Title: **The treatment effect of rivaroxaban on clot characteristics in patients who present acutely with first time Deep Vein Thrombosis**

Running header: **Validation of a biomarker of clot structure in anticoagulation treatment**

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Abstract (Max 250 words)

BACKGROUND: The acute vascular disease deep vein thrombosis (DVT) requires oral anticoagulants to prevent progression. Monitoring therapeutic efficacy of direct oral anticoagulants (DOAC), including rivaroxaban, is problematic as no reliable test is available. Advances in rheometry have led to the development of a functional coagulation biomarker using Gel Point (GP) analysis which assesses clot structure formation. The biomarker measures incipient clot formation time (T_{GP}) and quantifies fibrin clot structure in terms of fractal dimension (d_f).

OBJECTIVE: This study aimed to investigate clot structure formation in first time DVT and the effect of rivaroxaban treatment.

METHODS: This prospective observational cohort study measured the GP and standard laboratory markers at three sample points: pre-treatment and at 20 and 60 days following 15mg BD and 20mg OD rivaroxaban respectively.

RESULTS: Forty DVT patients (mean age 64 years [SD±14.8]; 23 males, 17 female) were recruited. The results show that DVT vs non-DVT patients did not have a significantly different GP profile (d_f: 1.72±0.06 vs 1.70±0.06 and T_{GP}: 267±68sec vs 262±73sec) with both within the defined healthy index. In addition, rivaroxaban therapy increased T_{GP} to 392s (±135s) after 20 days, and subsequently increased to 395s (±194s) at 60 days but did not significantly increase d_f (from 1.69±0.05 to 1.71±0.06).

CONCLUSIONS: The results indicate in this cohort of DVT patients there was no underlying hypercoagulable effect as determined by gel point analysis. Furthermore, the anticoagulant effect of rivaroxaban prolonged clotting, suggesting a protective effect against clot formation, without significantly reducing clot microstructural properties.

What does this paper add?

- First time DVTs do not demonstrate abnormal clot characteristics
- Gel point analysis quantifies the effect of rivaroxaban and alteration in dosage
- Rivaroxaban's effect on clotting time remained consistently prolonged.

Main Article

Introduction

Deep vein thrombosis (DVT) is a serious and potentially life-threatening condition that affects up to 1 million people annually in Europe.[1,2] Prompt diagnosis and effective management of DVT are the key goals in improving outcome in these patients.[3-5] Risk of recurrence remains a problematic issue particularly in unprovoked DVT, with a risk of recurrence approximately 10% in the first year post event, raising to 30% in 5 years.[6-7] Current guidelines recommend the use of oral anticoagulants as first line therapies in the management of DVT.[8,9] The use of anticoagulation prevents the ongoing development of the thromboembolism and reduces the risk of complications over the acute phase of the disease.[9] Increasingly, the use of Direct Oral Anticoagulants (DOACs), such as rivaroxaban and apixaban, are replacing longer established anticoagulants such as warfarin. The advantages of DOACs over warfarin include fixed dosing regimens and reduced clinical monitoring.[10] This has resulted in DOAC prescribing in England, as a proportion of total anticoagulant prescribing, increasing from 9% in 2014 to 74% in 2019.[11] One of the challenges faced by clinicians in the management of unprovoked DVT, is in deciding which patients benefit from time limited anticoagulation, and which require longer term anticoagulation.[12] DVT patients are a heterogeneous group, complicated by multiple risk factors for bleeding or thromboembolism, including age, renal function, body weight, frailty, and the presence of comorbid disease which all may influence the response to DOACs and the subsequent safety of these agents.[13,14] This promotes a difficult clinical question in how to risk stratify patients into whether they continue anticoagulation or not.

Despite the clear advantages of DOACs over the older oral anticoagulants, bleeding remains a concern for those requiring long term anticoagulation.[15,16] Furthermore, there is currently no readily available bedside test that accurately measures the therapeutic effect of DOACs. Ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) is the standard for accurate quantification of serum DOAC levels but is limited by slow turnaround times and methodologically demanding processes, making it clinically impractical.[16] Drug specific chromogenic anti-Xa assays are recommended as a means of quantifying drug levels of the direct factor Xa inhibitors (apixaban, edoxaban, and rivaroxaban), but are limited by poor reliability, long turnaround time and relatively high cost.[10] There are currently several tools to predict VTE recurrence (e.g.ERDOO2, Vienna, Dash) and bleeding risk (e.g. VTE bleed, HASBLED), however, most of these tools have limitations or have not been validated.[17,18] As a result, there is a growing need for the development of other effective tools and techniques to better assess the risk benefit/ratio for oral anticoagulation in DVT and evaluate its management.

