

BMJ Open

BMJ Open is committed to open peer review. As part of this commitment we make the peer review history of every article we publish publicly available.

When an article is published we post the peer reviewers' comments and the authors' responses online. We also post the versions of the paper that were used during peer review. These are the versions that the peer review comments apply to.

The versions of the paper that follow are the versions that were submitted during the peer review process. They are not the versions of record or the final published versions. They should not be cited or distributed as the published version of this manuscript.

BMJ Open is an open access journal and the full, final, typeset and author-corrected version of record of the manuscript is available on our site with no access controls, subscription charges or pay-per-view fees (<http://bmjopen.bmj.com>).

If you have any questions on BMJ Open's open peer review process please email info.bmjopen@bmj.com

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-029164
Article Type:	Research
Date Submitted by the Author:	15-Jan-2019
Complete List of Authors:	Sajeev, Jithin; Monash University Eastern Health Clinical School, ; Eastern Health, Department of Cardiology Koshy, Anoop; Monash University Eastern Health Clinical School; Monash University Eastern Health Clinical School Dewey, Helen; Eastern Health, Neurosciences; Monash University Eastern Health Clinical School Kalman, Jonathan; Royal Melbourne Hospital, Rajakariar, Kevin; Eastern Health, Department of Cardiology Tan, Mae; Eastern Health, Department of Cardiology Street, Maryann; Deakin University, School of Nursing and Midwifery; Eastern Health, Eastern Health - Deakin University Nursing and Midwifery research Centre Roberts, Louise; Monash University Eastern Health Clinical School; Eastern Health, Department of Cardiology Cooke, Jennifer; Eastern Health, Department of Cardiology; Monash University Eastern Health Clinical School Wong, Michael; Eastern Health, Department of Cardiology Frost, Tanya; Eastern Health, Department of Neurosciences Teh, Andrew W.; Monash University Eastern Health Clinical School; Eastern Health, Department of Cardiology
Keywords:	Pacing & electrophysiology < CARDIOLOGY, Stroke medicine < INTERNAL MEDICINE, Cardiology < INTERNAL MEDICINE

SCHOLARONE™
Manuscripts

1
2
3 **The association between excessive premature atrial complexes and cryptogenic stroke:**
4 **results of a case – control study.**
5

6 Jithin K. Sajeev, MBChB^{1,2}, Anoop N. Koshy, MBBS¹, Helen Dewey, MBBS, PhD^{1,3},
7
8 Jonathan M. Kalman, MBBS, PhD⁵, Kevin Rajakariar, MBBS², Mae C. Tan, MBBS²,
9
10 Maryann Street, PhD⁴, Louise Roberts, PhD^{1,2}, Jennifer C. Cooke, MBBS^{1,2}, Michael C.
11
12 Wong, MBBS, PhD², Tanya Frost, RN³, Andrew W. Teh, MBBS, PhD^{1,2}
13
14
15

16 **Affiliations**
17
18

19 ¹Eastern Health Clinical School, Monash University, Victoria, Australia
20
21

22 ²Department of Cardiology, Eastern Health, Victoria, Australia
23
24

25 ³Department of Neuroscience, Eastern Health, Victoria, Australia
26
27

28 ⁴School of Nursing and Midwifery, Deakin University, Victoria, Australia
29
30

31 ⁵Department of Cardiology, The Royal Melbourne Hospital, Victoria, Australia
32
33

34 **Corresponding Author:**
35
36

37 A/Prof. Andrew W Teh
38
39

40 Department of Cardiology, Box Hill Hospital,
41
42

43 Level 2, Building B, 8 Arnold Street, Box Hill,
44
45

46 VIC 3128, Australia
47
48

49 Phone: +61 3 9895 4833
50
51

52 Email: andrew.teh@easternhealth.org.au
53
54
55
56
57

58 **Word Count: 2207**
59
60

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 stroke¹. 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

1
2
3 database that covered three tertiary, university hospitals. Stroke subtypes were determined
4
5 according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification¹².
6
7

8
9 Inclusion criteria were: 1) age ≥ 18 years; 2) adjudicated to have had an ischaemic stroke/TIA
10
11 by the stroke service and 3) underwent 24-hour Holter monitoring following their index
12
13 stroke/TIA. Exclusion criteria were: 1) history of atrial fibrillation, atrial flutter or a
14
15 subsequent diagnosis of these arrhythmias on inpatient telemetry, Holter monitoring or during
16
17 follow up; 2) underlying severe cardiomyopathy with an ejection fraction $< 35\%$; 3) previous
18
19 coronary artery bypass grafting; 4) recent myocardial infarction or 5) severe chronic
20
21 obstructive airways disease.
22
23

24
25 Eligible patients were compared to a group of age-matched controls, without prior history of
26
27 stroke or AF and underwent outpatient Holter monitoring. The control group were composed
28
29 of patients that underwent investigation of chest pain, syncope, pre-syncope and palpitations.
30
31 The stroke/TIA and control groups were age matched with the same exclusion criteria
32
33 applied.
34
35

36 37 *Clinical assessment and outcome measures* 38 39

40
41 All included patients adjudicated as having a stroke/TIA underwent investigation and
42
43 treatment as per the national stroke guidelines recommendation for standard of care.¹³ This
44
45 included physical examination, blood measurements, 12 lead ECG, pulse oximetry, computed
46
47 tomography of the brain (CTB), inpatient cardiac monitoring and vascular assessment. All
48
49 patients underwent Holter monitoring with 24 hours of continuous rhythm capture utilising
50
51 the SEER Light Holter Monitor (GE Healthcare, Milwaukee, USA). Data were analysed
52
53 offline upon completion of the monitoring period by cardiac technicians and subsequently
54
55 reviewed by a cardiac electrophysiologist blinded to the study hypothesis. Rhythm analysis
56
57
58
59
60

1
2
3 was conducted using MARS Ambulatory ECG Analysis System (GE Healthcare, Milwaukee,
4
5 USA).

6
7
8 Baseline demographic and clinical data were collected from electronic health records along
9
10 with vascular risk factors to allow for calculation of a CHA₂DS₂VASc score, medication use
11
12 and to identify presentations with recurrent stroke or TIA. Based on prior literature, we
13
14 defined excessive PAC burden as ≥ 200 PACs/24 hours and a long atrial run as ≥ 20 beats.^{14 15}

17 18 *Statistical methods*

19
20
21 Demographic data, disease status and outcome measures are presented as proportions and
22
23 summarised by descriptive statistics. Data were tested for normality and parametric or non-
24
25 parametric tests applied as appropriate. Correlation trends were analysed using Spearman's
26
27 rho for non-parametric data. A p-value <0.05 was deemed statistically significant and a 95%
28
29 confidence interval (CI) is presented where applicable. Markers associated with stroke/TIA
30
31 and excessive PACs were identified by univariate and multivariate logistical regression. Any
32
33 variable with a p value <0.25 on univariate analysis was included in multivariate analyses.

34
35
36 All statistical analysis was performed with SPSS Statistics 24.0 (IBM, USA).

