Cronfa - Swansea University Open Access Repository

This is an author produced version of a paper published in:
Atherosclerosis

Cronfa URL for this paper:
http://cronfa.swan.ac.uk/Record/cronfa27876

Paper:
Lawrence, M., Sabra, A., Thomas, P., Obaid, D., D'Silva, L., Morris, R., Hawkins, K., Brown, M., Williams, P., et. al. (2015). Fractal dimension: A novel clot microstructure biomarker use in ST elevation myocardial infarction patients. *Atherosclerosis, 240*(2), 402-407.
http://dx.doi.org/10.1016/j.atherosclerosis.2015.04.012

This item is brought to you by Swansea University. Any person downloading material is agreeing to abide by the terms of the repository licence. Copies of full text items may be used or reproduced in any format or medium, without prior permission for personal research or study, educational or non-commercial purposes only. The copyright for any work remains with the original author unless otherwise specified. The full-text must not be sold in any format or medium without the formal permission of the copyright holder.

Permission for multiple reproductions should be obtained from the original author.

Authors are personally responsible for adhering to copyright and publisher restrictions when uploading content to the repository.

http://www.swansea.ac.uk/library/researchsupport/ris-support/

Validation of a clot microstructure biomarker in the treatment of ST-segment-elevation myocardial infarction

Measuring changes in clot quality

Authors: Matthew J. Lawrence[a,b], Ahmed Sabra[a,b], 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†], Alexander J. Chase[c], David Smith[c], Phillip A. Evans[a,b]

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

Corresponding Author: Professor Phillip Adrian Evans, Professor of Emergency Medicine and Haemostasis, NISCHR Haemostasis Biomedical Research Unit, Morriston Hospital, ABMU Health Board, Swansea, SA6 6NL, United Kingdom, Tel: +44 1792 70 3418 Fax: +44 1792 53 2446 Email: phillip.evans2@wales.nhs.uk

Word Count: **3503**

Number of Figures: 3

Number of Tables: 2

†since moved to Roche Diagnostics International Ltd, Forrenstrasse 2, 6343 Rotkreuz, Switzerland

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 clot microstructure (d_f), clot strength ($G`_{GP}$), and clot formation time (T_{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 24h after admission for assessment of the new biomarkers, standard coagulation tests and scanning electron microscopy (SEM).

Results: d_f after pPCI was significantly lower than d_f on admission (mean 1.631 [SD 0.063] vs 1.751 [0.052], p<0.000001) whereas d_f at 24h was similar to that on admission. $G`_{GP}$ also showed similar trend to $d_f$ (p<0.001). $T_{GP}$ was significantly 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 $G`_{GP}$ reflect the 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 reproducible point of care test can be safely performed at pPCI and demonstrates a quantifiable response to clinical therapies.

Key words: Percutaneous coronary intervention, acute myocardial infarction, drug monitoring, fractal dimension, gel point, coagulation

Introduction

Changes in clot microstructure and viscoelastic properties are increasingly recognized as important mechanisms underlying pro-thrombotic disease.[1-4] We have recently reported a novel point of care viscoelastic 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 and suffers of 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-ischemic 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.

Materials and Methods

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 hours. 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 300mg of aspirin, 600mg of clopidogrel and between 2000-5000 IU of unfractionated heparin. A bolus of Bivalirudin (0.75mg/kg) was given followed by an infusion (1.75mg/kg/h). 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 75mg of aspirin and 75mg of clopidogrel commencing 24 hours after the procedure.

For the study three samples of blood were taken from the patients, the first taken on admission following the 300mg 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 (75mg) aspirin and clopidogrel had been administered (point C).

Blood Sampling

One 20ml 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 *Rheometric Measurements: The Gel Point*). The remaining aliquots were used to perform standard coagulation screens, complete blood counts (CBC) and platelet function analysis. A small aliquot (20µl) of blood was taken to obtain SEM images of whole blood mature clots formed ex vivo.

