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The Impact of Patient Characteristics and Antiplatelet Regimes on Clot Microstructure in Patients Treated for ST Elevation Myocardial Infarction: Clot Microstructure can Evaluate Therapeutic Efficacy

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Abstract

Background: Unfavourable clot microstructure is associated with adverse outcomes in ST elevation myocardial infarction (STEMI). We investigated the effect of comorbidities and anti-platelet treatment on clot microstructure in STEMI patients using fractal dimension (d_f), a novel biomarker of clot microstructure derived from the visco-elastic properties of whole blood.
Methods: Patients with STEMI (n = 187) were recruited sequentially receiving aspirin with Clopidogrel (n = 157) then Ticagrelor (n = 30). Patient characteristics and blood for rheological analysis obtained. We quantified d_f using sequential frequency sweep tests to obtain the phase angle of the Gel Point which is synonymous with the clot microstructure.
Results: Higher d_f was observed in males (1.755 ± 0.068) versus females (1.719 ± 0.061, $p = .001$), in patients with diabetes (1.786 ± 0.067 vs 1.743 ± 0.046, $p < .001$), hypertension (1.760 ± 0.065 vs 1.738 ± 0.069, $p = .03$) and previous MI (1.787 ± 0.073 vs 1.744 ± 0.066, $p = .011$) compared to without. Patients receiving Ticagrelor had lower d_f than those receiving Clopidogrel (1.708 ± 0.060 vs 1.755 ± 0.067, $p < .001$). Significant correlation with d_f was found with haematocrit ($r = 0.331$, $p < .0001$), low-density lipoprotein (LDL) ($r = 0.155$, $p = .046$) and fibrinogen ($r = 0.182$, $p = .014$). Following multiple regression analysis, diabetes, LDL, fibrinogen and haematocrit remained associated with higher d_f while treatment with Ticagrelor remained associated with lower d_f.
Conclusions: The biomarker d_f uniquely evaluates the effect of interactions between treatment and underlying disease on clot microstructure. STEMI patients with diabetes and elevated LDL had higher d_f, indicating denser clot. Ticagrelor resulted in a lower d_f than Clopidogrel signifying a less compact clot.

Keywords

ST elevation myocardial infarction (STEMI), clot microstructure, fractal analysis, lipids, diabetes

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Introduction

Coronary thrombosis is well established as a central feature of acute myocardial infarction[1] and the risk of myocardial infarction is increased by both inherited and acquired hypercoagulable states.[2] Most myocardial infarctions are precipitated by rupture of vulnerable atherosclerotic plaque with exposure of the subendothelial matrix to blood leading to promotion of platelet activity and coagulation pathway activation resulting in arterial thrombosis, vessel occlusion and myocyte necrosis.[3]

In the treatment of ST-elevation myocardial infarction (STEMI) early revascularisation with primary percutaneous intervention is the priority to restore perfusion and limit cell necrosis, significantly improving morbidity and mortality.[4] Following revascularisation, treatment of co-morbid risk factors such as diabetes, hypertension, hyperlipidaemia and smoking is required and a dual antiplatelet regime including aspirin and a second antiplatelet agent is recommended to maintain stent patency and prevent further thrombus related ischaemic events.[5]

Recent studies have shown the importance of the mechanical properties of the clot in both the effectiveness of treatment and patient outcome, with poor outcome associated with clot formation of highly dense and tightly packed fibrin microstructures which are resistant to deformation and therapeutic breakdown.[6,7] Current techniques such as electron microscopy that are used for assessing clot microstructure are not suitable for routine use in a clinical setting.[8] A novel biomarker of clot quality has been developed that assesses the viscoelastic properties of coagulating blood to quantify its clot microstructure in terms of a fractal dimension (d_f) with increasing values representing denser, more complex clots.[8] The measurement of d_f uses whole blood and can be performed in a near patient setting thus providing a rapid assessment of coagulation.[8] It has been shown that patients with atherosclerosis are associated with rapidly forming, denser, and more compact clots and that clot quality can be modified by anti-thrombotic and antiplatelet therapy.[9,10]

In this study we aimed to investigate the effect of comorbidities and treatment on clot microstructure in a cohort of STEMI patients using the novel biomarker d_f.