Current research has identified a prothrombotic plasma fibrin clot phenotype associated with DVT and PE.[19-22] This fibrin clot phenotype shows a relationship between risk of recurrence and altered clot microstructural properties measured by clot lysis time (CLT) and clot structure permeability. Reduced clot structure permeability and prolonged CLT are predictive of DVT recurrence following withdrawal of anticoagulation. It has also been previously shown that VTE patients taking rivaroxaban have improved blood viscoelastic properties compared to healthy controls, which are known to be some of the most sensitive measures of clot formation.[23-26] Advanced viscoelastic techniques can also be used to link changes in viscoelasticity to features of clot structure formation.[27] This technique uses Gel Point (GP)

analysis and has been investigated and validated in a number of disease states.[28-30] The GP biomarker can provide a point of care, rapid assessment of viscoelastic and microstructural properties in coagulating blood. Previous studies have shown that GP analysis is a potential indicator of anticoagulation effectiveness and increased risk of VTE recurrence in patients with a first time DVT.[27,31]

We had previously shown that GP analysis was a significant marker of clot formation in the treatment of VTE. In anticoagulation we hypothesised that the GP analysis will also be capable of identifying the forgoing effects of rivaroxaban in first time DVT patients. To investigate this hypothesis, the present study aimed to determine changes in clot structure in a cohort of patients with suspected first time DVT compared to non-DVT patients. We also aimed to investigate the effect of anticoagulation with rivaroxaban on those with confirmed DVT.

Materials and Methods

Study design and population group

This study complies with the declaration of Helsinki and ethical favourable opinion was gained from Hampstead Research Ethics Committee (MREC no: 14/LO/1231). Adults≥18 years attending the Acute GP Unit (AGPU) for investigation of possible DVT were screened for eligibility. Informed written consent was obtained from all recruited participants and confirmation of ongoing consent was reiterated at each sample point. Exclusion criteria included: 1) Previous DVT or PE; 2) Dual antiplatelets or long-term non-steroidal anti-inflammatory drugs; 3) Current oral anticoagulation therapy; 4) pregnancy; 5) active cancer; 6) any systemic disease known to affect the coagulation system; 7) intravenous drug users; 8) known to have HIV or hepatitis; 9) liver disease; 10) severe renal impairment – stage 4-5 chronic kidney disease; 11) known hereditary clotting disorders; 12) previous family history of VTE.

Following informed consent, a blood sample was taken at the same time as standard investigational blood tests. The patients under the care of the AGPU followed the investigational protocol of the hospital including completion of the Wells Score and subsequent D-dimer and/or Doppler ultrasound scan as indicated. For patients who did not have a DVT, they entered the non-DVT group for analysis. Patients with a confirmed DVT were started on a treatment and regime appropriate for their diagnosis i.e. 3 months for provoked and 6 months for unprovoked DVT. Patients who commenced on the DOAC rivaroxaban were followed up within 21 days to provide the change of dose, and again approximately 1 month after. For both follow up appointments further blood sampling was performed.

Blood sampling

A 7ml baseline sample (Sample A) was collected from all participants prior to any treatment at the initial assessment for possible DVT following fully informed consent and meeting of inclusion and exclusion criteria. Participants with a confirmed DVT by doppler ultrasound who commenced rivaroxaban treatment had a 7ml blood sample taken at approximately 20 days (Sample B) whilst on the 15mg BD dose of rivaroxaban. The dose change of rivaroxaban to 20mg OD occurs post day 21 of treatment. The final sample of 7ml (Sample C) was taken 60-90 days from the initial commencement of treatment. Blood testing as part of the patient's standard care was taken at the same time to minimise venepuncture for the patient. Standard testing included full blood count (FBC), coagulation testing, urea and electrolytes. The full range of testing was performed on all patients at each sample point. The rate of attrition of the study was very low with a 100% follow up at sample B and 95% in sample C. Each 7ml sample underwent haemorheological testing (6.6ml) and in addition 21µl was used to obtain SEM images of whole blood mature clots formed ex vivo.

