MONASH University

Atrial Myopathy: Analysing the Link to Ischaemic Stroke

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A thesis submitted for the degree of *Doctor of Philosophy.*

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Abbreviations

ESUS	Embolic Stroke of Undetermined Source
AF	Atrial Fibrillation
BMI	Body Mass Index
$PTFV_1$	P Wave Terminal Force V_1
PAC	Premature Atrial Complexes
CS	Cryptogenic Stroke
CMR	Cardiac Magnetic Resonance
ECG	Electrocardiography
LA	Left Atrium
LV	Left Ventricle
LAA	Left Atrial Appendage
LGE	Late Gadolinium Enhancement
BNP	Brain Natriuretic Peptide
CRP	C-Reactive Protein
hsTrop	High Sensitivity Troponin
hsCRP	High Sensitivity C-Reactive Protein
ANP	Atrial Natriuretic Peptide
PVI	Pulmonary Vein Isolation
ACE2	Angiotensin Converting Enzyme 2
EA	Electroanatomic

Thesis Abstract

Ischaemic stroke is a leading cause of mortality and disability in Australia. Despite attempts to identify a mechanism, up to one quarter of ischaemic strokes are labelled as an embolic stroke of undetermined source (ESUS). Cardiac thromboembolism is thought to contribute to a significant proportion of these strokes. Atrial fibrillation (AF), the most common cardiac arrhythmia, is well described in the pathogenesis of cardioembolic strokes. A significant proportion of patients with ESUS are thought to have occult AF resulting in embolic strokes.

AF is a well described marker of atrial myopathy, associated with concurrent atrial structural and functional abnormalities. Emerging evidence casts doubt on the hypothesis that cardioembolic strokes occur solely due to AF. Studies in patients with implantable cardiac devices showed a lack of temporal association between AF and stroke. Trials of oral anticoagulation in an unselected cohort with ESUS failed to derive benefit. There is an emerging need to identify novel reproducible cardiac markers to guide patient selection and inform treatment choices following ESUS.

In this thesis, we reviewed the existing literature and advanced the hypothesis for an atrial myopathy that could lead to cardioembolic strokes. Atrial myopathy is described by abnormal electrical, structural, biochemical and histological changes. We evaluated various diagnostic modalities to identify and assess plausible markers of atrial myopathy and ischaemic stroke. We also assessed the association with ischaemic stroke subtypes, including cryptogenic stroke and the recently defined entity labelled as ESUS.

Non-fibrillatory electrical markers were assessed. The association between P wave terminal force V_1 ($PTFV_1$), measured from a 12 lead ECG, and ischaemic stroke was characterised. Prior studies indicated an association between elevated $PTFV_1$ and ischaemic stroke. However, we demonstrated a significant reduction in reliability due to inter observer and inter ECG variability in measurements. Furthermore, assessment of electroanatomical atrial substrate failed to demonstrate a significant association between elevated $PTFV_1$ and abnormal atrial substrate.

We also evaluated non fibrillatory electrical markers on continuous ambulatory monitoring associated with ischaemic stroke. Our data indicated that excessive premature atrial complexes (PAC) quantified through 24-hour Holter monitoring were independently associated with ischaemic stroke and the subset with cryptogenic stroke. There was also a significant rise in PAC burden with increasing vascular risk factors.

The current evidence for novel consumer smart devices generating single lead ECGs for detection of AF to prevent ischaemic stroke was also evaluated. Significant limitations of the current generation of devices for population screening of AF was evident due to high false positive rates. This may result in healthcare resource over-utilisation due to further confirmatory testing. We also assessed the accuracy of a smartphone based single lead ECG platform for detecting AF and proposed a novel workflow for clinical utilisation that minimised secondary clinical overread while maintaining the accuracy of the diagnosis.

Finally, biochemical and structural markers of an atrial myopathy in ESUS were assessed by comparing a patient cohort with ESUS against a risk factor matched control group without AF or stroke. Angiotensin converting enzyme 2 (ACE2), an integral membrane protein associated with abnormal atrial electroanatomical substrate and fibrosis was assessed. We demonstrated a novel association between ACE2 activity and ESUS. Atrial volume and phasic function were also significantly different between the groups.

