Atherosclerosis 240 (2015) 402–407

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Fractal dimension: A novel clot microstructure biomarker use in ST elevation myocardial infarction patients

Matthew J. Lawrence [a, b, 2], Ahmed Sabra [a, b, 2], Phillip Thomas [c], Daniel R. Obaid [c], Lindsay A. D'Silva [a, b], Roger H.K. Morris [d], Karl Hawkins [a, b], Martin R. Brown [e], Phylip R. Williams [e], Simon J. Davidson [f, 1], Alexander J. Chase [c], David Smith [c], Phillip A. Evans [a, b, *]

[a] NISCHR Haemostasis Biomedical Research Unit, Morriston Hospital, ABMU Health Board, Swansea SA6 6NL, UK
[b] College of Medicine, Swansea University, Swansea SA2 8PP, UK
[c] Cardiac Centre, Morriston Hospital, ABMU Health Board, Swansea SA6 6NL, UK
[d] School of Applied Science, Cardiff Metropolitan University, Cardiff CF5 2YB, UK
[e] College of Engineering, Swansea University, Swansea SA2 8PP, UK
[f] Department of Haematology, Royal Brompton Hospital, Royal Brompton and Harefield NHS Foundation Trust, London SW3 6NP, UK

ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form
12 March 2015
Accepted 6 April 2015
Available online 13 April 2015

Keywords:
Percutaneous coronary intervention
Acute myocardial infarction
Drug monitoring
Fractal dimension
Gel Point
Coagulation

ABSTRACT

Objectives: Changes in clot microstructure are increasingly implicated in the pathology of atherosclerosis although most data are from techniques in the remote laboratory using altered blood. We validate the novel biomarker Gel Point in STEMI patients and assess therapeutic interventions. Gel Point marks the transition of blood from a visco-elastic liquid to visco-elastic solid and is rapidly measured using unadulterated blood. The Gel Point provides measurements of three parameters to reflect clot microstructure (fractal dimension (d_f)), real-time clot formation time (T_{GP}) and blood clot strength (elasticity at the Gel Point (G'_{GP})).

Methods: We prospectively recruited 38 consecutive patients with STEMI undergoing primary percutaneous coronary intervention (pPCI). Venous blood samples were collected on admission, after pPCI and 24 h after admission for assessment of the new biomarkers, standard coagulation tests and scanning electron microscopy (SEM).

Results: d_f after pPCI was lower than d_f on admission (mean 1.631 [SD 0.063] vs 1.751 [0.052], $p < 0.000001$) whereas d_f at 24 h was similar to that on admission. G'_{GP} also showed similar trend to d_f ($p < 0.001$). T_{GP} was prolonged at after-PCI measurement compared with admission (median 854 s [IQR 581–1801] vs 217 [179–305], $p < 0.00001$). Changes in the values of d_f and G'_{GP} were consistent with changes in the SEM images of the mature clot.

Conclusions: We characterise Gel Point derived markers of clot microstructure in patients admitted with emergency arterial thrombosis. This point of care test can potentially be used to assess the efficacy of therapeutic interventions by measuring changes in clot microstructure.

1. Introduction

Changes in clot microstructure and visco-elastic properties are increasingly recognized as important mechanisms underlying prothrombotic disease [1–4]. We have recently reported a novel point of care visco-elastic technique that provides three related biomarkers all calculated from one measurement at the Gel Point (GP); clot formation time (T_{GP}), clot strength (G'_{GP}) and clot microstructure quantified by fractal dimension (d_f) [2,5,6]. Importantly, in contrast to many assays in routine clinical use, the measurement is performed using unadulterated whole blood, in a near patient setting and provides rapid assessment of coagulation. This technique has been validated in healthy volunteers outpatients with

* Corresponding author. NISCHR Haemostasis Biomedical Research Unit, Morriston Hospital, ABMU Health Board, Swansea SA6 6NL, UK.
E-mail address: phillip.evans2@wales.nhs.uk (P.A. Evans).
[1] Since moved to Roche Diagnostics International Ltd, Forrenstrasse 2, 6343 Rotkreuz, Switzerland.
[2] Matthew J. Lawrence & Ahmed Sabra claim joint first authorship.

http://dx.doi.org/10.1016/j.atherosclerosis.2015.04.012
0021-9150/

venous thromboembolism [2,5], however, has yet to be investigated in arterial thrombosis and the hospital inpatient emergency setting.

In myocardial infarction abnormal thrombus microstructure formation has been associated with both premature coronary atherothrombosis and adverse events following primary percutaneous coronary intervention (pPCI) [7–10]. Previous studies of clot microstructure however have relied mostly on multiple analytical and imaging techniques on processed samples in remote laboratories, limiting potential for translation into clinical use. In contrast, the GP biomarkers provide a rapid single test in unadulterated blood.

The current gold standard treatment of ST elevated myocardial infarction (STEMI) is to restore coronary flow by mechanical thrombus disruption or removal in combination with adjunctive antithrombotic therapy which includes a variety of anti-platelet and anticoagulants [11]. Balancing the inherent bleeding risk of potent antithrombotics against their proven anti-ischaemic benefits remains a major challenge to the cardiac community [12].

The present study investigates whether GP biomarkers can be safely and feasibly obtained in the setting of emergency time-dependent clinical care and reproducibly measure the effect of antithrombotic therapy in pPCI.

