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Apixaban enhances endogenous fibrinolysis in patients with atrial fibrillation

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Aims Approximately 20% of ischaemic stroke patients exhibit spontaneous arterial recanalization, attributable to endogenous fibrinolysis, which strongly relates to improved functional outcome. The impact of oral anticoagulants on endogenous fibrinolysis is unknown. Our aim was to test the hypothesis that apixaban enhances endogenous fibrinolysis in non-valvular atrial fibrillation (NVAF).

Methods and results In a prospective cross-sectional analysis, we compared endogenous fibrinolysis in NVAF patients ($n = 180$) taking aspirin, warfarin, or apixaban. In a prospective longitudinal study, patients were tested before and after apixaban ($n = 80$). Endogenous fibrinolysis was assessed using the Global Thrombosis Test (GTT) and thromboelastography (TEG). Endogenous fibrinolysis [measured by GTT lysis time (LT)] was shorter on apixaban compared with warfarin or aspirin [median 1850 (IQR 1591–2300) vs. 2758 (2014–3502) vs. 2135 (1752–2463) s, $P < 0.0001$]. Among TEG indices, a small but significant difference in clot lysis time (CLT) was observed [apixaban 60.0 (45.0–61.0) vs. warfarin 61.0 (57.0–62.0) vs. aspirin 61.0 (59.0–61.0) min, $P = 0.036$]. Apixaban improved endogenous fibrinolysis measured using the GTT [LT pre-treatment 2204 (1779–2738) vs. on-treatment 1882 (1607–2374) s, $P = 0.0003$], but not by using TEG. Change in LT (ΔLT) with apixaban correlated with baseline LT ($r = 0.77$, $P < 0.0001$). There was weak correlation between ΔLT and ΔCLT in response to apixaban ($r = 0.28$, $P = 0.02$) and between on-apixaban LT and CLT ($r = 0.25$, $P = 0.022$).

Conclusion Apixaban enhances endogenous fibrinolysis, with maximal effect in those with impaired fibrinolysis pre-treatment. Apixaban-treated patients exhibit more favourable fibrinolysis profiles than those taking warfarin or aspirin. Whether apixaban may confer additional thrombotic risk reduction in NVAF patients with impaired fibrinolysis, compared to warfarin, merits further study.

Keywords Endogenous fibrinolysis • Thrombosis • Apixaban • Atrial fibrillation • Non-vitamin K antagonist oral anticoagulant

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What's new?

- Impaired endogenous fibrinolysis is a recently recognized risk factor for adverse cardiovascular events.
- There are currently no known oral medications which can enhance endogenous fibrinolysis.
- In a prospective study of 240 patients with non-valvular atrial fibrillation, we studied the effects of apixaban on endogenous fibrinolytic status.
- We demonstrate that apixaban enhances endogenous fibrinolysis, with maximal effect in those with impaired fibrinolysis pre-treatment. This is the first study showing favourable modulation of endogenous fibrinolysis using an oral medication.
- In a cross-sectional study, apixaban-treated patients exhibited more favourable fibrinolysis profile than patients taking warfarin or aspirin.
- Future studies are needed to assess whether enhancing fibrinolysis with apixaban when this is impaired, might translate into a reduction in ischaemic and thromboembolic events, and whether patients with impaired fibrinolysis may derive greater benefit from apixaban than warfarin or aspirin.

Introduction

Spontaneous fibrinolysis is an important defence mechanism against downstream infarction following occlusive arterial thrombosis. In patients with acute stroke, spontaneous arterial recanalization is observed in 17–24% of patients and is strongly related to improved functional outcomes. Importantly, *ex vivo* endogenous fibrinolysis has been shown to be significantly impaired in patients with stroke compared to normal volunteers.[1] Impaired endogenous fibrinolysis has also been shown to be marker of recurrent thrombotic risk in patients with acute coronary syndrome[2] and end-stage renal failure.[3]

Patients with atrial fibrillation (AF) frequently exhibit impaired endogenous fibrinolysis and preliminary data indicate that this state improves after successful restoration of sinus rhythm with radiofrequency ablation.[4] A review of the importance of endogenous fibrinolysis in determining clinical outcomes concluded that global assays, assessing proaggregatory, and fibrinolytic pathways, could aid in identifying impaired fibrinolysis as a potential target for pharmacological modulation.[3]

Currently, there is no available oral pharmacotherapy to favourably modulate fibrinolytic status where this is impaired. Beside the use of plasminogen activators to achieve acute thrombolysis in the setting of acute myocardial infarction and stroke, pharmacological options to manipulate the fibrinolytic state are limited. Our preliminary data indicate that the non-vitamin K antagonist oral anticoagulants (NOACs) may enhance endogenous fibrinolysis in patients with AF, with significant effect observed only with apixaban.[4] In our pilot data in 20 patients, apixaban enhanced endogenous fibrinolysis, evidenced by a significant reduction in endogenous fibrinolysis time. However, it is noteworthy that with all NOACs, there was a trend to favourably enhancing endogenous fibrinolysis and perhaps if the sample size had been sufficiently large, a significant effect may have been observed. Neither warfarin, nor aspirin or clopidogrel, have been shown to enhance endogenous fibrinolysis.

Nevertheless, the impact of pharmacotherapy on the effectiveness of the spontaneous endogenous fibrinolytic pathway has been difficult to measure, due to lack of available techniques. Factorial assays such as plasminogen activator inhibitor 1, tissue plasminogen activator and thrombin activatable fibrinolysis inhibitor cannot provide a reflection of the *overall* state of endogenous fibrinolysis.[3] There are currently two point-of-care techniques that provide a global assessment of thrombus formation and fibrinolysis, namely thromboelastography (TEG or ROTEM), which uses citrated or whole blood, and the Global Thrombosis Test (GTT), using non-anticoagulated blood. The determinants of the results of these global tests of fibrinolysis are the thrombus properties (clot strength, determined by the thickness, density, and pore size of fibrin strands) and the rate of fibrinolysis.

We hypothesized that there was beneficial effect of apixaban on endogenous fibrinolysis in patients with non-valvular atrial fibrillation (NVAF). To test this hypothesis, we performed a cross-sectional study of NVAF patients treated with apixaban, warfarin, or aspirin. Second, we assessed the impact of initiating apixaban.