37 38 39 *Patient and public involvement*

40
41
42 Public involvement was sought after the methods and outcome measures were identified. The
43
44 protocol and study design were reviewed by human research ethics committee, 45% of whom
45
46 were members of the public.
47
48

49 50 51 *Ethics approval and data sharing*

52
53
54 The research protocol was approved by the institutional Human Research Ethics Committee
55
56 and written informed consent was not deemed necessary by the committee (approval number:
57
58
59
60

1
2
3 LR09/2016). The raw data will be made available by the corresponding author upon
4
5 reasonable request.
6
7
8
9

10 11 **Results**

12
13
14 In total, 537 patients presented with a stroke/TIA during the study inclusion period and
15
16 underwent Holter monitoring. Following exclusions, 461 patients with a stroke/TIA were
17
18 compared against 251 age-matched patients that underwent Holter monitoring during the
19
20 same time period (Figure 1). The median time to Holter monitoring following the stroke or
21
22 TIA was 40 days.
23
24

25 26 *Ischaemic stroke and PAC burden*

27
28
29 Baseline characteristics stratified according to study groups are shown in Table 1. In both
30
31 groups, the mean age was 70 years and the majority of patients were male. Stroke/TIA
32
33 patients were significantly more likely to have comorbidities of hypertension, diabetes
34
35 mellitus, dyslipidaemia, peripheral vascular disease and a prior history of smoking. There
36
37 were 79 patients with a prior cerebrovascular event in the stroke/TIA group. On admission,
38
39 there was significantly higher use of statins in the stroke/TIA cohort, however no difference
40
41 was evident in the use of antiplatelet therapy or oral anticoagulants. The prevalence of
42
43 excessive PACs were significantly higher in the stroke/TIA group (25.6% vs 14.7%, $p =$
44
45 0.001), however atrial runs of ≥ 20 beats were not significantly different (Table 1).
46
47
48

49
50
51 Multivariate analysis showed female sex, hypertension, history of smoking and excessive
52
53 PACs were significantly associated with a stroke/TIA; excessive PACs conferred the highest
54
55 risk for stroke/TIA with an odds ratio of 1.97(CI: 1.29 – 3.02) (Table 2). Analysis of PAC
56
57 burden revealed a skewed distribution, median PACs/24 hours and longest atrial ectopic runs
58
59
60

1
2
3 were significantly higher in the stroke/TIA group. However, number of beats in runs of
4
5 greater than 3 beats was not significantly different between the 2 groups.
6
7

8 *Cryptogenic Stroke and PAC burden*

10
11 One hundred and eighty-five patients with cryptogenic stroke, after excluding TIA, were
12
13 compared with 251 patients in the control group. The mean age was not significantly
14
15 different between the control and cryptogenic stroke group, 70.5 and 68.9 years respectively.
16
17

18 Cryptogenic stroke was significantly associated with excessive PAC burden, female sex,
19
20 hypertension, diabetes mellitus, smoking, obstructive sleep apnoea and a higher median
21
22 CHA₂DS₂VASc score on univariate analysis. Excessive PACs (OR: 1.95; CI: 1.16 – 3.28),
23
24 female sex (OR: 1.78; CI: 1.19 – 2.67) and hypertension (OR: 1.67; CI: 1.05 – 2.64)
25
26 maintained significant and independent associations with cryptogenic stroke subtype on
27
28 multivariate logistical regression (Table 2).
29
30
31
32

33 *Vascular risk factors and PAC burden*

34
35
36 Increasing CHA₂DS₂VASc score was associated with increasing median PACs in both groups
37
38 (Figure 2). A CHA₂DS₂VASc score greater than 3 in the control group and 2 in the stroke/TIA
39
40 group were associated with an excessive PAC burden. However, only a moderate to weak
41
42 correlation was evident between increasing CHA₂DS₂VASc score and increasing PACs/24 hours in
43
44 all patients ($r_s = 0.32$, $p < 0.001$), control patients ($r_s = 0.24$, $p < 0.001$) and stroke/TIA patients (r_s
45
46 $= 0.32$, $p < 0.001$) respectively.
47
48
49
50

51 In all patients, age, hypertension, diabetes mellitus and peripheral vascular disease were significant
52
53 univariate predictors of excessive PACs. However only age and hypertension remained significant
54
55 independent predictors of excessive PACs on regression analysis, with age ≥ 75 being the strongest
56
57 predictor for excessive PACs (Table 3).
58
59
60

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 AF¹⁷. 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

1
2
3 and lends further support to the hypothesis, that excessive PACs may be the manifestation or
4 marker of underlying atrial myopathy, that confers an increased risk for cryptogenic stroke.
5

6
7 Further, similar to AF, the risk for ischaemic stroke in the presence of an excessive PAC
8 burden appear to be modulated by vascular risk factors. A CHA₂DS₂VASc score of 2
9
10 conferred a similar risk for ischaemic stroke in patients with excessive PACs, as with AF.⁹
11

12
13 An increasing CHA₂DS₂VASc score was significantly associated with increasing median
14
15 PAC burden in both the control and stroke/TIA group. However, despite the positive
16
17 correlation between CHA₂DS₂VASc score and premature atrial complexes, the strength of the
18
19 correlation itself remained weak. This was suggestive of differential effects of the various
20
21 components of CHA₂DS₂VASc score in contributing to a high PAC burden.
22
23
24
25

26
27 To our knowledge, this is the first study to evaluate the independent association between
28
29 specific vascular risk factors and an excessive PAC burden. The independent contribution of
30
31 the various risk markers that make up CHA₂DS₂VASc score have not been assessed
32
33 previously.^{9 18 20} Delineation of these specific risk factors that contribute to excessive PACs
34
35 provides insights into the potential pathophysiological basis for excessive PAC.
36
37
38

39
40 Increasing age and hypertension were independently and significantly associated with
41
42 excessive PACs in the present study. This is consistent with electro-anatomical studies that
43
44 demonstrated slower conduction velocities and both global and regional reduction in atrial
45
46 voltages with increasing age and hypertension^{21 22}. Such areas corresponded to delayed
47
48 enhancement on magnetic resonance imaging and histological fibrosis^{23,24}. Thrombogenesis
49
50 associated with this underlying atrial remodelling may help explain a significant proportion
51
52 of strokes currently classified as cryptogenic. Both advancing age and hypertension are also
53
54 associated with small and large vessel stroke subtypes. In addition to atrial remodelling, it is
55
56 likely that vascular risk factors promote thrombogenesis and ischaemic stroke through
57
58
59
60

1
2
3 multiple pathways including arterial endothelial dysfunction, and atherosclerosis with
4
5 localised plaque rupture.²⁵
6
7

8 Our report of the independent association between excessive PACs and cryptogenic stroke
9
10 further implicates a risk factor driven atrial substrate abnormality in its pathogenesis. These
11
12 findings are clinically relevant as the results of a recently concluded large multicentre
13
14 randomised controlled trial failed to demonstrate a benefit for oral anticoagulation in an
15
16 unselected population with embolic stroke of undetermined source²⁶. This highlights the
17
18 heterogeneity of the pathophysiological mechanisms that lead to cryptogenic stroke. There is
19
20 an unmet clinical need to develop risk markers that identify the subset of patients with
21
22 cryptogenic stroke that occur as a result of cardio-embolism.
23
24
25

26
27 Studies have previously described serological and echocardiographic markers associated with
28
29 the recurrence of cryptogenic stroke²⁷. Similarly, excessive PACs are readily assessed and
30
31 may serve as a novel and reproducible marker to identify patients at high risk for the
32
33 cryptogenic stroke. It is unclear if excessive PACs directly promote thrombogenesis or if they
34
35 are simply a marker of adverse atrial remodelling that leads to thrombogenesis and stroke.
36
37
38 Regardless, the risk conferred by an elevated CHA₂DS₂VASc score in conjunction with
39
40 excessive PACs for ischaemic stroke remains significant⁹.
41
42
43

44 This study has limitations. The absence of prolonged monitoring with devices such as
45
46 implantable loop recorders could have led to an underestimation of incident AF. However,
47
48 we excluded all patients with a diagnosis of AF over 1.9 years of mean follow up. A higher
49
50 number of cryptogenic stroke patients were present in our population than previously
51
52 reported. This is likely due to referral bias, as patients were only included if they underwent
53
54 Holter monitoring. However, the higher prevalence of cryptogenic stroke improves the
55
56 strength of our findings in this specific subset of stroke patients.
57
58
59
60

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.

Acknowledgements

None.