2. Materials and methods

2.1. Study design and population group

This study complies with the declaration of Helsinki and was approved by a local Research Ethics Committee (Wales Research Ethics Committee 6; REC Number 07/WMW02/34). Adult (≥18 years) patients with STEMI admitted to undergo primary pPCI were recruited at a large teaching hospital in South Wales. For those unable to consent due to need of emergency treatment, consent was sought retrospectively. Informed written consent was obtained from all participants and confirmation of ongoing consent was reiterated at 24 h. Exclusion criteria included any other form of anticoagulation prior to hospital admission or those with any known blood dyscrasia.

Prior to pPCI all patients received 300 mg of aspirin and 600 mg of clopidogrel. A bolus of Bivalirudin (0.75 mg/kg) was given followed by an infusion (1.75 mg/kg/h). In addition patients received between 2000 and 5000 IU of unfractionated heparin according to treating physician preference to reduce risk of peri-procedural catheter thrombosis as per current practice. During catheter angiography the initial flow in the infarct related artery prior to any percutaneous intervention was noted using the TIMI grading system with grade 1 indicating no anterograde flow through the occlusion and grade 3 indicating normal flow [13]. All patients continued to receive 75 mg of aspirin and 75 mg of clopidogrel commencing 24 h after the procedure.

For the study three samples of blood were taken from the patients, the first taken on admission following the 300 mg of aspirin (usually given pre-hospital by ambulance paramedic protocol) but before administration of clopidogrel, heparin or Bivalirudin (point A). The second sample was taken immediately after the pPCI procedure (point B) with the third on the following day after the (75 mg) aspirin and clopidogrel had been administered (point C).

2.2. Blood sampling

One 20 ml sample of venous blood was obtained at point A, B and C respectively. For each of the 3 samples the blood was divided into several aliquots. One aliquot of whole venous blood was immediately transferred to a rheometer for testing (see Section 2.3). The remaining aliquots were used to perform standard coagulation screens, complete blood counts (CBC) and platelet function analysis. A small aliquot (20 μl) of blood was taken to obtain SEM images of whole blood mature clots formed ex vivo.

2.3. Rheometric measurements: the Gel Point

The haemorheological biomarkers (T_{GP}, G'_{GP} & d_f) which are the focus of this study are obtained from the Gel Point (GP) technique [14–18]. In the present study a 6.6 ml aliquot of whole unadulterated venous blood was loaded into a double-gap concentric cylinder measuring geometry of a TA Instruments AR-G2 (TA Instruments, New Castle, DE, USA) controlled-stress rheometer (at 37 °C ± 0.1 °C) in a near patient setting and tested immediately to obtain the GP, which identifies the transition of the blood from a visco-elastic liquid to a visco-elastic solid [6]. The GP technique has been previously validated for use with blood in several studies [2,5,6]. An expanded version of the GP measurement is provided in the supplementary material accompanying this article (see Supplemental 1). The GP provides measures of (i) the time taken to reach the GP (the incipient clot formation time), T_{GP}; (ii) the shear elastic modulus at the GP, G'_{GP}, which is a measure of the strength of the incipient clot; and (iii) the fractal dimension of the clot, d_f, which is a quantification of the clot structure.

2.4. Computational analysis

Previous studies using light scattering and microscopy have established that incipient fibrin clots have fractal properties, where the mass, M, is related to d_f by the following power law equation [$M \approx \varepsilon^{df}$, where ε is some length scale value in the range 100 nm to 10 μm] this relationship is used to illustrate the how changes in d_f relate to changes in mass [19].

2.5. Scanning electron microscopy (SEM)

SEM was used to image micrographs of mature formed clots at the three sample points for 3 individuals. Clots were fixed, dehydrated, critical point dried and coated with gold–palladium. All clots were prepared following a standardised protocol [20]. Samples at each time point were allowed to clot at 37 °C. All samples were then washed three times with 2 cocadylate buffer pH 7.2 for the removal of excess salt and fixed for a minimum of 4 h in 2% glutaraldehyde solution. The clots were rinsed with cocadylate buffer and dehydrated in a series of ethanol concentrations from 30 to 100%. The clots were critical point dried with hexamethyldisilazane for 45 min and placed in a fume hood for 24 h. They were then mounted to 0.5″ SEM stubs (agar scientific, UK) and sputter coated with gold palladium. All samples were investigated with a Hitachi S4800 scanning electron microscope (Hitachi, High-Technologies Corporation, Tokyo, Japan).

2.6. Laboratory markers

A 4 ml aliquot of blood was used for CBC analysis, samples being collected into plastic, full-draw dipotassium EDTA Vacuettes (Greiner Bio-One, Stonehouse, UK Ref: 454286). CBC was analysed using a Sysmex XE 2100 (Sysmex UK, Milton Keynes, UK) automated haematology analyser within 2 h of collection.

An additional 4.5 ml was used for routine coagulation studies, being transferred immediately into citrated silicone glass Vacutainers (0.109 M) (Becton–Dickinson, Plymouth, UK Ref: 367691). Prothrombin Time (PT), activated partial thromboplastin time (APTT) and Clauss fibrinogen were measured using a Sysmex CA1500 analyser within 2 h of collection. Fibrinogen calibration