GP Analysis

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^{o}C ± 0.1^{o}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 [27]. Figure 1 shows the quantification of the GP, where blood is loaded between two surfaces, to which small amplitude oscillatory stress measurements are taken (Figure 1 A). These measurements are used to measure the phase angle of the material. The phase angle measures viscoelasticity and has a range from zero to

ninety degrees. Phase angle measures whether the material deforms elastically like a solid (low phase angle result) or flows like a liquid (high phase angle result). To obtain the GP measurement the phase angle is measured over time as the blood clots (Figure 1 B). Four different frequencies are used (2Hz, 1.2Hz, 0.6Hz and 0.2Hz) to measure the changes in phase angle. The point where phase angle becomes frequency independent (crossover of the 4 frequencies) is the GP and is deemed the point where the blood first becomes a sample spanning haematological stable clot. It has been previously established that the phase angle at the GP is synonymous with the fibrin structure of the clot and can be quantified using fractal dimension, d_f.[26,27] Therefore, GP results provide several useful measures of clotting (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 elasticity of the incipient clot; and (iii) the fractal dimension of the clot, d_f, which is a quantification of the clot's fibrin structure. The GP technique has been previously validated for use with blood in several studies [2,5,6].

Scanning electron microscopy (SEM)

Scanning electron microscopy was performed on coagulated blood for a sample of the DVT patients receiving rivaroxaban. Samples at baseline, 21 days and 3 months were analysed to investigate the effect of anticoagulation on clot structure formation. Clots were washed with ca-codylate buffer and fixed with glutaraldehyde. Point-critical dehydration with ethanol (30-100%) and hexamethyldisiazane fixed the samples for imaging. The sample underwent chromium sputter and imaged using a Hitachi Ultra-high resolution FE-SEM S-4800.

Laboratory markers

Each participant had a full blood count measured at all sample points. Blood was collected in 4ml full-draw dipotassium EDTA Vacuettes (Becton Dickinson, Plymouth, UK Ref: 367839). Within 2 hours of sample collection the blood was analysed using the Sysmex XE 2100 (Sysmex UK, Milton Keynes, UK) automated haematology analyser. Each participant also had routine coagulation studies at all sample points. 2.7ml of blood was drawn into a citrated silicone glass Vacutainer (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. 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. D-dimer analysis was carried out in presenting patients with possible DVT. D-dimer analysis was carried out using latex immunoturbidimetric assay Hemosil HS d-dimer (Instrumentation Laboratory, Warrington, UK). The d-dimer assay was performed on an ACL TOP 500 (Instrumentation Laboratory, Warrington, UK). Urea and electrolytes were measured in all participants at all sample points, blood drawn into 5ml SST II vacutainers (BectonDickinson, Plymouth, UK Ref:367954).

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. Two sample t-test and Wilcoxon signed-rank were used to compare differences between two sampling points and one-way ANOVA with

Bonferroni analysis was used to compare differences across all sampling points. Spearman's correlation analysis was undertaken 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. IBM Statistical Package for Social Sciences (SPSS) for Windows, version 22.0 (Armonk, NY: IBM Corp.) and Minitab version 16 software (Havertown, PA) were used to perform the analysis. Analysis of covariance using a general linear model was also used to model the effect on T_{GP} of gender, sample point, haematocrit and fibrinogen.

Results

Patient group

A total of 217 were included in the study, 40 with confirmed DVT and 177 tested negative for DVT. Demographic information about the DVT and non-DVT groups can be found in Table 1. The age, BMI, social factors and co-morbidities were all comparable across the groups. There were a significantly higher number of females investigated for DVT than males, although more males were confirmed to have a DVT. Provoking factors of DVT were recorded in 9 of the confirmed DVT group including 3 relating to travel, 5 orthopaedic lower limb surgeries and 1 lower limb plaster cast. Any missing data was excluded from the analysis. For comparisons between DVT and non DVT only baseline samples were used. For DVT patients only those who completed all three samples were included in the analysis.

GP markers

Results of the GP markers can be observed in Table 2 and 3. Both T_{GP} and d_f were higher in the confirmed DVT group vs non-DVT group, however not significantly. Sampling at the 3 time points (baseline, rivaroxaban 15mg BD, and rivaroxaban 20mg OD) showed significant prolongation of the T_{GP} and a non-significant reduction in d_f. Baseline measurement of T_{GP} of 267.0 ± 63.3 in the DVT group was significantly increased with treatment raising significantly to 392.3 ± 135.7, this prolongation of T_{GP} at 395.5 ±194.2 is maintained at the final sample point with the reduced dose to 20mg OD.

Standard markers

Results of the standard markers can be observed in Table 2 and 3. On diagnosis markers of inflammation: white cell count, neutrophils and monocytes were significantly elevated in the

confirmed DVT group. Analysis of the DVT group indicate that the fibrinogen concentration, white cell count, neutrophils and monocytes were all significantly raised compared to the non DVT group. The standard laboratory markers over the 3 time points in the DVT group indicated significant reduction of the white cell count and fibrinogen concentration at time points B and C compared to baseline.