Methods

We conducted a prospective, observational (non-randomized) study in 200 stable outpatients with NVAF, approved by the National Research Ethics Service and the UK Health Research Authority (ClinicalTrials.gov identifier: NCT03199521). All subjects gave written informed consent and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

The study comprised two arms; a longitudinal arm ($n = 80$) and a cross-sectional arm ($n = 180$), with 60 patients on apixaban taking part in both. In the prospective longitudinal study, 80 patients with newly diagnosed NVAF were recruited, who were scheduled to start anticoagulation with apixaban for thromboprophylaxis of stroke and systemic embolism and who were studied before and during apixaban treatment. In the cross-sectional study, 180 patients with known NVAF already established on treatment with one of aspirin ($n = 60$), warfarin ($n = 60$) or apixaban ($n = 60$) for thromboprophylaxis of stroke and systemic embolism, were studied in an observational study, where drug allocation was by physician choice, and not randomized.

The following exclusion criteria were applied: age <18 years; significant hepatic or renal impairment likely to cause a bleeding diathesis; patients taking antiplatelet or anticoagulant therapy (except for patients taking part in the cross-sectional study), systemic steroids or immunosuppression; known active malignancy; bleeding diathesis; blood dyscrasia [platelets $<70 \times 10^9$/L, haemoglobin <80 g/L, international normalized ratio (INR) >1.4; activated partial thromboplastin time (aPTT) more than twice upper limit of normal; and leucocyte count $<3.5 \times 10^9$/L, neutrophil count $<1 \times 10^9$/L); active alcohol; or substance abuse; those involved in another investigational trial of a medicine or medical device; those unable or unwilling to provide consent.

Antithrombotic therapy

The choice of oral antithrombotic therapy was decided by the clinical care team. Apixaban was given at a dose of 5 mg b.i.d. (or 2.5 mg b.i.d. in patients with two or more of the following: age ≥80 years, weight ≤60 kg, and serum creatinine ≥133 μmol/L). In the longitudinal study, blood samples were taken before (i.e. non-anticoagulated) and after patients were established on uninterrupted apixaban treatment for at least 4 weeks. For the cross-sectional study, blood samples were obtained after patients had

been established on treatment for at least 4 weeks. Patients taking warfarin were tested after at least three previous consecutive INR readings within the therapeutic range (2.0–3.0).

Blood sampling

Venous blood samples were obtained from an antecubital vein using an 18-G butterfly cannula and a two-syringe technique. The first 5 mL was used for routine blood tests and the second 10 mL used for assessment of thrombotic status and apixaban levels measured using drug-specific calibrators (anti-Xa [FXa] level). Blood samples were taken 4–6 h after the last dose of apixaban. Fasting was not required.

Assessment of thrombotic status including endogenous fibrinolysis

Global thrombosis test

Thrombotic status was assessed using the point-of-care GTT (Thromboquest Ltd, London, UK), which assesses overall thrombotic status, including platelet reactivity, coagulation and endogenous (spontaneous) thrombolysis.[2] The test was performed on native, non-anticoagulated whole blood within 15 s of withdrawal. The instrument assesses firstly the time taken to form an occlusive thrombus under high shear, which is a marker of platelet reactivity (occlusion time, OT). Shorter OT represents enhanced platelet reactivity. Following the arrest of flow due to the formation of an occlusive platelet thrombus cross-linked by fibrin strands. Following a short stabilization period, the instrument records the time required to dissolve the thrombus formed in the first phase, through endogenous fibrinolysis, which manifests in restart of flow (lysis time, LT). Longer LTs represent less effective endogenous fibrinolysis. In addition, the GTT measures thrombus stability by measuring the number of rebleeds (number of drops, D) after OT until the compete occlusion and final arrest of flow. The intra-assay and inter-assay coefficients of variation (CV) for OT and LT were assessed in 10 subjects on repeated sampling and also running samples in parallel.

Thromboelastography

Venous blood was also assessed with TEG (TEG 5000 Hemostasis Analyser System, Haemonetics, Watford, UK). Two tests were performed in parallel for each patient, one using non-citrated whole blood tested immediately after withdrawal and one using non-citrated whole blood with the addition of kaolin activator after four minutes, according to the manufacturer's instructions. The TEG assesses platelet-independent clot formation, fibrinogen contribution to clot integrity (R, K, A, MA, and TMA) as well as the primary fibrinolytic potential of the clot (LY30, LY60, and CLT) (*Table 3*). The intra-assay and inter-assay CV for all TEG parameters were assessed in 10 subjects on repeated sampling and running samples in parallel for the whole blood alone and with kaolin activator.

Apixaban (anti-FXa) levels

In patients taking apixaban, samples were taken for apixaban level at the same time as blood draw for thrombotic status assessment. Apixaban level was measured using HemosIL Liquid Heparin kit (Werfen UK, Birchwood, Warrington, Cheshire, UK) on ACL TOP500 analyser using drug-specific calibrators. Based on population pharmacokinetics studies on patients taking apixaban 5 mg b.i.d. for stoke prevention in NVAF, peak and trough levels of apixaban (measured as anti-FXa levels) have been reported as 171 ng/mL (91–321) and 103 ng/mL (41–230), respectively.[5] Anti-FXa levels were assessed by an independent investigator blinded to GTT and TEG results.

Study endpoints

The primary endpoint was the change in thrombotic status, in particular endogenous fibrinolysis, in response to apixaban in the longitudinal study. The secondary endpoint was a comparison of endogenous fibrinolysis in patients established on different antithrombotic treatments (apixaban, warfarin, and aspirin) in the cross-sectional study.

Sample size and statistical analysis

Results from our pilot study[4] showed a 24% relative reduction in endogenous fibrinolysis time with apixaban with a medium effect size ($r = 0.4$, $z = 2.763$, $P = 0.006$). Using a two-tailed t test, we calculated that a sample size of 78 patients would be required to detect a 24% relative reduction with 90% power based on an effect size of 0.4 and $\alpha = 0.05$ (longitudinal study). For the cross-sectional study, it was assumed that endogenous fibrinolysis in patients taking warfarin and aspirin would be similar, based on our earlier pilot showing no change in endogenous fibrinolysis in response to warfarin.[6] Assuming a one-tailed independent group comparison and an effect size of 0.4, a total of 180 patients were required to give $\alpha = 0.05$ to achieve 80% power.

