phospholipid, did not detect PS in VLDL from both normal individuals (n=5) and patients (n=10). PC, SM and cerebrosides were present in all VLDL (Fig. 4A). The only compositional difference was a lack of PE in biphasic waveform patients only (Fig. 4B). This was a reproducible finding that was specific for patients with the biphasic waveform as ITU patients with normal waveforms had findings similar to that of normal (data not shown).

VLDL from biphasic waveform patients show procoagulant activity in clotting assays of normal plasma

Following on from the above findings, we examined for procoagulant cofactor activity of VLDL in the plasma milieu using a modified aPTT clotting assay to assess the number of phospholipid dependent reactions in the intrinsic pathway of coagulation. As seen in figure 5A, the addition into normal plasma of isolated VLDL from patients with the biphasic waveform showed significant shortening of clot time (p=0.000) when compared with addition into the same plasma of equivolume VLDL from normal individuals. The mean clot time was 203 seconds (SD 2.52) for VLDL from 7 normal unrelated donors and this decreased to 178 seconds (SD 8.55) for VLDL from 7 patients with biphasic waveforms (TL18 77-85). The box plot shows the interquartile ranges from 25th to 75th percentile and median clot times of 205 and 179 seconds respectively for normal or biphasic VLDL addition. This clear difference could not be accounted for by differences in total TG content with considerable overlap between normal (range of 0.4 to 1.5 mM) and patients (range 0.5 to 1.8 mM). Fig. 5B demonstrates the dose-dependence of this effect. Increasing
proportional ratios of VLDL from patients admixed to normal VLDL shortened the clot time proportionately in all 6 cases with a plateauing of effect in 2 experiments. This leads us to conclude that VLDL from patients with the biphasic waveform show significant procoagulant cofactor activity.

**Discussion**

The initial serendipitous observation of the biphasic aPTT waveform in patients with DIC led to two parallel lines of investigation. One, in the clinical diagnostic utility of this analysis within the ITU setting and the other, in the isolation of its molecular mechanism. Knowledge that its mechanism is as a result of complex formation between two established bio-markers in inflammation and lipid metabolism, namely CRP and VLDL respectively (13, 14), enabled us to show that its clinical prediction for adverse clinical outcomes is superior to that by either component alone. This observation provides insight into possible functional mechanisms of the CRP-VLDL complex *in vivo*. Whilst complex formation can be induced *in vitro* at physiological calcium concentrations of approximately 2.5 mM, optimal conditions are at higher calcium concentrations that are more likely to exist at sites local to tissue injury (21, 22). Such a mechanism that could optimise and localise complex formation to relevant parts of the microvasculature would be particularly important for the function of the complex and/or each of its components. In this respect, we were able to demonstrate that VLDL from patients with the biphasic waveform had over twice the thrombin generating potential of normal VLDL and that this was not due to oxidation or microparticle contamination. Whilst previous studies have established that normal VLDL can generate physiological levels of thrombin albeit with considerable donor variability (12, 13), this differential increase was consistent in patients with the biphasic waveform within the large sample set investigated.

The enhanced procoagulant aspect of VLDL from patients with the biphasic waveform is demonstrated through the significant clot-time shortening in a modified aPTT assay. In this experimental set up, the added normal or patient VLDL provided the only differentiating source of phospholipid surface provision for the coagulation reaction. This demonstration may also be one explanation for the finding that shorter aPTT clot times are associated with adverse outcomes in a general hospital setting (23).

Because phospholipase A₂ abrogates the activity, phospholipid appears essential for this procoagulant action of VLDL. More specific assessment was made using annexin V; a calcium dependant phospholipid binding protein with high affinity for negatively charged phospholipid surfaces and high specificity for PS (20). This identified that VLDL from patients with the biphasic waveform has increased anionic phospholipid exposure as assessed by flow cytometry. Further confirmation was via blocking thrombin generation using increasing annexin V, to which biphasic VLDL was far more sensitive. We were however unable to demonstrate increased PS by TLC studies, which would have been sensitive to detecting 0.5 µg of PS. Deguchi et al have however shown a small amount of PS (0.4%) in normal VLDL and such trace amounts might possibly be responsible for the extent of prothrombinase activity observed (24). Nonetheless, the presence of PS per se cannot be equated with procoagulant potential as low density lipoproteins (LDL) have the highest distribution of PS amongst circulating lipoproteins [81% as compared with 9% in VLDL] (24) and are not attributed with physiological levels of thrombin generation,
unless modified by oxidation (13). Specific assessment of VLDL oxidation confirmed that the VLDL particles as a whole were not oxidised.