Scanning Electron Microscopy

The images in Figure 2 show the fibrin network of an anticoagulated sample receiving the maximum dose of 15mg BD with a d_f of 1.69 alongside a non-anticoagulated sample with a d_f of 1.73. Comparison between the two images can be objectively made regarding fibre width, branching and openness of fibres. The sample with rivaroxaban appears to have thinner fibres with increased branching but with increased porosity.

DVT group – with confirmed PE

Within the DVT group, 3 individuals received investigation into pulmonary embolism, all were confirmed as positive. Non-parametric analysis comparing these 3 PE to the rest of the group was non-significant although as further investigations to rule out or diagnose PE were not performed on the 37 other DVT patients it is not possible to comment on this further.

Table 1: Table of demographics and comorbidities for confirmed Deep Vein Thrombosis and non-Deep Vein Thrombosis patient groups. Results include mean ± standard deviation, or number (percentage of the representative group). Significance differences determined using independent t-tests. * indicates significance. (BMI - Body mass index, AF - Atrial fibrillation)

	DVT	Non-DVT	Significance value
N	40	177	
Sex M/F	24/16	66/111	0.008*
Age (years ± SD)	64 ± 14.9	61 ± 16.6	0.359
BMI (± SD)	29.3 ± 5.5	31.3 ± 7.0	0.1114
Current smoker	10 (25%)	32 (18%)	0.319
Current alcohol use	35 (87.5%)	140 (79%)	0.226
Comorbidities			
Hypertension	14 (35%)	77 (43.5%)	0.314
Diabetes	4 (10%)	24 (13.6%)	0.606
Hypercholesterolaemia	8 (20%)	36 (20.3%)	0.949
AF	1 (2.5%)	1 (0.5%)	0.252

Table 2: Viscoelastic, haematological and coagulation biomarkers for Deep Vein Thrombosis and non-Deep Vein Thrombosis groups at presentation of symptoms. Viscoelastic markers (d_f – fractal dimension, T_{GP}(s) – Time to gel point in seconds). Coagulation markers (PT – prothrombin time, APTT - activated partial thromboplastin time) Values reported as mean ± Standard Deviation. Significance differences determined using independent t-tests. * indicates significance.

	DVT	Non-DVT	Significance value
d_f	1.717 ± 0.059	1.705 ± 0.059	0.290
T_{GP}(s)	267 ± 68	262 ± 73	0.755
Platelets (x10^9/L)	241 ±73	259 ± 66	0.121
Haematocrit (l/l)	0.429 ± 0.048	0.423 ± 0.046	0.474
White Blood Cells(x10^9/L)	9.2 ± 2.8	7.7 ± 2.1	0.001*
Neutrophils(x10^9/L)	6.3 ± 2.4	4.9 ± 1.8	0.001*
PT (s)	10.8 ± 0.6	10.6 ± 0.6	0.010*
APTT (s)	24.3 ± 2.3	24.6 ± 1.9	0.495
Fibrinogen (g/l)	4.0 ± 0.9	3.5 ± 0.9	0.014*

Table 3: **Viscoelastic, haematological and coagulation biomarkers for DVT group at baseline and during treatment**. Viscoelastic markers (d_f – fractal dimension, T_{GP}(s) – Time to gel point in seconds). Coagulation markers (PT – prothrombin time, APTT - activated partial thromboplastin time). Values reported as mean ± Standard Deviation. Significance between baseline, 15mg and 20mg doses assessed using one-way ANOVA. * indicates significance.

Coagulation markers	Baseline	Rivaroxaban 15mg BD dose	Rivaroxaban 20mg OD dose	Significance value
d_f	1.72 ± 0.059	1.69 ± 0.051	1.71 ± 0.06	0.136
T_{GP} (s)	267.1 ± 68.0[§]	392.3 ± 135.7	395.5 ± 194.2	<0.001*
Platelets (x10^9/L)	241 ±73	261 ± 93	255 ± 70	0.289
Haematocrit (l/l)	0.429 ± 0.048	0.436 ± 0.055	0.441 ± 0.053	0.335
White Blood Cells (x10^9/L)	9.2 ± 2.8[§]	7.8 ± 2.3	7.6 ± 2.1	<0.001*
PT (s)	10.8 ± 0.6[§]	13.6 ± 1.7	12.7 ± 1.4	<0.001*
APTT (s)	24.3 ± 2.3[§]	35.3 ± 5.3	33.6 ± 6.4	<0.001*
Fibrinogen (g/l)	4.0 ± 0.9[¥]	3.4 ± 0.7	3.1 ± 0.6	<0.001*

[¥]Significantly different from the Rivaroxaban 20mg OD dose only after Bonferroni correction.