Data are presented as mean ± standard deviation when normally distributed, or as median (interquartile range), when non-normally distributed. Dichotomous variables were compared using the χ^2 test. Paired comparison between groups was evaluated with paired *t*-test and Wilcoxon rank sum test. The analysis of variance or Kruskal–Wallis test was used to assess differences between groups. To investigate the relationship between the change in fibrinolysis in response to apixaban and baseline characteristics, univariate and multivariate regression models were used. All variables were analysed using univariate regression; clinically relevant parameters and those shown to be significant ($P \leq 0.05$) were entered into the multivariate model. In a *post hoc* analysis, the model's assumptions were tested and the residuals were normally distributed. Regression models were used to illustrate the linear prediction between change in LT (ΔLT) and baseline LT. Correlations were analysed using Pearson's and Spearman's methods. Analyses were performed with Stata version 15.1 (StataCorp, College Station, TX, USA).

Results

Between June 2017 and May 2018, 270 patients were screened and 200 patients recruited. Clinical characteristics of patients in the longitudinal study are presented in *Table 1* and those in the cross-sectional study in *Table 2*. For the GTT, the intra-assay CV for OT was 6% and for LT 8%, and the inter-assay CV was 7% for OT and 9% for LT. The average intra-assay CV for TEG was 22% using native blood and 20% for native blood with kaolin.

Effect of apixaban on thrombotic status including endogenous fibrinolysis

Distributions of OT and LT pre- and during apixaban treatment are shown in *Figure 1*. Compared to baseline, apixaban significantly prolonged OT (361 ± 112 vs. 463 ± 124 s, $P < 0.0001$). The number of drops (D) did not change in response to apixaban treatment (2.5 ± 1.7 vs. 3.1 ± 1.6, $P = 0.173$). There was moderate inverse correlation between the change in OT in response to apixaban (ΔOT) and baseline OT ($r = -0.4$, $P = 0.0002$).

Compared to baseline, LT on apixaban was significantly shorter [2204 (1779–2738) vs. 1882 (1607–2374) s, $P = 0.0003$]. There was

Table 1 Baseline clinical characteristics of patients in longitudinal study

	Whole group ($n = 80$)	Baseline LT 0–1500 ($n = 9$)	Baseline LT 1501–3000 ($n = 53$)	Baseline LT 3001–4500 ($n = 11$)	Baseline LT 4501–6000 ($n = 7$)	*P*-value
Age (years)	69.5 ± 13.6	70.2 ± 11.7	69.0 ± 15.0	67.3 ± 11.6	76.0 ± 7.0	0.675
Male	43 (54)	2 (22)	33 (62)	6 (55)	2 (29)	0.074
Weight (kg)	83.28 ± 26.64	67.57 ± 19.61	83.39 ± 25.78	95.57 ± 35.46	83.31 ± 18.11	0.211
Height (cm)	169.00 ± 14.21	163.77 ± 9.47	172.00 ± 11.35	160.90 ± 25.79	165.71 ± 7.86	0.085
BMI	27.93 ± 6.50	24.80 ± 5.25	27.68 ± 6.73	30.31 ± 5.96	30.00 ± 6.01	0.819
Current smoker	5 (6.3)	1 (11)	3 (5.6)	1 (9)	0 (0)	0.798
AF type						
Paroxysmal	71 (88.8)	7 (78)	47 (89)	10 (90)	7 (100)	0.567
Persistent	9 (11.2)	2 (22)	6 (11)	1 (10)	0 (0)	0.512
Hypertension	48 (60)	4 (44)	30 (56)	9 (82)	5 (71)	0.293
Diabetes mellitus	11 (13.8)	2 (22)	7 (13)	2 (18)	0 (0)	0.603
Hyperlipidaemia	29 (36.3)	3 (33)	20 (38)	5 (45)	1 (14)	0.583
Prior CAD	5 (6.25)	0 (0)	4 (7.5)	1 (9)	0 (0)	0.712
Prior MI	2 (2.5)	0 (0)	1 (2)	1 (9)	0 (0)	0.484
Prior PCI	2 (2.5)	0 (0)	2 (3.7)	0 (0)	0 (0)	0.790
Renal impairment	3 (3.8)	1 (11)	2 (3.7)	0 (0)	0 (0)	0.562
Prior major bleeding	2 (2.5)	0 (0)	2 (3.75)	0 (0)	0 (0)	0.790
Prior CVA	3 (3.8)	0 (0)	3 (5.5)	0 (0)	0 (0)	0.662
LV impairment						
None	72 (90)	7 (77)	47 (89)	11 (100)	9 (100)	0.308
Mild	5 (6)	1 (11)	3 (5.5)	0 (0)	0 (0)	0.636
Moderate	2 (2.5)	1 (11)	1 (1.8)	0 (0)	0 (0)	0.350
Severe	1 (1.5)	0 (0)	2 (3.7)	0 (0)	0 (0)	0.790
CHA_2DS_2VASc score	3 (1–4)	3 (2–3)	2 (1–4)	3 (2–3)	3 (2–3)	0.193
HASBLED score	1 (1–1)	1 (1–1)	1 (1–1)	1 (0–1)	1 (1–2)	0.128
Concomitant medication						
Statin	29 (36)	4 (44)	20 (38)	4 (36)	1 (14)	0.621
Beta blocker	21 (26)	1 (11)	14 (26)	5 (45)	1 (14)	0.298
CCB blocker	23 (28.75)	2 (22)	12 (23)	5 (45)	4 (57)	0.144
PPI	17 (21.25)	2 (22)	11 (21)	2 (18)	2 (29)	0.960
Metformin	6 (7.5)	1 (11)	4 (7.5)	1 (9)	0 (0)	0.855
Baseline blood tests						
Haemoglobin (g/L)	138 ± 19	127 ± 18	139 ± 20	144 ± 9	134 ± 19	0.118
Haematocrit (%)	41 ± 5	38 ± 5	41 ± 5	43 ± 3	40 ± 7	0.151
Platelet count ($\times 10^9$/L)	257 ± 85	296 ± 76	248 ± 85	249 ± 28	281 ± 141	0.259
White cell count ($\times 10^9$/L)	8.7 ± 2.7	7.7±1.8	8.8 ± 2.4	8.4 ± 3.2	9.1 ± 5.6	0.583
eGFR	72 ± 16	74 ± 21	71 ± 16	77 ± 14	65 ± 11	0.261
Fibrinogen (g/L)	4.5 ± 1.5	3.6 ± 0.5	4.4 ± 1.5	4.8 ± 0.9	5.8 ± 1.7	0.122
PT (s)	12.0 ± 1.4	11.5 ± 1.3	12.0 ± 1.4	12.1 ± 1.7	11.5 ± 0.9	0.748
aPTT (s)	28.6 ± 3.3	28.6 ± 4.0	28.0 ± 2.2	31.0 ± 6.2	29.2 ± 3.0	0.411
CRP (mg/L)	4.8 ± 6.6	2.3 ± 2.2	5.4 ± 7.7	3.2 ± 2.8	7 ± 5	0.182
OT at baseline (s)	361 ± 112	377 ± 126	360 ± 110	376 ± 109	324 ± 130	0.740
LT at baseline (s)	2204 (1779–2738)	1425 (1350–1454)	2114 (1800–2347)	3427 (3099–3667)	5166 (4843–6000)	**<0.001**
OT on treatment (s)	463 ± 124	501 ± 98.4	452 ± 118	542 ± 151	376 ± 88	**0.038**
LT on treatment (s)	1882 (1607–2374)	1367 (1206–1607)	1866 (1631–2312)	2322 (1975–3345)	2707 (1710–3285)	**<0.001**