Sources of funding

This research received no specific project grant from any funding agency in the public, commercial or not-for-profit sectors. Andrew W. Teh is the recipient of an Early Career Fellowship from the National Health and Medical Research Council of Australia (NHMRC). Jithin K. Sajeev is the recipient of an Australian Government Research Training Program Scholarship. Anoop N. Koshy is the recipient of a Postgraduate Research Scholarships from the NHMRC, National Heart Foundation of Australia and The Royal Australasian College of Physicians CRB Blackburn Scholarship. Jonathan M. Kalman is supported by a Practitioner Fellowship from the NHMRC.

Competing interests

None.

References

1. Royal College of Physicians Sentinel Stroke National Audit Programme (SSNAP). National clinical audit annual results portfolio March 2016-April 2017 2017 [Available from: <http://bit.ly/1NHylqH> accessed 24 December 2018].
2. Hart RG, Diener HC, Coutts SB, et al. Embolic strokes of undetermined source: the case for a new clinical construct. *Lancet Neurol* 2014;13(4):429-38. doi: 10.1016/S1474-4422(13)70310-7
3. Gladstone DJ, Spring M, Dorian P, et al. Atrial fibrillation in patients with cryptogenic stroke. *N Engl J Med* 2014;370(26):2467-77. doi: 10.1056/NEJMoa1311376 [published Online First: 2014/06/26]
4. Ntaios G, Papavasileiou V, Milionis H, et al. Embolic strokes of undetermined source in the Athens stroke registry: a descriptive analysis. *Stroke* 2015;46(1):176-81. doi: 10.1161/STROKEAHA.114.007240
5. Liao J, Khalid Z, Scallan C, et al. Noninvasive cardiac monitoring for detecting paroxysmal atrial fibrillation or flutter after acute ischemic stroke: a systematic review. *Stroke* 2007;38(11):2935-40. doi: 10.1161/STROKEAHA.106.478685
6. Brachmann J, Morillo CA, Sanna T, et al. Uncovering Atrial Fibrillation Beyond Short-Term Monitoring in Cryptogenic Stroke Patients: Three-Year Results From the Cryptogenic Stroke and Underlying Atrial Fibrillation Trial. *Circ Arrhythm Electrophysiol* 2016;9(1):e003333. doi: 10.1161/CIRCEP.115.003333 [published Online First: 2016/01/15]

- 1
2
3 7. Conen D, Adam M, Roche F, et al. Premature atrial contractions in the general population:
4
5 frequency and risk factors. *Circulation* 2012;126(19):2302-8. doi:
6
7 10.1161/CIRCULATIONAHA.112.112300 [published Online First: 2012/10/11]
8
9
- 10 8. Healey JS, Connolly SJ, Gold MR, et al. Subclinical atrial fibrillation and the risk of
11
12 stroke. *N Engl J Med* 2012;366(2):120-9. doi: 10.1056/NEJMoa1105575
13
14
- 15 9. Larsen BS, Kumarathurai P, Falkenberg J, et al. Excessive Atrial Ectopy and Short Atrial
16
17 Runs Increase the Risk of Stroke Beyond Incident Atrial Fibrillation. *J Am Coll*
18
19 *Cardiol* 2015;66(3):232-41. doi: 10.1016/j.jacc.2015.05.018 [published Online First:
20
21 2015/07/18]
22
23
- 24 10. Marinheiro R, Parreira L, Amador P, et al. Excessive atrial ectopic activity as an
25
26 independent risk factor for ischemic stroke. *Int J Cardiol* 2017;249:226-30. doi:
27
28 10.1016/j.ijcard.2017.08.054
29
30
- 31 11. Tereshchenko LG, Shah AJ, Li Y, et al. Electrocardiographic deep terminal negativity of
32
33 the P wave in V1 and risk of mortality: the National Health and Nutrition
34
35 Examination Survey III. *J Cardiovasc Electrophysiol* 2014;25(11):1242-8. doi:
36
37 10.1111/jce.12453
38
39
- 40 12. Adams HP, Jr., Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute
41
42 ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of
43
44 Org 10172 in Acute Stroke Treatment. *Stroke* 1993;24(1):35-41.
45
46
- 47 13. Clinical guidelines for stroke management 2010. *National Stroke Foundation Melbourne*
48
49 *Australia* 2010
50
51
- 52 14. Todo K, Moriwaki H, Saito K, et al. Frequent premature atrial contractions in stroke of
53
54 undetermined etiology. *Eur Neurol* 2009;61(5):285-8. doi: 10.1159/000206853
55
56
- 57 15. Engstrom G, Hedblad B, Juul-Moller S, et al. Cardiac arrhythmias and stroke: increased
58
59 risk in men with high frequency of atrial ectopic beats. *Stroke* 2000;31(12):2925-9.
60

- 1
2
3 16. Acharya T, Tringali S, Bhullar M, et al. Frequent Atrial Premature Complexes and Their
4
5 Association With Risk of Atrial Fibrillation. *Am J Cardiol* 2015;116(12):1852-7. doi:
6
7 10.1016/j.amjcard.2015.09.025 [published Online First: 2015/11/28]
8
9
10 17. Murakoshi N, Xu D, Sairenchi T, et al. Prognostic impact of supraventricular premature
11
12 complexes in community-based health checkups: the Ibaraki Prefectural Health Study.
13
14 *Eur Heart J* 2015;36(3):170-8. doi: 10.1093/eurheartj/ehu407 [published Online First:
15
16 2014/11/02]
17
18
19 18. Binici Z, Intzilakis T, Nielsen OW, et al. Excessive supraventricular ectopic activity and
20
21 increased risk of atrial fibrillation and stroke. *Circulation* 2010;121(17):1904-11. doi:
22
23 10.1161/CIRCULATIONAHA.109.874982
24
25
26 19. Pinho J, Braga CG, Rocha S, et al. Atrial ectopic activity in cryptogenic ischemic stroke
27
28 and TIA: a risk factor for recurrence. *J Stroke Cerebrovasc Dis* 2015;24(2):507-10.
29
30 doi: 10.1016/j.jstrokecerebrovasdis.2014.09.029
31
32
33 20. Kamel H, Elkind MS, Bhave PD, et al. Paroxysmal supraventricular tachycardia and the
34
35 risk of ischemic stroke. *Stroke* 2013;44(6):1550-4. doi:
36
37 10.1161/STROKEAHA.113.001118 [published Online First: 2013/05/02]
38
39
40 21. Kistler PM, Sanders P, Fynn SP, et al. Electrophysiologic and electroanatomic changes in
41
42 the human atrium associated with age. *J Am Coll Cardiol* 2004;44(1):109-16. doi:
43
44 10.1016/j.jacc.2004.03.044
45
46
47 22. Medi C, Kalman JM, Spence SJ, et al. Atrial electrical and structural changes associated
48
49 with longstanding hypertension in humans: implications for the substrate for atrial
50
51 fibrillation. *J Cardiovasc Electrophysiol* 2011;22(12):1317-24. doi: 10.1111/j.1540-
52
53 8167.2011.02125.x
54
55
56 23. Oakes RS, Badger TJ, Kholmovski EG, et al. Detection and quantification of left atrial
57
58 structural remodeling with delayed-enhancement magnetic resonance imaging in
59
60

1
2
3 patients with atrial fibrillation. *Circulation* 2009;119(13):1758-67. doi:

4
5 10.1161/CIRCULATIONAHA.108.811877
6
7

8 24. Diez J. Mechanisms of cardiac fibrosis in hypertension. *J Clin Hypertens (Greenwich)*
9
10 2007;9(7):546-50.
11

12 25. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction
13
14 in hypertensive patients. *Circulation* 2001;104(2):191-6.
15
16

17 26. Hart RG, Sharma M, Mundl H, et al. Rivaroxaban for Stroke Prevention after Embolic
18
19 Stroke of Undetermined Source. *N Engl J Med* 2018;378(23):2191-201. doi:
20
21 10.1056/NEJMoa1802686 [published Online First: 2018/05/17]
22
23

24 27. Yaghi S, Moon YP, Mora-McLaughlin C, et al. Left atrial enlargement and stroke
25
26 recurrence: the Northern Manhattan Stroke Study. *Stroke* 2015;46(6):1488-93. doi:
27
28 10.1161/STROKEAHA.115.008711
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 **Figure Legend**
4

5
6 **Figure 1.**
7

8
9 Patient selection: inclusions and exclusions.