Patients and Methods

Study Design and Population Group

This study complied with the declaration of Helsinki and was approved by a local Research Ethics Committee (Wales Research Ethics Committee 6; REC reference number 13/WA/0366). Adult (≥18 years) patients who had suffered a STEMI and received primary PCI (PPCI) were recruited at a tertiary centre. Informed written consent was obtained for all participants the morning after (12 h) admission of PPCI. Exclusion criteria included any known malignancy, blood dyscrasia, anticoagulation use prior to hospital admission or requirement for prolonged anticoagulation or GP IIb/IIIa antagonist use following PPCI.

Prior to PPCI all patients received 300 mg of aspirin and a dose of P2Y12 inhibitor (either 600 mg of Clopidogrel for the initial 157 patients then following a change in departmental

policy 30 patients received 180 mg of Ticagrelor). During PCI, anticoagulation was achieved via a bolus of Bivalirudin (0.75 mg/kg) followed by an infusion (1.75 mg/kg/hr) or unfractionated heparin given as a weight adjusted dose (80-100 mcg/kg) depending on the operator's preference. All patients continued to receive 75 mg of aspirin and either 75 mg of Clopidogrel or 90 mg of Ticagrelor commencing 12 h after the procedure. Routinely prescribed venous thromboprophylaxis was withheld until after blood sampling.

Demographic Variables

Demographics data was collected for each patient, including presence of diabetes, hypertension, smoking status, previous myocardial infarction, family history of ischaemic heart disease, statin usage and low-density lipoprotein level (LDL). In addition, ischaemic time (time from symptoms onset to restoration of coronary flow) was recorded. All cause mortality was obtained for all patients out to 2 years from the index PPCI.

Blood Sampling

Twenty ml of blood was drawn on the day following the PCI procedure (at least 12 h following the end of the PPCI) to allow effects of peri-procedural anticoagulation to have worn off and after the prophylactic dose of the P2Y12 inhibitor had been administered. Each blood sample was divided into several aliquots. One aliquot of whole venous blood was used immediately for viscoelastic measurements. The remaining aliquots were used to perform standard coagulation screens and full blood count (FBC). The maximum troponin T value during admission was also recorded.

Laboratory Markers

A 4 ml aliquot of blood was used for FBC 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 h of collection.

An additional 4.5 ml 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 h of collection. Fibrinogen calibration was verified against the second International Fibrinogen Standard Version 4 (NIBSC code 96/612). All reagents were obtained from Siemens, (Frimley, UK). All testing was performed within 2 h of sample collection.

Rheometric Measurements: the Gel Point

In this 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. Sequential frequency sweep tests were performed to obtain the Gel Point (GP), which identifies the transition of the blood from a visco-elastic liquid to a visco-elastic solid.[8] The instant where the phase angle becomes frequency independent (identified by crossover of phase angle at the 4 frequencies) is the GP and marks where the blood's fibrin network first becomes a sample spanning haematological stable clot ie capable of performing haemostatic functionality. The phase angle at the GP is synonymous with how the fibrin microstructure of the clot is organised and can be quantified using fractal dimension (d_f).[8] Full details of the technique are available as described in previous publications.[8–10]

Statistical Analysis

Statistical analysis was performed using Graph Pad Prism 9.0. Descriptive statistics of tests were calculated as mean and standard deviation (SD). Association between variables was explored using two-sample comparisons undertaken using the two-sample t-test to investigate differences in d_f. Pearson's correlation analysis was used to investigate significant relationships between d_f and continuous variables that were normally distributed. Multiple Regression was used to investigate significant relationships between d_f and the continuous predictors investigated and factors demonstrating significant association with d_f. Results were deemed significant when $p < .05$ and the absence of multicollinearity was confirmed using Variance Inflation.

Results

Influence of Patient Parameters on Fractal Dimension (d_f) of the Incipient Clot

In total, 187 patients with STEMI undergoing PPCI were included. The baseline demographic details are included in Table 1. The 2-year mortality for all patients was 4.8%. There was no difference in mortality in patients taking Clopidogrel (5.1%) and Ticagrelor (3.3%), p > .99. Our data (Table 2) demonstrated that d_f was significantly higher in male patients than females (1.755 ± 0.068 vs 1.719 ± 0.061, $p = .001$) and in those with diabetes compared with those without diabetes (Figures 1 and 2) (1.786 ± 0.067 vs 1.743 ± 0.046. $p < .001$). It was also higher in those with hypertension compared to without (1.760 ± 0.065 vs 1.738 ± 0.069, $p = .03$) and in those with a history of previous MI compared to without (1.787 ± 0.073 vs 1.744 ± 0.066, $p = 0.011$). No significant difference in d_f was observed between smokers and non-smokers, those receiving and not receiving statin therapy and those with and without a family history of ischaemic heart disease. Ischaemia time was separated into <12 h and prolonged ischaemic time (>12 h) and there was no difference in d_f between these two groups. Importantly, patients receiving the P2Y12 platelet inhibitor Ticagrelor had a significantly lower d_f than those receiving Clopidogrel (1.708 ± 0.060 vs 1.755 ± 0.067, $p < .001$).