In conclusion, we assessed indicators of an atrial myopathy and ischaemic stroke utilising various diagnostic modalities, including 12 lead ECG's, Holter monitoring smart devices and biomarkers. We demonstrated a novel association between ESUS and a biomarker. The utility of these various markers of atrial myopathy for predicting future cardioembolic stroke and guiding therapy requires further assessment.

Publications & awards during candidature

Publications arising from this thesis: Published

1. Sajeev JK, Kalman JM, Dewey H, Cooke JC, Teh AW. The Atrium and Embolic Stroke: Myopathy Not Atrial Fibrillation as the Requisite Determinant? **JACC Clin Electrophysiol** 2020;6:251-261.

2. Sajeev JK, Koshy AN, Dewey H et al. Poor reliability of P-wave terminal force V1 in ischemic stroke. **J Electrocardiol** 2019;52:47-52.

3. Sajeev JK, Koshy AN, Dewey H et al. Association between excessive premature atrial complexes and cryptogenic stroke: results of a case-control study. **BMJ Open** 2019;9:e029164.

4. Sajeev JK, Koshy AN, Teh AW. Wearable devices for cardiac arrhythmia detection: a new contender? **Intern Med J** 2019;49:570-573.

5. Koshy AN, Sajeev JK, Negishi K et al. Accuracy of blinded clinician interpretation of single-lead smartphone electrocardiograms and a proposed clinical workflow. **Am Heart J** 2018;205:149-15

Publications arising from this thesis: under review

6. Sajeev JK, Wong GR, Kalman JM, Teh AW. et al. P-wave Terminal Force V_1 is not associated with abnormal electrophysiological substrate.
   Submitted to **J Electrocardiol**

7. Sajeev JK, Dewey H, Kalman JM, Burrell LM, Teh AW. et al. Angiotensin converting enzyme 2 activity is associated with embolic stroke of undetermined source.
Submitted to **Stroke**

Publications unrelated to the thesis

8. Rajakariar K, Koshy AN, Sajeev JK, Nair S, Roberts L, Teh AW. Accuracy of a smartwatch based single-lead electrocardiogram device in detection of atrial fibrillation. **Heart** 2020

9. Al-Kaisey AM, Koshy AN, Ha FJ et al. Accuracy of wrist-worn heart rate monitors for rate control assessment in atrial fibrillation. **Int J Cardiol** 2020;300:161-164.

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Abstracts arising from this thesis

1. Sajeev J, Burrell L, Dewey H et al. P5740 ACE2 activity level is associated with embolic stroke of undetermined source. Eur Heart J 2019;40:ehz746. 0680.
   (Oral presentation: ESC, Paris 2019)

2. Sajeev J, Burrell L, Dewey H et al. Elevated Plasma Angiotensin Converting Enzyme 2 (ACE2) Activity is Associated with Embolic Stroke of Undetermined Source. Heart, Lung and Circulation 2019;28:S296.
   (New investigator poster prize (Finalist), CSANZ, Perth 2019)

3. Sajeev J, Dewey H, Koshy AN et al. Excessive premature atrial complexes predict cryptogenic stroke. Int J Stroke; 2018:9-9.
   (Oral presentation: Stroke, Sydney 2018)

4. Koshy AN, Sajeev J, Rajakariar K et al. HIGH ATRIAL ECTOPY IN STROKE: AN INDEPENDENT RISK FACTOR? J Am Coll Cardiol 2017;69:338.
   (Oral presentation: ACC, Washington DC 2017)

5. MacPherson M, Sajeev J, Wong G et al. An Elevated P Wave Terminal Force V1 is not Associated with Worsening Atrial Electroanatomic Substrate. Heart, Lung and Circulation 2019;28:S201.

6. Sajeev J, Bhatia M, Dewey H et al. The Utility of P-Wave Terminal Force V1 is Limited by its Reliability. Heart, Lung and Circulation 2018;27:S199.

7. Koshy A, Sajeev J, Wong M et al. Proposed Workflow for the Utilisation of a Single-Lead Smartphone-Based Electrocardiogram in Clinical Practice. Heart, Lung and Circulation 2018;27:S187.