10
11 AF = atrial fibrillation; CABG = coronary artery bypass grafting; COPD = chronic
12 obstructive pulmonary disease; TIA = transient ischaemic attack;
13
14
15

16
17 **Figure 2.**
18

19
20 Median PACs by CHA₂DS₂VASc score for control and stroke/TIA groups.
21

22
23 CHA₂DS₂VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 ₂ DS ₂ VASc 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

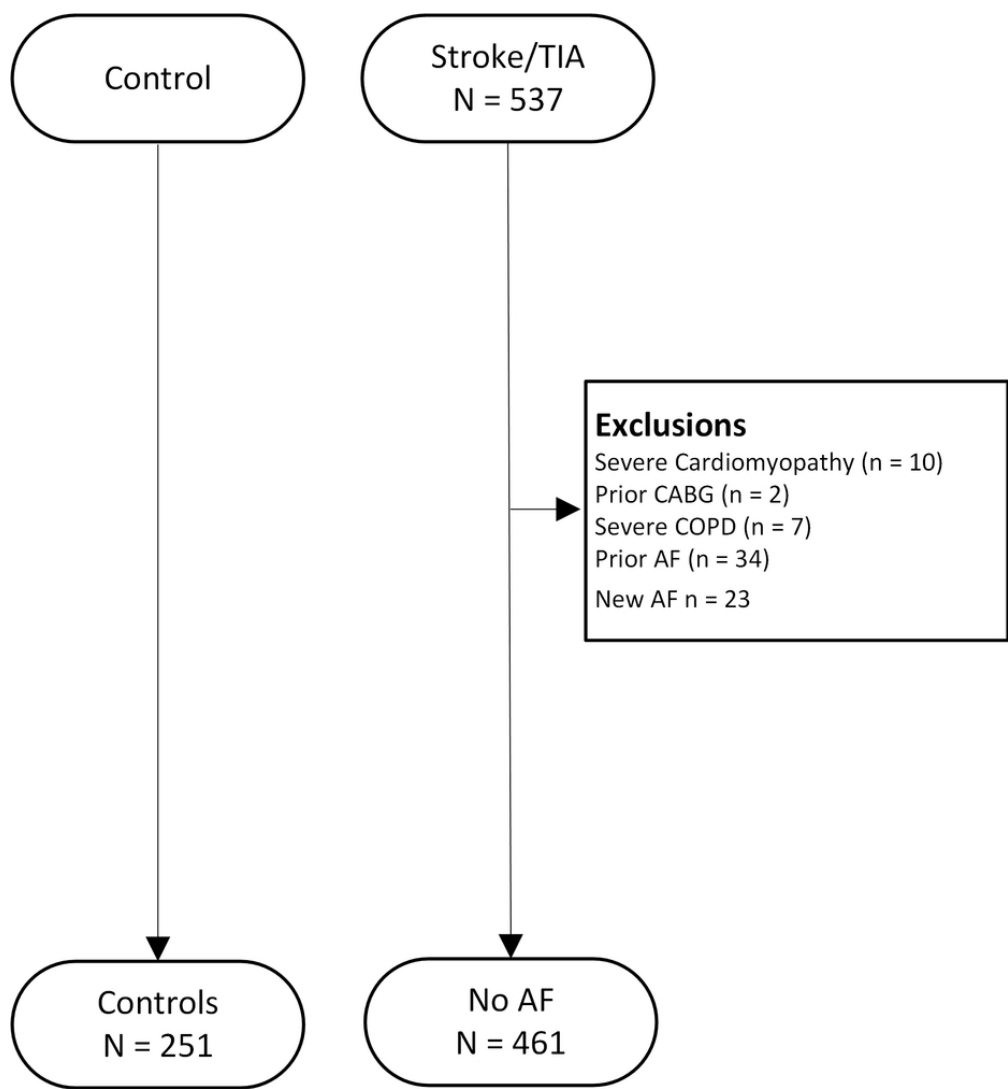
CI = confidence interval.

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

Author Statement

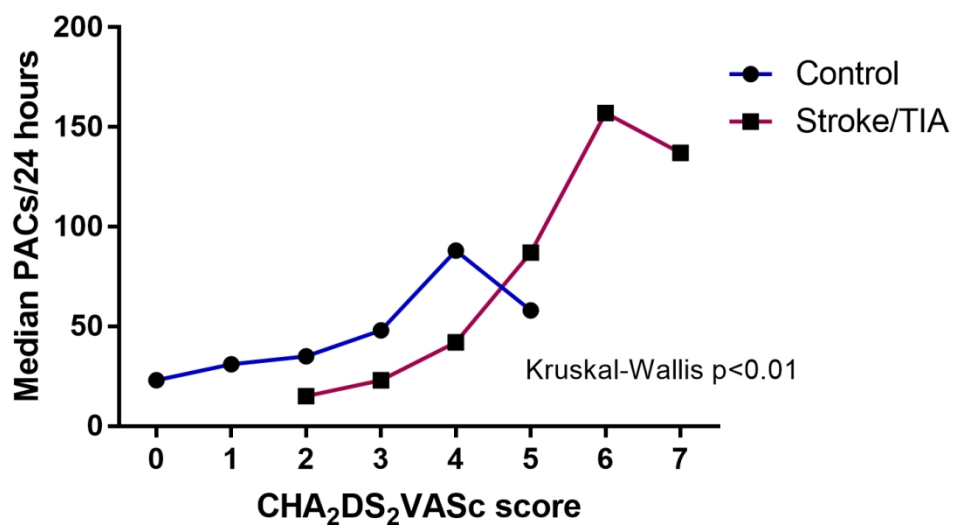
JS led and designed the study, collated and analysed data and wrote the manuscript. AK collated and analysed the data and revised the manuscript. HD contributed to study design and revised the manuscript. JK contributed to study design and revised the manuscript. KR collated and analysed the data and 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



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 CHA₂DS₂VASc score for control and stroke/TIA groups.
CHA₂DS₂VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;

127x74mm (300 x 300 DPI)

BMJ Open

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

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2019-029164.R1
Article Type:	Research
Date Submitted by the Author:	14-Mar-2019
Complete List of Authors:	Sajeev, Jithin; Monash University Eastern Health Clinical School, ; Eastern Health, Department of Cardiology Koshy, Anoop; Monash University Eastern Health Clinical School; Monash University Eastern Health Clinical School Dewey, Helen; Eastern Health, Neurosciences; Monash University Eastern Health Clinical School Kalman, Jonathan; Royal Melbourne Hospital, Rajakariar, Kevin; Eastern Health, Department of Cardiology Tan, Mae; Eastern Health, Department of Cardiology Street, Maryann; Deakin University, School of Nursing and Midwifery; Eastern Health, Eastern Health - Deakin University Nursing and Midwifery research Centre Roberts, Louise; Monash University Eastern Health Clinical School; Eastern Health, Department of Cardiology Cooke, Jennifer; Eastern Health, Department of Cardiology; Monash University Eastern Health Clinical School Wong, Michael; Eastern Health, Department of Cardiology Frost, Tanya; Eastern Health, Department of Neurosciences Teh, Andrew W.; Monash University Eastern Health Clinical School; Eastern Health, Department of Cardiology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Neurology
Keywords:	Pacing & electrophysiology < CARDIOLOGY, Stroke medicine < INTERNAL MEDICINE, Cardiology < INTERNAL MEDICINE