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 from which the fractal dimension of the clot is determined.[14-18] The GP technique has been previously validated for use with blood in several studies.[2,5,6] The GP marks the transition of the blood from a viscoelastic liquid to a viscoelastic solid. 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 ± 0.1ºC) in a near patient setting and tested immediately to obtain the GP.[6]

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 100nm to 10μm] this relationship is used to illustrate the how changes in d_f relate to changes in mass.[19]

Scanning Electron Microscopy (SEM)

SEM was used to image micrographs of mature formed clots at the three sample points for 3 individuals, clots that 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 hours 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 minutes and placed in a fume hood for 24 hours. 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).

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). FBC was analyzed using a Sysmex XE 2100 (Sysmex UK, Milton Keynes, UK) automated haematology analyser within 2 hours of collection.

An additional 4.5ml was used for routine coagulation studies, being transferred immediately into citrated silicone glass Vacutainers (0.109M) (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 hours of collection. Fibrinogen calibration was verified against the 2nd International Fibrinogen Standard Version 4 (NIBSC code 96/612). All reagents were obtained from Siemens, (Frimley, UK). All testing was performed within 2 hours of sample collection.

Platelet aggregation Measurements

Measurement of platelet aggregation was achieved using the Multiplate analyser (Dynabyte GmBH, Munich, Germany) to explore the effect of platelet function on d_f. An aliquot of whole blood (3ml) was transferred to hirudin tubes (Roche Diagnostics GmbH, Mannheim, Austria Ref: 06675751) and kept at room temperature for 30 minutes before testing. For analysis, 300 µl of whole hirudinated blood was added to 300 µl of saline pre-heated to 37°C and allowed to incubate for 3 minutes in individual test cells. Following incubation platelet activation was induced by addition of specific agonists, to respective test cells and electrical impedance was recorded. The agonists tested included adenosine diphosphate (ADP, 20 µl of 0.2mM stock solution) which triggers platelet activation via platelet ADP receptors. The most important receptor of ADP is P2Y12 receptor, which is inhibited by clopidogrel and other thienopyridines. The second was the ASPI test reagent (20 µl of 15 mM stock

solution) which contains arachidonic acid, a substrate of the platelet enzyme cyclooxygenase. Cyclooxygenase transforms arachidonic acid into thromboxane A2, a platelet activator. Aspirin blocks cyclooxygenase thereby inhibiting platelet activity and ASPI induced aggregation.

Statistical analysis

Descriptive analyses were performed to establish baseline characteristics. Results are reported as mean (±SD) unless otherwise stated. The normality was assessed using normal probability plots and Shapiro–Wilk test. Wilcoxon signed-rank and two sample t-test were used to compare differences between two time points and one way ANOVA with Tukey’s pairwise analysis was used to compare differences across all sampling points. Spearman's correlation analysis was done to explore any associations between various tests as defined from the start of symptoms to the time blood was taken. Differences were assumed to be significant at $p<0.05$ and actual probability values are quoted when deemed significant. Minitab version 16 software (Havertown, PA) was used to perform the analysis.

Results

Patient and angiographic data

Forty-five patients with suspected STEMI were screened between January 2012 and April 2014. Seven subjects were excluded: 2 not STEMI as diagnosis, 2 received anticoagulants in the lab prior to testing, one on established warfarin therapy, one technical failure and one withdrew consent for the second sample. Thirty-eight patients were confirmed to have STEMI and included in this analysis. Baseline characteristics of the patients and angiographic data are presented in Table 1.