Values are presented as mean ± SD or median (IQR) and *n* (%). Renal impairment defined as eGFR <60; AF type; left ventricular function classification: mild 45–55% ejection fraction, moderate 35–45% ejection fraction, and severe <35% ejection fraction. CHA_2DS_2VASc score in AF and HAS-BLED bleeding risk. Normal values: haemoglobin 130–180 g/L in males and 115–165 g/L in females; haematocrit 40–52% in males and 36–47% in females; platelet count 150–400 × 10^9/L; white cell count 4–11 × 10^9/L; eGFR >60; fibrinogen 1.8–5.4 g/L; PT 11–13.5 s; aPTT 25–35 s; and CRP 0–5 mg/L.

Statistically significant values $P < 0.05$ are set in bold.

AF, atrial fibrillation; aPTT, activated partial thromboplastin time; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blocker; CRP, C-reactive protein; CVA, cerebrovascular accident; eGFR, estimated glomerular filtration rate; IQR, interquartile range; LT, lysis time; MI, myocardial infarction; PCI, percutaneous coronary intervention; PPI, proton pump inhibitor; SD, standard deviation.

Table 2 Baseline clinical characteristics of patients in cross-sectional study

	Whole group ($n = 180$)	Apixaban ($n = 60$)	Warfarin ($n = 60$)	Aspirin ($n = 60$)	*P*-value
Age (years)	73.8 ± 12.1	69.6 ± 13.4	74.6 ± 9.7	77.1 ± 12.0	0.248
Male	105 (58)	33 (55)	35 (58)	37 (62)	0.636
Weight (kg)	82.37 ± 21.22	83.70 ± 27.15	83.62 ± 18.01	79.80 ± 17.16	0.573
Height (cm)	169.48 ± 11.87	168.45 ± 15.68	171.11 ± 8.83	168.88 ± 9.97	0.567
BMI	28.00 ± 5.60	28.25 ± 7.08	28.13 ± 5.03	27.60 ± 4.35	0.652
Current smoker	11 (6)	5 (8)	4 (7)	2 (3)	0.598
AF type					
Paroxysmal	138 (76)	54 (90)	35 (60)	48 (80)	**0.001**
Persistent	28 (16)	6 (10)	12 (20)	10 (17)	0.115
Hypertension	122 (68)	37 (62)	42 (70)	43 (70)	0.454
Diabetes mellitus	33 (18)	9 (15)	12 (20)	12 (20)	0.716
Hyperlipidaemia	99 (55)	26 (43)	31 (52)	42 (70)	**0.011**
Prior CAD	44 (24)	3 (5)	20 (33)	21 (35)	**<0.001**
Prior MI	20 (11)	2 (3)	10 (17)	8 (13)	0.054
Prior PCI	15 (8)	2 (3)	9 (15)	4 (6)	0.059
Renal impairment	18 (10)	2 (3)	7 (12)	9 (15)	0.090
Prior major bleeding	7 (4)	2 (3)	5 (8)	0 (0)	0.059
Prior CVA	25 (14)	3 (5)	11 (18)	11 (18)	0.051
Prior LV impairment					
None	150 (83)	54 (90)	47 (78)	49 (82)	0.090
Mild	15 (8)	3 (5)	6 (10)	6 (10)	0.253
Moderate	9 (5)	1 (2)	6 (10)	2 (3)	0.147
Severe	6 (4)	2 (3)	1 (2)	3 (5)	1.000
CHA_2DS_2VASc score	3 (2–4)	3 (1–4)	4 (2–5)	4 (3–5)	0.945
HASBLED score	1 (1–2)	1 (1–1)	1 (1–2)	1 (1–2)	0.388
Concomitant medication					
Statin	107 (59)	25 (42)	40 (66)	42 (70)	**0.003**
Beta blocker	92 (51)	15 (25)	11 (18)	15 (25)	**<0.001**
CCB	44 (24)	18 (30)	11 (18)	15 (25)	0.329
PPI	68 (38)	13 (22)	26 (43)	29 (48)	**0.006**
Metformin	19 (11)	5 (8)	7 (12)	7 (12)	0.790
Baseline blood tests					
Haemoglobin (g/L)	134 ± 17	138 ± 18	132 ± 15	133 ± 16	0.428
Haematocrit (%)	40 ± 5	41 ± 5	40 ± 4	40 ± 4	0.743
Platelet count ($\times 10^9$/L)	227 ± 72	235 ± 55	215 ± 85	229 ± 72	0.052
White cell count ($\times 10^9$/L)	7.6 ± 2.0	7.9 ± 2.1	7.1 ± 1.9	7.7 ± 2.0	0.093
eGFR	67 ± 16	70 ± 16	66 ± 16	65 ± 17	0.139
Fibrinogen (g/L)	4.5 ± 1.1	4.4 ± 1.4	4.5 ± 1.0	4.5 ± 0.9	0.402
PT (s)	17.7 ± 9.1	12.0 ± 1.4	29.5 ± 6.7	12.3 ± 2.3	**<0.001**
aPTT (s)	32.0 ± 7.3	28.3 ± 3.3	38.8 ± 7.3	29.0 ± 5.4	**<0.001**
CRP (mg/L)	5.5 ± 10.6	4.7 ± 6.4	4.0 ± 3.6	7.4 ± 16	0.872