SCHOLARONE™
Manuscripts

1
2
3 **The association between excessive premature atrial complexes and cryptogenic stroke:**
4
5 **results of a case – control study.**
6
7

8 Jithin K. Sajeev, MBChB^{1,2}, Anoop N. Koshy, MBBS¹, Helen Dewey, MBBS, PhD^{1,3},
9
10 Jonathan M. Kalman, MBBS, PhD⁵, Kevin Rajakariar, MBBS², Mae C. Tan, MBBS²,
11
12 Maryann Street, PhD⁴, Louise Roberts, PhD^{1,2}, Jennifer C. Cooke, MBBS^{1,2}, Michael C.
13
14 Wong, MBBS, PhD², Tanya Frost, RN³, Andrew W. Teh, MBBS, PhD^{1,2}
15
16
17

18 **Affiliations**
19

20
21 ¹Eastern Health Clinical School, Monash University, Victoria, Australia
22

23
24 ²Department of Cardiology, Eastern Health, Victoria, Australia
25

26
27 ³Department of Neuroscience, Eastern Health, Victoria, Australia
28

29
30 ⁴School of Nursing and Midwifery, Deakin University, Victoria, Australia
31

32
33 ⁵Department of Cardiology, The Royal Melbourne Hospital, Victoria, Australia
34
35

36 **Corresponding Author:**
37

38
39 A/Prof. Andrew W Teh
40

41
42 Department of Cardiology, Box Hill Hospital,
43

44
45 Level 2, Building B, 8 Arnold Street, Box Hill,
46

47
48 VIC 3128, Australia
49

50
51 Phone: +61 3 9895 4833
52

53
54 Email: andrew.teh@easternhealth.org.au
55
56
57
58
59

60 **Word Count: 2370**

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 stroke¹. 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 myopathy⁸. 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 stroke¹⁵. 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

1
2
3 database that covered three tertiary, university hospitals. Stroke subtypes were determined
4
5 according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification¹⁶.
6
7

8
9 Inclusion criteria were: 1) age ≥ 18 years; 2) adjudicated to have had an ischaemic stroke/TIA
10
11 by the stroke service and 3) underwent 24-hour Holter monitoring following their index
12
13 stroke/TIA. Exclusion criteria were: 1) history of atrial fibrillation, atrial flutter or a
14
15 subsequent diagnosis of these arrhythmias on inpatient telemetry, Holter monitoring or during
16
17 follow up; 2) underlying severe cardiomyopathy with an ejection fraction $< 35\%$; 3) previous
18
19 coronary artery bypass grafting; 4) recent myocardial infarction or 5) severe chronic
20
21 obstructive airways disease.
22
23

24
25 Eligible patients were compared to a group of age-matched controls, without prior history of
26
27 stroke or AF and underwent outpatient Holter monitoring. The control group were composed
28
29 of patients that underwent investigation of chest pain, syncope, pre-syncope and palpitations.
30
31 The stroke/TIA and control groups were age matched with the same exclusion criteria
32
33 applied.
34
35

36 37 *Clinical assessment and outcome measures*

38
39
40 All included patients adjudicated as having a stroke/TIA underwent investigation and
41
42 treatment as per the national stroke guidelines recommendation for standard of care.¹⁷ This
43
44 included physical examination, blood measurements, 12 lead ECG, pulse oximetry, computed
45
46 tomography of the brain (CTB), inpatient cardiac monitoring and vascular assessment. All
47
48 patients underwent Holter monitoring with 24 hours of continuous rhythm capture utilising
49
50 the SEER Light Holter Monitor (GE Healthcare, Milwaukee, USA). Data were analysed
51
52 offline upon completion of the monitoring period by cardiac technicians and subsequently
53
54 reviewed by a cardiac electrophysiologist blinded to the study hypothesis. Rhythm analysis
55
56
57
58
59
60

1
2
3 was conducted using MARS Ambulatory ECG Analysis System (GE Healthcare, Milwaukee,
4 USA).
5
6

7
8 Baseline demographic and clinical data were collected from electronic health records along
9 with vascular risk factors to allow for calculation of a CHA₂DS₂VASc score, medication use
10 and to identify presentations with recurrent stroke or TIA. Based on prior literature, we
11 defined excessive PAC burden as ≥ 200 PACs/24 hours and a long atrial run as ≥ 20 beats.^{10 18}
12
13
14

15
16
17
18
19
20
21

Statistical methods

22
23 Demographic data, disease status and outcome measures are presented as proportions and
24 summarised by descriptive statistics. Data were tested for normality and parametric or non-
25 parametric tests applied as appropriate. Correlation trends were analysed using Spearman's
26 rho for non-parametric data. A p-value < 0.05 was deemed statistically significant and a 95%
27 confidence interval (CI) is presented where applicable. Markers associated with stroke/TIA
28 and excessive PACs were identified by univariate and multivariate logistical regression. Any
29 variable with a p value < 0.25 on univariate analysis was included in multivariate analyses.
30
31
32

33 All statistical analysis was performed with SPSS Statistics 24.0 (IBM, USA).
34
35
36
37
38
39

Patient and public involvement

40
41
42
43 Public involvement was sought after the methods and outcome measures were identified. The
44 protocol and study design were reviewed by human research ethics committee, 45% of whom
45 were members of the public.
46
47
48
49
50
51

Ethics approval and data sharing

52
53
54 The research protocol was approved by the institutional Human Research Ethics Committee
55 and written informed consent was not deemed necessary by the committee (approval number:
56
57
58
59
60

1
2
3 LR09/2016). The raw data will be made available by the corresponding author upon
4
5 reasonable request.
6
7

8 **Results**

9

10
11 In total, 537 patients presented with a stroke/TIA during the study inclusion period and
12
13 underwent Holter monitoring. 23 out of 537 (4.2%) patients had AF identified on Holter
14
15 monitoring and were excluded. Four patients with AF had an excessive PAC burden (17%).
16
17

18
19 Following exclusions, 461 patients with a stroke/TIA were compared against 251 age-
20
21 matched patients that underwent Holter monitoring during the same time period (Figure 1).
22
23

24 The median time to Holter monitoring following the stroke or TIA was 40 days.
25
26

27 *Ischaemic stroke and PAC burden*

28
29

30 Baseline characteristics stratified according to study groups are shown in Table 1. In both
31
32 groups, the mean age was 70 years and the majority of patients were male. Stroke/TIA
33
34 patients were significantly more likely to have comorbidities of hypertension, diabetes
35
36 mellitus, dyslipidaemia, peripheral vascular disease and a prior history of smoking. There
37
38 were 79 patients with a prior cerebrovascular event in the stroke/TIA group. On admission,
39
40 there was significantly higher use of statins in the stroke/TIA cohort, however no difference
41
42 was evident in the use of antiplatelet therapy or oral anticoagulants. The prevalence of
43
44 excessive PACs were significantly higher in the stroke/TIA group (25.6% vs 14.7%, $p =$
45
46 0.001), however atrial runs of ≥ 20 beats were not significantly different (Table 1).
47
48
49

50
51 Multivariate analysis showed female sex, hypertension, history of smoking and excessive
52
53 PACs were significantly associated with a stroke/TIA. Excessive PACs conferred the highest
54
55 risk for stroke/TIA with an odds ratio of 1.97(CI: 1.29 – 3.02), but the difference was not
56
57 significant when compared with other risk factors associated with stroke/TIA (Table 2).
58
59
60

1
2
3 Multivariate analysis with various definitions of excessive PACs based on prior literature
4 yielded similar results, with a significant association between Excessive PACs and
5 stroke/TIA (Supplementary file).
6
7
8

9
10 Analysis of PAC burden revealed a skewed distribution, median PACs/24 hours and longest
11 atrial ectopic runs were significantly higher in the stroke/TIA group. However, number of
12 beats in runs of greater than 3 beats was not significantly different between the 2 groups.
13
14
15
16

