Thrombosis Research 264 (2026) 109772

Contents lists available at ScienceDirect

Thrombosis Research

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

Full Length Article

Thrombus formation, endogenous fibrinolysis and atrial fibrillation states in patients with ischaemic stroke: An assessment using global thrombosis test profiles

Yang Chen [a,b], Ying Gue [b], Sandra Elsheikh [a,b], Coleen Ditchfield [c], Sogol Koolaji [d], Andrew M. Hill [a,b,c], Garry McDowell [b,e], Diana A. Gorog [d,f,g,h], Gregory Y.H. Lip [b,i,j,*]

[a] Department of Cardiovascular and Metabolic Medicine, Institute of Life Course and Medical Sciences, University of Liverpool, Liverpool, United Kingdom
[b] Liverpool Centre for Cardiovascular Science at University of Liverpool, Liverpool John Moores University and Liverpool Heart and Chest Hospital, Liverpool, United Kingdom
[c] Department of Medicine, Whiston Hospital, Mersey and West Lancashire Teaching Hospitals NHS Trust, Prescot, United Kingdom
[d] Department of Cardiology, East and North Hertfordshire NHS Trust, Stevenage, Hertfordshire, United Kingdom
[e] School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, United Kingdom
[f] Faculty of Medicine, National Heart and Lung Institute, Imperial College, London, United Kingdom
[g] University of Hertfordshire, Hatfield, Hertfordshire, United Kingdom
[h] School of Cardiovascular and Metabolic Medicine & Sciences, Kings College London, London, United Kingdom
[i] Danish Centre for Health Services Research, Department of Clinical Medicine, Aalborg University, Aalborg, Denmark
[j] Department of Cardiology, Lipidology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

ARTICLE INFO

Keywords:
Atrial fibrillation
Ischaemic stroke
Global thrombosis test
Occlusion time
Lysis time
Thrombosis
Fibrinolysis

ABSTRACT

Background: Atrial fibrillation (AF) is often first detected during or after ischaemic stroke (IS), yet many cases remain undetected. We evaluated whether thrombotic and fibrinolytic profiles vary with AF status in IS and whether these markers may help identify AF.

Methods: We conducted a prospective observational study of adults with confirmed IS, with blood sampling during index admission after withholding pre-admission anticoagulant therapy where applicable. Patients were classified as AF or non-AF, with AF subtyped as known AF (KAF, $N = 20$), AF detected concurrently with stroke (AFDCS, $N = 10$), or detected after stroke (AFDNCS, $N = 6$). Thrombotic status was assessed using Global Thrombosis Test-derived occlusion time (OT) and lysis time (LT). Multivariable linear regression analyses were performed to account for demographic, clinical, and anticoagulant-related factors. An exploratory cohort excluding KAF, AFDCS, and thrombolysis was used to evaluate AFDNCS prediction.

Results: 115 patients with IS (mean [SD] age: 68.5 ± 13.5 years; 57.4% male) were included. AF patients were older and had a higher burden of comorbidities, but most laboratory parameters were similar between groups. Despite normal coagulation tests, AF patients had longer OT than non-AF patients (474 ± 214 vs. 373 ± 176 s, $P = 0.009$), while LT did not differ. OT varied significantly across AF states ($P = 0.002$), longest in KAF and shortest in AFDNCS, whereas LT did not differ. In multivariable analyses, the association between AF and longer OT was attenuated after accounting for prior anticoagulant exposure. In the exploratory cohort ($N = 72$) with 5 AFDNCS cases during 6-month follow-up, OT showed discrimination for predicting AFDNCS (AUC = 0.737, 95% CI 0.620–0.834).

Conclusions: We identify distinct haemostatic patterns across AF states in patients with IS. Short OT in patients with AFDNCS may identify a cohort at increased ischaemic risk of future events, and requires further study.

* Corresponding author at: Liverpool Centre for Cardiovascular Science at University of Liverpool, Liverpool John Moores University and Liverpool Heart and Chest Hospital, William Henry Duncan Building, 6 West Derby Street, Liverpool, L7 8TX, United Kingdom.
E-mail address: gregory.lip@liverpool.ac.uk (G.Y.H. Lip).

https://doi.org/10.1016/j.thromres.2026.109772
Received 20 April 2026; Received in revised form 15 June 2026; Accepted 6 July 2026
Available online 7 July 2026

1. Introduction

The epidemiological and clinical burden of ischaemic stroke (IS) and atrial fibrillation (AF) has been well established [1,2]. While each condition carries significant individual burden, growing evidence highlights the importance of their intersection. AF is not only a leading cause of cardioembolic stroke but is also frequently diagnosed for the first time during or after an acute stroke, conversely, stroke may unmask latent AF [3]. Recent estimates from the Global Burden of Disease study indicate that, in 2019, the global prevalence of coexisting AF and IS was estimated at approximately 6.9 million people (95% uncertainty interval: 6.5–8.7 million) [4]. That year also saw an estimated 700,000 new cases globally, a 35% increase compared with 2009 [4], highlighting the rising clinical and public health burden associated with AF and IS.

In patients with IS, AF status is often categorised into known AF (KAF), identified before the index IS, and AF detected after stroke, which includes AF diagnosed concurrently with stroke (AFDCS) or AF diagnosed non-concurrently with stroke (AFDNCS) [5,6]. In a large-scale cohort study involving over 800,000 patients admitted with IS, 5.5% had KAF, while 2.3% were newly diagnosed with AFDAS [7]. Current detection relies largely on single-time ECG testing, and variation in monitoring intensity leads to differences in AF detection rates. One meta-analysis demonstrated a clear gradient in AF detection, increasing from 7.7% with single ECG to 10.7% with short-term Holter and 16.9% with mobile telemetry or implantable loop recorders [8]. While extended monitoring or advanced detection technologies may improve AF detection, they also impose significant resource burdens [9]. Therefore, scalable approaches are needed to identify patients at highest risk and to target intensive monitoring where it is most likely to provide clinical benefit.

Emerging evidence indicates that thrombosis and endogenous fibrinolysis are mechanistically relevant to both AF and IS, involving coordinated interactions between platelet activation, endothelial function, and the coagulation–fibrinolytic systems [10]. Although biomarkers such as thrombin, plasmin, tissue plasminogen activator and plasminogen activator inhibitor-1 capture discrete steps in the coagulation–fibrinolytic pathways, they do not represent overall haemostatic function [10,11]. This has led to a growing interest in global assessments of thrombotic and fibrinolytic function. The Global Thrombosis Test (GTT) assesses thrombus formation and endogenous fibrinolysis in native whole blood, offering a physiologically appropriate evaluation for arterial thrombotic risk [11,12].

While global thrombotic status is related to ongoing cardiovascular risk in patients with acute coronary syndromes and ST-segment elevation myocardial infarction [13,14], whether global thrombotic status can identify patients with IS at highest ischemic risk, in particular patients with concomitant AF, is unknown. We aimed to investigate GTT-derived thrombosis and fibrinolysis parameters in IS patients, first comparing these measures between AF and non-AF groups, then across AF diagnostic categories, and finally exploring whether GTT indices could identify AFDNCS.

