Alterations in clot architecture are associated with low fibrinolytic potential in acute myocardial infarction

Short Title: Endogenous Fibrinolysis and Fibrin Clot in STEMI

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Introduction

The likelihood of coronary thrombosis culminating in arterial occlusion is dependent on the effectiveness of endogenous fibrinolysis in breaking up the forming thrombus(1). Reduced fibrinolytic potential increases the risk of lasting occlusion. Laboratory studies have shown abnormally dense fibrin thrombi in populations at risk of arterial thrombosis, such as acute coronary syndrome (ACS), stent thrombosis, diabetes, renal failure and those with family history of premature MI(2). Impaired endogenous fibrinolysis is a recently-described risk factor for adverse cardiovascular events in ACS patients(3-5). How structural thrombus characteristics relate to endogenous fibrinolytic potential, is unknown. We aimed to identify structural alterations in thrombus composition that underlie resistance to endogenous fibrinolysis.

Methods

A prospective observational study was undertaken, approved by National Research Ethics Service and the UK Health Research Authority (ClinicalTrials.gov identifier: NCT02562690) and conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients gave written informed consent.

We screened 50 patients with acute ST-segment elevation myocardial infarction (STEMI) to assess thrombotic status, to identify 15 patients who were included. Patients with STEMI presenting with a view to primary percutaneous coronary intervention (PPCI) were recruited. All patients received dual antiplatelet therapy loading in the ambulance before arrival. Patients with known coagulation disorders, active malignancy, sepsis or blood dyscrasias (platelet count<100x10^9/L, haemoglobin<80g/L) or on anticoagulants were excluded.
Assessment of endogenous fibrinolysis

Thrombotic status was assessed using the point-of-care Global Thrombosis Test (GTT, Thromboquest Ltd., London, UK). Venous blood was drawn upon arrival, before PPCI or heparin administration, and immediately (within 15 sec of withdrawal) inserted into the GTT. The instrument assesses the time for the formation of an occlusive thrombus (occlusion time OT, sec) under high shear, and in the second phase, measures the time until restart of flow due to endogenous fibrinolysis (lysis time LT, sec) (5). Thrombotic status was assessed in duplicate (two samples per patient run in parallel channels). In one channel, the measurement was allowed to proceed as normal, with occlusion time and endogenous fibrinolysis time measured. In the other channel, the measurement was terminated after occlusion, the thrombus extracted, fixed and analysed.

Scanning Electron Microscopy

The thrombus formed in the GTT was carefully extracted, washed several times in Na-cacodylate buffer, fixed in 2.5% glutaraldehyde for 1 hour, critically dried with increasing alcohol concentrations (5-100%) followed by hexamethyldisilazane, sputter-coated with gold with Quorum 150(6), and imaged using a Phenom ProX (EDS) scanning electron microscope (SEM) (Lambda Photometrics Ltd., Harpenden, UK). Fibrin density and clot architecture were assessed at x3500-9000 magnification. Thickness of fibrin fibres (n=100) from nine different areas per thrombus were measured and compared. Fibrin density was calculated using a Scentis Database Image software with a Multiple Phase Percentage Processing package, based on differential light intensities on SEM, to derive total fibrin fibre area (% per visual field [p.v.f.]) indicating fibrin density and void space-gap (% p.v.f.), an indirect measure of clot density. SEM analysis was performed blinded to thrombotic status results.

Statistical analysis
To relate differences in clot architecture to \textit{in vitro} endogenous fibrinolysis, patients were divided into 4 quartiles based on LT, and clot characteristics compared between groups. Kruskal-Wallis test was used to assess differences between groups and Wilcoxon signed-rank test to investigate differences in different areas within clots. Correlation was assessed with Spearman’s method. Analyses were performed with Stata V15.1 (StataCorp, Texas, USA).

\textbf{Results}

There was a significant association between effectiveness of endogenous fibrinolysis and fibrin fibre thickness ($p=0.0001$). As LT increased (less efficient fibrinolysis), the fibrin network of the \textit{in vitro} thrombus was significantly more compact and dense, with thinner fibrin fibres and smaller gaps (Figure). Fibrin fibre thickness correlated inversely with LT ($r=-0.89$, $p=0.001$)

Clinical characteristics including age, sex, cardiovascular risk factors, haemoglobin, haematocrit, platelet count, fibrinogen, peak troponin and pain-to-door time were not related to LT or fibrin fibre thickness. OT was not different between quartiles ($p=0.688$). The relationship between LT and fibrin fibre thickness was further examined by creating a multivariable regression model, built on clinical parameters known to impact on endogenous fibrinolysis (age, hypertension, diabetes, haemoglobin, platelet count, neutrophil count, creatinine and fibrinogen). After accounting for these variables, the relationship between LT and fibrin fibre thickness remained significant ($p=0.005$).

\textbf{Conclusion}

Patients with STEMI who exhibit impaired endogenous fibrinolysis, create \textit{in vitro} thrombi with much denser fibrin meshwork, thinner fibrin fibres and smaller gaps between fibres, than patients with effective endogenous fibrinolysis. This difference in clot architecture may
underlie the resistance to endogenous fibrinolysis observed in some ACS patients and predispose to ongoing thrombosis risk. This is the first study correlating a point-of-care measure of endogenous fibrinolysis with \textit{in vitro} fibrin clot characteristics. The evidence base linking dense thrombus architecture to cardiovascular risk could now be translated from an off-line laboratory association to a clinically meaningful, near-patient assessment, with the potential to modify risk.
Conflict of Interest

None declared.
References


Figure. Representative SEM images of in vitro thrombus from patients with increasing fibrinolysis time (panels A→D), at increasing magnification (1→3), and differential light intensities (A4→D4). Images relate directly to LT quartiles in table of clot characteristics (median [interquartile range]) below.
<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>Fibrin fibre thickness (average of all areas)</th>
<th>Fibrin fibre thickness - Core section (nm)</th>
<th>Fibrin fibre thickness - Periphery section (nm)</th>
<th>Total fibrin fibre area (% p.v.f.)</th>
<th>Gap size (% p.v.f.)</th>
<th>LT (sec)</th>
<th>Total (all patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT 0-1500</td>
<td>388.0</td>
<td>371.5</td>
<td>388.4</td>
<td>57.6</td>
<td>91.0</td>
<td>2903</td>
<td>0.0001</td>
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<tr>
<td>LT 1501-3000</td>
<td>570.3</td>
<td>671.6</td>
<td>388.4</td>
<td>3.2</td>
<td>32.7</td>
<td>1322</td>
<td>0.024</td>
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<tr>
<td>LT 3001-4500</td>
<td>444.4</td>
<td>454.1</td>
<td>371.5</td>
<td>55.1</td>
<td>9.5</td>
<td>2465</td>
<td>0.018</td>
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<td>LT 4501-6000</td>
<td>331.3</td>
<td>316.8</td>
<td>315.6</td>
<td>92.8</td>
<td>4.8</td>
<td>3419</td>
<td>0.022</td>
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**Figure(s)**