Values are presented as mean ± SD or median (IQR) and *n* (%). Renal impairment defined as eGFR <60; AF type; left ventricular function classification: mild 45–55% ejection fraction, moderate 35–45% ejection fraction, and severe <35% ejection fraction. CHA_2DS_2VASc score in AF and HAS-BLED bleeding risk. Normal values: haemoglobin 130–180 g/L in males and 115–165 g/L in females; haematocrit 40–52% in males and 36–47% in females; platelet count 150–400 $\times$ 10^9/L; white cell count 4–11 $\times$ 10^9/L; eGFR >60; fibrinogen 1.8–5.4 g/L; PT 11–13.5 s; aPTT 25–35 s; and CRP 0–5 mg/L.

Statistically significant values $P < 0.05$ are set in bold.

AF, atrial fibrillation; aPTT, activated partial thromboplastin time; BMI, body mass index; CAD, coronary artery disease; CCB, calcium channel blocker; CRP, C-reactive protein; CVA, cerebrovascular accident; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MI, myocardial infarction; PCI, percutaneous coronary intervention; PPI, proton pump inhibitor; SD, standard deviation.

no correlation between baseline OT and baseline LT ($P = 0.740$) or between on-treatment OT and on-treatment LT ($P = 0.241$).

Apixaban did not alter TEG indices, except for a small reduction in the rate of clot formation with kaolin (68.4° vs. 67°, $P = 0.026$) (*Tables 3* and *4*).

Baseline OT and LT did not correlate with any of the baseline TEG indices with or without kaolin. In particular, baseline fibrinolysis assessment (LT) with the GTT did not correlate with any of the baseline TEG indices of fibrinolysis (LY30, LY60, and CLT). On-treatment OT did not correlate with any on-treatment TEG indices. There was a significant correlation between ΔOT and the change in reaction time (R) with kaolin in response to apixaban ($r = 0.54$, $P < 0.0001$). There was a weak correlation between on-treatment LT in GTT correlated with clot lysis time (CLT) in the TEG ($r = 0.25$, $P = 0.022$). There was weak correlation between ΔLT and the change in kaolin CLT (ΔCLT) in response to apixaban ($r = 0.28$, $P = 0.02$).

Magnitude of effect of apixaban in relation to baseline fibrinolysis

The change in LT in response to apixaban (ΔLT) correlated closely with baseline LT ($r = 0.77$, $P < 0.0001$). The magnitude of effect of apixaban on reducing LT was greatest in those with the longest LT at baseline (*Figure 2*). Patients on apixaban were grouped into quartiles based on baseline LT to assess the magnitude of change in LT with apixaban (*Table 1*). The ΔLT between the four groups was significantly different ($P < 0.0001$).

Cross-sectional comparisons of apixaban, warfarin, and aspirin

Measures of thrombotic status assessed with GTT and TEG are shown in *Table 5*. LT was significantly lower in patients taking apixaban than other medications. From the TEG indices, only native CLT was significantly lower in the apixaban group compared to patients on warfarin and aspirin. Multivariable regression models were applied to account for baseline differences in the three groups. After accounting for these variables (*Table 2*), both OT and LT remained significantly different between the apixaban and the warfarin arms ($P < 0.0001$).

Relationship of thrombotic effect to apixaban levels

Apixaban levels in patients taking 5 mg b.i.d. was C_{max} 152.9 ng/mL (32.9–317.9 ng/mL), and in patients taking the 2.5 mg b.i.d. dose was C_{max} 125.85 ng/mL (40.6–344.6 ng/mL). Apixaban levels correlated weakly with OT on apixaban ($r = 0.27$, $P = 0.022$) but not with LT.

There were no significant relationships between apixaban levels and TEG parameters.

Discussion

In this study, our principal findings are as follow: (i) apixaban significantly improved endogenous fibrinolysis, and the effect of apixaban was greatest in those patients with the longest LT at baseline; and (ii) in comparison to patients on warfarin or aspirin, patients taking apixaban exhibited more rapid (more effective) endogenous fibrinolysis.

Table 3 TEG indices

R (min)	Measures the time from the start of a sample run until the first significant level of detectable clot formation. R reduces in hypercoagulable conditions
K (min)	Measures the time from R until a fixed level of clot strength is reached. K is shortened in hypercoagulable conditions.
Angle	Represents the rate of clot formation and reflects fibrinogen activity. Angle relates to K. Both represent the rate of clot formation. Angle is larger in hypercoagulable conditions
MA (mm)	Represents whole clot strength and reflects many aspects of clot formation including platelet number and function as well as the fibrin contribution to clot strength. MA is larger by hypercoagulable conditions
LY30 (%)	Represents the percentage of clot which has lysed after 30 min of MA
LY60 (%)	Represents the percentage of clot which has lysed after 60 min of MA
TMA (min)	Measures the time to form maximum clot strength
CLT (min)	Measures the time to 2 mm amplitude reduction from MA

CLT, clot lysis time; K, kinetics; MA, maximum amplitude; R, reaction time; TEG, thromboelastography; TMA, time to maximum amplitude.

These observations may have clinical relevance, given that effectiveness of endogenous fibrinolysis is an important determinant of the clinical outcome of a thrombotic stimulus. Whether apixaban may confer additional thrombotic risk reduction in NVAF patients who have impaired fibrinolysis, compared to warfarin, requires further study. Importantly, the finding that apixaban-treated patients exhibit more favourable fibrinolysis profiles than those taking warfarin or aspirin does not mean that apixaban by itself exhibits a more favourable fibrinolysis profile as compared to aspirin and warfarin.

