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The association between excessive premature atrial complexes and cryptogenic stroke: results of a case – control study.

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Abstract

Objective: Recent anticoagulation trials in all-comer cryptogenic stroke patients have yielded equivocal results, reinvigorating the focus on identifying reproducible markers of an atrial myopathy. We investigated the role of excessive premature atrial complexes (PACs) in ischaemic stroke, including cryptogenic stroke and its association with vascular risk factors.

Methods and Results: A case control study was conducted utilising a multi-centre institutional stroke database to compare 461 patients with an ischaemic stroke or transient ischaemic attack (TIA) with a control group consisting of age matched patients without prior history of ischaemic stroke/TIA. All patients underwent 24-hour Holter monitoring during the study period and atrial fibrillation was excluded.

An excessive PAC burden, defined as ≥ 200 PACs/24 hours, was present in 25.6% and 14.7% ($p < 0.01$), of stroke/TIA and control patients respectively. On multivariate regression, excessive PACs conferred the highest risk for ischaemic stroke/TIA (OR 1.97; 95% confidence interval (CI): 1.29 – 3.02; $p < 0.01$) and exceeded risk conferred by smoking (OR 1.58; CI: 1.06 – 2.36; $p < 0.05$) and hypertension (OR 1.53; CI: 1.07 – 2.17; $p < 0.05$). Excessive PACs remained the strongest independent risk factor for the cryptogenic stroke subtype (OR 1.95; CI: 1.16 – 3.28; $p<0.05$). Vascular risk factors that promote atrial remodelling, increasing age (≥ 75 years OR 3.64; CI: 2.08 – 6.36; $p < 0.01$) and hypertension (OR 1.54; CI: 1.01 – 2.34; $p < 0.05$) were independently associated with excessive PACs.

Conclusions: Excessive PACs are independently associated with cryptogenic stroke and may be a reproducible marker of atrial myopathy. Prospective studies assessing their utility in guiding stroke prevention strategies are warranted.

Keywords: Stroke; Atrial premature complexes; Cryptogenic stroke; Embolic stroke of undetermined source.

Strength and limitations of this study

- This study employed a case control design to compare atrial rhythm abnormalities during Holter monitoring in patients with ischaemic stroke and an aged match control group.
- This study analysed the association between premature atrial complexes and cryptogenic stroke subtype, after excluding atrial fibrillation.
- This study analysed the association between specific vascular risk factors and premature atrial complexes.
- This study is limited by a lack of prolonged continuous cardiac monitoring that may have led to an underestimation of the presence of atrial fibrillation.

Introduction

Approximately 100,000 strokes occur every year in the United Kingdom, with 1 in 4 survivors experiencing another stroke[1]. While 85% of all strokes are ischaemic in nature, 25% - 35% of these are labelled cryptogenic, as a clear cause is not identified.[2] Subclinical paroxysmal AF is postulated to be the cause for a significant proportion of these cryptogenic strokes.[3 4] However, despite prolonged rhythm monitoring, occult AF occur in only a small proportion of patients.[5 6]

With the recent equivocal results of randomised controlled anticoagulation trials in all-comer patients with embolic stroke of undetermined source, there has been a heightened focus in identifying reproducible markers of an atrial myopathy. Premature atrial complexes have been thought to be a benign phenomenon with a prevalence in the general population that ranges from 6% - 29%.[7] A limited number of studies have shown a significant association between excessive PACs and ischaemic stroke, suggesting their relevance as a marker of atrial myopathy.[8-10] However, these have not delineated whether excessive PACs confer an increased risk for the cryptogenic stroke subtype. In addition, no studies have assessed the pathophysiological basis of an increased PAC burden and whether individual vascular risk factors that promote stroke independently correlate with excessive PAC burden.[11] We sought to determine the association between excessive PACs and ischaemic stroke, including the cryptogenic stroke subtype and their relationship to conventional risk factors.

Methods

A multicentre case-control study was conducted among consecutive patients who presented with an ischaemic stroke or TIA between May 2011 and December 2015. Patients within the stroke/TIA group were identified through a prospectively maintained institutional stroke

database that covered three tertiary, university hospitals. Stroke subtypes were determined according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification[12].

Inclusion criteria were: 1) age ≥18 years; 2) adjudicated to have had an ischaemic stroke/TIA by the stroke service and 3) underwent 24-hour Holter monitoring following their index stroke/TIA. Exclusion criteria were: 1) history of atrial fibrillation, atrial flutter or a subsequent diagnosis of these arrhythmias on inpatient telemetry, Holter monitoring or during follow up; 2) underlying severe cardiomyopathy with an ejection fraction <35%; 3) previous coronary artery bypass grafting; 4) recent myocardial infarction or 5) severe chronic obstructive airways disease.

Eligible patients were compared to a group of age-matched controls, without prior history of stroke or AF and underwent outpatient Holter monitoring. The control group were composed of patients that underwent investigation of chest pain, syncope, pre-syncope and palpitations. The stroke/TIA and control groups were age matched with the same exclusion criteria applied.

Clinical assessment and outcome measures

All included patients adjudicated as having a stroke/TIA underwent investigation and treatment as per the national stroke guidelines recommendation for standard of care.[13] This included physical examination, blood measurements, 12 lead ECG, pulse oximetry, computed tomography of the brain (CTB), inpatient cardiac monitoring and vascular assessment. All patients underwent Holter monitoring with 24 hours of continuous rhythm capture utilising the SEER Light Holter Monitor (GE Healthcare, Milwaukee, USA). Data were analysed offline upon completion of the monitoring period by cardiac technicians and subsequently reviewed by a cardiac electrophysiologist blinded to the study hypothesis. Rhythm analysis

was conducted using MARS Ambulatory ECG Analysis System (GE Healthcare, Milwaukee, USA).

Baseline demographic and clinical data were collected from electronic health records along with vascular risk factors to allow for calculation of a CHA_2DS_2VASc score, medication use and to identify presentations with recurrent stroke or TIA. Based on prior literature, we defined excessive PAC burden as ≥ 200 PACs/24 hours and a long atrial run as ≥20 beats.[14 15]

Statistical methods

Demographic data, disease status and outcome measures are presented as proportions and summarised by descriptive statistics. Data were tested for normality and parametric or non-parametric tests applied as appropriate. Correlation trends were analysed using Spearman's rho for non-parametric data. A p-value <0.05 was deemed statistically significant and a 95% confidence interval (CI) is presented where applicable. Markers associated with stroke/TIA and excessive PACs were identified by univariate and multivariate logistical regression. Any variable with a p value <0.25 on univariate analysis was included in multivariate analyses. All statistical analysis was performed with SPSS Statistics 24.0 (IBM, USA).

Patient and public involvement

Public involvement was sought after the methods and outcome measures were identified. The protocol and study design were reviewed by human research ethics committee, 45% of whom were members of the public.