2. Methods

2.1. Study design and ethical approval

We conducted a prospective, observational study involving patients admitted with suspected acute IS, who were subsequently confirmed to have IS based on clinical evaluation and/or neuroimaging during hospitalisation. The study was conducted across two UK hospitals (Whiston Hospital, Cheshire and Lister Hospital, Stevenage). The study was designed and conducted in accordance with the Declarations of Helsinki 1964 and its subsequent revisions, and complied with Good Clinical Practice guidelines. The study was approved by the UK Health Research Authority Research Ethics Service (IRAS number: 316736). All patients gave written informed consent.

2.2. Study population

Recruitment was conducted within the stroke units of the participating hospitals from July 2023 to April 2025. We enrolled patients who fulfilled the inclusion criteria of (1) age $\geq$ 18 years; (2) current inpatient at the time of baseline data collection for recent IS, and (3) confirmed to have IS by a stroke physician and/or imaging (computed tomography or magnetic resonance imaging). Exclusion criteria were: (1) inability or unwilling to provide informed consent; and (2) receiving palliative or end-of-life care at the time of screening. All included patients were managed by the clinical teams according to best standard care with guideline-directed antiplatelet therapy and standard secondary prevention following IS.

2.3. Global thrombosis test and sampling procedures

The GTT is an automated point-of-care whole-blood assay that provides functional measures of thrombus formation and endogenous fibrinolysis, and has been described previously [12,15]. OT reflects the time required for shear-induced thrombus formation to arrest flow, whereas LT reflects the time needed for endogenous fibrinolysis to restore flow.

Venous blood was collected by trained clinical staff (doctor or research nurse) using standard aseptic venepuncture, within 24 h of informed consent and before hospital discharge. Where thrombolysis was administered, blood sampling was performed at least 24 h after thrombolytic therapy. For patients with prior anticoagulant exposure, anticoagulant therapy had been withheld before blood sampling following index stroke admission. Thrombotic status was assessed using 4 mL of freshly drawn venous blood, which was introduced into the device within 15 s of blood draw, after which the assay proceeded automatically.

2.4. Study outcomes and follow-up

The outcomes of interest were AF status and its temporal subtypes among patients with IS. Based on clinical records and monitoring during admission and follow-up, participants with any documented AF were classified into those with AF (comprising KAF, AFDCS and AFDNCS), while those without evidence of AF throughout the study period were classified as the non-AF group. For the predictive analysis, we specifically examined whether baseline thrombotic status measures (OT, LT) were related to subsequent AFDNCS.

All participants were followed for up to 6 months post-enrolment, or until death, with outcome information obtained through hospital electronic records, general practitioner records, or telephone follow-up.

2.5. Covariates and data collection

We extracted baseline variables across several domains, including demographics, medical and drug history, stroke severity assessed using the National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale (mRS) and routine laboratory measurements on admission. Details of all collected variables are provided in Table 1. These baseline characteristics were pre-specified and consistently collected across study sites.

2.6. Sample size estimation

There is a lack of published data on the use of GTT in patients with both IS and AF. Utilising data available in the literature from AF population, preliminary sample size estimates were generated to inform recruitment targets and ensure adequate statistical power for detecting meaningful group differences. For LT, Spinthakis et al. reported a mean and SD of 1900 $\pm$ 460 s in anticoagulated patients with AF [15]. Assuming a 20% relative difference between groups and a 1:1 allocation

Table 1
Baseline characteristics of IS patients between AF and non-AF patients.

	All	Non-AF	AF	P
N	115	79	36	
Age, years	68.5 ± 13.5	65.6 ± 14.2	74.7 ± 9.0	0.001
Male, n (%)	66 (57.4)	46 (58.2)	20 (55.6)	0.840
White, n (%)	113 (98.3)	77 (97.5)	36 (100.0)	1.000
BMI, kg/m2	28.3 ± 5.7	27.9 ± 5.8	29.1 ± 5.4	0.270
SBP, mmHg	146.2 ± 18.8	145.1 ± 19.9	148.6 ± 16.2	0.359
DBP, mmHg	80.6 ± 13.8	80.5 ± 14.9	80.8 ± 11.2	0.912
Smoking status, n (%)				0.019
Non-smoker	76 (66.1)	46 (58.2)	30 (83.3)	
Ex-smoker	17 (14.8)	13 (16.5)	4 (11.1)	
Current smoker	22 (19.1)	20 (25.3)	2 (5.6)	
mRS on admission	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	1.0 (0.0, 1.0)	0.004
NIHSS on admission	3.0 (2.0, 6.0)	3.0 (2.0, 6.0)	3.0 (2.0, 6.0)	0.718
Comorbidity, n (%)				
Hypertension	69 (60.0)	43 (54.4)	26 (72.2)	0.100
Hypercholesterolaemia	19 (16.5)	12 (15.2)	7 (19.4)	0.595
Diabetes mellitus	26 (22.6)	19 (24.1)	7 (19.4)	0.639
Coronary artery disease	9 (7.8)	4 (5.1)	5 (13.9)	0.136
Congestive heart failure	4 (3.5)	1 (1.3)	3 (8.3)	0.090
Ischemic heart disease	5 (4.3)	2 (2.5)	3 (8.3)	0.176
Prior Stroke/TIA	17 (14.8)	9 (11.4)	8 (22.2)	0.159
Chronic kidney disease	12 (10.4)	5 (6.3)	7 (19.4)	0.048
Cardiomyopathy	3 (2.6)	0 (0.0)	3 (8.3)	0.029
Lung disease	19 (16.5)	13 (16.5)	6 (16.7)	1.000
Laboratory test				
APTT, second	23.8 ± 2.8	23.6 ± 3.3	23.7 ± 2.4	0.957
PT, second	11.4 ± 1.0	11.2 ± 1.0	11.7 ± 0.9	0.011
TC, mmol/L	4.5 ± 1.1	4.6 ± 1.2	4.2 ± 0.9	0.052
HDL-C, mmol/L	1.3 ± 0.4	1.3 ± 0.5	1.3 ± 0.4	0.927
LDL-C, mmol/L	2.6 ± 0.9	2.7 ± 1.0	2.4 ± 0.8	0.091
Triglycerides, mmol/L	1.4 ± 0.8	1.5 ± 0.9	1.2 ± 0.6	0.037
WBC, × 10^9/L	8.8 ± 2.9	8.9 ± 3.0	8.5 ± 2.7	0.476
Platelet, × 10^9/L	257.0 ± 95.9	261.7 ± 100.6	247.5 ± 80.2	0.457
Hemoglobin, g/dL	14.7 ± 11.8	15.2 ± 13.9	13.5 ± 2.0	0.448
Treatment, n (%)				
Pre-admission anticoagulant use	20 (17.4)	2 (2.5)	20 (55.6)	< 0.001
Oral anticoagulant at discharge	37 (32.2)	8 (10.1)	29 (80.6)	< 0.001
Thrombolysis	18 (15.7)	12 (15.2)	6 (16.7)	1.000
ACEIs/ARBs	42 (36.5)	29 (36.7)	13 (36.1)	1.000
Beta blockers	33 (28.7)	12 (15.2)	21 (58.3)	< 0.001
Diuretics	14 (12.2)	6 (7.6)	8 (22.2)	0.034
DHP-CCB	38 (33.0)	27 (34.2)	11 (30.6)	0.831
Statins	95 (82.6)	65 (82.3)	30 (83.3)	1.000