[§]Significantly different from the Rivaroxaban 20mg OD and 15mg doses after Bonferroni correction

The role of gender in DVT patients

A significantly greater proportion of males in the DVT group as compared to the non-DVT was observed (Table 1). We explored for gender differences in the rheological markers in the DVT group and the analysis is presented in Table 4. T_{GP} was found to be significantly increased after treatment with rivaroxaban and that this was the case regardless of gender. Furthermore, the relationship between d_f and T_{GP} in both the females and males showed a significant negative correlation between d_f and T_{GP} in females (Pearson correlation coefficient r= -0.401 $p<0.05$) but this relationship was not observed in males. At all the timepoints analysed females had a significantly reduced d_f as compared with males (baseline: 1.69±0.06 vs 1.73±0.05 (p=0.03) 15mg BD dose: 1.67±0.05 vs 1.70±0.05, p=0.04) 20mg OD dose: 1.68±0.05 vs 1.73±0.06 (p=0.01)). These results indicate that males have a significantly higher d_f than females throughout and that this is associated with a shortening of the T_{GP}. Furthermore, although rivaroxaban therapy in males reduces d_f and shortens T_{GP} at the second time point (15mg BD dose), at the third time point (20mg OD dose) d_f returns to the baseline value suggesting that rivaroxaban therapy is unable to regulate clot structure and hence thrombotic risk as effectively in males as in females.

	Baseline	Rivaroxaban 15mg BD dose	Rivaroxaban 20mg OD dose	Significance value
d_f				
Male	1.73 ± 0.05§	1.70 ± 0.05	1.73 ± 0.06§	0.114
Female	1.69 ± 0.06§	1.67 ± 0.05	1.68 ± 0.05§	0.587
T_{GP}(s)				
Male	284.3 ± 72.5	388.3 ± 110.8	330.8 ± 108.3§	0.003*
Female	241.6 ± 54.4	399.1 ± 173.7	494.9 ± 256.9§	0.002*

Table 4: d_f and T_{GP} in males and females with DVT. DVT group at baseline and on treatment for males (n=24) and females (n=16). Values reported as mean ± Standard Deviation. Significance values between male and females was assessed using a two-way ANOVA (§ indicates significance $p<0.05$ between males and females) and (* indicates within group significance).

DVT recurrence

Follow up data was collected at 3.5 years for each of the 40 confirmed DVT patients. Eleven recurrences of DVT were reported after 3.5 years. All of the recurrent DVT patients had an unprovoked original DVT. The location of the recurrent DVT occurred in the same leg in eight of the incidences, with four of these in the same region and four in a different location of the same leg. Given the significant role of both d_f and T_{GP} we observed in subjects with DVT, we specifically investigated if significant differences existed between these biomarkers in subjects with a recurrence of DVT at each time treatment point within 3.5 years of admission. The results are presented in Table 5. At baseline d_f and T_{GP} was significantly higher in the

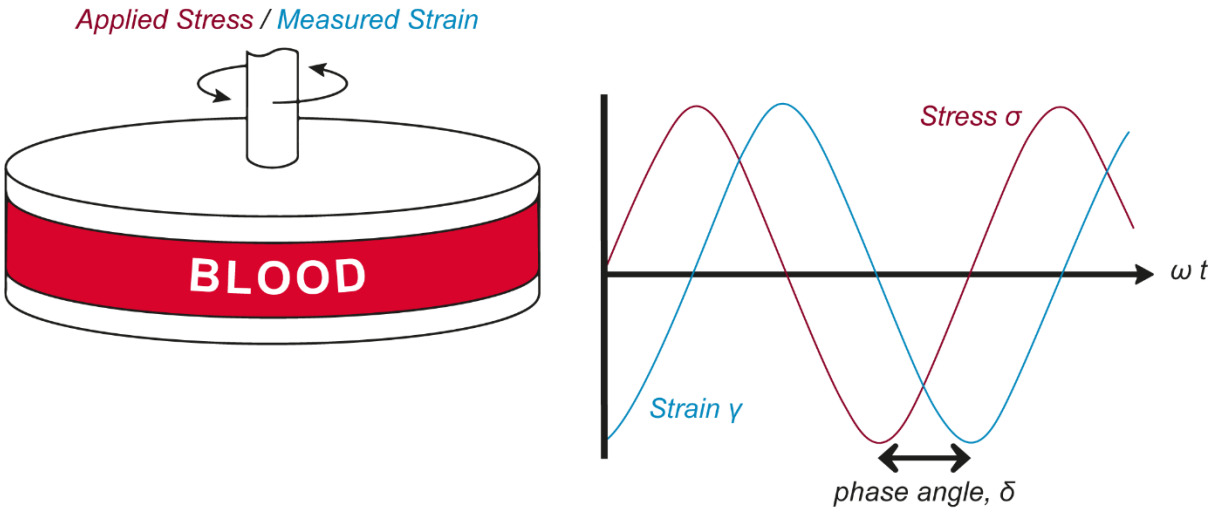
recurrent group as compared to the non-recurrent group. The results in dose regimes of rivaroxaban in the recurrent and non-recurrent groups shows a prolongation of T_{GP} and reduction in d_f.