Correlation of continuous variables are shown in Table 3. The strongest correlation with d_f was with haematocrit (Hct)

Table 1. Baseline Demographics with Mean Values of the Total Population.

Patient characteristics, n = 187	
Male	76%
Age	63.1 ± 12.4
Diabetes	14%
Smoker	45%
Hypertension	45%
Previous myocardial infarction	11%
Family History ischaemic heart disease	13%
Statin therapy	32%
Ticagrelor	16%
d_f	1.748 ± 0.068
Ischaemic time >12 h	12%
LDL (mmol/L)	3.1 ± 1.3
Maximum Troponin T (ng/L)	2828 ± 2347
Platelets	259 ± 70
PT	10.67 ± 0.95
APTT	24.36 ± 7.05
Fibrinogen (g/L)	4.03 ± 0.80
Haematocrit	0.43 ± 0.05

Table 2 Differences in d_f in Subjects following PPCI for STEMI Acute Coronary Syndrome. All Comparisons Involved the Two-Sample T-Test as All the Data was Shown to be Normally Distributed.

Variable	n	Mean (SD)	Significance
Female	44	1.719 (0.61)	$p = .001$
Male	147	1.757 (0.068)	$p = .025$
No Diabetes	162	1.743 (0.046)	$p = .886$
Diabetes	25	1.786 (0.067)	$p = .031$
Non-Smoker	99	1.750 (0.063)	$p = .011$
Smoker	88	1.748 (0.068)	$p = .283$
No Hypertension	105	1.738 (0.069)	$p = .180$
Hypertension	82	1.760 (0.065)	$p < .001$
Previous MI	18	1.787 (0.073)	$p = .314$
No Previous MI	169	1.744 (0.066)	
Family History IHD	24	1.762 (0.083)	
No Family History IHD	163	1.746 (0.066)	
Statin therapy	55	1.758 (0.076)	
No Statin therapy	132	1.743 (0.064)	
Clopidogrel	157	1.755 (0.067)	
Ticagrelor	30	1.708 (0.060)	
Ischaemic Time (<12hrs)	23	1.745 (0.070)	
Ischaemic Time (>12hrs)	164	1.758 (0.053)	

($r = 0.331$, $p < .0001$). Significant associations with d_f were also seen with increasing low density lipoprotein (LDL) ($r = 0.155$, $p = .046$) and plasma fibrinogen ($r = 0.182$, $p = .014$). No significant association was observed with age, platelet count, maximum troponin T or standard coagulation assays (PT and APTT) in our patients.