In our current study, 23% of patients with NVAF had significantly prolonged LT before the start of anticoagulation (defined as baseline LT >3000 s, based on prior data).[3] Such impaired endogenous fibrinolysis in patients with acute coronary syndromes has been associated with increased risk of cardiovascular death and recurrent MI.[2,3,5] Dual antiplatelet therapy post-ACS did not appear to reduce LT. The average 15% reduction in fibrinolysis time with apixaban seen here is potentially clinically significant, but even greater effect, up to 48% reduction in LT was seen in those with the most impaired fibrinolysis at baseline. If apixaban were to exert similar reduction in LT in patients with ACS, as that observed here in NVAF, that would be expected to reduce the risk of major adverse cardiovascular event in those with prolonged LT at baseline. Our observations suggest that further study in controlled trials could investigate whether apixaban may confer additional thrombotic risk reduction in NVAF patients with impaired fibrinolysis, compared to warfarin.

The effect of improving fibrinolytic status with apixaban appears not to be simply an anticoagulant effect, since patients on apixaban had more rapid fibrinolysis than patients therapeutically anticoagulated with warfarin, and there was no relation between apixaban

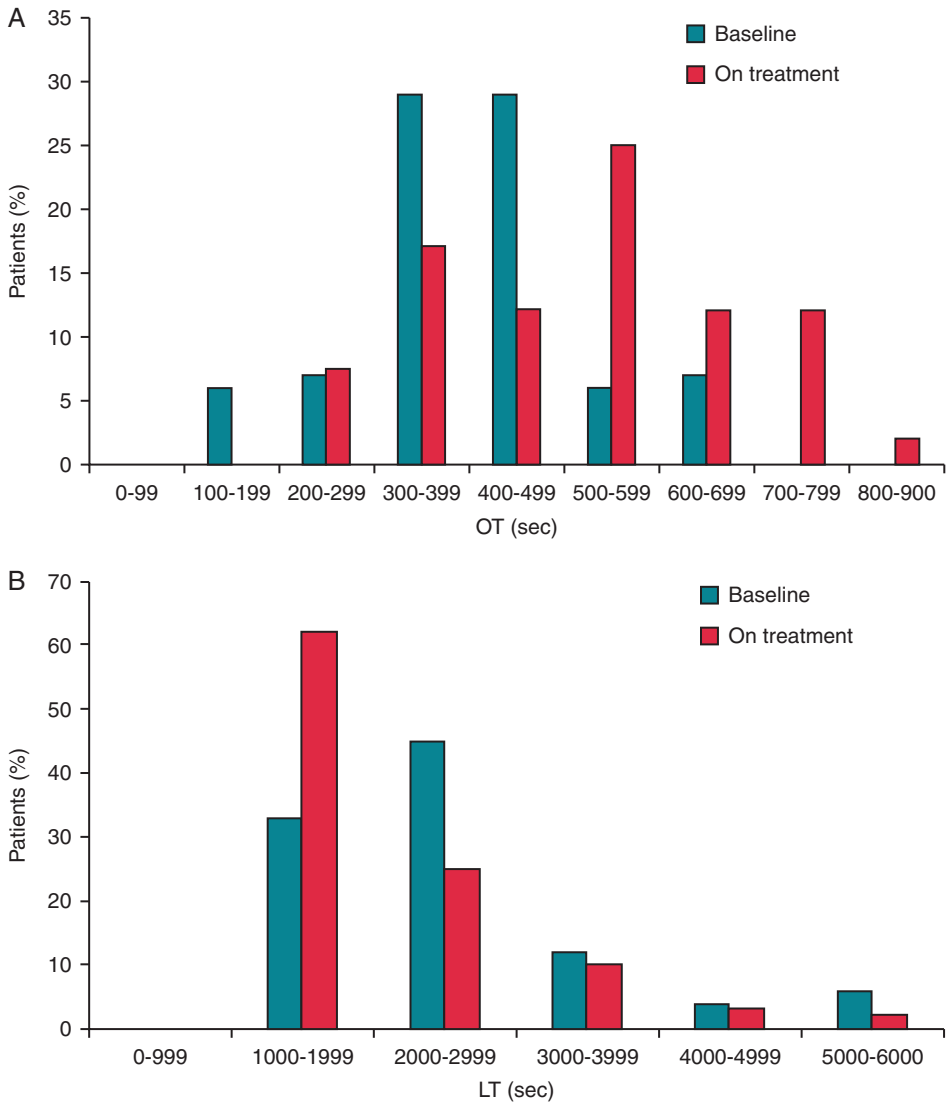

Figure 1 Effect of apixaban on occlusion time and lysis time. (*A*) Distribution of OT before and after apixaban treatment. (*B*) Distribution of LT before and after apixaban treatment. Apixaban significantly prolonged OT as evidenced by rightward shift (reduction in platelet reactivity). LT was significantly reduced as evidenced by leftward shift (representing faster lysis). LT, lysis time; OT, occlusion time.

levels and LT. Apixaban levels in our study were similar to those previously reported[5] and are known to correlate closely with anti-FXa activity. In the ARISTOTLE trial, apixaban was as effective as warfarin in reducing the risk of ischaemic stroke and systemic embolism in NVAF, whilst the AVERROES study showed that apixaban was superior to aspirin.[7] Both ARISTOTLE and AVERROES suggest a greater benefit of apixaban, compared to warfarin, in reducing ischaemic stroke and systemic embolism in patients with $CHADS_2$ score ≥3 than in patients with lower $CHADS_2$ scores, suggesting apixaban may have additional advantages in the highest risk patients. Although in the ARISTOTLE trial comparing apixaban with warfarin in NVAF, the secondary endpoint of combined ischaemic or uncertain type (not clearly haemorrhagic or ischaemic) stroke was non-significantly different with both anticoagulants,[8] a subgroup analysis of patients with previous stroke or transient ischaemic attack showed the rate of stroke or systemic embolism was significantly lower with apixaban than with warfarin, suggesting that the absolute benefits of apixaban might be even greater in high-risk patients.[6] In the largest real-world retrospective analysis of ~77 000 patients with NVAF, apixaban use was associated with significantly lower risk of stroke and systemic embolism than warfarin.[9]

Apixaban is a direct inhibitor of free and clot- or prothrombinase-bound FXa, thereby prevents thrombin generation. Thrombin is not only a key protein in fibrin clot formation, but also the most potent activator of platelet aggregation *in vivo*[10] and an important determinant of the strength and stability of the fibrin clot and its resistance to fibrinolysis.[11] Apart from reduced thrombin generation, FXa inhibition with apixaban might also impact platelet haemostasis by blocking the direct effects of FXa via protease activator receptor signalling.[10]