17 *Cryptogenic Stroke and PAC burden*

18
19 One hundred and eighty-five patients with cryptogenic stroke, after excluding TIA, were
20 compared with 251 patients in the control group. The mean age was not significantly
21 different between the control and cryptogenic stroke group, 70.5 and 68.9 years respectively.
22
23 Cryptogenic stroke was significantly associated with excessive PAC burden, female sex,
24 hypertension, diabetes mellitus, smoking, obstructive sleep apnoea and a higher median
25 CHA₂DS₂VASc score on univariate analysis. Excessive PACs (OR: 1.95; CI: 1.16 – 3.28),
26 female sex (OR: 1.78; CI: 1.19 – 2.67) and hypertension (OR: 1.67; CI: 1.05 – 2.64)
27 maintained significant and independent associations with cryptogenic stroke subtype on
28 multivariate logistical regression (Table 2).
29
30
31
32
33
34
35
36
37
38
39
40
41
42

43 *Vascular risk factors and PAC burden*

44
45 Increasing CHA₂DS₂VASc score was associated with increasing median PACs in both groups
46 (Figure 2). A CHA₂DS₂VASc score greater than 3 in the control group and 2 in the stroke/TIA
47 group were associated with an excessive PAC burden. However, only a moderate to weak
48 correlation was evident between increasing CHA₂DS₂VASc score and increasing PACs/24 hours in
49 all patients ($r_s = 0.32$, $p < 0.001$), control patients ($r_s = 0.24$, $p < 0.001$) and stroke/TIA patients (r_s
50 $= 0.32$, $p < 0.001$) respectively.
51
52
53
54
55
56
57
58
59
60

1
2
3 In all patients, age, hypertension, diabetes mellitus and peripheral vascular disease were significant
4 univariate predictors of excessive PACs. However only age and hypertension remained
5
6 independently associated with excessive PACs on regression analysis, with age ≥ 75 being the
7
8 strongest marker associated with excessive PACs (Table 3).
9
10
11
12

13 **Discussion**

14
15
16 In this study, we compared the differences in PAC burden between patients with a stroke/TIA
17
18 and an age-matched control population, after excluding AF. Excessive PAC burden was
19
20 significantly more common in the stroke/TIA group. An important new finding in our study
21
22 was that excessive PACs demonstrated an independent association for the cryptogenic stroke
23
24 subtype, after adjusting for conventional risk factors (OR: 1.95; CI: 1.16 – 3.28). There was a
25
26 stepwise rise in PAC burden with increasing number of vascular risk factors; age and
27
28 hypertension were independent risk factors associated with excessive PACs.
29
30
31
32

33
34 Investigators have previously demonstrated a significantly higher PAC burden in patients that
35
36 develop incident AF and ischemic stroke.^{18 20} Existing longitudinal studies from Engstrom et
37
38 al. that demonstrated a high PAC burden conferred a 1.9 times higher risk for ischaemic
39
40 stroke.¹⁹ Despite the differences in methodology, the current study showed 1.97 times rise in
41
42 odds for ischaemic stroke. Prior studies have also shown an association between runs of
43
44 PACs and ischaemic stroke in patients without documented AF²¹. The Copenhagen Holter
45
46 study, a cohort study that analysed the risk for stroke with an elevated PAC burden, defined
47
48 excessive supraventricular ectopic activity as a composite of either >30 PACs/hour or a run
49
50 of >20 PACs, and found a positive correlation with increased stroke and death.^{10 13} In
51
52 contrast, we did not show a significant difference in PAC runs >20 beats between the two
53
54 groups.¹⁵ This apparent discrepancy is likely due to a lack of standardised definitions for
55
56
57
58
59
60

1
2
3 excessive PACs and treating atrial premature runs ≥ 20 beats as a standalone variable in the
4
5 current study, instead of a composite measure.
6
7

8 The present study specifically analysed the association between excessive PACs and
9
10 cryptogenic stroke subtype. It demonstrated an independent association between cryptogenic
11
12 stroke subtype and excessive PACs with an odds ratio of 1.95. This is an important finding
13
14 and lends further support to the hypothesis, that excessive PACs may be the manifestation or
15
16 marker of underlying atrial myopathy, that confers an increased risk for cryptogenic stroke.
17
18

19 Further, similar to AF, the risk for ischaemic stroke in the presence of an excessive PAC
20
21 burden appear to be modulated by vascular risk factors. A CHA₂DS₂VASc score of 2
22
23 conferred a similar risk for ischaemic stroke in patients with excessive PACs, as with AF.¹⁰
24
25

26 An increasing CHA₂DS₂VASc score was significantly associated with increasing median
27
28 PAC burden in both the control and stroke/TIA group. However, despite the positive
29
30 correlation between CHA₂DS₂VASc score and premature atrial complexes, the strength of the
31
32 correlation itself remained weak. This was suggestive of differential effects of the various
33
34 components of CHA₂DS₂VASc score in contributing to a high PAC burden. The independent
35
36 contribution of the various risk markers that make up CHA₂DS₂VASc score have not been
37
38 assessed previously.^{10 13 22} Delineation of these specific risk factors that contribute to
39
40 excessive PACs provides insights into the potential pathophysiological basis for excessive
41
42 PAC.
43
44
45
46
47

48 Increasing age and hypertension were independently and significantly associated with
49
50 excessive PACs in the present study. This is consistent with electro-anatomical studies that
51
52 demonstrated slower conduction velocities and both global and regional reduction in atrial
53
54 voltages with increasing age and hypertension^{23 24}. Such areas corresponded to delayed
55
56 enhancement on magnetic resonance imaging and histological fibrosis^{25,26}. Thrombogenesis
57
58
59
60

1
2
3 associated with this underlying atrial remodelling may help explain a significant proportion
4 of strokes currently classified as cryptogenic. Both advancing age and hypertension are also
5 associated with small and large vessel stroke subtypes. In addition to atrial remodelling, it is
6 likely that vascular risk factors promote thrombogenesis and ischaemic stroke through
7 multiple pathways including arterial endothelial dysfunction, and atherosclerosis with
8 localised plaque rupture.²⁷
9

10
11 Our report of the independent association between excessive PACs and cryptogenic stroke
12 further implicates a risk factor driven atrial substrate abnormality in its pathogenesis. These
13 findings are clinically relevant as the results of a recently concluded large multicentre
14 randomised controlled trial failed to demonstrate a benefit for oral anticoagulation in an
15 unselected population with embolic stroke of undetermined source⁸. This highlights the
16 heterogeneity of the pathophysiological mechanisms that lead to cryptogenic stroke. There is
17 an unmet clinical need to develop risk markers that identify the subset of patients with
18 cryptogenic stroke that occur as a result of cardio-embolism.
19

20
21 Studies have previously described serological and echocardiographic markers associated with
22 the recurrence of cryptogenic stroke²⁸. Similarly, excessive PACs are readily assessed and
23 may serve as a novel and reproducible marker to identify patients at high risk for the
24 cryptogenic stroke. It is unclear if excessive PACs directly promote thrombogenesis or if they
25 are simply a marker of adverse atrial remodelling that leads to thrombogenesis and stroke.
26
27 Regardless, the risk conferred by an elevated CHA₂DS₂VASc score in conjunction with
28 excessive PACs for ischaemic stroke remains significant¹⁰.
29