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; APTT, activated partial thromboplastin time; ARB, angiotensin II receptor blocker; DHP-CCB, dihydropyridine calcium channel blocker; HDL—C, high-density lipoprotein cholesterol; IS, ischaemic stroke; LDL-C, low-density lipoprotein cholesterol; mRS, modified Rankin scale; NIHSS, NIH Stroke Scale; PT, prothrombin time; TC, total cholesterol; WBC, white blood cell.

ratio, a two-sample *t*-test (80% power, two-sided $\alpha = 0.05$) indicated that 20 participants per group (40 in total) would be required. For OT, using an assumed mean of 460 s and SD of 125 s [15], a similar 20% difference would require 15 participants in each group (30 participants in total). Sample size calculations followed standard methods for comparing two independent means, as outlined by Chow et al. [16] These calculations assumed equal group sizes, whereas AF is unevenly distributed in patients withIS. Accordingly, beyond meeting minimum sample size requirements for AF versus non-AF comparisons, a larger cohort was recruited to support exploratory subgroup analyses based on the temporal relationship between AF and IS diagnosis.

2.7. Statistical analysis

Baseline missing data across all study variables is summarised in Supplementary Table S1. To minimise potential bias arising from incomplete data, multivariate imputation by chained equations was applied to generate imputed datasets under the assumption of missing at random. Continuous variables were checked for normality using the Shapiro–Wilk test within groups. Normally distributed variables are expressed as mean with SD and compared using the Student's *t*-test or one-way analysis of variance, while non-normally distributed variables are presented as median with interquartile range (IQR) and compared with the Mann–Whitney U or Kruskal–Wallis tests. Categorical variables are reported as counts with percentages and compared using the Chi-square or Fisher tests.

The OT and LT between patients with and without AF were compared. This initial step established whether AF itself is associated with altered thrombotic or fibrinolytic function. The distribution of OT and LT was then examined separately in males and females to explore potential sex-related variations in thrombogenicity, given that sex differences have been reported in stroke [17].

Subsequent analyses examined OT and LT across non-AF and three AF diagnostic categories (KAF, AFDCS, and AFDNCS) to assess whether thrombotic and fibrinolytic profiles differ across AF groups defined by diagnostic timing.

To explore whether the observed associations between AF and GTT-derived parameters were independent of baseline clinical differences between AF and non-AF patients, additional multivariable linear regression analyses were performed with OT and LT as continuous dependent variables. Given the modest sample size, a parsimonious adjustment strategy was adopted, including demographic factors, clinically relevant baseline comorbidities that differed between groups, and their combined adjustment. Because prior anticoagulant exposure may also influence OT and LT measurements and was more common among patients with KAF, additional models incorporating prior anticoagulant use were constructed. Multicollinearity was assessed using variance inflation factors.

To assess the potential impact of treatment-related confounding, exploratory sensitivity analyses were performed by stratifying patients according to prior anticoagulant exposure, statin use, and thrombolysis status, given the potential influence of these therapies on thrombotic and fibrinolytic parameters. Comparisons of OT and LT were then performed between AF and non-AF patients within each exposure stratum.

To explore potential factors associated with OT and LT, exploratory correlation analyses were performed between GTT-derived parameters and routinely collected clinical and laboratory variables in the overall stroke cohort using Spearman correlation coefficients, to further inform interpretation of the findings.

Exploratory analyses assessed GTT-based prediction of AFDNCS in patients without KAF or AFDCS and without exposure to intravenous thrombolysis before blood sampling, to minimise potential treatment-related effects, despite blood collection occurring >24 h after thrombolysis. The discriminative ability of OT and LT for identifying AFDNCS was assessed using receiver operating characteristic (ROC) analysis, with area under the curve (AUC) and 95% confidence interval (CI) calculated to quantify performance. Optimal cut-offs were determined using the Youden index, with corresponding sensitivity, specificity, positive predictive value, and negative predictive value reported. Logistic regression models were then used to determine whether adding OT or LT to a basic clinical model of age and sex improved risk

classification, with incremental value quantified using categorical and continuous Net Reclassification Improvement (NRI) with 95% CIs.

All statistical analyses were two-sided, and a P value <0.05 was considered statistically significant. All analyses were conducted using SPSS (version 31.0, IBM Corp., Armonk, NY, USA, https://www.ibm.com/products/spss-statistics) and R (version 4.3.1, R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org).

3. Results

3.1. Baseline characteristics

A total of 121 patients were initially enrolled, 3 withdrew and 3 were lost to follow-up, leaving 115 participants for analysis (mean [SD] age: 68.5 ± 13.5 years, 66 [57.4%] males), and the study flow is shown in Fig. 1.

Baseline characteristics of IS patients with and without AF are summarised in Table 1. Patients with AF (N = 36, mean [SD] age: 74.7 ± 9.0 years, 20 [55.6%] males) were older than those without AF (N = 79, mean [SD] age: 65.6 ± 14.2 years, 46 [58.2%] males), while the proportion of males was similar. Body mass index, blood pressure, and admission NIHSS did not differ significantly, although pre-stroke disability (mRS) was higher in the AF group (P = 0.004). Cardiomyopathy (8.3% vs 0%, P = 0.029) and chronic kidney disease (19.4% vs 4.0%, P = 0.048) were more frequent in AF patients, while other comorbidities were comparable between groups. Laboratory parameters were largely similar, except for a slightly prolonged prothrombin time (PT) in AF patients (P = 0.011).

Baseline characteristics across AF diagnostic timing groups (KAF [N = 20], AFDCS [N = 10] and AFDNCS [N = 6]) [Supplementary Table S2] with generally similar clinical profile apart from hypercholesterolaemia was more common in AFDNCS compared with KAF and AFDCS.

3.2. Comparison of GTT-derived parameters between AF and non-AF patients

Comparisons of OT and LT between AF and non-AF patients are shown in Fig. 2. In the full cohort, OT was significantly longer in AF than in non-AF patients (474.2 ± 214.1 vs 372.6 ± 176.1 s, P = 0.009). Similar findings were observed in women (505.3 ± 209.5 vs. 378.6 ± 188.9 s, P = 0.039), whereas the difference in males did not reach significance (449.4 ± 219.7 vs 368.4 ± 168.8 s, P = 0.108). LT did not differ between AF and non-AF patients in the overall cohort (2408 [1782–3991] vs. 2237 [1894–3260] seconds, P = 0.993) nor within sex-specific analyses (all P > 0.05).