The most appropriate composition of phospholipids for activating the prothrombin complex in vivo is still a matter of debate. The importance of PS stems from in vitro studies under static conditions where synthetic vesicles containing PC:PS ratios of 3:1 appear most similar to the procoagulant effect of activated platelets (25). By contrast, under flow conditions, vesicles containing PC alone can cause the highest increase in procoagulant activity as measured by fibrin deposition (26). Rosing et al have also shown the ability of positively charged membranes to enhance prothrombin activation in the presence of a small amount of PS (27). The findings that PE was absent specifically from VLDL of patients with the biphasic waveform are intriguing especially in light of literature suggesting pro as well as the anticoagulant properties of PE. Both in purified systems and in plasma, PE was found to be essential for optimal expression of activated protein C inactivation of factor Va (28).

Amongst lipoproteins, the PE:PS ratio is highest in high-density lipoproteins (HDL), which has been demonstrated to promote protein C-dependent anticoagulation in plasma (29). In addition, PE blockade by anti-phospholipid antibodies promotes the procoagulant activity of thrombin (30). However, PE in a purified system can increase tenase activity especially on membranes with sub-optimal PS (31). In terms of prothrombinase activation in vitro, PE does not contribute at optimal PS concentrations but can reduce PS requirements by up to ten fold (32).

PE within VLDL can also accelerate the rate of fibrin formation in whole blood through a factor XII dependent manner (33). In our demonstration of the differential VLDL effect within the modified aPTT assay, the plasma used and its factor XII level were not variables.

Conformational changes could also account for functionally distinct differences between pro and anticoagulant activities of these phospholipid-presenting surfaces. The apoprotein contribution can be relevant especially as these differences between HDL and VLDL probably account for their contrasting effects on coagulant function, as the relative distribution of the various phospholipids in both is quite similar (29). The apo B skeleton appears essential to the presentation of these anionic surfaces and the loss of prothrombinase activity through its immunoadsorption is likely to be through disruption of lipoprotein integrity. Apo E removal had no direct effect on thrombin generation. It is a much smaller constituent protein, which is functionally involved in the uptake of remnant particles by the LDL receptor and LDL receptor related protein (33). However it was noted that VLDL from patients with the biphasic waveform frequently had low levels of apo E and this may have relevance to impaired clearance of such particles from the circulation and ultimately, to the ability to sustain thrombin generation.

Whilst significant efforts will be needed to identify the biochemistry and structural properties of VLDL that are responsible for the procoagulant properties observed, it does not detract from the major finding here that VLDL from patients manifesting the biphasic waveform can significantly enhance thrombin generation. Moreover, the calculation of total thrombin generating capacity from the quantitative and qualitative changes in VLDL within serial samples of patients with sepsis and DIC show a direct positive correlation with clinical progression. This further supports the relevance of thrombin as a major player in the pathophysiology of sepsis (6, 8). Whilst its primary role may be as part of the acute phase “protective” response, the protracted and enhanced response fuelled by VLDL could lead to deleterious procoagulant consequences. This would be compounded by the severe reduction during severe sepsis of HDL, with its associated anticoagulant properties (29, 35). As such, formation of the CRP-VLDL complex could be an indication of transition from an adaptive to a maladaptive response in the critically ill. This would be consistent with the well-recognised contribution of DIC to increased mortality and multi-organ failure (6).

In conclusion, this paper describes a mechanism that could link the detection of a biphasic aPTT waveform with clinical DIC. The findings consolidate the diagnostic utility of waveform analysis by providing mechanistic evidence via increased thrombin generation from its VLDL component. Furthermore, this is of potential therapeutic relevance as a biomarker of increased procoagulant potential to better target anticoagulant based therapies in this setting. In addition, it provides insight into mechanisms other than by way of microparticles and cell surfaces that can enhance and abnormally sustain thrombin generation in vivo. Lastly, its further pathogenic understanding holds promise for better unravelling this multifaceted area of critical care biology.

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