30
31 This study has limitations. The absence of prolonged monitoring with devices such as
32 implantable loop recorders could have led to an underestimation of incident AF. However,
33 we excluded all patients with a diagnosis of AF over 1.9 years of mean follow up. A higher
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 number of cryptogenic stroke patients were present in our population than previously
4 reported. This is likely due to referral bias, as patients were only included if they underwent
5 Holter monitoring. All patients included in the study had guideline-based referral for Holter
6 monitoring. However, as Holter monitoring was an inclusion criterion, we do not have data
7 on patient who may have received their Holter monitoring at an external institution.
8
9 However, the higher prevalence of cryptogenic stroke improves the strength of our findings
10 in this specific subset of stroke patients. The time to Holter monitoring following the stroke,
11 based on routine institutional clinical waiting periods, could have introduced unintended
12 variables such as neurologically mediated cardiac modelling with resultant excessive PACs
13 and reverse causality. The higher burden of PACs was noted in a highly selective patient
14 cohort with ischaemic stroke and a high burden of vascular risk factors. Despite the use of
15 multivariate regression analysis, unrecognised confounders cannot be excluded in a cross-
16 sectional case control study, therefore these findings should not be extrapolated to other
17 patient cohorts.
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

36 **Conclusions**

37
38 Excessive PACs are significantly associated with cryptogenic stroke. Vascular risk factors,
39 increasing age and hypertension, were independently associated with excessive PACs. The
40 utility of novel and reproducible cardiac markers to guide preventative strategies in
41 cryptogenic stroke warrant further evaluation.
42
43
44
45
46
47
48

49 **Acknowledgements**

50
51 None.
52
53
54

55 **Sources of funding**

56
57 This research received no specific project grant from any funding agency in the public,
58 commercial or not-for-profit sectors.
59
60

Competing interests

None.

Data Sharing

Data can be made available by contacting the corresponding author.

References

1. Royal College of Physicians Sentinel Stroke National Audit Programme (SSNAP). National clinical audit annual results portfolio March 2016-April 2017 2017 [Available from: <http://bit.ly/1NHylqH> accessed 24 December 2018.
2. Hart RG, Diener HC, Coutts SB, et al. Embolic strokes of undetermined source: the case for a new clinical construct. *Lancet Neurol* 2014;13(4):429-38. doi: 10.1016/S1474-4422(13)70310-7
3. Benjamin EJ, Blaha MJ, Chiuve SE, et al. Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association. *Circulation* 2017;135(10):e146-e603. doi: 10.1161/CIR.0000000000000485 [published Online First: 2017/01/27]
4. Gladstone DJ, Spring M, Dorian P, et al. Atrial fibrillation in patients with cryptogenic stroke. *N Engl J Med* 2014;370(26):2467-77. doi: 10.1056/NEJMoa1311376 [published Online First: 2014/06/26]
5. Ntaios G, Papavasileiou V, Milionis H, et al. Embolic strokes of undetermined source in the Athens stroke registry: a descriptive analysis. *Stroke* 2015;46(1):176-81. doi: 10.1161/STROKEAHA.114.007240
6. Liao J, Khalid Z, Scallan C, et al. Noninvasive cardiac monitoring for detecting paroxysmal atrial fibrillation or flutter after acute ischemic stroke: a systematic review. *Stroke* 2007;38(11):2935-40. doi: 10.1161/STROKEAHA.106.478685

- 1
2
3 7. Brachmann J, Morillo CA, Sanna T, et al. Uncovering Atrial Fibrillation Beyond Short-
4
5 Term Monitoring in Cryptogenic Stroke Patients: Three-Year Results From the
6
7 Cryptogenic Stroke and Underlying Atrial Fibrillation Trial. *Circ Arrhythm*
8
9 *Electrophysiol* 2016;9(1):e003333. doi: 10.1161/CIRCEP.115.003333 [published
10
11 Online First: 2016/01/15]
12
13
- 14
15 8. Hart RG, Sharma M, Mundl H, et al. Rivaroxaban for Stroke Prevention after Embolic
16
17 Stroke of Undetermined Source. *N Engl J Med* 2018;378(23):2191-201. doi:
18
19 10.1056/NEJMoa1802686 [published Online First: 2018/05/17]
20
21
- 22
23 9. Conen D, Adam M, Roche F, et al. Premature atrial contractions in the general population:
24
25 frequency and risk factors. *Circulation* 2012;126(19):2302-8. doi:
26
27 10.1161/CIRCULATIONAHA.112.112300 [published Online First: 2012/10/11]
28
29
- 30
31 10. Larsen BS, Kumarathurai P, Falkenberg J, et al. Excessive Atrial Ectopy and Short Atrial
32
33 Runs Increase the Risk of Stroke Beyond Incident Atrial Fibrillation. *J Am Coll*
34
35 *Cardiol* 2015;66(3):232-41. doi: 10.1016/j.jacc.2015.05.018 [published Online First:
36
37 2015/07/18]
38
39
- 40
41 11. Marinheiro R, Parreira L, Amador P, et al. Excessive atrial ectopic activity as an
42
43 independent risk factor for ischemic stroke. *Int J Cardiol* 2017;249:226-30. doi:
44
45 10.1016/j.ijcard.2017.08.054
46
47
- 48
49 12. Himmelreich JCL, Lucassen WAM, Heugten M, et al. Frequent premature atrial
50
51 contractions are associated with atrial fibrillation, brain ischaemia, and mortality: a
52
53 systematic review and meta-analysis. *Europace* 2018 doi: 10.1093/europace/euy276
54
55 [published Online First: 2018/12/07]
56
57
- 58
59 13. Binici Z, Intzilakis T, Nielsen OW, et al. Excessive supraventricular ectopic activity and
60
increased risk of atrial fibrillation and stroke. *Circulation* 2010;121(17):1904-11. doi:
10.1161/CIRCULATIONAHA.109.874982

- 1
2
3 14. Sejr MH, Riahi S, Larsen TB, et al. Premature atrial complexes in an ischemic stroke
4
5 population and risk of recurrent stroke: a systematic review. *Expert Rev Cardiovasc*
6
7 *Ther* 2017;15(6):447-55. doi: 10.1080/14779072.2017.1332992 [published Online
8
9 First: 2017/05/24]
10
11
- 12 15. Pinho J, Braga CG, Rocha S, et al. Atrial ectopic activity in cryptogenic ischemic stroke
13
14 and TIA: a risk factor for recurrence. *J Stroke Cerebrovasc Dis* 2015;24(2):507-10.
15
16 doi: 10.1016/j.jstrokecerebrovasdis.2014.09.029
17
18
- 19 16. Adams HP, Jr., Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute
20
21 ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of
22
23 Org 10172 in Acute Stroke Treatment. *Stroke* 1993;24(1):35-41.
24
25
- 26 17. Clinical guidelines for stroke management 2010. *National Stroke Foundation Melbourne*
27
28 *Australia* 2010
29
30
- 31 18. Todo K, Moriwaki H, Saito K, et al. Frequent premature atrial contractions in stroke of
32
33 undetermined etiology. *Eur Neurol* 2009;61(5):285-8. doi: 10.1159/000206853
34
35
- 36 19. Engstrom G, Hedblad B, Juul-Moller S, et al. Cardiac arrhythmias and stroke: increased
37
38 risk in men with high frequency of atrial ectopic beats. *Stroke* 2000;31(12):2925-9.
39
- 40 20. Acharya T, Tringali S, Bhullar M, et al. Frequent Atrial Premature Complexes and Their
41
42 Association With Risk of Atrial Fibrillation. *Am J Cardiol* 2015;116(12):1852-7. doi:
43
44 10.1016/j.amjcard.2015.09.025 [published Online First: 2015/11/28]
45
46
- 47 21. Murakoshi N, Xu D, Sairenchi T, et al. Prognostic impact of supraventricular premature
48
49 complexes in community-based health checkups: the Ibaraki Prefectural Health Study.
50
51 *Eur Heart J* 2015;36(3):170-8. doi: 10.1093/eurheartj/ehu407 [published Online First:
52
53 2014/11/02]
54
55
56
57
58
59
60