Ethics approval and data sharing

The research protocol was approved by the institutional Human Research Ethics Committee and written informed consent was not deemed necessary by the committee (approval number:

LR09/2016). The raw data will be made available by the corresponding author upon reasonable request.

Results

In total, 537 patients presented with a stroke/TIA during the study inclusion period and underwent Holter monitoring. Following exclusions, 461 patients with a stroke/TIA were compared against 251 age-matched patients that underwent Holter monitoring during the same time period (Figure 1). The median time to Holter monitoring following the stroke or TIA was 40 days.

Ischaemic stroke and PAC burden

Baseline characteristics stratified according to study groups are shown in Table 1. In both groups, the mean age was 70 years and the majority of patients were male. Stroke/TIA patients were significantly more likely to have comorbidities of hypertension, diabetes mellitus, dyslipidaemia, peripheral vascular disease and a prior history of smoking. There were 79 patients with a prior cerebrovascular event in the stroke/TIA group. On admission, there was significantly higher use of statins in the stroke/TIA cohort, however no difference was evident in the use of antiplatelet therapy or oral anticoagulants. The prevalence of excessive PACs were significantly higher in the stroke/TIA group (25.6% vs 14.7%, p = 0.001), however atrial runs of ≥20 beats were not significantly different (Table 1).

Multivariate analysis showed female sex, hypertension, history of smoking and excessive PACs were significantly associated with a stroke/TIA; excessive PACs conferred the highest risk for stroke/TIA with an odds ratio of 1.97(CI: 1.29 – 3.02) (Table 2). Analysis of PAC burden revealed a skewed distribution, median PACs/24 hours and longest atrial ectopic runs

were significantly higher in the stroke/TIA group. However, number of beats in runs of greater than 3 beats was not significantly different between the 2 groups.

Cryptogenic Stroke and PAC burden

One hundred and eighty-five patients with cryptogenic stroke, after excluding TIA, were compared with 251 patients in the control group. The mean age was not significantly different between the control and cryptogenic stroke group, 70.5 and 68.9 years respectively. Cryptogenic stroke was significantly associated with excessive PAC burden, female sex, hypertension, diabetes mellitus, smoking, obstructive sleep apnoea and a higher median CHA_2DS_2VASc score on univariate analysis. Excessive PACs (OR: 1.95; CI: 1.16 – 3.28), female sex (OR: 1.78; CI: 1.19 – 2.67) and hypertension (OR: 1.67; CI: 1.05 – 2.64) maintained significant and independent associations with cryptogenic stroke subtype on multivariate logistical regression (Table 2).

Vascular risk factors and PAC burden

Increasing CHA_2DS_2VASc score was associated with increasing median PACs in both groups (Figure 2). A CHA_2DS_2VASc score greater than 3 in the control group and 2 in the stroke/TIA group were associated with an excessive PAC burden. However, only a moderate to weak correlation was evident between increasing CHA_2DS_2VASc score and increasing PACs/24 hours in all patients ($r_s = 0.32$, $p<0.001$), control patients ($r_s = 0.24$, $p<0.001$) and stroke/TIA patients ($r_s = 0.32$, $p<0.001$) respectively.

In all patients, age, hypertension, diabetes mellitus and peripheral vascular disease were significant univariate predictors of excessive PACs. However only age and hypertension remained significant independent predictors of excessive PACs on regression analysis, with age ≥75 being the strongest predictor for excessive PACs (Table 3).

Discussion

In this study, we compared the differences in PAC burden between patients with a stroke/TIA and an age-matched control population, after excluding AF. Excessive PAC burden was significantly more common in the stroke/TIA group. An important new finding in our study was that excessive PACs demonstrated the strongest independent association for the cryptogenic stroke subtype, after adjusting for conventional risk factors (OR: 1.95; CI: 1.16 – 3.28). There was a stepwise rise in PAC burden with increasing number of vascular risk factors; age and hypertension were independent risk factors associated with excessive PACs.

Investigators have previously demonstrated a significantly higher PAC burden in patients that develop incident AF and ischemic stroke.[14 16] Our work builds on existing work from Engstrom et al. that demonstrated a high PAC burden conferred a 1.9 times higher risk for ischaemic stroke. [15] The current study similarly showed 1.97 times rise in odds for ischaemic stroke. Prior studies have also shown an association between runs of PACs and ischaemic stroke in patients without documented AF[17]. The Copenhagen Holter study, a cohort study that analysed the risk for stroke with an elevated PAC burden, defined excessive supraventricular ectopic activity as a composite of either >30 PACs/hour or a run of >20 PACs, and found a positive correlation with increased stroke and death.[9 18] In contrast, we did not show a significant difference in PAC runs >20 beats between the two groups.[19] This apparent discrepancy is likely due to a lack of standardised definitions for excessive PACs and treating atrial premature runs ≥20 beats as a standalone variable in the current study, instead of a composite measure.

The present study specifically analysed the association between excessive PACs and cryptogenic stroke subtype. It demonstrated an independent association between cryptogenic stroke subtype and excessive PACs with an odds ratio of 1.95. This is an important finding

and lends further support to the hypothesis, that excessive PACs may be the manifestation or marker of underlying atrial myopathy, that confers an increased risk for cryptogenic stroke. Further, similar to AF, the risk for ischaemic stroke in the presence of an excessive PAC burden appear to be modulated by vascular risk factors. A CHA_2DS_2VASc score of 2 conferred a similar risk for ischaemic stroke in patients with excessive PACs, as with AF.[9]

An increasing CHA_2DS_2VASc score was significantly associated with increasing median PAC burden in both the control and stroke/TIA group. However, despite the positive correlation between CHA_2DS_2VASc score and premature atrial complexes, the strength of the correlation itself remained weak. This was suggestive of differential effects of the various components of CHA_2DS_2VASc score in contributing to a high PAC burden.

To our knowledge, this is the first study to evaluate the independent association between specific vascular risk factors and an excessive PAC burden. The independent contribution of the various risk markers that make up CHA_2DS_2VASc score have not been assessed previously.[9 18 20] Delineation of these specific risk factors that contribute to excessive PACs provides insights into the potential pathophysiological basis for excessive PAC.