	Baseline	Rivaroxaban 15mg BD dose	Rivaroxaban 20mg OD dose
d_f			
Non-recurrent	1.71 ± 0.06§	1.70 ± 0.05	1.69 ± 0.06
Recurrent	1.75 ± 0.06§	1.69 ± 0.05	1.72 ± 0.05
T_{GP}(s)			
Non-recurrent	281.5 ± 69.84*	406.4 ± 139.54	384.6 ± 154.29
Recurrent	227.7 ± 38.94*	354.4 ± 108.40	335.5 ± 72.80

Table 5: d_f and T_{GP} in subjects with recurrent and non-recurrent DVT. DVT group at baseline and on treatment for recurrence (n=11) and non-recurrence (n=29). Values reported as mean ± Standard Deviation. Significance values between recurrence (n=11) and non-recurrence at each treatment point was assessed using a two-sample t-test (§indicates significance $p<0.01$ between recurrent and non-recurrent subjects) and (* indicates significance at $p<0.05$).

Figure 1: Diagrammatic representation of the GP experiment: Figure 1A represents blood contained between two testing surfaces where small amplitude oscillatory stress measurements are performed and the resultant strain waveform is recorded. The differences in phase between the stress and strain waveforms are recorded as the phase angle. Figure 1B represents a typical gel point experiment result showing the change in phase angle for 4 different testing frequencies with respect to time. The GP is located where the 4 frequencies crossover. The GP defines the transition of the blood from a liquid to a solid and is the first point where a sample spanning haematological stable clot is formed. The T_{GP} (Time to GP) assesses the kinetic pathways during the liquid phase of clot formation and is a measure of clotting time. The phase angle at the GP is synonymous with clot structure and can be converted to fractal dimension. Where a high phase angle equals a low d_f value which indicates a loose/open fibrin clot structure and a low phase angle equals a high d_f value indicating a dense/tight fibrin clot structure.