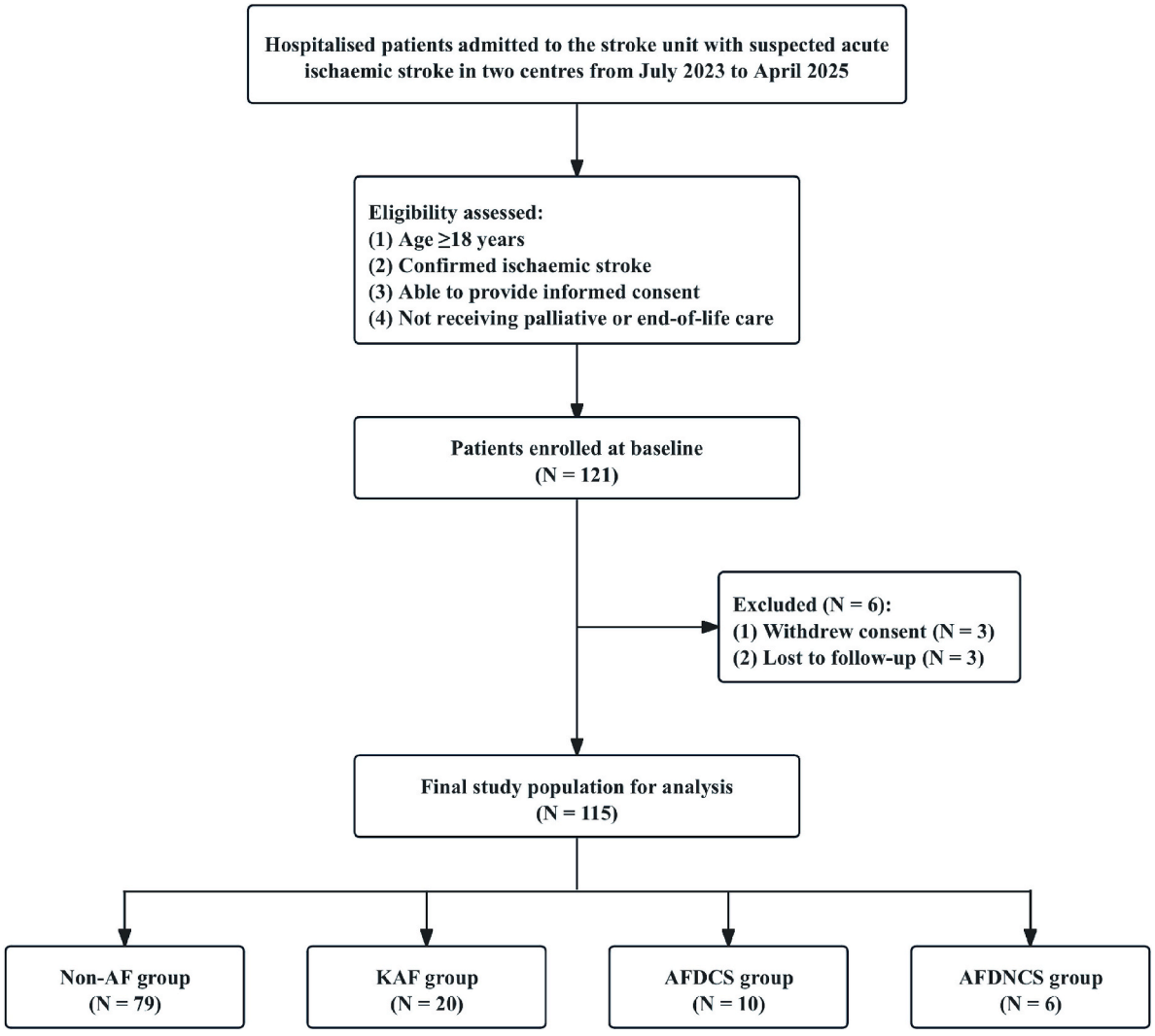
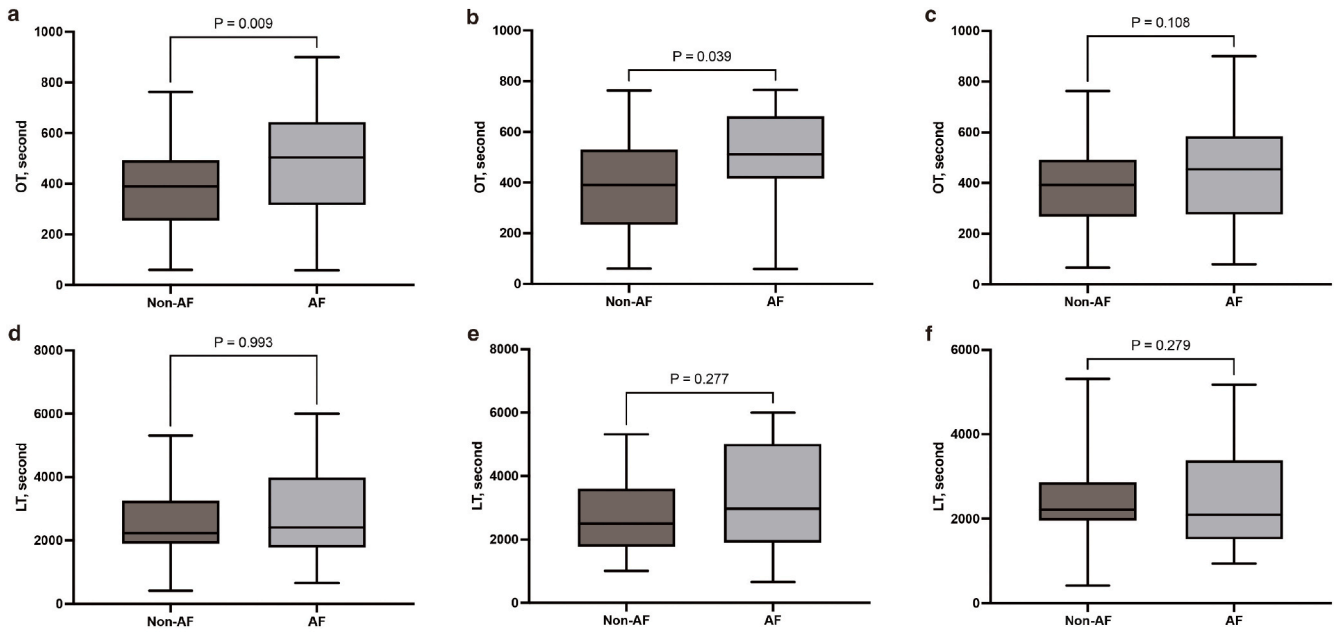


Fig. 1. Flow diagram of patient recruitment and classification. AF, atrial fibrillation; AFDCS, atrial fibrillation detected concurrently with stroke; AFDNCS, atrial fibrillation detected non-concurrently with stroke; KAF, known atrial fibrillation.



	All	Non-AF	AF	P
Overall				
N	115	79	36	
OT, second	404.5 ± 193.9	372.6 ± 176.1	474.2 ± 214.1	0.009
LT, second	2237 (1864, 3551)	2237 (1894, 3260)	2408 (1782, 3991)	0.993
Female				
N	49	33	16	
OT, second	420.0 ± 202.8	378.6 ± 188.9	505.3 ± 209.5	0.039
LT, second	2530 (1835, 4043)	2503 (1770, 3604)	2971 (1899, 5014)	0.277
Male				
N	66	46	20	
OT, second	392.9 ± 187.7	368.4 ± 168.8	449.4 ± 219.7	0.108
LT, second	2208 (1864, 2869)	2218 (1957, 2863)	2095 (1513, 3382)	0.279

Fig. 2. Comparison of OT and LT between AF and non-AF patients overall and by sex. Panels (a, d) represent the overall cohort, (b, e) females, and (c, f) males. AF, atrial fibrillation; LT, lysis time; OT, occlusion time.