Increasing age and hypertension were independently and significantly associated with excessive PACs in the present study. This is consistent with electro-anatomical studies that demonstrated slower conduction velocities and both global and regional reduction in atrial voltages with increasing age and hypertension [21 22]. Such areas corresponded to delayed enhancement on magnetic resonance imaging and histological fibrosis [23,24]. Thrombogenesis associated with this underlying atrial remodelling may help explain a significant proportion of strokes currently classified as cryptogenic. Both advancing age and hypertension are also associated with small and large vessel stroke subtypes. In addition to atrial remodelling, it is likely that vascular risk factors promote thrombogenesis and ischaemic stroke through

multiple pathways including arterial endothelial dysfunction, and atherosclerosis with localised plaque rupture.[25]

Our report of the independent association between excessive PACs and cryptogenic stroke further implicates a risk factor driven atrial substrate abnormality in its pathogenesis. These findings are clinically relevant as the results of a recently concluded large multicentre randomised controlled trial failed to demonstrate a benefit for oral anticoagulation in an unselected population with embolic stroke of undetermined source[26]. This highlights the heterogeneity of the pathophysiological mechanisms that lead to cryptogenic stroke. There is an unmet clinical need to develop risk markers that identify the subset of patients with cryptogenic stroke that occur as a result of cardio-embolism.

Studies have previously described serological and echocardiographic markers associated with the recurrence of cryptogenic stroke[27]. Similarly, excessive PACs are readily assessed and may serve as a novel and reproducible marker to identify patients at high risk for the cryptogenic stroke. It is unclear if excessive PACs directly promote thrombogenesis or if they are simply a marker of adverse atrial remodelling that leads to thrombogenesis and stroke. Regardless, the risk conferred by an elevated CHA_2DS_2VASc score in conjunction with excessive PACs for ischaemic stroke remains significant[9].

This study has limitations. The absence of prolonged monitoring with devices such as implantable loop recorders could have led to an underestimation of incident AF. However, we excluded all patients with a diagnosis of AF over 1.9 years of mean follow up. A higher number of cryptogenic stroke patients were present in our population than previously reported. This is likely due to referral bias, as patients were only included if they underwent Holter monitoring. However, the higher prevalence of cryptogenic stroke improves the strength of our findings in this specific subset of stroke patients.

Conclusions

Excessive PACs are significantly associated with cryptogenic stroke. Vascular risk factors, increasing age and hypertension, were independently associated with excessive PACs. The utility of novel and reproducible cardiac markers to guide preventative strategies in cryptogenic stroke warrant further evaluation.

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Competing interests

None.

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Figure Legend

Figure 1.

Patient selection: inclusions and exclusions.

AF = atrial fibrillation; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; TIA = transient ischaemic attack;

Figure 2.

Median PACs by CHA_2DS_2VASc score for control and stroke/TIA groups.

CHA_2DS_2VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;

Tables

Table 1. Baseline characteristics of study participants.

Characteristics	Control n = 251 n (%)	Stroke/TIA n = 461 n (%)	P value
Age, y (SD)	70.5 (11.6)	69.8 (12.5)	0.45
Sex, Female	88 (35.1)	195 (42.3)	0.06
Stroke Subtypes			
Large vessel atherosclerosis	-	82 (17.8)	-
Small vessel occlusion	-	86 (18.7)	-
Cryptogenic	-	291(63.1)	-
Stroke of other determined aetiology	-	2 (0.4)	-
Vascular Risk Factors			
Hypertension	130 (51.8)	294 (63.8)	0.01
Dyslipidaemia	91 (36.3)	209 (45.3)	0.02
Diabetes Mellitus	38 (15.1)	113 (24.5)	0.01
Any Smoking	44 (17.5)	118 (25.6)	0.01
Previous Stroke/TIA	0 (0)	79 (17.1)	<0.001
Myocardial Infarction	40 (15.9)	82 (17.8)	0.53
Peripheral Vascular Disease	5 (2.0)	26 (5.6)	0.02
Sleep Apnoea	14 (5.6)	13 (2.8)	0.07

History of Heart Failure	11 (4.4)	25 (5.4)	0.55
CHA_2DS_2VASc score median (IQR)	2 (1 – 3)	5 (4 – 5)	<0.001
Medications			
Warfarin	4 (1.6)	4 (0.9)	0.38
Direct Oral Anticoagulant	4 (1.6)	1 (0.2)	-
Antiplatelet Therapy	81 (32.3)	165 (35.8)	0.35
Beta blocker	48 (19.1)	82 (17.8)	0.66
Ace Inhibitor	105 (41.8)	224 (48.6)	0.08
Statin	79 (31.5)	187 (40.6)	0.02
Premature Atrial Complexes			
PACs/24 hours median (IQR)	37 (13 - 115)	62 (20 - 208)	<0.01
Longest atrial run median (IQR)	3 (0 – 7)	3 (0 – 8)	<0.01
Atrial runs >3 beats median (IQR)	1 (0 – 2)	1 (0 – 4)	0.07
≥ 200 PACs/24 hours	37 (14.7)	118 (25.6)	<0.001
≥20 beats in runs	13 (5.2)	27 (5.9)	0.71

SD = standard deviation; TIA = transient ischaemic attack; IQR = interquartile range; PAC = premature atrial complexes; The data is presented as n (%), unless otherwise stated.

Table 2. Multivariate analysis: risk factors associated with Stroke/TIA and cryptogenic stroke.

Characteristic	**Stroke/TIA**		**Cryptogenic Stroke**	
	Odds Ratio (95% CI)	*P value*	*Odds Ratio (95% CI)*	*P value*
Female	1.51 (1.09 - 2.11)	<0.05	1.78 (1.19 – 2.67)	<0.01
Hypertension	1.53 (1.07 - 2.17)	<0.05	1.67 (1.05 – 2.64)	<0.05
Smoking	1.58 (1.06 - 2.36)	<0.05	1.55 (0.95 – 2.53)	0.08
≥200 PACs	1.97 (1.29 - 3.02)	<0.01	1.95 (1.16 – 3.28)	<0.05

CI = confidence interval; PAC = premature atrial complexes.

Variables adjusted in the multivariate model: Age, sex, excessive premature atrial complexes, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease and sleep apnoea

Table 3. Multivariate analysis: Vascular risk factors predictive of excessive PACs in all patients.