Gel Point Biomarker

A significant change ($p<0.001$) in d_f is observed between point A, pre-pPCI (1.751 ± 0.052) and point B, immediately post-pPCI (1.634 ± 0.058). At point C, 24hours later, the d_f returns to a value similar to point A (1.742 ± 0.041) (Figure 1). This effect is mirrored in the changes observed for $G`_{GP}$ (Table 2), with a significant difference at point B compared to point A ($p<0.001$). The T_{GP} shows a significant increase in value ($p<0.001$) at point B (from 238 ± 93 sec at point A to 1310 ± 1014 sec at point B), before returning to 245 ± 95 sec at Point C. There is a significant change in d_f associated with treatment ($p<0.0005$, one-way ANOVA) with Point B being significantly lower than both the first and the 3rd when the data was analysed by Tukey's pairwise method. Exactly the same can be said about $G`_{GP}$. However, for T_{GP}, Point B is significantly greater than both A and C.

Table 1: Patient Baseline Characteristics and Procedural Details

Patient Characteristics	
Age	69.3±14.6
Male	23(60.5%)
Current Smoker	7(18.4%)
Hypertension	16(42.1%)
Diabetes Mellitus	6(15.8)%)
Dyslipidaemia	13(34.2%)
Previous MI	2(5.3%)
Cardiogenic Shock	1(2.6%)
Procedural Details	
Infarct Related Artery	
LAD	17(44.7%)
LCX	8(21.1%)
RCA	13(34.2%)
Ischaemia Time (mins)	240 (240-345)
Syntax Score	19.9(±13.5)
Lesion Success (residual stenosis <50%/TIMI≥2)	34(89.5%)
Thrombus Aspiration	19(50.0%)
No of Stents (%Drug Eluting)	1.1(±0.7) (77.5%)
Occluded artery (TIMI 0) at presentation	19(50.0%)
TIMI 3 flow at presentation	11(28.9%)
TIMI 3 flow after PCI	27(71.1%)
Gp2b3A Inhibitor use	1(2.6%)
28 Day Mortality	4(10.5%)

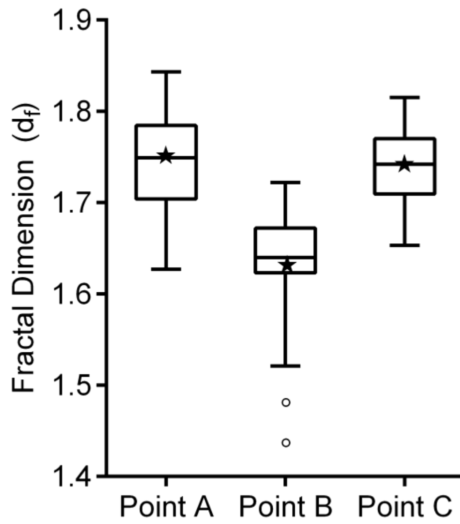

Figure 1: Fractal Dimension, d_f. box and whisker plot illustrating the change in d_f for the three different time points measured for the STEMI patients (n=38); Point A (pre-pPCI), Point B (post-pPCI) and Point C (24 hours). The Star's represents the mean value of the data for each time point (Point A:1.751 ± 0.052; Point B: 1.634 ± 0.058 and Point C 1.742 ± 0.041). *denotes a significant change in d_f associated with treatment ($p<0.0005$, one-way ANOVA) with Point B being significantly lower than both Point A and Point C when the data was analysed by Tukey's pairwise method.

Table 2: Table of the Gel Point, standard markers of haemostasis and platelet aggregometry. * denotes a significant deviations from the baseline Point A values as detected by one way ANOVA and Tukey's pairwise analysis; where p<0.05.