Awards during candidature

1. Finalist New Investigator Poster Prize–PhD candidate (CSANZ Perth, 2019)

2. Best of Emerging Researchers nomination, Monash Research Symposium, 2018

3. Royal Australian College of Physicians Research Prize (co-author) -(2017, 2019, Runner up 2018)

4. Henry Krum Memorial Research Prize 2017 (Co-first author)

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within Eastern Health and Eastern Health Clinical School, Monash University under the *primary supervision of Associate Professor Andrew W Teh and co supervised by Professor Helen Dewey and Professor Jonathan Kalman*

This thesis includes 5 original papers published in peer reviewed journals and 2 submitted publications. The core theme of the thesis is the association between markers of atrial dysfunction/myopathy and ischaemic stroke.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 1 - 7 my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status** *(published, in press, accepted or returned for revision, submitted)*	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author(s), Monash student Y/N***
1	The Atrium and Embolic Stroke. Myopathy Not Atrial Fibrillation as the Requisite Determinant?	Published	75%. Literature review, concept, drafting article and final approval	All authors provided input to manuscript through either data collection, manuscript review and editing totalling 25% of contribution 1. J.Kalman – 5% 2. H. Dewey – 5% 3. J.Cooke – 5% 4. A.Teh – 10%	No
2	Poor reliability of P-wave terminal force V1 in ischemic stroke	Published	70%. Literature review, concept, data collection, analysis,	All authors provided input to manuscript, through either data collection, manuscript review and editing totalling 30% of contribution	No

			manuscript writing.	1. H. Dewey – 2% 2. J. Kalman – 2% 3. A. Koshy – 6% 4. J. Cooke – 2% 5. A. Teh – 10% 6. M. Bhatia – 2% 7. L. Roberts – 2% 8. T. Frost – 2% 9. R. Denver – 2%	
3	P-wave Terminal Force V_1 is not associated with abnormal electrophysiological substrate	Submitted	60%. Literature review, concept, data collection, analysis, manuscript writing.	All authors provided input to manuscript, through either data collection, manuscript review and editing totalling 40% of contribution 1. G. Wong – 18% 2. M. Macpherson – 2% 3. A, Koshy – 5% 4. J. Kalman – 2% 5. H. Dewey – 2% 6. L. Roberts – 2% 7. A. Koshy – 2% 8. J. Cooke – 2% 9. A. Teh – 5%	No
4	Association between excessive premature atrial complexes and cryptogenic stroke: results of a case-control study.	Published	70%. Literature review, concept, data collection, analysis, manuscript writing.	All authors provided input to manuscript, through either data collection, manuscript review and editing totalling 30% of contribution 1. A.Koshy – 6% 2. K Rajakariar – 2% 3. M. Tan – 2% 4. M. Street– 2% 5. T. Frost– 2% 6. L. Roberts– 2% 7. M. Wong– 2% 8. H. Dewey– 5% 9. J. Kalman– 2% 10. A. Teh – 5%	No
5	Wearable devices for cardiac arrhythmia	Published	70%. Literature review,	All authors provided input to manuscript, through either	No

	detection: a new contender?		concept, drafting article.	manuscript review and editing totalling 30% of contribution 1. A. Koshy – 15% 2. A. Teh – 15%	
6	Accuracy of blinded clinician interpretation of single-lead smartphone electrocardiograms and a proposed clinical workflow	Published	50% Literature review, concept, data collection and analysis, drafting article and final approval	All authors provided input to manuscript, through either manuscript review and editing totalling 50% of contribution 1. A. Koshy – 26% 2. K. Negishi – 2% 3. M. Wong – 2% 4. A.Teh – 10% 5. J. Cooke – 2% 6. C. Pham – 2% 7. S. Cooray – 2% 8. Y. Khavar – 2% 9. L. Roberts – 2%	No
7	Angiotensin converting enzyme 2 activity is associated with embolic stroke of undetermined source.	Submitted	70%. concept, patient recruitment, data collection and analysis, drafting article and final approval	All authors provided input to manuscript, through either manuscript review, editing or tehcnical support totalling 30% of contribution 1. H. Dewey – 2% 2. J. Kalman – 2% 3. L. Burrell – 6% 4. J. Cooke – 2% 5. L. Roberts – 2% 6. S. Patel – 2% 7. M. Gould – 2% 8. J. Ngoh – 2% 9. A. Teh – 10%	No

**If no co-authors, leave fields blank*

I have not renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Jithin K Sajeev

Student signature: **Date:** 14/04/2020

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Andrew W Teh

Main Supervisor signature: **Date:** 14/04/2020

Acknowledgements

If I wrote “This thesis has been a result of the blood, sweat and tears poured into it”, I would have taken a liberal creative license. In reality, the last three years have been extremely rewarding, sufficiently productive and at times testing. I had some trepidation in becoming the first higher degree candidate through the department, a ‘guinea pig’ of sorts. The fact that it remained on track is a testament to the support, mentorship and opportunities I have been afforded by those that surrounded me.