3.3. OT and LT distributions across non-AF and AF groups

As shown in Fig. 3, OT differed significantly across the four groups ($P = 0.002$). Patients with KAF had the longest OT (526.4 ± 193.1 s), followed by AFDCS (483.4 ± 218.2 s), both longer than non-AF patients (372.6 ± 176.1 s), while AFDNCS showed the shortest OT (285.3 ± 198.9 s). LT did not differ significantly overall ($P = 0.358$), although median values varied across groups, being 2719 [1783–4765] seconds in KAF, 1973 [1066–2802] seconds in AFDCS, 2881 [1886–4231] seconds in AFDNCS, and 2237 [1894–3260] seconds in non-AF patients.

3.4. Additional multivariable analyses

Multivariable linear regression analyses (Supplementary Table S3) showed that the association between AF and longer OT remained significant after adjustment for demographic factors (age and sex), and baseline comorbidities that were more prevalent in the AF group (hypertension, coronary artery disease, congestive heart failure, ischaemic heart disease, prior stroke/transient ischaemic attack, chronic kidney disease, and cardiomyopathy), but was attenuated after inclusion of prior anticoagulant use and in the fully adjusted model. No significant association between AF and LT was observed.

3.5. Exploratory therapy-related sensitivity analyses

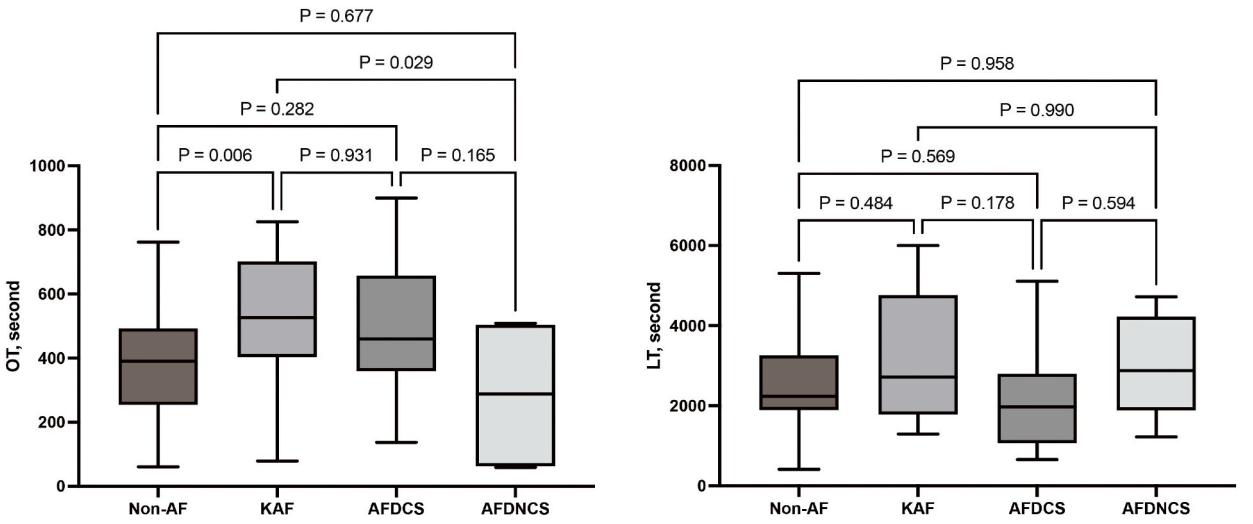
All documented prior anticoagulant exposure involved direct oral anticoagulants, with no prior warfarin users (Supplementary Table S4). Coagulation parameters at sampling were largely within normal ranges irrespective of prior anticoagulant history (Supplementary Table S5). Stratified analyses according to prior anticoagulant exposure, statin use, and thrombolysis status showed broadly consistent directional patterns, although subgroup sizes were limited in some strata (Supplementary Table S6).

3.6. Exploratory correlations of OT and LT with clinical and laboratory variables

Exploratory correlation analyses (Supplementary Fig. S1) showed only weak associations between GTT-derived parameters and routinely collected clinical/laboratory variables, with no consistent pattern apart from a weak inverse correlation between LT and diastolic blood pressure.

3.7. Exploratory analysis of OT and LT for predicting AFDNCS

After excluding patients with KAF or AFDCS and those exposed to thrombolysis before blood sampling, a total of 72 patients remained for



	Non-AF	KAF	AFDCS	AFDNCS	P
N	79	20	10	6	
OT, second	372.6 ± 176.1	526.4 ± 193.1	483.4 ± 218.2	285.3 ± 198.9	0.002
LT, second	2,237 (1,894, 3,260)	2,719 (1,783, 4,765)	1,973 (1,066, 2,802)	2,881 (1,886, 4,231)	0.358

Fig. 3. OT and LT across non-AF and AF diagnostic categories. AF, atrial fibrillation; AFDCS, atrial fibrillation detected concurrently with stroke; AFDNCS, atrial fibrillation detected non-concurrently with stroke; KAF, known atrial fibrillation; LT, lysis time; OT, occlusion time.

analysis. The predictive value of GTT parameters for newly detected AFDNCS ($N = 5$) was then evaluated. Within this exploratory cohort, OT was numerically shorter in patients who subsequently developed AFDNCS, whereas LT did not show a clear difference, although neither

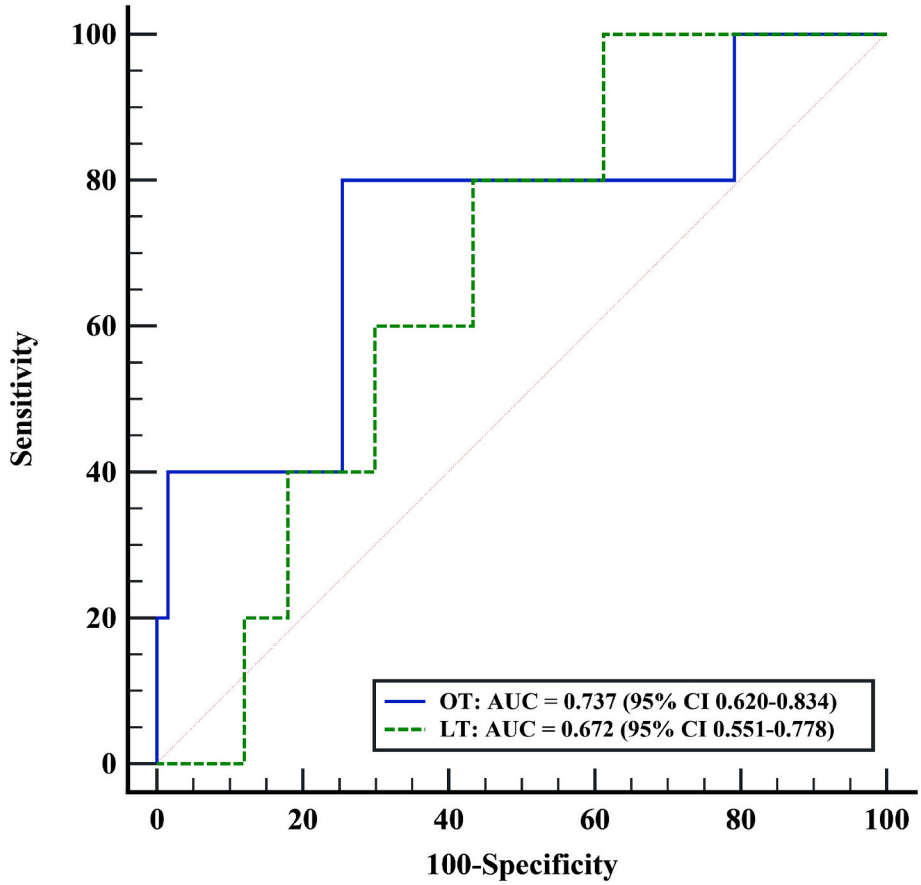


Fig. 4. Predictive value of OT and LT for newly detected AFDNCS after exclusion of patients with KAF, AFDCS, and thrombolysis before blood sampling. AFDCS, atrial fibrillation detected concurrently with stroke; AFDNCS, atrial fibrillation detected non-concurrently with stroke; AUC, area under the curve; CI, confidence interval; KAF, atrial fibrillation; LT, lysis time; OT, occlusion time.