Multiple Regression Analysis

We further examined the data using Multiple Regression Analysis (Table 4). Only the variables showing significant difference or correlation were fitted in the regression
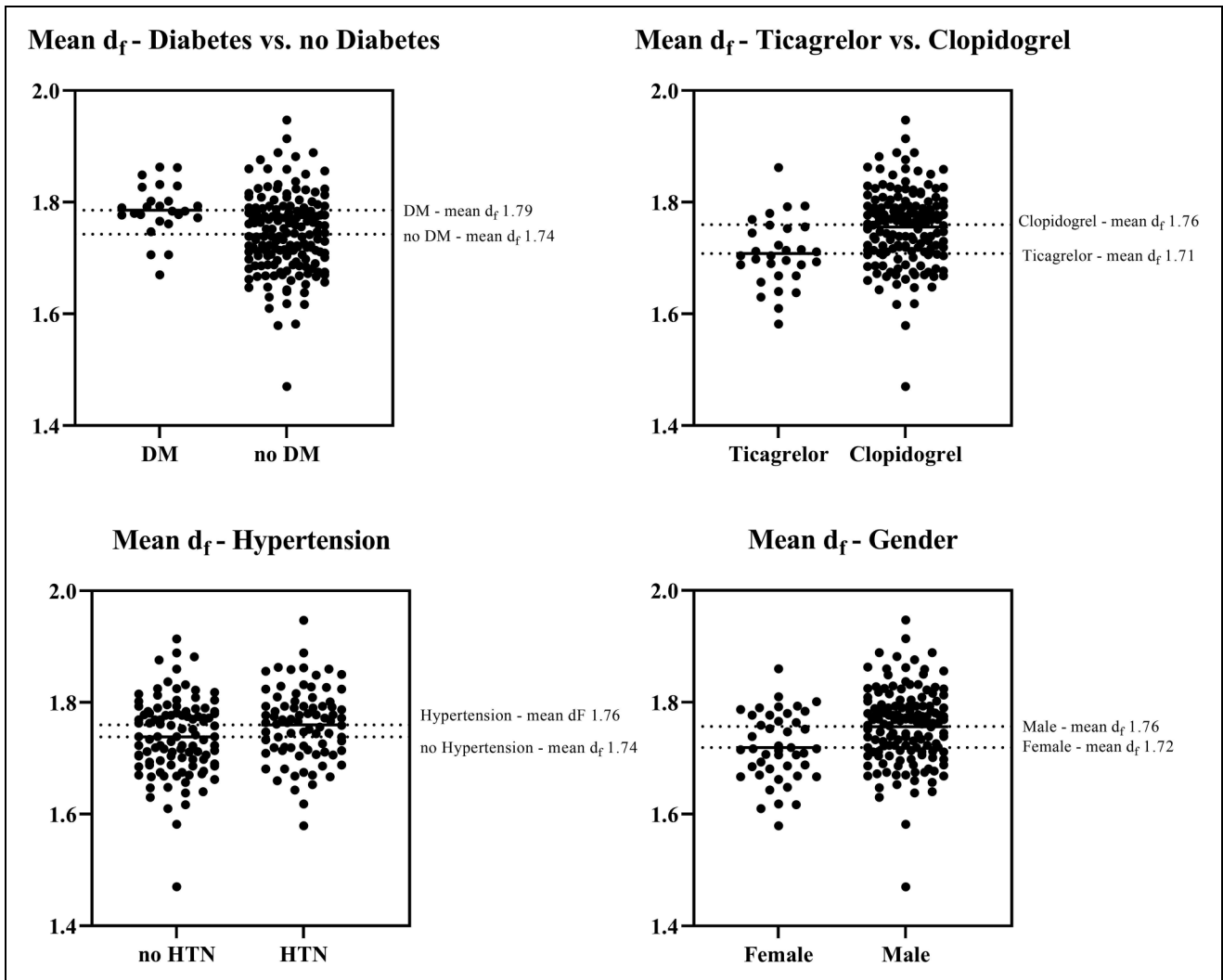
Figure I. Comparison of mean d_f of patients with diabetes (1.79 ± 0.07) and without (1.74 ± 0.05) $p < .001$, following treatment with ticagrelor (1.71 ± 0.06) and clopidogrel (1.76 ± 0.07) $p < .001$, hypertension (1.76 ± 0.07) and no hypertension (1.74 ± 0.07) $p = .03$ and male (1.76 ± 0.07) with female (1.72 ± 0.06) $p = .001$.

model. This confirmed that d_f in these STEMI patients was significantly determined after multiple regression analysis from the plasma LDL level, fibrinogen and Hct. Presence of diabetes also significantly influencing d_f but not the presence of hypertension or a history of previous MI. Male patients had demonstrated significantly higher d_f than females on univariate analysis but following multiple regression analysis (accounting for the higher haematocrit levels in males, 0.43 ± 0.04 than females, 0.39 ± 0.05, $p = .014$) gender became non-significant. Treatment with Ticagrelor remained associated with a lower d_f than Clopidogrel after multiple regression analysis. No multi-collinearity was observed in this model, as confirmed by the Variance Inflation values. In addition, the adjusted R-square was explored to determine the explanatory power of the model in determining d_f for this model the value was 29%