Nevertheless, studies assessing the effect of FX inhibition on platelet reactivity are few, but appear to show consistency in reduction of tissue-factor/platelet-dependent thrombin generation and thrombus formation. Blood spiked with rivaroxaban *ex vivo* showed reduced platelet aggregation induced by tissue factor and to a lesser extent induced by thrombin.[12,13] Even at the very low dose of 2.5 mg b.i.d., rivaroxaban reduced platelet-dependent thrombin generation and coagulation-dependent thrombus-formation in patients treated with aspirin plus $P2Y_{12}$ inhibitor, whereas pure platelet-dependent thrombus formation was not affected.[14] Indeed, FXa inhibition appears to have no significant effect on platelets[14] including in response to adenosine diphosphate, collagen, thrombin receptor-activating peptide, or arachidonic acid.[15] Apixaban may therefore favourably enhance endogenous fibrinolysis through reduction in platelet-dependent and non-platelet-dependent thrombin generation, which directly impact on the structure and stability of the thrombus and its resistance to fibrinolysis.

Table 4 Difference in GTT and TEG parameters in response to apixaban

	Baseline (n = 80)	Apixaban (n = 80)	P-value
OT (s)	361 ± 112	463 ± 124	**<0.0001**
LT (s)	2204 (1779–2738)	1882 (1607–2374)	**0.0003**
R (min) native	7.8 (5.4–11.4)	8.9 (6.1–12.2)	0.398
R (min) kaolin	4.2 (2.6–5.6)	4.8 (3.2–6.3)	0.159
K (min) native	3.6 (2.6–5.8)	4.2 (3.0–7.2)	0.096
K (min) kaolin	1.4 (1.1–2.2)	1.7 (1.4–2.3)	0.113
Angle (°) native	43.0 (39.0–47.0)	42.0 (38.0–45.0)	0.552
Angle (°) kaolin	68.4 (65.0–72.0)	67.0 (58.0–70.0)	**0.026**
MA (mm) native	34.5 (27.3–48.3)	34.6 (29.6–49.8)	0.535
MA (mm) kaolin	21.0 (17.8–25.0)	23.3 (20.0–25.9)	0.068
LY30 (%) native	0.1 (0.0–3.1)	0.0 (0.0–0.4)	0.276
LY30 (%) kaolin	1.9 (0.3–5.4)	0.4 (0.0–2.0)	0.066
LY60 (%) native	1.4 (0.0–5.8)	0.5 (0.0–3.0)	0.405
LY60 (%) kaolin	4.2 (1.9–9.9)	2.6 (1.0–4.6)	0.067
TMA (min) native	34.5 (27.3–48.3)	34.6 (29.6–49.8)	0.535
TMA (min) kaolin	21.0 (17.8–25.0)	23.3 (20.0–25.9)	0.068
CLT (min) native	60.6 (59.4–61.5)	60.15 (57.7–61.2)	0.155
CLT (min) kaolin	60.8 (59.5–61.7)	60.8 (59.7–61.5)	0.780

Statistically significant values $P < 0.05$ are set in bold.
CLT, clot lysis time; GTT, Global Thrombosis Test; K, kinetics; LT, lysis time; MA, maximum amplitude; OT, occlusion time; R, reaction time; TEG, thromboelastography; TMA, time to maximum amplitude.

The benefit of apixaban on endogenous fibrinolysis was only observed with the GTT and not with TEG. The fundamental difference between these techniques is that the GTT employs high shear to stimulate thrombus formation resulting in platelet activation and thrombin generation, whereas TEG is a haemostatic assay that measures the global viscoelastic properties of whole blood clot formation under low shear. This results in significant differences in the clot formed and therefore, also in what the 'lysis' assays measure. The GTT assesses the lysis and stability of a platelet thrombus, whereas in the TEG it reflects clot lysis. The GTT is particularly well-adapted to investigate the role of thrombin inhibitors such as NOACs, primarily because thrombin generation from shear-activated platelets and fibrin stabilization of the initial platelet aggregates play a major role in determining the measured OT,[3] whereas platelet-dependent thrombin generation is much less likely at the low shear rates in the TEG.

Nevertheless, the effect of NOACs on TEG parameters is contentious. Whilst some small studies reported that apixaban had minimal effect on TEG parameters, and that for the patients on apixaban, mean R value was within reference range representative of a normal population,[16] others have shown that spiking of blood with apixaban *in vitro* increased R time and time to maximal thrombus growth and coagulation,[17] prolonged clotting time and time to maximum

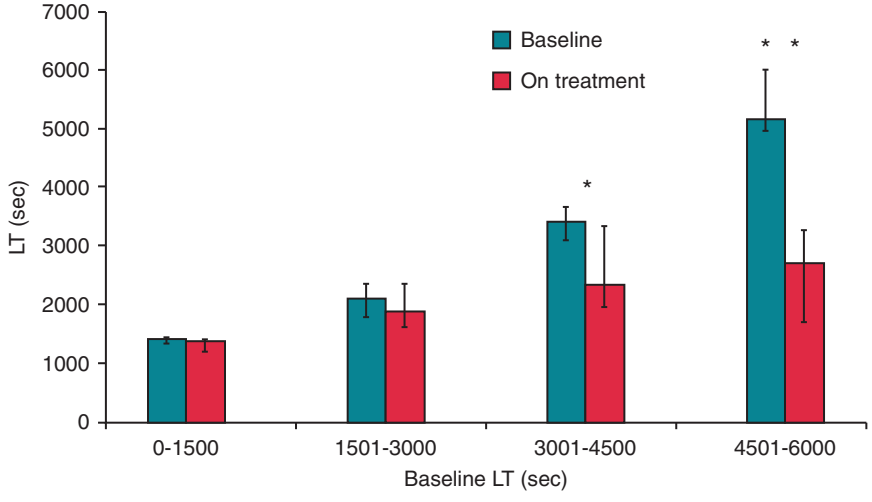

Figure 2 Effect of apixaban on lysis time according to baseline LT. The effect of apixaban on LT was particularly marked in patients with longest baseline LT. *P = 0.040, **P = 0.018. LT, lysis time.