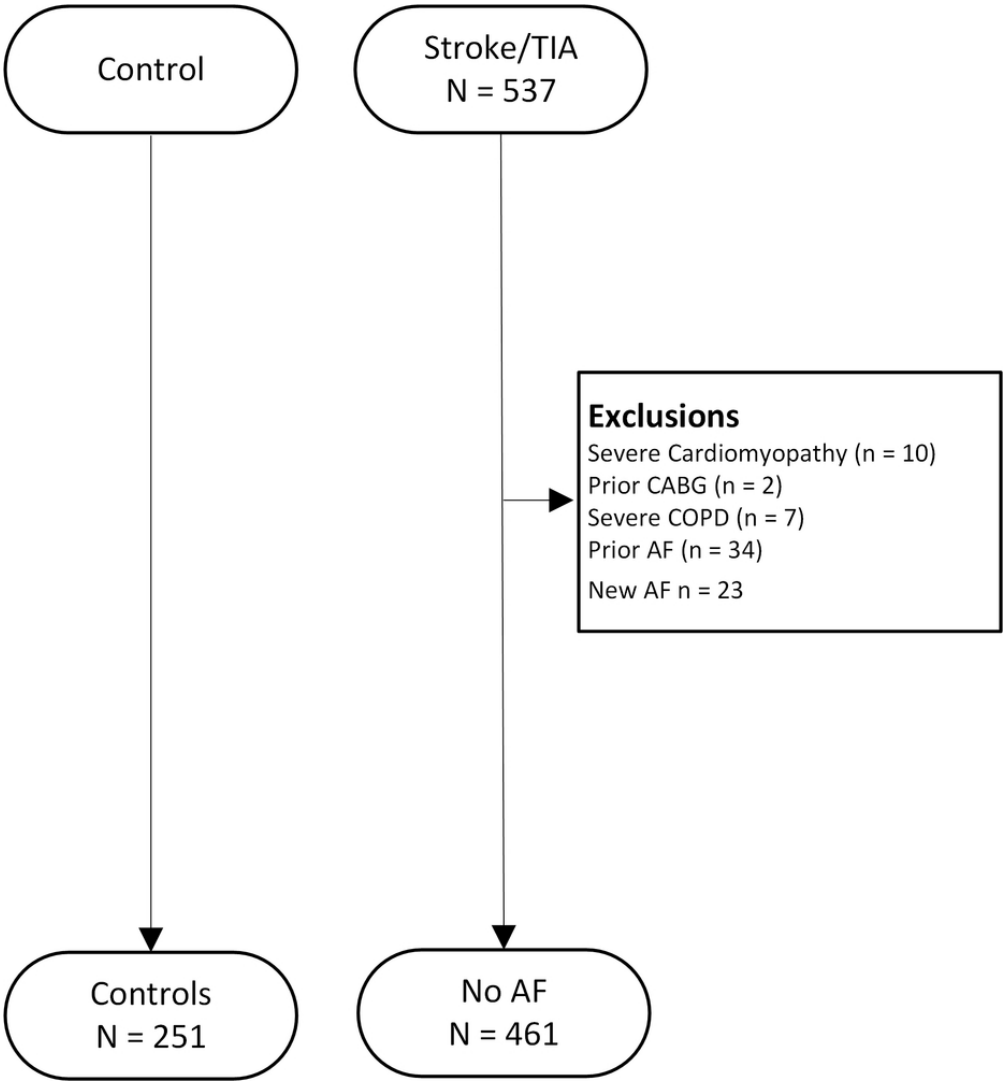
Risk factor	Odds Ratio (95% CI)	P value
Age		
65 – 74	2.52 (1.42 - 4.45)	<0.01
≥ 75	3.64 (2.08 - 6.36)	<0.01
Hypertension	1.54 (1.01 - 2.34)	<0.05
Diabetes Mellitus	1.41 (0.91 - 2.20)	0.13

CI = confidence interval.

Variables adjusted in the multivariate model: Age: <65, 65-74, ≥75, gender, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease, sleep apnoea.

Author Statement

JS led and designed the study, collated and analysed data and wrote the manuscript. AK collated and analysed the data and revised the manuscript. HD contributed to study design and revised the manuscript. JK contributed to study design and revised the manuscript. KR collated and analysed the data and revised the manuscript. MT collated and analysed the data and revised the manuscript. MS analysed the data and revised the manuscript. LR contributed to study design and revised the manuscript. JC contributed to study design and revised the manuscript. MW contributed to study design and revised the manuscript. TF collated and analysed the data and revised the manuscript. AT supervised and designed the study revised the manuscript. All authors read and approved the final manuscript.



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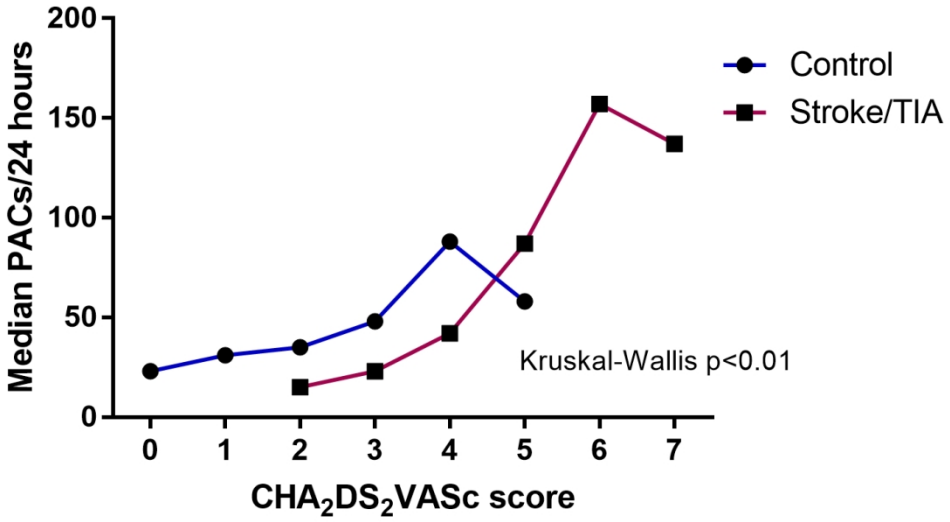
AF = atrial fibrillation; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; TIA = transient ischaemic attack;

78x84mm (300 x 300 DPI)



Median PACs by CHA_2DS_2VASc score for control and stroke/TIA groups.
CHA_2DS_2VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;

127x74mm (300 x 300 DPI)

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The association between excessive premature atrial complexes and cryptogenic stroke: results of a case – control study.

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Abstract

Objective: Recent anticoagulation trials in all-comer cryptogenic stroke patients have yielded equivocal results, reinvigorating the focus on identifying reproducible markers of an atrial myopathy. We investigated the role of excessive premature atrial complexes (PACs) in ischaemic stroke, including cryptogenic stroke and its association with vascular risk factors.

Methods and Results: A case control study was conducted utilising a multi-centre institutional stroke database to compare 461 patients with an ischaemic stroke or transient ischaemic attack (TIA) with a control group consisting of age matched patients without prior history of ischaemic stroke/TIA. All patients underwent 24-hour Holter monitoring during the study period and atrial fibrillation was excluded.

An excessive PAC burden, defined as ≥ 200 PACs/24 hours, was present in 25.6% and 14.7% ($p < 0.01$), of stroke/TIA and control patients respectively. On multivariate regression, excessive PACs (OR 1.97; 95% confidence interval (CI): 1.29 – 3.02; $p < 0.01$), smoking (OR 1.58; CI: 1.06 – 2.36; $p < 0.05$) and hypertension (OR 1.53; CI: 1.07 – 2.17; $p < 0.05$) were independently associated with ischaemic stroke/TIA. Excessive PACs remained the strongest independent risk factor for the cryptogenic stroke subtype (OR 1.95; CI: 1.16 – 3.28; $p<0.05$). Vascular risk factors that promote atrial remodelling, increasing age (≥ 75 years OR 3.64; CI: 2.08 – 6.36; $p < 0.01$) and hypertension (OR 1.54; CI: 1.01 – 2.34; $p < 0.05$) were independently associated with excessive PACs.