	Pre-PCI (Point A)	Post-PCI (Point B)	24 hour (Point C)
GelPoint (Median(IQR)			
T_{GP} (sec)	217(179-305)	854(581-1801)*	225(181-288)
$G`_{GP}$ (Pa)	0.044(0.040-0.054)	0.027(0.019-0.032)*	0.044(0.039-0.056)
Coagulation Screen			
PT (sec)	10.9±0.86	43.5±19.57*	10.6±0.59
APTT (sec)	27.3±11.87	157.7±79.22*	26.6±5.89
Fib(Clauss) (g/l)	3.5±0.67	2.7±0.63*	3.9±0.61
Full Blood Count			
Platelet Count ($x10^9$)	249±86	241±78*	265±76
Haemoglobin (g/l)	13.8±1.57	12.9±1.29	13.3±1.57
Haematocrit (l/l)	0.409±0.036	0.391±0.034*	0.398±0.045
Multiplate (Median(IQR))			
ADP AUC	94(76-123)	100(72-125)	28(16-55)*
ASPI AUC	24(16-43)	25(15-39)	12(8-16)

Standard coagulation markers

The standard coagulation, FBC and fibrinolysis markers are presented in Table 2. A significant reduction in fibrinogen concentration and a significant prolongation of PT and APTT were observed at point B compared to point A and C.

Platelet aggregometry

The platelet aggregometry results are shown in Table 2. All patients received aspirin at least 60 minutes before first sample collected. The ASPI AUC results at point A and B were both found to me well below the stated normal range quoted by the manufacturer (75-115U) indicating the high loading dose of aspirin (300mg) given to patients prior to admission to hospital is reducing platelet activity. We found no evidence of low responders to aspirin at point C (24 hours) in this study with all patients showing an ASPI AUC result of less than 40U.[21] Clopidegral was given just before sampling point B, as a result no reduction in the ADP AUC values were recorded at points A & B as the drug is either absent or not had sufficient time to work. The ADP AUC values are reduced at point C in the majority of patients, however there are 8 patients that would be classified as low responders. Having ADP AUC values above 47U, previously classified as a cut off for low responders to clopidegral in patients who have received PCI.[22]

Comparison between SEM images of mature clot microstructure and templating effect of d_f

Analysis of the SEM micrographs was based on visual inspection of the images of a particular patient at Point A, B & C (See Figure 2) The images show little difference between point A and C; with fibre width, fibre density and pore space all being

similar. The image of point B (Figure 2) in comparison to Point A & C illustrates reduced fibre width and density. The results of the rheological analysis corresponding to these images are: A) d_f = 1.739 ($G`_{GP}$= 0.061Pa; $T_{GP}$=247), B) $d_f$ = 1.650 ($G`_{GP}$= 0.021Pa; T_{GP}=3120sec) and C) d_f = 1.727 ($G`_{GP}$= 0.056Pa; T_{GP}=247).

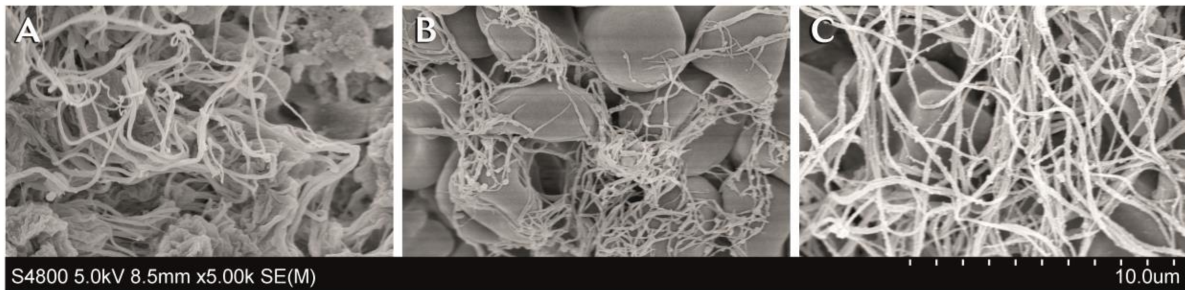

Figure 2 – Representative SEM micrographs of fully formed blood clots taken from the same individual at three different time intervals: Point A (pre-pPCI), Point B (post-pPCI) and Point C (24 hours). The images show a change in the clot microstructure characteristics, where at point B the clot has reduced fibre width and density when compared to Point A and C. The scale bar applies to all three images and is 10 μm long. The image at Point A corresponds to a d_f of 1.739, with a d_f of 1.650 at Point B and 1.727 at Point C.