comparison reached statistical significance (Supplementary Table S7). As shown in Fig. 4, OT demonstrated greater discriminative ability than LT for identifying AFDNCS (AUC = 0.737, 95% CI 0.620–0.834 vs. AUC = 0.672, 95% CI 0.551–0.778), with shorter OT values associated with subsequent AFDNCS. The optimal OT cut-off derived using the Youden index was 290.9 s, corresponding to a sensitivity of 80.0%, specificity of 74.6%, positive predictive value of 19.0%, and negative predictive value of 98.0% (Supplementary Table S8).

When added to a basic model including age and sex (Table 2), OT significantly improved risk reclassification ($NRI_{categorical}$ = 63.6%, 95% CI 35.2%–92.1%, $P < 0.001$; $NRI_{continuous}$ = 94.3%, 95% CI 20.7%–168.0%, $P = 0.012$), whereas LT provided no significant incremental value ($P = 0.127$ and $P = 0.230$, respectively).

4. Discussion

This study represents the first investigation to evaluate thrombotic and fibrinolytic function in patients with IS in relation to AF status, and several important findings emerged. First, AF patients exhibited significantly longer OT than non-AF patients, while LT differences were not significant, suggesting that thrombus formation is more affected than fibrinolysis in the presence of AF. Additional multivariable analyses accounting for baseline demographic and comorbidity differences showed that this association remained broadly consistent. Although attenuation was observed after inclusion of prior anticoagulant exposure, all anticoagulants had been withheld before GTT sampling, suggesting that this may reflect both treatment history and the longer-standing AF clinical context it represents. Second, although LT did not differ significantly across AF diagnostic categories, descriptive variation was observed across groups, which should be interpreted cautiously given the small subgroup sizes. Third, in patients without prior AF and without thrombolysis exposure, shorter baseline OT showed a potential discriminatory signal for identifying AFDNCS in exploratory analysis, although the limited event number requires cautious interpretation. Collectively, these findings suggest that GTT-derived parameters may reflect differences in haemostatic profiles across AF trajectories, although the exploratory findings, particularly for AFDNCS, require cautious interpretation.

The paradoxical prolongation of OT in IS patients with AF may reflect the complex interplay between haemodynamic disturbance and pharmacological modulation. AF is a recognised prothrombotic condition, arising from the combined effects of blood stasis, endothelial dysfunction, and abnormal platelet and coagulation activity [18,19]. In our cohort, differences in OT across AF categories appear to reflect heterogeneity in AF status and disease stage rather than a simple gradient of anticoagulant exposure or intrinsic haemostatic properties alone. Patients with KAF exhibited the longest OT, likely reflecting the combined effects of chronic atrial arrhythmia, long-standing haemodynamic disturbance, and prior anticoagulant therapeutic exposure. Importantly, routine coagulation parameters, including prothrombin time (median 11.7 s, IQR 11.0–12.4) and activated partial thromboplastin time (median 24 s, IQR 23–25), were within normal ranges and comparable between groups at the time of sampling, suggesting that any residual anticoagulant effect was limited. This raises the possibility that the observed differences may be more closely related to underlying AF-associated pathophysiology and disease chronicity rather than direct pharmacological effects, particularly given the broadly consistent findings across exploratory treatment-stratified analyses, although this warrants further investigation. Exploratory treatment-stratified analyses yielded broadly similar directional findings, although subgroup sizes were limited. In contrast, patients with AFDCS, who were generally not anticoagulated at the time of GTT measurement, demonstrated intermediate OT values. Patients with AFDNCS showed shorter OT, consistent with a haemostatic profile that had not yet been substantially modified by sustained AF-related haemodynamic perturbation or long-term treatment, although this observation should be interpreted cautiously given the very small subgroup size and exploratory nature of the analysis.

These findings suggest that OT prolongation in AF may be influenced by the chronicity of the arrhythmic state. In patients with established AF, long-standing haemodynamic disturbance together with prior therapeutic exposure may contribute to a more pronounced prolongation of OT. In contrast, newly diagnosed AF in the acute stroke setting may represent an early phase of AF-related haemostatic alteration, prior to the development of more stable, chronic changes. However, the number of patients with AFDCS in our cohort was small ($N = 10$), and this observation should therefore be interpreted with caution. While previous studies have shown that anticoagulant therapy suppresses thrombus formation in AF, as reflected by prolonged OT following treatment exposure [20,22], our results indicate that differences in OT across AF diagnostic categories cannot be explained by anticoagulation alone. These findings suggest that OT may capture different haemostatic states across the AF continuum, with shorter OT potentially representing an exploratory signal associated with subsequent AF detection, and prolonged OT reflecting chronic haemodynamic and treatment-related effects in established AF.

Although LT did not differ significantly across AF diagnostic subgroups, descriptive differences in LT were observed and may provide exploratory insight into potential heterogeneity in fibrinolytic dynamics. The relatively shorter LT observed in patients with AFDCS may reflect a transient enhancement of endogenous fibrinolytic activity during the early phase of AF and acute ischaemic stroke. Acute haemodynamic disturbance, heightened sympathetic activation, and stress-related endothelial responses in newly diagnosed AF may promote a compensatory increase in fibrinolytic responsiveness [23–25], resulting in shorter LT under high-shear conditions. By comparison, patients in the KAF group likely had a longer history of AF and were chronically anticoagulated. Although AF duration was not systematically recorded in our cohort, the observed prolongation of LT in this group may be consistent with the possibility that chronic AF is associated with altered fibrinolytic function. This pattern may be consistent with the concept that increasing AF burden is associated with greater thrombotic risk, suggesting that impaired fibrinolysis may represent a mechanistic link between AF chronicity and stroke risk, even in anticoagulated patients. This descriptive pattern may warrant further investigation, although mechanistic inference cannot be made from the present data. This may, in part, reflect a more advanced atrial substrate and progressive endothelial dysfunction in chronic AF, as evidenced by elevated plasma von Willebrand factor levels, reduced nitric oxide bioavailability, increased asymmetric dimethylarginine, and impaired flow-mediated vasodilation [26,27]. All these may reflect sustained vascular injury and diminished fibrinolytic capacity [28], which may worsen with disease duration. These mechanisms remain biologically plausible but were not directly assessed in the present study. Spinthakis et al. have previously shown differences in LT between apixaban and warfarin [15]. In addition, analyses from the ARISTOTLE trial have suggested that pre-treatment

Table 2
Risk reclassification improvement for AFDNCS with OT or LT in patients without KAF, AFDCS, or thrombolysis.