Conclusions: Excessive PACs are independently associated with cryptogenic stroke and may be a reproducible marker of atrial myopathy. Prospective studies assessing their utility in guiding stroke prevention strategies may be warranted.

Keywords: Stroke; Atrial premature complexes; Cryptogenic stroke; Embolic stroke of undetermined source.

Strength and limitations of this study

- This study employed a case control design to compare the burden of premature atrial complexes in ischaemic stroke, including the cryptogenic stroke subtype and an age matched control group.
- All patients underwent 24 hours of ambulatory Holter monitoring to exclude atrial fibrillation and to document the burden of premature atrial complexes.
- This study describes the association between vascular risk factors and premature atrial complexes and used multivariate analysis to reduce confounding.
- The study is limited by its cross-sectional, case control design and causality cannot be inferred from the associations.

Introduction

Approximately 100,000 strokes occur every year in the United Kingdom, with 1 in 4 survivors experiencing another stroke[1]. While 87% of all strokes are ischaemic in nature, 25% - 35% of these are labelled cryptogenic, as a clear cause is not identified.[2 3] Subclinical paroxysmal AF is postulated to be the cause for a significant proportion of these cryptogenic strokes.[4 5] However, despite prolonged rhythm monitoring, occult AF occur in only a small proportion of patients.[6 7]

With the recent equivocal results of randomised controlled anticoagulation trials in all-comer patients with embolic stroke of undetermined source, there has been a heightened focus in identifying reproducible markers of an atrial myopathy[8]. Premature atrial complexes have been thought to be a benign phenomenon with a prevalence in the general population that ranges from 6% - 29%.[9] A limited number of studies have shown a significant association between excessive PACs and ischaemic stroke, suggesting their relevance as a marker of atrial myopathy.[10-14] While another study has shown an elevated risk for recurrent stroke in patients with excessive PACs, following a cryptogenic stroke[15]. However, these have not delineated whether baseline excessive PACs confer an increased risk for the cryptogenic stroke subtype. In addition, it is unclear whether vascular risk factors that promote stroke, independently and uniformly lead to atrial remodelling that result in excessive PAC burden. We sought to determine the association between excessive PACs and ischaemic stroke, including the cryptogenic stroke subtype and their relationship to conventional risk factors.

Methods

A multicentre case-control study was conducted among consecutive patients who presented with an ischaemic stroke or TIA between May 2011 and December 2015. Patients within the stroke/TIA group were identified through a prospectively maintained institutional stroke

database that covered three tertiary, university hospitals. Stroke subtypes were determined according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification[16].

Inclusion criteria were: 1) age ≥18 years; 2) adjudicated to have had an ischaemic stroke/TIA by the stroke service and 3) underwent 24-hour Holter monitoring following their index stroke/TIA. Exclusion criteria were: 1) history of atrial fibrillation, atrial flutter or a subsequent diagnosis of these arrhythmias on inpatient telemetry, Holter monitoring or during follow up; 2) underlying severe cardiomyopathy with an ejection fraction <35%; 3) previous coronary artery bypass grafting; 4) recent myocardial infarction or 5) severe chronic obstructive airways disease.

Eligible patients were compared to a group of age-matched controls, without prior history of stroke or AF and underwent outpatient Holter monitoring. The control group were composed of patients that underwent investigation of chest pain, syncope, pre-syncope and palpitations. The stroke/TIA and control groups were age matched with the same exclusion criteria applied.

Clinical assessment and outcome measures

All included patients adjudicated as having a stroke/TIA underwent investigation and treatment as per the national stroke guidelines recommendation for standard of care.[17] This included physical examination, blood measurements, 12 lead ECG, pulse oximetry, computed tomography of the brain (CTB), inpatient cardiac monitoring and vascular assessment. All patients underwent Holter monitoring with 24 hours of continuous rhythm capture utilising the SEER Light Holter Monitor (GE Healthcare, Milwaukee, USA). Data were analysed offline upon completion of the monitoring period by cardiac technicians and subsequently reviewed by a cardiac electrophysiologist blinded to the study hypothesis. Rhythm analysis

was conducted using MARS Ambulatory ECG Analysis System (GE Healthcare, Milwaukee, USA).

Baseline demographic and clinical data were collected from electronic health records along with vascular risk factors to allow for calculation of a CHA_2DS_2VASc score, medication use and to identify presentations with recurrent stroke or TIA. Based on prior literature, we defined excessive PAC burden as ≥ 200 PACs/24 hours and a long atrial run as ≥20 beats.[10 18 19]

Statistical methods

Demographic data, disease status and outcome measures are presented as proportions and summarised by descriptive statistics. Data were tested for normality and parametric or non-parametric tests applied as appropriate. Correlation trends were analysed using Spearman's rho for non-parametric data. A p-value <0.05 was deemed statistically significant and a 95% confidence interval (CI) is presented where applicable. Markers associated with stroke/TIA and excessive PACs were identified by univariate and multivariate logistical regression. Any variable with a p value <0.25 on univariate analysis was included in multivariate analyses. All statistical analysis was performed with SPSS Statistics 24.0 (IBM, USA).

Patient and public involvement

Public involvement was sought after the methods and outcome measures were identified. The protocol and study design were reviewed by human research ethics committee, 45% of whom were members of the public.

Ethics approval and data sharing

The research protocol was approved by the institutional Human Research Ethics Committee and written informed consent was not deemed necessary by the committee (approval number:

LR09/2016). The raw data will be made available by the corresponding author upon reasonable request.

Results

In total, 537 patients presented with a stroke/TIA during the study inclusion period and underwent Holter monitoring. 23 out of 537 (4.2%) patients had AF identified on Holter monitoring and were excluded. Four patients with AF had an excessive PAC burden (17%).

Following exclusions, 461 patients with a stroke/TIA were compared against 251 age-matched patients that underwent Holter monitoring during the same time period (Figure 1).

The median time to Holter monitoring following the stroke or TIA was 40 days.

Ischaemic stroke and PAC burden

Baseline characteristics stratified according to study groups are shown in Table 1. In both groups, the mean age was 70 years and the majority of patients were male. Stroke/TIA patients were significantly more likely to have comorbidities of hypertension, diabetes mellitus, dyslipidaemia, peripheral vascular disease and a prior history of smoking. There were 79 patients with a prior cerebrovascular event in the stroke/TIA group. On admission, there was significantly higher use of statins in the stroke/TIA cohort, however no difference was evident in the use of antiplatelet therapy or oral anticoagulants. The prevalence of excessive PACs were significantly higher in the stroke/TIA group (25.6% vs 14.7%, p = 0.001), however atrial runs of ≥20 beats were not significantly different (Table 1).