Discussion

Clot Microstructure in Myocardial Infarction

The mechanical structure of the clot and its development plays an important part in the treatment and outcome of myocardial infarction.[7] To date, studies have not investigated clot microstructure using a dynamic technique that measures clot microstructure and mechanical properties in a real time whole blood bedside test. We have shown previously that the d_f of the incipient clot is higher in patients with coronary artery disease both in stable and acute presentations, reflecting denser clots with abnormal morphology that are mechanically stronger and harder to break down.[9] This present study determined the effect of demographic factors such as gender and age, risk factors such as diabetes and elevated LDL and antiplatelet treatment on the clot microstructure of patients following acute myocardial infarction.
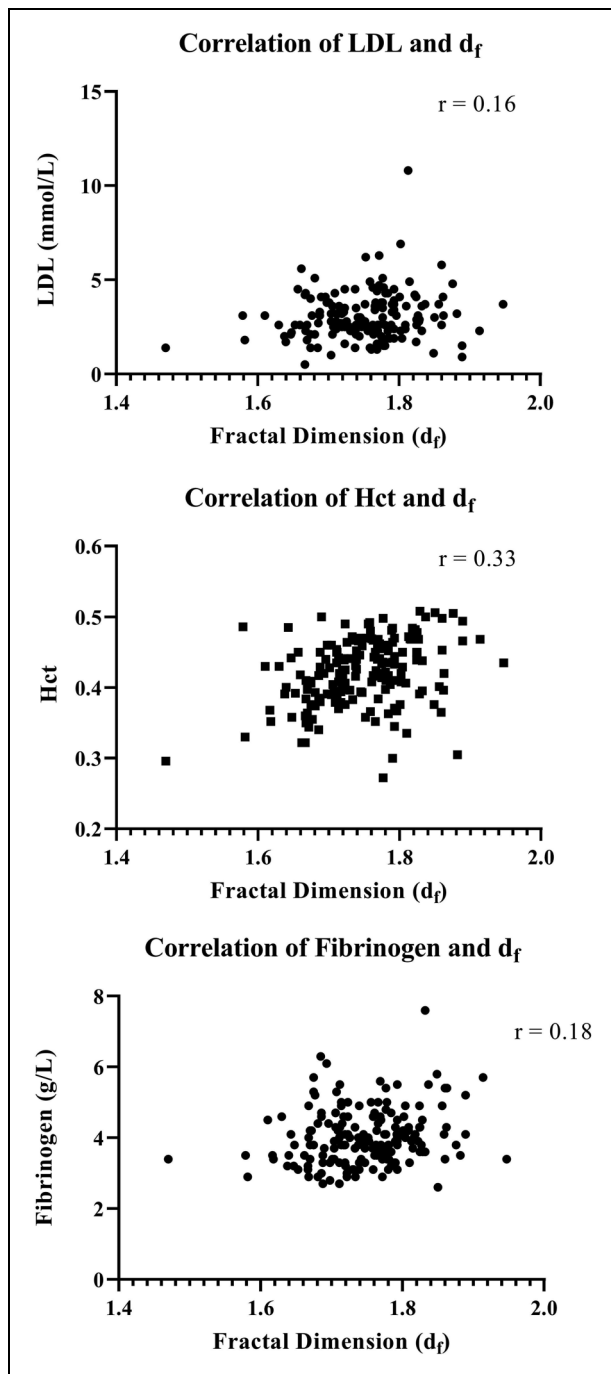
Correlation of LDL and d_f

Correlation of Hct and d_f

Correlation of Fibrinogen and d_f

Figure 2. Correlation with d_f of LDL ($r = 0.16$, $p = .046$), hct ($r = 0.33$, $p < .001$) and fibrinogen ($r = 0.18$, $p = .014$).

Anti-Platelet Treatment and Clot Microstructure Following Acute Myocardial Infarction

Platelet activation and aggregation plays a key role in acute myocardial infarction and the routine addition of Clopidogrel to Aspirin treatment reduces adverse vascular events and mortality.[11] However, the use of dual anti-platelet therapy does come with an increased risk of bleeding.[12] A significant proportion of patients (up to 25%) undergoing treatment with coronary

Table 3. Pearson's Correlations Versus d_f in STEMI Subjects.

	Correlation versus d_f	Significance
Age (years)	−0.071	$p = .332$
Maximum Troponin	−0.001	$p = .985$
Platelets	−0.070	$p = .353$
PT	0.029	$p = .698$
APTT	−0.033	$p = .660$
LDL	0.155	$p = .046$
Fibrinogen	0.182	$p = .014$
Haematocrit	0.331	$p < .0001$

Table 4. Multiple Regression to Determine the Variables and Factors Related to d_f in Patients with STEMI. VIF Refers to Variance Inflation and was Used to Determine the Absence of Multicollinearity in the Model.