Discussion

The GP biomarkers may have potential as a future tool in cardiac risk stratification, by providing a single test to better assess the effects of antithrombotic therapy which may improve patient outcome by providing a more individually tailored therapeutic approach. We present the first data investigating a novel biomarker of clot microstructure, *Gel Point*, in the hospital setting of STEMI patients undergoing pPCI. Gel Point biomarkers can be safely and rapidly obtained in emergency clinical areas including the cardiac catheter laboratory and coronary care unit, without disruption or time delay to clinical care pathways, demonstrating potential as a useful clinical tool.

Therapeutic intervention using anti-platelets and thrombin inhibitors results in a significant reduction in d_f (from 1.751 ± 0.052 at point A to 1.634 ± 0.058 at point B, $p<0.000010$). This is consistent with our previous observations of a reduction of d_f in healthy volunteer blood by the addition of heparin in vitro and in venous thrombo-embolism (VTE) outpatients receiving oral anticoagulants.[2,5] On day 1 post pPCI following clearance of peri-procedural anti-thrombotic d_f increases to 1.742 ± 0.041.

To fully appreciate what these changes in d_f represent, it is useful to consider a relationship between d_f and the amount of fibrin mass structurally organized within the clot.[19] Figure 3 illustrates the non-linear relationship between d_f and mass, where a clot with a d_f of 1.84 (the highest single value of d_f obtained in the study) incorporates approximately 4 times the amount of mass than a clot formed with a d_f of 1.74. Whereas, a reduction in d_f from 1.75 (Point A) to 1.63 (point B- post treatment) would result in a 75% decrease in clot mass. The structural and mass

changes between point A and B would result in very different structures being formed, we illustrate this with the images shown in Figure 3. This is an important finding considering previous studies have shown the significant role clot microstructure has on fibrinolysis. [23,24] The images provided in Figure 3 are representative of different fractal structures with d_f values of 1.63, 1.75 and 1.84. These images clearly show a significant difference in the structures of the clots formed over this range, the high density of red (nodes) in the 1.84 images indicates a higher density of mass in that area with a large amount of inter connectivity creating a very strong clot. As d_f is reduced the number of these dense areas (red) will reduce where at 1.75 there are only a handful of red areas and at 1.63 there are barely any. Consequently these clots will mechanically weaker and more highly friable. These observations are supported by the by the significant positive correlation between d_f and $G`_{GP}$ ($p<0.001$), the highest values of $d_f$ corresponding to the highest values of clot strength, taking the elastic modulus, $G`_{GP}$, as analogous to that of clot *'strength'*.