Multivariate analysis showed female sex, hypertension, history of smoking and excessive PACs were significantly associated with a stroke/TIA. Excessive PACs conferred the highest risk for stroke/TIA with an odds ratio of 1.97(CI: 1.29 – 3.02), but the difference was not significant when compared with other risk factors associated with stroke/TIA (Table 2).

Multivariate analysis with various definitions of excessive PACs based on prior literature yielded similar results, with a significant association between Excessive PACs and stroke/TIA (Supplementary file).

Analysis of PAC burden revealed a skewed distribution, median PACs/24 hours and longest atrial ectopic runs were significantly higher in the stroke/TIA group. However, number of beats in runs of greater than 3 beats was not significantly different between the 2 groups.

Cryptogenic Stroke and PAC burden

One hundred and eighty-five patients with cryptogenic stroke, after excluding TIA, were compared with 251 patients in the control group. The mean age was not significantly different between the control and cryptogenic stroke group, 70.5 and 68.9 years respectively. Cryptogenic stroke was significantly associated with excessive PAC burden, female sex, hypertension, diabetes mellitus, smoking, obstructive sleep apnoea and a higher median CHA_2DS_2VASc score on univariate analysis. Excessive PACs (OR: 1.95; CI: 1.16 – 3.28), female sex (OR: 1.78; CI: 1.19 – 2.67) and hypertension (OR: 1.67; CI: 1.05 – 2.64) maintained significant and independent associations with cryptogenic stroke subtype on multivariate logistical regression (Table 2).

Vascular risk factors and PAC burden

Increasing CHA_2DS_2VASc score was associated with increasing median PACs in both groups (Figure 2). A CHA_2DS_2VASc score greater than 3 in the control group and 2 in the stroke/TIA group were associated with an excessive PAC burden. However, only a moderate to weak correlation was evident between increasing CHA_2DS_2VASc score and increasing PACs/24 hours in all patients ($r_s = 0.32$, $p<0.001$), control patients ($r_s = 0.24$, $p<0.001$) and stroke/TIA patients ($r_s = 0.32$, $p<0.001$) respectively.

In all patients, age, hypertension, diabetes mellitus and peripheral vascular disease were significant univariate predictors of excessive PACs. However only age and hypertension remained independently associated with excessive PACs on regression analysis, with age ≥75 being the strongest marker associated with excessive PACs (Table 3).

Discussion

In this study, we compared the differences in PAC burden between patients with a stroke/TIA and an age-matched control population, after excluding AF. Excessive PAC burden was significantly more common in the stroke/TIA group. An important new finding in our study was that excessive PACs demonstrated an independent association for the cryptogenic stroke subtype, after adjusting for conventional risk factors (OR: 1.95; CI: 1.16 – 3.28). There was a stepwise rise in PAC burden with increasing number of vascular risk factors; age and hypertension were independent risk factors associated with excessive PACs.

Investigators have previously demonstrated a significantly higher PAC burden in patients that develop incident AF and ischemic stroke.[18 20] Existing longitudinal studies from Engstrom et al. that demonstrated a high PAC burden conferred a 1.9 times higher risk for ischaemic stroke. [19] Despite the differences in methodology, the current study showed 1.97 times rise in odds for ischaemic stroke. Prior studies have also shown an association between runs of PACs and ischaemic stroke in patients without documented AF[21]. The Copenhagen Holter study, a cohort study that analysed the risk for stroke with an elevated PAC burden, defined excessive supraventricular ectopic activity as a composite of either >30 PACs/hour or a run of >20 PACs, and found a positive correlation with increased stroke and death.[10 13] In contrast, we did not show a significant difference in PAC runs >20 beats between the two groups.[15] This apparent discrepancy is likely due to a lack of standardised definitions for

excessive PACs and treating atrial premature runs ≥20 beats as a standalone variable in the current study, instead of a composite measure.

The present study specifically analysed the association between excessive PACs and cryptogenic stroke subtype. It demonstrated an independent association between cryptogenic stroke subtype and excessive PACs with an odds ratio of 1.95. This is an important finding and lends further support to the hypothesis, that excessive PACs may be the manifestation or marker of underlying atrial myopathy, that confers an increased risk for cryptogenic stroke. Further, similar to AF, the risk for ischaemic stroke in the presence of an excessive PAC burden appear to be modulated by vascular risk factors. A CHA_2DS_2VASc score of 2 conferred a similar risk for ischaemic stroke in patients with excessive PACs, as with AF.[10]

An increasing CHA_2DS_2VASc score was significantly associated with increasing median PAC burden in both the control and stroke/TIA group. However, despite the positive correlation between CHA_2DS_2VASc score and premature atrial complexes, the strength of the correlation itself remained weak. This was suggestive of differential effects of the various components of CHA_2DS_2VASc score in contributing to a high PAC burden. The independent contribution of the various risk markers that make up CHA_2DS_2VASc score have not been assessed previously.[10 13 22] Delineation of these specific risk factors that contribute to excessive PACs provides insights into the potential pathophysiological basis for excessive PAC.

Increasing age and hypertension were independently and significantly associated with excessive PACs in the present study. This is consistent with electro-anatomical studies that demonstrated slower conduction velocities and both global and regional reduction in atrial voltages with increasing age and hypertension [23 24]. Such areas corresponded to delayed enhancement on magnetic resonance imaging and histological fibrosis [25,26]. Thrombogenesis

associated with this underlying atrial remodelling may help explain a significant proportion of strokes currently classified as cryptogenic. Both advancing age and hypertension are also associated with small and large vessel stroke subtypes. In addition to atrial remodelling, it is likely that vascular risk factors promote thrombogenesis and ischaemic stroke through multiple pathways including arterial endothelial dysfunction, and atherosclerosis with localised plaque rupture.[27]

Our report of the independent association between excessive PACs and cryptogenic stroke further implicates a risk factor driven atrial substrate abnormality in its pathogenesis. These findings are clinically relevant as the results of a recently concluded large multicentre randomised controlled trial failed to demonstrate a benefit for oral anticoagulation in an unselected population with embolic stroke of undetermined source[8]. This highlights the heterogeneity of the pathophysiological mechanisms that lead to cryptogenic stroke. There is an unmet clinical need to develop risk markers that identify the subset of patients with cryptogenic stroke that occur as a result of cardio-embolism.