	Coefficient	Standard Error	Significance	VIF
Constant	1.456	0.05369	$p < .0001$	
LDL	0.008742	0.003729	$p = .0205$	1.060
Fibrinogen	0.01710	0.006021	$p = .0052$	1.018
Haematocrit	0.4313	0.1148	$p = .0002$	1.266
Gender	0.01958	0.01267	$p = .1245$	1.244
Diabetes	0.03090	0.01456	$p = .0356$	1.126
Hypertension	0.01130	0.01041	$p = .2797$	1.202
Ticagrelor	−0.04260	0.01388	$p = .0026$	1.023

stenting during acute myocardial infarction do not respond to Clopidogrel and this is associated with adverse outcomes.[13] The P2Y12 inhibitor Ticagrelor has been shown more effective at reducing ischemic events than Clopidogrel.[14] Previously, in healthy subjects we have shown that d_f could differentiate between different antiplatelet drugs in either mono or dual therapy.[15] In that previous study, Aspirin created a negligible alteration in d_f, in comparison to P2Y12 inhibitors, which caused more significant reductions in d_f and clot mass. This study adds to that in showing that patients receiving the combination of Aspirin and Clopidogrel had a higher d_f than those patients receiving Aspirin and Ticagrelor. The effect on thrombus microstructure that corresponds to the difference in d_f between the anti-platelet regimes is illustrated in Figure 3. The nonlinear relationship between d_f and mass means that although a thrombus with a d_f of 1.71 (mean d_f in patients treated with Ticagrelor) is numerically only slightly lower than a thrombus with a d_f of 1.76 (mean d_f in patients treated with Clopidogrel), the latter incorporates approximately twice the amount of mass. This results in a thrombus with denser microstructure, more likely to be resistant to degradation.

Patient Characteristics and Fractal Dimension

Consistent with previous work on patients with stable coronary disease, we found age had no effect on d_f.[10] The standard coagulation screen (PT and APTT) were not correlated with d_f in this study, however the majority of PT and APTT values were within the normal range as patients still on anticoagulation
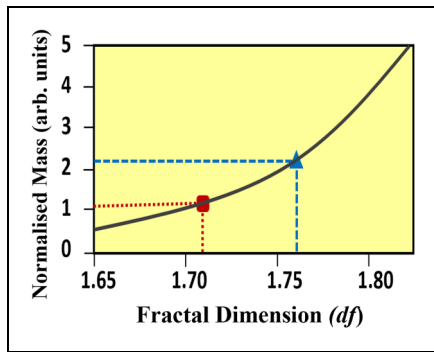
Figure 3. Difference in the amount of normalised mass incorperated into the incipient clot at square (mean d_f of ticagrelor treatment) and triangle (mean d_f of clopidgrel treatment).

when the sample was taken were excluded. Components of clot formation and physiological markers haematocrit and fibrinogen were associated with a higher value of d_f indicating altered mechanical clot properties. Interestingly, increased haematocrit and fibrinogen have been associated with a poor clot contraction process and poorer outcome.[16] Long Ischaemic time before presentation was not associated with increased d_f. That is in contrast to a previous study that demonstrated the in-situ thrombus become more dense over longer ischaemic times.[6] Our study implies that while formed thrombus becomes more organised over longer ischaemic times, this does not affect the structure of subsequent de-novo thrombus.[17]

Risk Factors for Abnormal Clot Microstructure in Myocardial Infarction

Following multi variable regression analysis the established cardiovascular risk factors smoking and hypertension had no effect on d_f. The effect of smoking is surprising given smoke exposure has previously been shown to increase thrombogenicity if examined immediately.[18] It is worth noting that we analysed d_f in patients the day following their acute MI when they would have abstained from smoking for at least 12 h. We did find that d_f was significantly higher in patients with diabetes and correlated significantly with LDL. Thrombus formation is effected by inflammation and oxidative stress.[19] Diabetes is a pro inflammatory and pro thrombotic state.[20] Patients with diabetes are recognised to have higher rates of MI, greater recurrence of MI and stent occlusions and poorer outcomes.[21] Diabetes is known to be associated with thrombus having altered mechanical properties and being more resistant to lysis.[22] This is consistent with the results of the present study, which shows that diabetes was significantly associated with an increased d_f, and indicates that people with diabetes form larger clots with more incorporated mass. The more potent oral P2Y12 receptor inhibitor, Ticagrelor, has shown increased relative benefits with higher absolute risk reductions in patients with diabetes as compared with Clopidogrel.[23] The near bedside capability of d_f testing might have potential to be exploited to tailor anti ischaemic therapy in future studies.