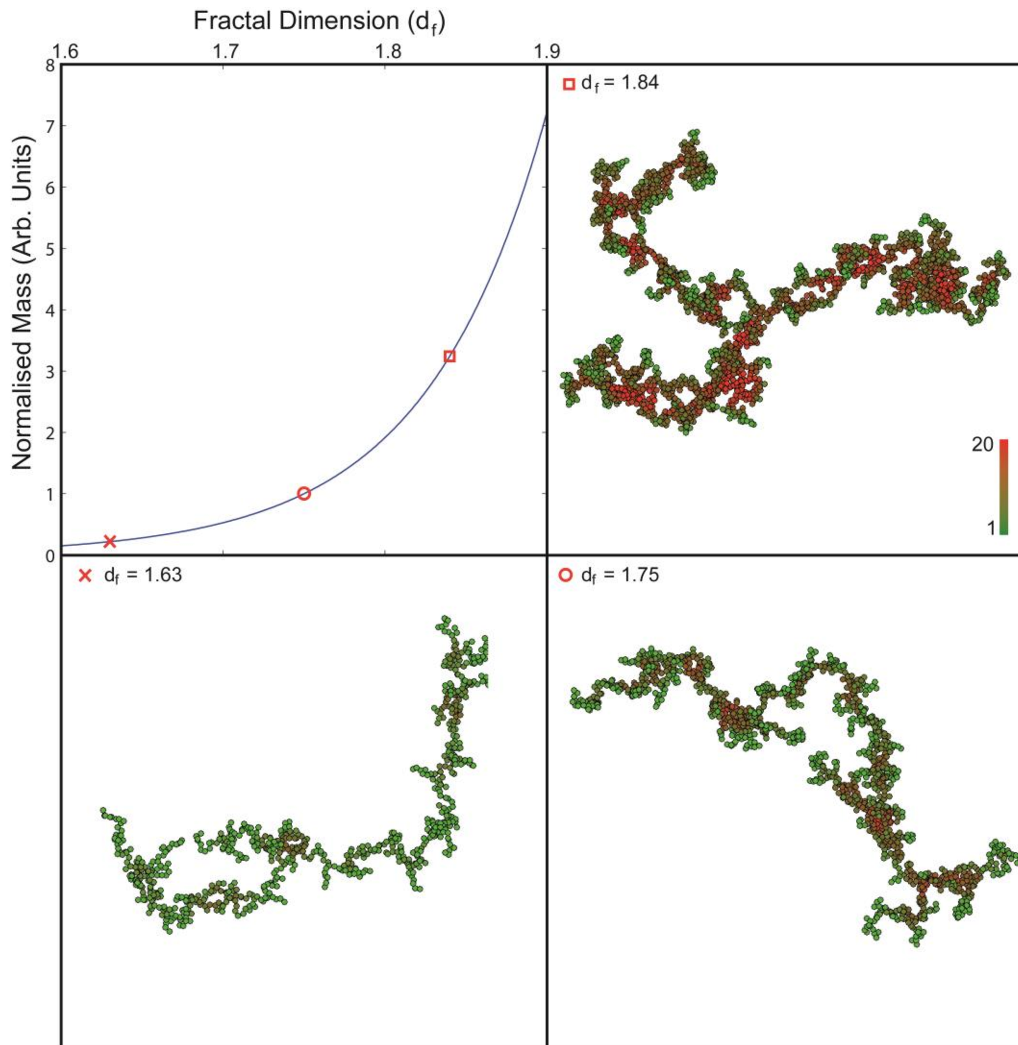

Figure 3 – Graph of *d_f* *vs.* Mass. Illustration of the non-linear relationship between d_f and the amount of mass, incorporated into the structure. Substantial increases in mass are required to generate small increments of d_f. The mass value on the y-axis is normalised for the healthy index value of d_f (=1.74). Illustrations of different incipient clot microstructures at particular values of d_f are provided (cross = 1.63, circle = 1.75 and square = 1.84 respectively). The colour of each node (unit sphere) within the fractal represents the local density of constituent nodes within a sphere of radii 5 units, the colour ranges from green (1 neighbouring node) to red (20 neighbouring nodes).

It has been previously suggested that only some 15-20% of the total available fibrinogen is estimated to be incorporated within the *incipient clot,* an important consideration when taking into account further clot development.[25] A recent study involving analysis of viscoelastic and imaging data has established that the incipient clot acts as a template for ensuing clot development.[17] The results herein show that incipient clot microstructure (d_f) mirror the changes observed in the mature clot (SEM images). Little change is observed when comparing the images Figure 2a & 2c (point A and C), the values of d_f and $G`_{GP}$ recorded at these points were also similar (1.739 to 1.727 and 0.061Pa to 0.059Pa respectively). In contrast the clot shown in Figure 2b (point B) illustrates a change to thinner fibres and significantly less crosslinking and density which corresponds to a decrease in both $d_f$ and $G`_{GP}$ (1.650, 0.021Pa) and an increase in T_{GP} from 247sec to 3120sec.