	$NRI_{Categorical}$ (95% CI)	P	$NRI_{Continuous}$ (95% CI)	P
Basic model	Reference		Reference	
Basic model + OT	63.6% (35.2%, 92.1%)	< 0.001	94.3% (20.7%, 168.0%)	0.012
Basic model + LT	46.7% (−13.3%, 106.6%)	0.127	54.3% (−34.5%, 143.1%)	0.230

Basic model was included age and sex.
Abbreviations: AFDCS, atrial fibrillation detected concurrently with stroke; AFDNCS, atrial fibrillation detected non-concurrently with stroke; CI, confidence interval; KAF, atrial fibrillation; LT, lysis time; NRI, net reclassification improvement; OT, occlusion time.

fibrinolytic profiles may be associated with bleeding risk in anticoagulated patients with AF, further supporting the clinical relevance of fibrinolytic heterogeneity in this population [29]. In our cohort, the majority of anticoagulated patients received direct OAC therapy (97.2%), although the present sample was not designed to evaluate treatment-specific fibrinolytic effects. However, given the limited number of patients in both the KAF and AFDCS subgroups any interpretation of these descriptive LT patternsshould be interpreted with caution and warrant confirmation in larger studies.

More broadly, interpretation of GTT-derived findings in an acute ischaemic stroke cohort requires some caution, given the clinical heterogeneity of stroke patients, the influence of concurrent therapies, and the complex physiological scope of the assay itself. As a native whole-blood assay performed under high shear conditions, OT and LT are likely influenced by multiple interacting determinants, including platelet function, blood rheology, and endogenous fibrinolysis activity. In exploratory correlation analyses, no strong or consistent associations were observed between GTT-derived parameters and routinely collected clinical or laboratory variables, suggesting that these measures are not readily explained by conventional single-marker assessments alone. While routinely collected laboratory parameters relevant to haemostatic physiology were available in the current cohort, dedicated mechanistic biomarkers of coagulation and fibrinolysis were not systematically measured, limiting direct mechanistic interpretation.

From a clinical perspective, the exploratory discriminative performance of OT for identifying AFDNCS in patients without prior AF or exposure to thrombolysis highlights the potential translational value of GTT in stroke care. Within this exploratory cohort, OT was numerically shorter in patients who subsequently developed AFDNCS, whereas LT did not show a clear difference, although neither comparison reached statistical significance. Therefore, shorter OT values should be interpreted as a potential haemostatic signal associated with subsequent AF detection rather than definitive evidence of a pre-existing prothrombotic phenotype. Given its simplicity, low cost, and minimal blood requirement (only 4 mL), the GTT can be conveniently performed at the bedside to evaluate both thrombotic and fibrinolytic capacity under physiological shear conditions. When used in combination with conventional ECG monitoring, it could help refine post-stroke AF detection strategies and risk stratification. Prospective validation is warranted to determine whether integrating GTT-derived parameters into clinical workflows can improve early AF diagnosis and guide personalised antithrombotic management in acute stroke populations.

4.1. Limitations

This study has several limitations. First, although the overall design was observational and causality cannot be definitively established, the analysis examining the association between baseline GTT parameters and subsequent development of newly diagnosed AFDNCS among patients free from KAF, AFDCS, or any thrombolytic therapy has a prospective structure. This component reflects a forward-looking approach that strengthens the temporal relationship between exposure and outcome. Second, the modest overall sample size, particularly within subgroups and the exploratory cohort used to evaluate OT as a predictor of AFDNCS, may limit statistical power and contribute to imprecise estimates or unstable effect sizes. Third, GTT measurements were obtained at a single time point, which may not capture dynamic changes in thrombotic or fibrinolytic function following IS. Fourth, concomitant therapies, including antithrombotic treatment, statin use, and thrombolysis exposure, were heterogeneous and not fully standardised, which may have influenced GTT-derived parameters despite exploratory sensitivity analyses. Fifth, echocardiographic assessment was unavailable in a substantial proportion of patients, limiting systematic evaluation of atrial structural remodelling and its relationship with GTT-derived parameters. Sixth, although all anticoagulants were withheld before GTT sampling and routine coagulation parameters were within normal ranges at the time of sampling, residual confounding related to prior anticoagulant exposure, treatment duration, and inter-individual variability in anticoagulant effects cannot be fully excluded. Seventh, although GTT provides an integrated assessment of whole-blood haemostatic function, dedicated mechanistic biomarkers directly involved in coagulation and fibrinolysis pathways were not systematically measured in the current cohort, which limits direct mechanistic interpretation of the observed associations. Eighth, AF detection was based on routine clinical documentation and follow-up, without the use of prolonged or continuous ECG monitoring. This approach may have resulted in underdiagnosis of asymptomatic or paroxysmal AF episodes, particularly post-discharge, leading to a lower observed incidence of AF. Consequently, the true burden of newly diagnosed AF after stroke may be underestimated in this study. Lastly, this study was conducted at two sites in the UK, and the vast majority of the enrolled participants were of White ethnicity, the generalisability of our findings to other ethnic or geographic populations may be limited. Future external validation studies in more diverse cohorts and across different healthcare settings will be essential to confirm the applicability and robustness of GTT-based AF risk stratification post stroke.

5. Conclusions

We show heterogeneity in GTT-derived whole-blood haemostatic function across AF diagnostic categories in patients with IS. Baseline OT tested during the index IS admission, particularly shorter OT, showed promising discriminative performance among AF-free patients for identifying patients who develop AFDNCS, indicating a prothrombotic phenotype preceding clinically detectable AF and supporting its potential role as an exploratory marker for predicting subsequent AF post-stroke. Although LT did not differ significantly across AF subgroups, descriptive variation was observed and warrants further investigation in larger studies. As a physiological, near-patient assay, the GTT provides a practical way of characterising global haemostatic status in the acute stroke setting. The potential value of incorporating GTT-derived parameters into post-stroke risk stratification and AF surveillance strategies warrants further evaluation in larger, adequately powered studies.

CRediT authorship contribution statement

Yang Chen: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation. **Ying Gue:** Writing – review & editing, Validation, Supervision, Formal analysis, Conceptualization. **Sandra Elsheikh:** Writing – review & editing, Data curation. **Coleen Ditchfield:** Writing – review & editing, Data curation. **Sogol Koolaji:** Writing – review & editing, Data curation. **Andrew M. Hill:** Writing – review & editing. **Garry McDowell:** Writing – review & editing, Supervision, Conceptualization. **Diana A. Gorog:** Writing – review & editing, Supervision. **Gregory Y.H. Lip:** Writing – review & editing, Supervision, Methodology, Conceptualization.

Pre-registered clinical trial number

Not available.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. Ethical approval was granted by the UK Health Departments Research Ethics Service (IRAS 316736), and written informed consent was obtained from all participants.

Funding

This study was funded by University of Liverpool (No. UoL001706).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Yang Chen gratefully acknowledges financial support from the China Scholarship Council (CSC) (No. 202308890013).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.thromres.2026.109772.