A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B

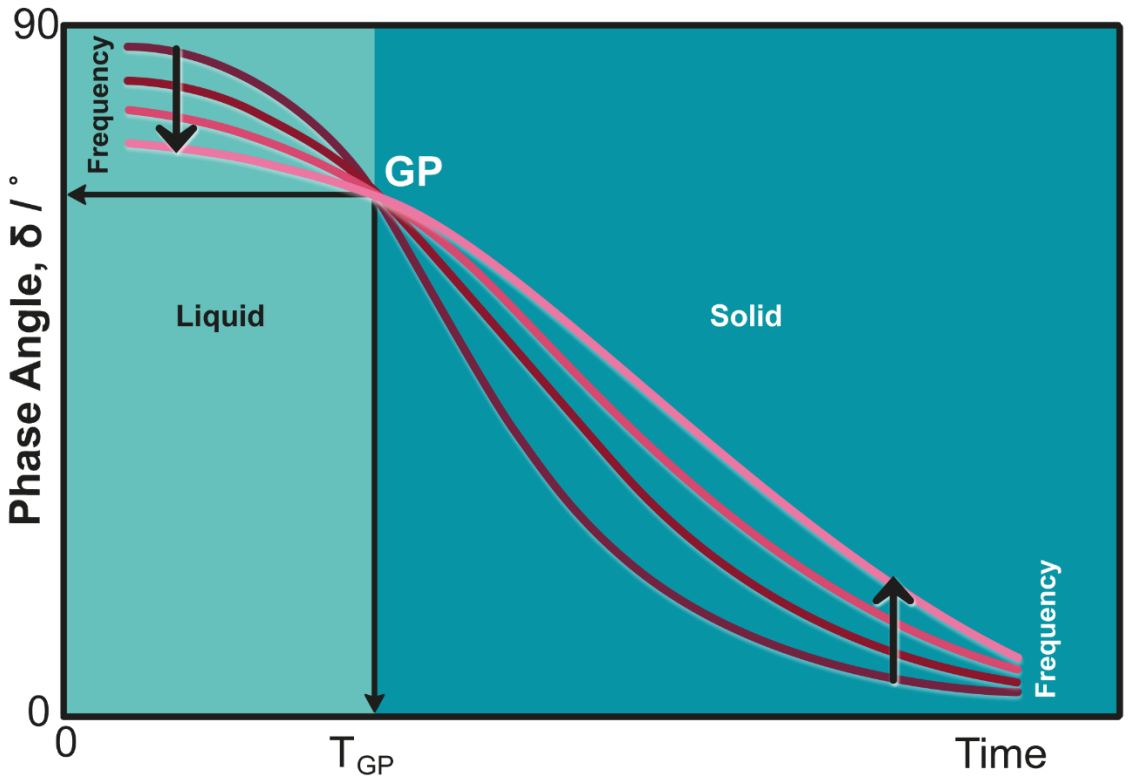
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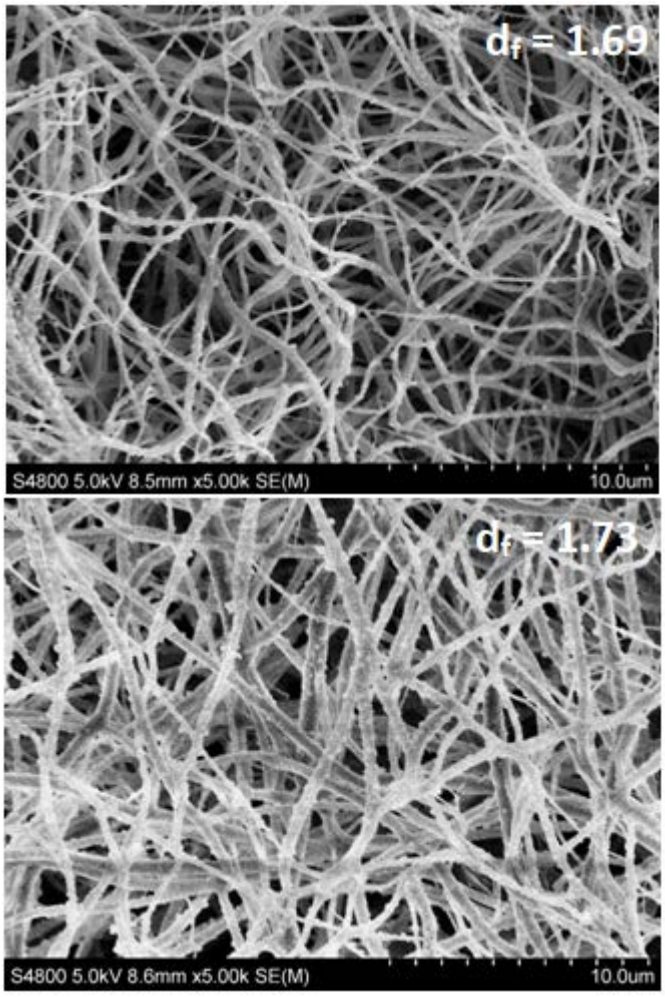
Figure 2: Scanning Electron Microscopy imaging of the fibrin network of an anticoagulated sample receiving the maximum dose of 15mg BD with a d_f of 1.69 alongside a non-anticoagulated sample with a d_f of 1.73.



Discussion

In this study we assessed the effect of rivaroxaban on viscoelastic and microstructural properties of the blood using GP analysis in first time DVT patients. The results for d_f in the DVT positive group show a trend to the formation of looser more open clot structure, especially during the maximum dose (15mg BD) given at the second time point. The changes in d_f would appear to be small but we have previously reported that these changes correlate with large changes in clot mass observed through SEM imaging.[30] Rivaroxaban increases the permeability and degradability to lysis of the fibrin clot, a feature consistent with a reduced d_f.[39] However, our initial analysis shows the d_f returns to its near original level on the reduced dose (20mg OD). [21] In contrast, the effect of rivaroxaban on T_{GP} shows a significant prolongation at both dose regimes.

Interestingly, further analysis demonstrated that gender was a significant predictor of response to rivaroxaban therapy in terms of d_f and T_{GP}. We show that females at baseline had a significantly lower d_f and end up with a longer T_{GP}, it is possible that rivaroxaban has a greater effect on females over males. The fact that males are showing significantly higher values of d_f suggests that males have a different clotting profile resulting in a different clot morphology than females. It has been reported that males in the wider population are at increased risk of DVT and thromboembolism.[40] A previous study in VTE patients fully anticoagulated with warfarin demonstrated that those patients who were found to have a higher d_f despite anticoagulation had a higher incidence of thromboembolic recurrence.[31] In the present study we show that rivaroxaban reduces d_f and prolongs T_{GP} in males at the 15mg BD dose, however, these effects are almost completely reversed at the 20mg OD dose, returning the GP clotting characteristics back to the baseline results. In comparison, females