In addition to diabetes, abnormal blood lipids are also known to be associated with a higher incidence and recurrence of MI.[24] Hyperlipidaemia is also known to effect coagulation with oxidised LDL causing platelet activation[25] and stimulating tissue factor expression.[26] Targeted LDL reduction with PCSK9 inhibitor has been shown to reduce cardiovascular events.[27] This was corroborated in our study by the significant association between LDL levels and higher df.

Limitations

This study has some limitations that may affect its interpretation. Firstly, no baseline measurement of d_f was obtained prior to intervention. Due to this, the effect of a patients underlying d_f on the subsequent response to treatment is not known. Secondly, in this study patients were not randomised to their antiplatelet strategy, rather they were recruited sequentially receiving initially Clopidogrel then Ticagrelor following a change in clinical practice. Although we attempted to adjust for possible confounding factors with multiple regression analysis, in the absence of randomisation, some form of selection bias cannot be ruled out. It is reassuring however that our findings confirmed a lower value of d_f with Ticagrelor, which is corroborated by our previous work in healthy subjects.[15] Thirdly, d_f values are known to be influenced acutely by therapeutic anticoagulation in the catheter laboratory.[10] To account for this d_f values were taken at least 12 h after the last administration of heparin or Bivalirudin to allow any effect to wear off. Finally, this is a single centre study so caution should be employed before generalising any findings. While we found no difference in mortality between Clopidogrel and Ticagrelor usage the study contained relatively small numbers and individual bleeding events were not recorded which should be considered during interpretation given Ticagrelor has been associated with more bleeding than Clopidogrel.[28]

Conclusions

In this study, we investigated the role of patient characteristics and the effect of antiplatelet treatment on clot microstructure as determined by the fractal dimension (d_f) in STEMI patients. Patients with diabetes mellitus and elevated LDL had higher d_f indicating denser clot. Treatment with Ticagrelor as compared to Clopidogrel resulted in a reduction in d_f value linked to a looser, less compact clot. This novel biomarker is unique in that it evaluates the overall effect and endpoint of a complex interaction between different drugs and underlying disease states on the clot at a microstructural level. Further randomised controlled studies to investigate the effect of d_f on subsequent clinical outcomes are required.

Author Contributions

Declaration of Conflicting Interests

Ethics Statement

Funding

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References

1. Ridolfi RL, Hutchins GM. The relationship between coronary artery lesions and myocardial infarcts: Ulceration of atherosclerotic plaques precipitating coronary thrombosis. *Am Heart J.* 1977;93(4):468-486.

2. Chan MY, Andreotti F, Becker RC. Hypercoagulable states in cardiovascular disease. *Circulation.* 2008;118(22):2286-2297.

3. Palasubramaniam J, Wang X, Peter K. Myocardial infarction-from atherosclerosis to thrombosis. *Arterioscler Thromb Vasc Biol.* 2019;39(8):e176-ee85.

4. Zijlstra F, Hoorntje JC, de Boer MJ, et al. Long-term benefit of primary angioplasty as compared with thrombolytic therapy for acute myocardial infarction. *N Engl J Med.* 1999;341(19):1413-1419.

5. O'Gara PT, Kushner FG, Ascheim DD, et al. 2013 ACCF/AHA guideline for the management of ST-elevation myocardial infarction: Executive summary: A report of the American college of cardiology foundation/American heart association task force on practice guidelines. *J Am Coll Cardiol.* 2013;61(4):485-510.

6. Silvain J, Collet JP, Nagaswami C, et al. Composition of coronary thrombus in acute myocardial infarction. *J Am Coll Cardiol.* 2011;57(12):1359-1367.

7. Sumaya W, Wallentin L, James SK, et al. Fibrin clot properties independently predict adverse clinical outcome following acute coronary syndrome: A PLATO substudy. *Eur Heart J.* 2018;39(13):1078-1085.

8. Evans PA, Hawkins K, Morris RH, et al. Gel point and fractal microstructure of incipient blood clots are significant new markers of hemostasis for healthy and anticoagulated blood. *Blood.* 2010;116(17):3341-3346.

9. Lawrence MJ, Sabra A, Thomas P, et al. Fractal dimension: A novel clot microstructure biomarker use in ST elevation myocardial infarction patients. *Atherosclerosis.* 2015;240(2):402-407.