Table 5 Thrombotic parameters in patients taking apixaban, warfarin and aspirin

	Apixaban ($n = 60$)	Warfarin ($n = 60$)	Aspirin ($n = 60$)	Three group comparison *P*-value	Apixaban vs. warfarin + aspirin *P*-value	Apixaban + warfarin vs. aspirin *P*-value	Apixaban vs. warfarin *P*-value
OT (s)	463 ± 131	589 ± 154	430 ± 121	0.126	0.054	0.025	**<0.0001**
LT (s)	1850 (1591–2300)	2758 (2014–3502)	2135 (1752–2463)	**0.0001**	**<0.0001**	0.465	**<0.0001**
R (min) native	8.8 (5.7–12.3)	13.0 (8.7–17.8)	8.8 (6.0–12.2)	**0.001**	0.059	0.067	**0.0007**
R (min) kaolin	4.7 (3.0–6.8)	6.5 (4.9–10.4)	3.0 (2.0–5.0)	**0.0001**	0.843	0.0002	**0.006**
K (min) native	4.4 (3.0–7.2)	5.4 (3.4–7.5)	4.0 (2.6–6.1)	0.076	0.954	0.0517	0.212
K (min) kaolin	1.7 (1.4–2.2)	2.0 (1.5–2.7)	1.5 (1.2–2.7)	0.152	0.789	0.0367	0.265
Angle (°) native	45.3 (30.7–52.1)	33.0 (26.2–47.9)	46.0 (33.0–59.0)	**0.009**	0.793	0.011	0.050
Angle (°) kaolin	70.0 (57.0–69.0)	61.0 (47.0–69.0)	67.3 (60.0–72.0)	0.152	0.839	0.084	0.265
MA (mm) native	70.0 (64.0–76.0)	69.0 (56.0–75.0)	69.0 (62.0–75.0)	0.673	0.386	0.830	0.347
MA (mm) kaolin	76.0 (72.0–80.0)	75.0 (72.0–79.0)	75.0 (73.0–79.0)	0.872	0.632	0.819	0.703
LY30 (%) native	0.0 (0.0–0.9)	0.0 (0.0–1.3)	0.0 (0.0–1.1)	0.841	0.810	0.744	0.980
LY30 (%) kaolin	0.4 (0.0–2.0)	0.6 (0.0–3.9)	1.2 (0.3–2.4)	0.872	0.264	0.293	0.520
LY60 (%) native	0.6 (0.0–3.1)	0.5 (0.0–5.6)	1.3 (0.1–4.7)	0.744	0.598	0.542	0.993
LY60 (%) kaolin	2.6 (1.0–5.0)	3.0 (1.0–9.5)	4.2 (2.1–5.9)	0.588	0.321	0.388	0.532
TMA (min) native	35.0 (30.0–50.0)	37.0 (32.0–49.0)	32.0 (26.0–43.0)	0.064	0.874	**0.037**	0.253
TMA (min) kaolin	24.0 (20.0–26.0)	26.0 (22.0–30.0	21.0 (16.0–23.0)	**0.001**	0.798	**0.001**	0.0511
CLT (min) native	60.0 (45.0–61.0)	61.0 (57.0–62.0)	61.0 (59.0–61.0)	**0.036**	**0.011**	0.100	0.055
CLT (min) kaolin	61.0 (60.0–62.0)	61.0 (60.0–62.0)	60.8 (60.4–61.4)	0.998	0.956	0.889	0.883

Statistically significant values $P < 0.05$ are set in bold.
CLT, clot lysis time; LT, lysis time; MA, maximal amplitude; OT, occlusion time; R, reaction time; TMA, time to maximal amplitude.

velocity.[18] In the largest study assessing patients with NVAF with TEG, patients taking NOAC developed clot that was quicker to lyse than patients taking warfarin, and the rate of clot dissolution was faster in those on apixaban than in rivaroxaban, with 11 of 16 TEG indices showing a difference between those on aspirin, warfarin, or a NOAC.[19]

Clinical implications

Our finding that that apixaban significantly improves endogenous fibrinolysis, particularly in patients with the longest LT at baseline, is significant and may be clinically important. The current data indicate that apixaban may have additional advantages over vitamin K antagonist (VKA) or aspirin in patients with impaired endogenous fibrinolysis, although since we did not assess patients pre- and post-VKA or aspirin, we cannot be sure of the relative effects of apixaban on fibrinolysis compared to VKA or aspirin.

Our findings support the signals from clinical trials, showing that apixaban (rather than VKA or aspirin) may have additional advantages in high-risk patients. Our data suggest that patients with impaired fibrinolysis may benefit more from apixaban to improve fibrinolysis, than warfarin. Future studies are required to confirm whether patients with NVAF and impaired fibrinolysis are at increased risk of ischaemic stroke and systemic embolism, than those with effective fibrinolysis. If this is confirmed in large prospective studies, then patients with NVAF and impaired fibrinolysis may gain additional benefits from treatment with apixaban to favourably modulate endogenous fibrinolysis, than from VKA or aspirin.

Whether enhancing endogenous fibrinolysis with apixaban when this is impaired in patients with NVAF or even in acute coronary syndromes, can translate into a reduction in ischaemic events, and whether patients with prolonged LT derive greater benefit from apixaban than warfarin or other NOAC, requires further study.

Limitations

The main limitations of our study are the non-randomized, observational study design, and the relatively small number of participants. The baseline LT in patients subsequently treated with apixaban was not uniform and therefore a greater effect of apixaban on LT may have been observed had we included more patients with impaired fibrinolysis at baseline. In the cross-sectional study, there were differences in clinical characteristics between the groups. Since fibrinolysis was not assessed before aspirin or warfarin treatment, we do not know the absolute magnitude of effect of these drugs on fibrinolysis, which may confound conclusion drawn about the observed shorter LT in patients on apixaban than on warfarin or aspirin. However, in contemporary clinical practice, few patients in the UK with NVAF are being started on VKA, and therefore, it would not have been logistically easy to compare patients pre- and post-VKA. Furthermore, whilst compliance was assessed in the apixaban and warfarin arms, it was not assessed in patients taking aspirin.

Conclusion

In conclusion, apixaban enhances endogenous fibrinolysis, with maximal effect in those with impaired fibrinolysis pre-treatment. Apixaban-treated patients exhibit more favourable fibrinolysis profiles than those taking warfarin or aspirin. Whether apixaban may

confer additional thrombotic risk reduction in NVAF patients with impaired fibrinolysis, compared to warfarin, merits further study.

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