Studies have previously described serological and echocardiographic markers associated with the recurrence of cryptogenic stroke[28]. Similarly, excessive PACs are readily assessed and may serve as a novel and reproducible marker to identify patients at high risk for the cryptogenic stroke. It is unclear if excessive PACs directly promote thrombogenesis or if they are simply a marker of adverse atrial remodelling that leads to thrombogenesis and stroke. Regardless, the risk conferred by an elevated CHA_2DS_2VASc score in conjunction with excessive PACs for ischaemic stroke remains significant[10].

This study has limitations. The absence of prolonged monitoring with devices such as implantable loop recorders could have led to an underestimation of incident AF. However, we excluded all patients with a diagnosis of AF over 1.9 years of mean follow up. A higher

number of cryptogenic stroke patients were present in our population than previously reported. This is likely due to referral bias, as patients were only included if they underwent Holter monitoring. All patients included in the study had guideline-based referral for Holter monitoring. However, as Holter monitoring was an inclusion criterion, we do not have data on patient who may have received their Holter monitoring at an external institution. However, the higher prevalence of cryptogenic stroke improves the strength of our findings in this specific subset of stroke patients. The time to Holter monitoring following the stroke, based on routine institutional clinical waiting periods, could have introduced unintended variables such as neurologically mediated cardiac modelling with resultant excessive PACs and reverse causality. The higher burden of PACs was noted in a highly selective patient cohort with ischaemic stroke and a high burden of vascular risk factors. Despite the use of multivariate regression analysis, unrecognised confounders cannot be excluded in a cross-sectional case control study, therefore these findings should not be extrapolated to other patient cohorts.

Conclusions

Excessive PACs are significantly associated with cryptogenic stroke. Vascular risk factors, increasing age and hypertension, were independently associated with excessive PACs. The utility of novel and reproducible cardiac markers to guide preventative strategies in cryptogenic stroke warrant further evaluation.

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Competing interests

None.

Data Sharing

Data can be made available by contacting the corresponding author.

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Figure Legend

Figure 1.

Patient selection: inclusions and exclusions.

AF = atrial fibrillation; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; TIA = transient ischaemic attack;

Figure 2.

Median PACs by CHA_2DS_2VASc score for control and stroke/TIA groups.

CHA_2DS_2VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;

Tables

Table 1. Baseline characteristics of study participants.

Characteristics	**Control** **n = 251** **n (%)**	**Stroke/TIA** **n = 461** **n (%)**	**P value**
Age, y (SD)	70.5 (11.6)	69.8 (12.5)	0.45
Sex, Female	88 (35.1)	195 (42.3)	0.06
Stroke Subtypes			
Large vessel atherosclerosis	-	82 (17.8)	-
Small vessel occlusion	-	86 (18.7)	-
Cryptogenic	-	291(63.1)	-
Stroke of other determined aetiology	-	2 (0.4)	-
Vascular Risk Factors			
Hypertension	130 (51.8)	294 (63.8)	0.01
Dyslipidaemia	91 (36.3)	209 (45.3)	0.02
Diabetes Mellitus	38 (15.1)	113 (24.5)	0.01

Any Smoking	44 (17.5)	118 (25.6)	0.01
Previous Stroke/TIA	0 (0)	79 (17.1)	<0.001
Myocardial Infarction	40 (15.9)	82 (17.8)	0.53
Peripheral Vascular Disease	5 (2.0)	26 (5.6)	0.02
Sleep Apnoea	14 (5.6)	13 (2.8)	0.07
History of Heart Failure	11 (4.4)	25 (5.4)	0.55
CHA_2DS_2VASc score median (IQR)	2 (1 – 3)	5 (4 – 5)	<0.001
Medications			
Warfarin	4 (1.6)	4 (0.9)	0.38
Direct Oral Anticoagulant	4 (1.6)	1 (0.2)	-
Antiplatelet Therapy	81 (32.3)	165 (35.8)	0.35
Beta blocker	48 (19.1)	82 (17.8)	0.66
Ace Inhibitor	105 (41.8)	224 (48.6)	0.08
Statin	79 (31.5)	187 (40.6)	0.02
Premature Atrial Complexes			
PACs/24 hours median (IQR)	37 (13 - 115)	62 (20 - 208)	<0.01
Longest atrial run median (IQR)	3 (0 – 7)	3 (0 – 8)	<0.01
Atrial runs >3 beats median (IQR)	1 (0 – 2)	1 (0 – 4)	0.07

≥ 200 PACs/24 hours	37 (14.7)	118 (25.6)	<0.001
≥20 beats in runs	13 (5.2)	27 (5.9)	0.71

SD = standard deviation; TIA = transient ischaemic attack; IQR = interquartile range; PAC = premature atrial complexes; The data is presented as n (%), unless otherwise stated.

Table 2. Multivariate analysis: risk factors associated with Stroke/TIA and cryptogenic stroke.

Characteristic	**Stroke/TIA**		**Cryptogenic Stroke**	
	Odds Ratio (95% CI)	*P value*	*Odds Ratio (95% CI)*	*P value*
Female	1.51 (1.09 - 2.11)	<0.05	1.78 (1.19 – 2.67)	<0.01
Hypertension	1.53 (1.07 - 2.17)	<0.05	1.67 (1.05 – 2.64)	<0.05
Smoking	1.58 (1.06 - 2.36)	<0.05	1.55 (0.95 – 2.53)	0.08
≥200 PACs	1.97 (1.29 - 3.02)	<0.01	1.95 (1.16 – 3.28)	<0.05

CI = confidence interval; PAC = premature atrial complexes.

Variables adjusted in the multivariate model: Age, sex, excessive premature atrial complexes, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease and sleep apnoea

Table 3. Multivariate analysis: Vascular risk factors associated with excessive PACs in all patients.