- 1
2
3 22. Kamel H, Elkind MS, Bhave PD, et al. Paroxysmal supraventricular tachycardia and the
4 risk of ischemic stroke. *Stroke* 2013;44(6):1550-4. doi:
5
6 10.1161/STROKEAHA.113.001118 [published Online First: 2013/05/02]
7
8
9
10 23. Kistler PM, Sanders P, Fynn SP, et al. Electrophysiologic and electroanatomic changes in
11 the human atrium associated with age. *J Am Coll Cardiol* 2004;44(1):109-16. doi:
12
13 10.1016/j.jacc.2004.03.044
14
15
16
17 24. Medi C, Kalman JM, Spence SJ, et al. Atrial electrical and structural changes associated
18 with longstanding hypertension in humans: implications for the substrate for atrial
19 fibrillation. *J Cardiovasc Electrophysiol* 2011;22(12):1317-24. doi: 10.1111/j.1540-
20
21 8167.2011.02125.x
22
23
24
25
26 25. Oakes RS, Badger TJ, Kholmovski EG, et al. Detection and quantification of left atrial
27 structural remodeling with delayed-enhancement magnetic resonance imaging in
28 patients with atrial fibrillation. *Circulation* 2009;119(13):1758-67. doi:
29
30 10.1161/CIRCULATIONAHA.108.811877
31
32
33
34
35 26. Diez J. Mechanisms of cardiac fibrosis in hypertension. *J Clin Hypertens (Greenwich)*
36 2007;9(7):546-50.
37
38
39
40 27. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction
41 in hypertensive patients. *Circulation* 2001;104(2):191-6.
42
43
44
45 28. Yaghi S, Moon YP, Mora-McLaughlin C, et al. Left atrial enlargement and stroke
46 recurrence: the Northern Manhattan Stroke Study. *Stroke* 2015;46(6):1488-93. doi:
47
48 10.1161/STROKEAHA.115.008711
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18 **Figure Legend**

19
20
21 **Figure 1.**

22
23
24 Patient selection: inclusions and exclusions.

25
26
27 AF = atrial fibrillation; CABG = coronary artery bypass grafting; COPD = chronic
28 obstructive pulmonary disease; TIA = transient ischaemic attack;

29
30
31
32 **Figure 2.**

33
34
35 Median PACs by CHA₂DS₂VASc score for control and stroke/TIA groups.

36
37
38 CHA₂DS₂VASc = risk score for ischaemic stroke; TIA = transient ischaemic attack;

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18 **Tables**
19
20

21 **Table 1.** Baseline characteristics of study participants.
22
23

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

24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 ₂ DS ₂ VASc 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

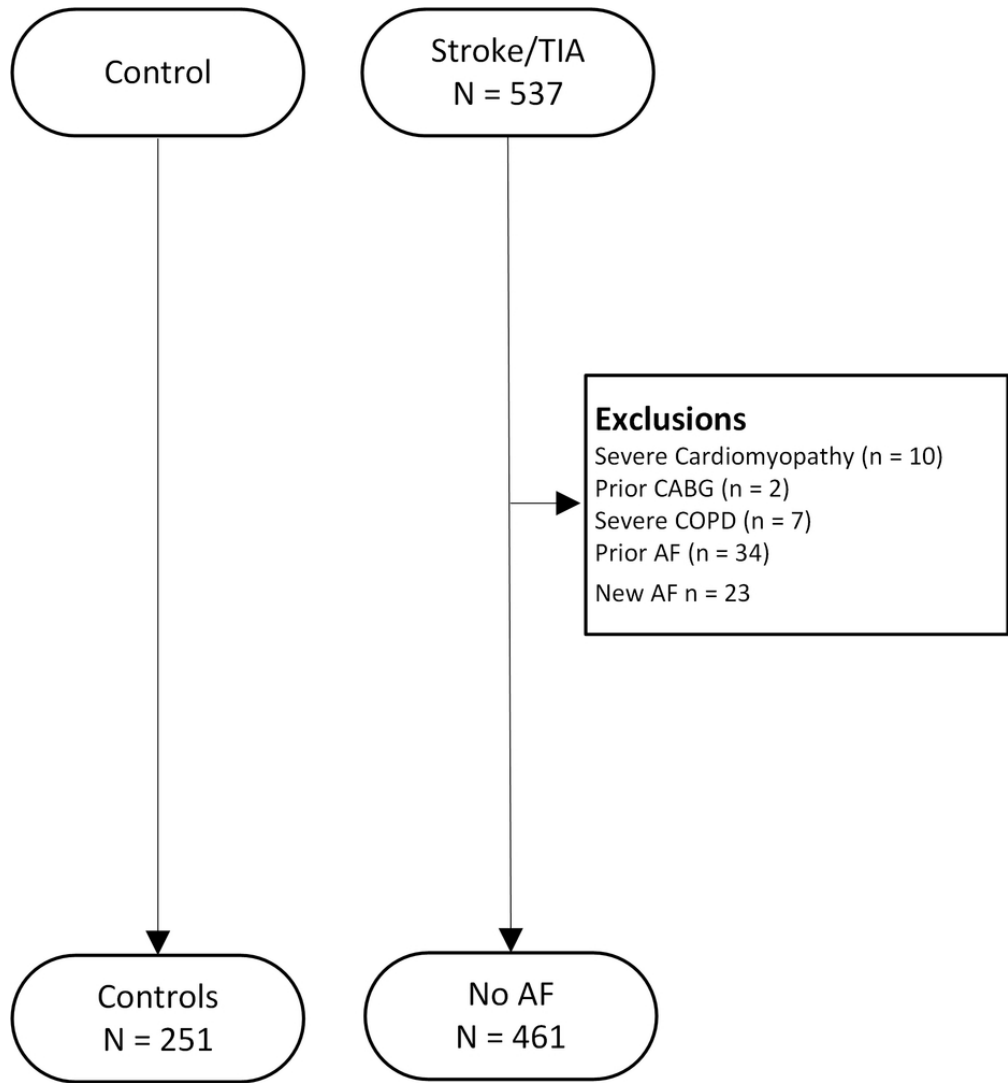
CI = confidence interval.

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

Author Statement

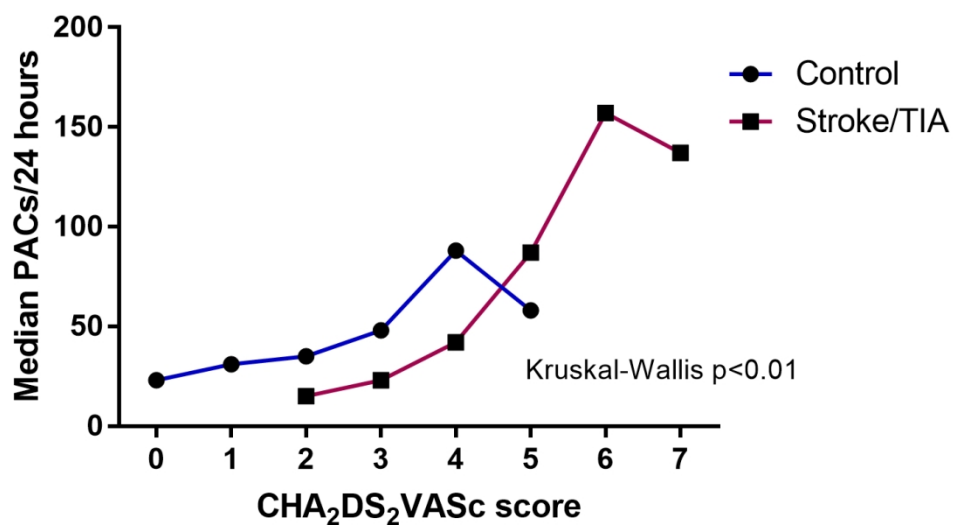
JS led and designed the study, collated and analysed data and wrote the manuscript. AK collated and analysed the data and revised the manuscript. HD contributed to study design and revised the manuscript. JK contributed to study design and revised the manuscript. KR collated and analysed the data and 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



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 CHA₂DS₂VASc score for control and stroke/TIA groups.
CHA₂DS₂VASc = 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