Data availability

The data underlying this study are not publicly available as they contain confidential clinical information and cannot be shared.

References

[1] X.-J. Dong, B.-B. Wang, F.-F. Hou, Y. Jiao, H.-W. Li, S.-P. Lv, F.-H. Li, Global burden of atrial fibrillation/atrial flutter and its attributable risk factors from 1990 to 2019, Europace : European Pacing, Arrhythmias, Cardiac Electrophysiol.: J. Work. Groups Cardiac Pacing, Arrhythmias, Cardiac Cell. Electrophysiol. Eur. Soc. Cardiol. 25 (2023) 793–803.
[2] Global, regional, and national burden of stroke and its risk factors, 1990-2021: a systematic analysis for the global burden of disease study 2021, Lancet Neurol. 23 (2024).
[3] H. Kamel, J.S. Healey, Cardioembolic stroke, Circ. Res. 120 (2017) 514–526.
[4] S. Hewage, A. Jadamba, D. Brain, W. Parsonage, S. McPhail, S. Kularatna, Global and regional burden of ischemic stroke associated with atrial fibrillation, 2009–2019, Prev. Med. 173 (2023) 107584.
[5] A. Mitaine, G. Duloquin, T. Pommier, C. Vergely, C. Guenancia, Y. Béjot, Prevalence and characteristics of known versus newly detected atrial fibrillation in ischemic stroke: a population-based study, Neuroepidemiology 58 (2024) 284–291.
[6] H.J. Ahn, Y.H. Go, S.R. Lee, J. Choi, K.Y. Lee, S. Kwon, et al., Comparative clinical profiles and outcomes of prior vs. concurrently diagnosed atrial fibrillation in acute ischaemic stroke: the implication of diagnosis timing, Europace 27 (2025).
[7] J. Guo, Z. Li, H. Gu, K. Yang, Y. Liu, J. Lu, et al., Prevalence, risk factors and prognostic value of atrial fibrillation detected after stroke after haemorrhagic versus ischaemic stroke, Stroke Vasc. Neurol. 9 (2024) 652–659.
[8] L.A. Sposato, L.E. Cipriano, G. Saposnik, E.R. Vargas, P.M. Riccio, V. Hachinski, Diagnosis of atrial fibrillation after stroke and transient ischaemic attack: a systematic review and meta-analysis, Lancet Neurol. 14 (2015) 377–387.
[9] D.S. Chew, E. Rennert-May, F.R. Quinn, B. Buck, M.D. Hill, E. Spackman, et al., Economic evaluation of extended electrocardiogram monitoring for atrial fibrillation in patients with cryptogenic stroke, Int. J. Stroke 16 (2021) 809–817.
[10] D.A. Gorog, G.Y.H. Lip, Impaired spontaneous/endogenous fibrinolytic status as new cardiovascular risk factor?: JACC review topic of the week, J. Am. Coll. Cardiol. 74 (2019) 1366–1375.
[11] Y. Chen, Y. Gue, G. McDowell, D.A. Gorog, G.Y.H. Lip, Impaired endogenous fibrinolysis status: a potential prognostic predictor in ischemic stroke, Minerva Med. 115 (2024) 364–379.
[12] J. Yamamoto, N. Inoue, K. Otsui, H. Ishii, D.A. Gorog, Global thrombosis test (GTT) can detect major determinants of haemostasis including platelet reactivity, endogenous fibrinolytic and thrombin generating potential, Thromb. Res. 133 (2014) 919–926.
[13] M. Farag, N. Spinthakis, Y.X. Gue, M. Srinivasan, K. Sullivan, D. Wellsted, D. A. Gorog, Impaired endogenous fibrinolysis in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention is a predictor of recurrent cardiovascular events: the RISK PPCI study, Eur. Heart J. 40 (2019) 295–305.
[14] S. Saraf, C. Christopoulos, I.B. Salha, D.J. Stott, D.A. Gorog, Impaired endogenous thrombolysis in acute coronary syndrome patients predicts cardiovascular death and nonfatal myocardial infarction, J. Am. Coll. Cardiol. 55 (2010) 2107–2115.
[15] N. Spinthakis, Y. Gue, M. Farag, M. Srinivasan, D. Wellsted, D.R.J. Arachchillage, et al., Apixaban enhances endogenous fibrinolysis in patients with atrial fibrillation, Europace 21 (2019) 1297–1306.
[16] S.-C. Chow, J. Shao, H. Wang, Y. Lokhnygina, Sample Size Calculations in Clinical Research, Chapman and Hall/CRC, 2017.
[17] M.J. Reeves, C.D. Bushnell, G. Howard, J.W. Gargano, P.W. Duncan, G. Lynch, et al., Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes, Lancet Neurol. 7 (2008) 915–926.
[18] A.A. Khan, G.Y. Lip, The prothrombotic state in atrial fibrillation: pathophysiological and management implications, Cardiovasc. Res. 115 (2019) 31–45.
[19] W.Y. Ding, D. Gupta, G.Y. Lip, Atrial fibrillation and the prothrombotic state: revisiting Virchow's triad in 2020, Heart 106 (2020) 1463–1468.
[20] C. Voukalis, G.Y. Lip, E. Shantsila, Effects of antithrombotic drugs on the prothrombotic state in patients with atrial fibrillation: the West Birmingham atrial fibrillation project, Thromb. Res. 200 (2021) 149–155.
[22] M. Farag, M. Niespialowska-Steuden, O. Okafor, B. Artman, M. Srinivasan, A. Khan, et al., Relative effects of different non-vitamin K antagonist oral anticoagulants on global thrombotic status in atrial fibrillation, Platelets 27 (2016) 687–693.
[23] N.A. Bosch, J. Cimini, A.J. Walkey, Atrial fibrillation in the ICU, Chest 154 (2018) 1424–1434.
[24] R. Carnagarin, M.G. Kiuchi, J.K. Ho, V.B. Matthews, M.P. Schlaich, Sympathetic nervous system activation and its modulation: role in atrial fibrillation, Front. Neurosci. 12 (2019) 1058.
[25] S. Qin, M. Boidin, B.J. Buckley, G.Y. Lip, D.H. Thijssen, Endothelial dysfunction and vascular maladaptation in atrial fibrillation, Eur. J. Clin. Investig. 51 (2021) e13477.
[26] H. Kottkamp, Human atrial fibrillation substrate: towards a specific fibrotic atrial cardiomyopathy, Eur. Heart J. 34 (2013) 2731–2738.
[27] A.A. Khan, G.N. Thomas, G.Y.H. Lip, A. Shantsila, Endothelial function in patients with atrial fibrillation, Ann. Med. 52 (2020) 1–11.
[28] N. Black, F. Mohammad, K. Saraf, G. Morris, Endothelial function and atrial fibrillation: a missing piece of the puzzle? J. Cardiovasc. Electrophysiol. 33 (2022) 109–116.
[29] W.A.E. Parker, T.A. Nelson, J. Lee, H.M. Judge, R.A. Ajjan, J. Westerbergh, et al., Pre-treatment lysis time of plasma-derived fibrin clots and bleeding in patients on oral anticoagulants for atrial fibrillation in the ARISTOTLE trial, Eur. Heart J. 47 (2026) 1949–1960.