10. Sabra A, Lawrence MJ, Aubrey R, et al. Characterisation of clot microstructure properties in stable coronary artery disease. *Open Heart.* 2017;4(2):e000562.

11. Chen ZM, Jiang LX, Chen YP, et al. Addition of clopidogrel to aspirin in 45,852 patients with acute myocardial infarction: Randomised placebo-controlled trial. *Lancet.* 2005;366(9497):1607-1621.

12. Berger PB, Bhatt DL, Fuster V, et al. Bleeding complications with dual antiplatelet therapy among patients with stable vascular disease or risk factors for vascular disease: Results from the clopidogrel for high atherothrombotic risk and ischemic stabilization, management, and avoidance (CHARISMA) trial. *Circulation.* 2010;121(23):2575-2583.

13. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation.* 2004;109(25):3171-3175.

14. Wallentin L, Becker RC, Budaj A, et al. Ticagrelor versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med.* 2009;361(11):1045-1057.

15. Knowles RB, Lawrence MJ, Ferreira PM, et al. Platelet reactivity influences clot structure as assessed by fractal analysis of viscoelastic properties. *Platelets.* 2018;29(2):162-170.

16. Tutwiler V, Peshkova AD, Andrianova IA, Khasanova DR, Weisel JW, Litvinov RI. Contraction of blood clots is impaired in acute ischemic stroke. *Arterioscler Thromb Vasc Biol.* 2017;37(2):271-279.

17. Koskinas KC, Siontis GC, Piccolo R, et al. Impact of diabetic Status on outcomes after revascularization with drug-eluting stents in relation to coronary artery disease complexity: Patient-level pooled analysis of 6081 patients. *Circ Cardiovasc Interv.* 2016;9(2):e003255.

18. Barua RS, Sy F, Srikanth S, et al. Effects of cigarette smoke exposure on clot dynamics and fibrin structure: An ex vivo investigation. *Arterioscler Thromb Vasc Biol.* 2010;30(1):75-79.

19. Undas A, Szuldrzynski K, Stepien E, et al. Reduced clot permeability and susceptibility to lysis in patients with acute coronary syndrome: Effects of inflammation and oxidative stress. *Atherosclerosis.* 2008;196(2):551-557.

20. Aras R, Sowers JR, Arora R. The proinflammatory and hypercoagulable state of diabetes mellitus. *Rev Cardiovasc Med.* 2005;6(2):84-97.

21. Machecourt J, Danchin N, Lablanche JM, et al. Risk factors for stent thrombosis after implantation of sirolimus-eluting stents in diabetic and nondiabetic patients: The EVASTENT matched-cohort registry. *J Am Coll Cardiol.* 2007;50(6):501-508.

22. Viswanathan GN, Marshall SM, Balasubramaniam K, Badimon JJ, Zaman AG. Differences in thrombus structure and kinetics in patients with type 2 diabetes mellitus after non ST elevation acute coronary syndrome. *Thromb Res.* 2014;133(5):880-885.

23. James S, Angiolillo DJ, Cornel JH, et al. Ticagrelor vs. Clopidogrel in patients with acute coronary syndromes and diabetes: A substudy from the PLATelet inhibition and patient outcomes (PLATO) trial. *Eur Heart J.* 2010;31(24):3006-3016.

24. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The framingham study. *Ann Intern Med*. 1971;74(1):1-12.

25. Carnevale R, Bartimoccia S, Nocella C, et al. LDL Oxidation by platelets propagates platelet activation via an oxidative stress-mediated mechanism. *Atherosclerosis*. 2014;237(1):108-116.

26. Rosenson RS, Lowe GD. Effects of lipids and lipoproteins on thrombosis and rheology. *Atherosclerosis*. 1998;140(2):271-280.

27. Murphy SA, Pedersen TR, Gaciong ZA, et al. Effect of the PCSK9 inhibitor evolocumab on total cardiovascular events in patients with cardiovascular disease: A prespecified analysis from the FOURIER trial. *JAMA Cardiol*. 2019;4(7):613-619.

28. Turgeon RD, Koshman SL, Youngson E, et al. Association of ticagrelor vs clopidogrel with Major adverse coronary events in patients with acute coronary syndrome undergoing percutaneous coronary intervention. *JAMA Intern Med*. 2020;180(3):420-428.