Non-response to anti-platelet therapy is an area of clinical concern with patient resistance up to 45% with aspirin and 35% with clopidogrel and an increased thrombotic risk.[26-28] In the present study we found no patients were aspirin resistant, however, at point C (24 hours after PCI) 8 patients are shown to be clopidegral low responders. Interestingly the mean d_f of these 8 patients was 1.761 ± 0.0331 compared to the rest of the group whose d_f was 1.735 ± 0.0428 (p=0.11).This highlights the need for larger studies assessing the link between platelet function and d_f, where ineffective antiplatelet therapy may be detected by d_f.

Limitations of the study included the inability to investigate the individual effect of the therapeutic interventions as many are given simultaneously or to catch the patients when they were treatment naive. Due to current care pathways each patient is given

a standard dose of 300mg aspirin en route to hospital. Thirdly, this study employed convenience sampling due to practical issues. However all patients were screened and considered for inclusion in the study when a researcher was available and baseline characteristics of the study participants is reassuring as it reflects what is expected in this patient population.

In summary we present the first data investigating Gel point biomarkers in STEMI patients undergoing pPCI. The management of acute coronary syndromes (ACS) requires a fine clinical balance of anticoagulation to treat pathological thrombus burden (efficacy) with the risks of bleeding, (safety).[12] Response to antiplatelet and anticoagulant therapy has significant inter-individual variation.[11] This study identifies that therapeutic intervention in STEMI patients with drugs given during pPCI has significant effects on clot microstructure, clot strength and the clot formation time as measured at the GP by d_f, $G`_{GP}$ and $T_{GP}$. Further investigations of bedside $d_f$, $G`_{GP}$ & T_{GP} in ACS patients are required to determine how this novel biomarker might have a role in determining optimum individual patient tailored therapy.

Acknowledgements and affiliations

We would like to acknowledge Steve Atherton, Medical Illustrations Department, Morriston Hospital & Dan Curtis, College of Engineering, Swansea University and to all the staff working in the Haemostasis Biomedical Research Unit, Coronary Care Unit and Catheterization Laboratory in Morriston Hospital. This work was supported by the National Institute of Social Care and Health Research Biomedical Research Unit grant scheme (grant number BRO1).

Conflict of Interest

None declared.

References

1. Weisel JW, Litvinov RI. Mechanisms of fibrin polymerization and clinical implications. *Blood* 2013;121:1712-9.
2. Lawrence MJ, Sabra A, Mills G, et al. A new biomarker quantifies differences in clot microstructure in patients with venous thromboembolism. *Br J Haematol* 2014 doi: 10.1111/bjh.13173 [Epub ahead of print]
3. Undas A, Zawilska K, Ciesla-Dul M, et al. Altered fibrin clot structure/function in patients with idiopathic venous thromboembolism and in their relatives. *Blood* 2009;114:4272-8
4. Undas A, Ariens RAS. Fibrin Clot Structure and Function: A Role in the Pathophysiology of Arterial and Venous Thromboembolic Diseases. *Arterioscler Thromb Vasc Biol* 2011;31:e88-e99
5. Evans PA, Hawkins K, Morris RHK, et al. Gel point and fractal microstructure of incipient clots are significant new markers of hemostasis for healthy and anticoagulated blood. *Blood* 2010;116:3341-4
6. Lawrence MJ, Kumar S, Hawkins K, et al. A new structural biomarker that quantifies and predicts changes in clot strength and quality in a model of progressive haemodilution. *Thromb Res* 2014;134:488-94
7. Collet JP, Allali Y, Lesty C, et al. Altered fibrin architecture is associated with hypofibrinolysis and premature coronary atherothrombosis. *Arterioscler Thromb Vasc Biol* 2006;26:2567-73.
8. Undas A, Zalewski J, Krochin M, et al. Altered plasma fibrin clot properties are associated with in-stent thrombosis. *Arterioscler Thromb Vasc Biol* 2010;30:276-82.