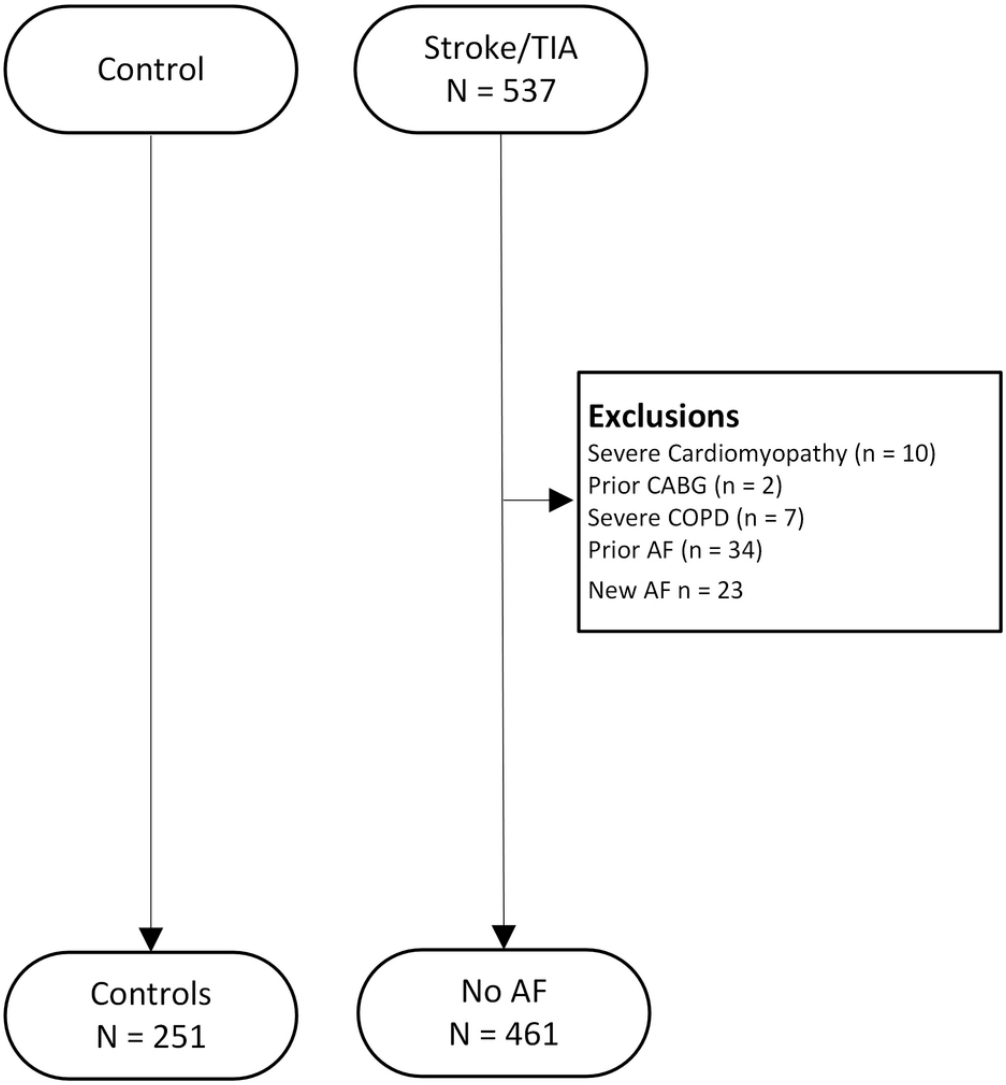
Risk factor	Odds Ratio (95% CI)	P value
Age		
65 – 74	2.52 (1.42 - 4.45)	<0.01
≥ 75	3.64 (2.08 - 6.36)	<0.01
Hypertension	1.54 (1.01 - 2.34)	<0.05
Diabetes Mellitus	1.41 (0.91 - 2.20)	0.13

CI = confidence interval.

Variables adjusted in the multivariate model: Age: <65, 65-74, ≥75, gender, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease, sleep apnoea.

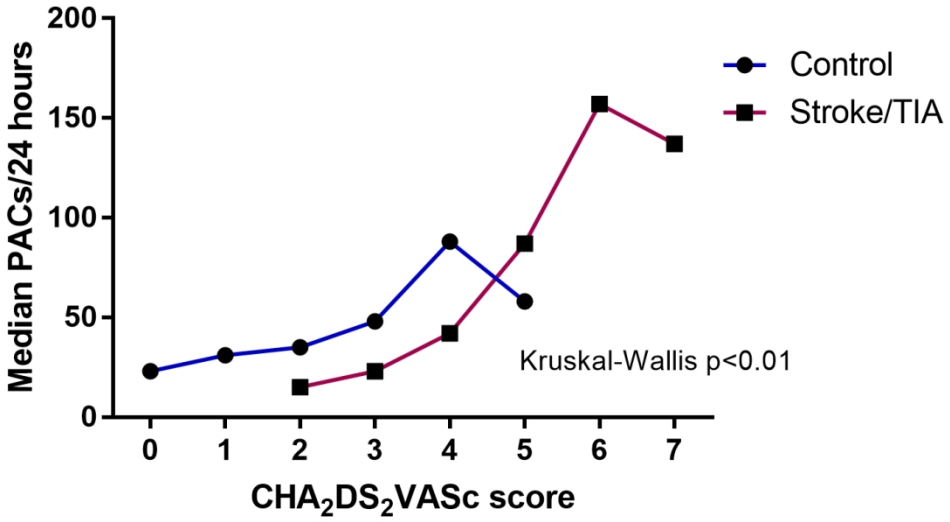
Author Statement

JS led and designed the study, collated and analysed data and wrote the manuscript. AK collated and analysed the data and revised the manuscript. HD contributed to study design and revised the manuscript. JK contributed to study design and revised the manuscript. KR collated and analysed the data and revised the manuscript. MT collated and analysed the data and revised the manuscript. MS analysed the data and revised the manuscript. LR contributed to study design and revised the manuscript. JC contributed to study design and revised the manuscript. MW contributed to study design and revised the manuscript. TF collated and analysed the data and revised the manuscript. AT supervised and designed the study revised the manuscript. All authors read and approved the final manuscript.



Patient selection: inclusions and exclusions.
AF = atrial fibrillation; CABG = coronary artery bypass grafting; COPD = chronic obstructive pulmonary disease; TIA = transient ischaemic attack;

78x84mm (300 x 300 DPI)



Median PACs by CHA_2DS_2VASc score for control and stroke/TIA groups.
CHA_2DS_2VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;

127x74mm (300 x 300 DPI)

Supplemental Data

Table 1. Multivariate analysis: risk factors associated with Stroke/TIA; Excessive PACs defined as >100 PACs/24 hours

Characteristic	**Stroke/TIA**	
	Odds Ratio (95% CI)	*P value*
Female	1.54 (1.10 - 2.14)	<0.05
Hypertension	1.46 (1.04 - 2.07)	<0.05
Smoking	1.57 (1.06 - 2.35)	<0.05
Excessive PACs	1.57 (1.10 - 2.25)	<0.05

CI = confidence interval; PAC = premature atrial complexes.

Variables adjusted in the multivariate model: Age, sex, excessive premature atrial complexes, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease and sleep apnoea

Table 2. Multivariate analysis: risk factors associated with Stroke/TIA; Excessive PACs defined as >700 PACs/24 hours or an atrial run >20 beats

Characteristic	**Stroke/TIA**	
	Odds Ratio (95% CI)	*P value*
Female	1.63 (1.16 - 2.27)	<0.01
Hypertension	1.45 (1.03 - 2.05)	<0.05
Smoking	1.59 (1.06 - 2.37)	<0.05
Excessive PACs	3.22 (1.78 - 5.83)	<0.01

CI = confidence interval; PAC = premature atrial complexes.

Variables adjusted in the multivariate model: Age, sex, excessive premature atrial complexes, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease and sleep apnoea