at both dosing regimens show a non-significant reduction in d_f and a significantly prolonged T_{GP}, with the largest change in T_{GP} seen at the 20mg OD dose. This suggests that rivaroxaban administration in DVT is potentially complex, with the 20mg OD dose seemingly more effective in females than males. This work provides evidence for the rationale of a personalised medicine approach where perhaps differences in gender (and other potential cofactors) should be considered when prescribing oral anticoagulants, as the effect of these agents may not be universal across all groups. This may be especially of clinical importance given the established higher risk of recurrent VTE seen in males compared to females.[40]

The significant prolongation of T_{GP} may be part of the success of rivaroxaban treatment as a long term VTE prophylaxis, especially with consideration of stasis as an initiator in this disease. Fibrin fibres densities and thicknesses obtained in previous studies suggested that rivaroxaban resulted in the formation of 'looser', thicker fibres but this was not confirmed in the present study.[21] This study suggests that the effectiveness of the rivaroxaban is to provide a protective effect from slowing any clot formation whilst also maintaining a normal clot microstructural template (maintain a d_f value within the normal range). This may also provide a rationale for why rivaroxaban is shown to have all the benefits of old oral anticoagulation (such as warfarin) but at a reduced bleeding risk.[17] Where clotting whilst on rivaroxaban may take longer to form there is still the ability to form a good quality clot. This is not necessarily true with other anticoagulants which have been shown to dramatically reduce clot microstructural properties.[27,31]

The viscoelastic and microstructural characteristics of these DVT patients suggests that there is no systemic hypercoagulable effect (Table 2), despite significant increases observed in

fibrinogen concentration and white blood cell. Furthermore, T_{GP} and d_f for both the DVT and non-DVT groups fall within the previously described normal healthy range.[27] The increase in fibrinogen concentration and white blood cells would be expected in thrombosis of the lower limbs, this is primarily a function of inflammation due to the thrombus formation.[32] However, in the non-DVT group the values were all within or marginally outside the normal range (fibrinogen concentration 1.5-4.5 g/L, WBC 4 and 11x10^9/L, neutrophils 2.0-7.5x10^9) [33] potentially supporting our finding of no significant difference in the markers of coagulation between the two studies. We would propose that any hypercoagulability in first time DVT is a localised effect close to the site of injury. Inflammation is considered causative of and reactive to thrombosis as thrombin stimulates inflammatory mediators (e.g. interleukin(IL-6, IL-8) that activate the coagulation cascade.[34,35] Localised coagulation, in response to endothelial injury, is also an established concept, with fibrinolysis, via the primary fibrinolysin plasmin, acting as a natural control mechanism to prevent systemic thrombotic syndromes.[36] Importantly, we also report that the baseline d_f and T_{GP} were both significantly higher and lower respectively, in the subjects with a recurrent DVT within 18 months, suggestive of a more rigid denser clot in this group.

Limitations of this study include the small sample size when considering subgroup analysis, in particular due to the small numbers it was not possible to analyse for gender in the recurrent and non-recurrent groups.

This study is the first to investigate first time DVT patients using GP analysis over the commencement of their oral anticoagulation treatment with rivaroxaban. In the study we show that for these patients first time DVT does not induce a systemic hypercoagulable state. In this study, we also have shown that the GP analysis is able to quantify the effects of

rivaroxaban on blood coagulation in terms of viscoelasticity and clot structure. Where rivaroxaban significantly prolongs clotting time (T_{GP}) but does not significantly alter clot microstructural properties. This would indicate that rivaroxaban provides the protection of oral anticoagulants whilst maintaining the ability of the blood to form stable healthy clots. Further work now needs to be carried out to investigate the effectiveness of GP analysis as a predictor of outcome or recurrence in DVT and comparison for different oral anticoagulants prescribed for the treatment of DVT with testing of anti-factor Xa levels or LMWH standards.

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Contributions

PAE conceived the study. PAE and VE coordinated the study. PAE, VE, KM, JW, KP, MJL drafted the article and interpreted the data. VE, CJ SP, KP recruited the patients and collected blood samples. VE performed the rheology tests. MJL provided rheological advice and analysis. VE, JW, SP, CJ and KP collected the patient data. VE, MJL and KM performed the statistical analyses and interpreted the data. PRW, KH, KP and PAE revised the manuscript critically for important intellectual content.

Declarations

The authors declare no competing financial conflicts of interest for the work detailed in this manuscript. The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper. This study was not funded by the Rivaroxaban manufacturer Bayer.

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