9. Mills JD, Ariëns RAS, Mansfield MW, Gran PJ. Altered fibrin clot structure in the healthy relatives of patients with premature coronary artery disease. *Circulation* 2002;106:1938-42.
10. Silvain J, Collet JP, Nagaswami C, et al. Composition of coronary thrombus in acute myocardial infarction. *J Am Coll Cardiol* 2011;57:1359-67.
11. O'Gara PT, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol.* 2013;61:e78-140.
12. Spencer FA, Moscucci M, Granger CB, et al. Does comorbidity account for the excess mortality in patients with major bleeding in acute myocardial infarction?. *Circulation* 2007;116:2793-801.
13. The TIMI Study group. The Thrombolysis in Myocardial Infarction (TIMI) Trial — Phase I Findings. *N Engl J Med* 1985;312:932-6
14. Winter HH, Chambon F. Analysis of linear viscoelasticity of a cross-linking polymer at the Gel-Point. *J Rheol* 1986;30:367-82
15. Scanlan JC, Winter HH. Composition dependence of the viscoelasticity of end-linked poly(dimethylsiloxane) at the gel point. *Macromolecules* 1991;24:47-54
16. Muthukumar M, Winter HH. Fractal dimension of a crosslinking polymer at the gel point. *Macromolecules* 1986;19:1284–5
17. Curtis DJ, Williams PR, Badiei N, et al. A study of microstructural templating in fibrin–thrombin gel networks by spectral and viscoelastic analysis. *Soft Matter* 2013;9:4883-9

18. Brown MR, Curtis DJ, Rees P, et al. Fractal discrimination of random fractal aggregates and its application in biomarker analysis for blood coagulation. *Chaos Solitons & Fractals* 2012;45:1025-32.
19. Curtis DJ, Brown MR, Hawkins K, et al. Rheometrical and molecular dynamics simulation studies of incipient clot formation in fibrin-thrombin gels: An activation limited aggregation approach. *J Nonnewton Fluid Mech* 2011;166:928-32
20. Langer BG, Weisel JW, Dinauer PA, et al. Deglycosylation of fibrinogen accelerates polymerization and increases lateral aggregation of fibrin fibers. *J Bio Chem* 1988;263:15056-63
21. Al-Azzam SI, Alzoubi KH, Khabour O, et al. The prevalence and factors associated with aspirin resistance in patients premedicated with aspirin. *Acta Cardiol* 2012;67:445-8.
22. Sibbing D, Braun S, Morath T, et al. Platelet reactivity after clopidogrel treatment assessed with point-of-care analysis and early drug-eluting stent thrombosis. *J Am Coll Cardiol* 2009;53:849-56.
23. Collet JP, Park D, Lesty C, et al. Influence of fibrin network conformation and fibrin fiber diameter on fibrinolysis speed: dynamic and structural approaches by confocal microscopy. *Arterio Thromb Vasc Biol* 2000;20:1354-61
24. Gabriel DA, Muga K, Boothroyd EM. The effect of fibrin structure on fibrinolysis. *J Biol Chem* 1992;267:24259-63
25. Weisel JW. The mechanical properties of fibrin for basic scientists and clinicians. *Biophys Chem* 2004;112:267-76.

26. Dorsch MP, Lee JS, Lynch DR, et al. Aspirin Resistance in Patients with Stable Coronary Artery Disease with and without a History of Myocardial Infarction. *Ann Pharmacother* 2007;41:737–41.
27. Nguyen TA, Diodati JG, Pharand C. Resistance to clopidogrel: A review of the evidence. *J Am Coll Cardiol* 2005;45:1157-64
28. Snoep, JD, Hovens MM, Eikenboom JC, et al. Association of laboratory-defined aspirin resistance with a higher risk of recurrent cardiovascular events: a systematic review and meta-analysis. *Arch Intern Med* 2007;167:1593-9.