The late Prof. Gishel New, was enthusiastic of my potential candidature through Eastern Health. I am grateful to her for this, as this instilled in me the necessary confidence. I am equally grateful to Dr. Jennifer Cooke, who I often turn to for advice. She has an aptitude for highlighting the realities of your choices and coming up with practical solutions for the problems. Once committed to doing a PhD, she ensured I was provided with every opportunity for success. Dr. Louise Roberts, research coordinator -extraordinaire, without whom I would have been lost navigating the necessary, but complicated red-tape that precedes research. To Dr. Bon Chou, thank you for the friendship and expertise you offered without condition. To Dr. Anoop Koshy, the countless hours on the phone (at times drawing the ire of our respective partners) perfecting ideas and pushing each other to stay on task has been invaluable.

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various presentations would not have been easy. I will forever be grateful for his patience and guidance; it has made me a better clinician and researcher. Over the past three years you have become a trusted mentor, colleague and friend.

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Chapter 1

The Atrium and Embolic Stroke

(A comprehensive review)

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STATE-OF-THE-ART REVIEW

The Atrium and Embolic Stroke

Myopathy Not Atrial Fibrillation as the Requisite Determinant?

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ABSTRACT

Atrial fibrillation (AF) is well-recognized in the pathophysiology of left atrial thrombogenesis and resultant cardioembolic stroke. Subclinical AF is believed to account for a significant proportion of embolic stroke. However, recent randomized control trials failed to demonstrate a significant benefit for oral anticoagulation, in an unselected population with embolic stroke of undetermined source. This has reinvigorated the focus on finding robust markers to identify patients at risk of cardioembolic stroke. Several nonfibrillatory atrial electrical markers, along with structural and biochemical abnormalities have been associated with ischemic stroke, independently of AF. An increasingly complex relationship exists among vascular risk factors, atrial remodeling, and thrombogenesis. Identifying robust markers of an underlying atrial myopathy may allow for early identification of patients at risk for cardioembolic stroke. This review outlines the inconsistencies in the evidence for AF as the prerequisite for left atrial thrombogenesis and embolic stroke. It will highlight the current evidence and controversies for adverse atrial remodeling, independent from rhythm, as a plausible mechanism for left atrial thrombogenesis and ischemic stroke. (J Am Coll Cardiol EP 2020;■:■–■) © 2020 by the American College of Cardiology Foundation.

Approximately 795,000 strokes occur every year in the United States with an associated cost of $33.9 billion/year (1). Atrial fibrillation (AF) is the most common cardiac arrhythmia and confers a significant risk for stroke (2–4). The commonly accepted mechanism for thromboembolism in AF alludes to blood stasis with resultant thrombus formation within the left atrial appendage (LAA) that lead to systemic embolization. In this context, the SPAF (Stroke Prevention in Atrial Fibrillation) trial investigators outlined the significant protective role of oral anticoagulation for stroke prevention in patients with AF (5).

However, one-third of all ischemic strokes are labeled cryptogenic or as an embolic stroke of undetermined source (ESUS), as no causative etiology is identified (6). Occult paroxysmal AF is believed to be the cause for a significant number of these strokes (7,8). This is an attractive hypothesis, as increasing rates of paroxysmal AF have been reported with prolonged ambulatory cardiac monitoring following episodes of cryptogenic stroke (7,9). Studies in patients with implantable cardiac devices with continuous monitoring, however, showed an apparent lack of temporal association between episodes of subclinical AF and subsequent strokes (10).

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the *JACC: Clinical Electrophysiology* author instructions page.

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ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

ANP = atrial natriuretic peptide

BNP = B-type natriuretic peptide

CMR = cardiac magnetic resonance

CRP = C-reactive protein

ECG = electrocardiography

ESUS = embolic stroke of undetermined source

Gal-3 = galectin 3

IL-6 = interleukin 6

LA = left atrium

LAA = left atrial appendage

PAC = premature atrial complexes

$PTFV_1$ = P-wave terminal force in lead V_1

TGFβ1 = transforming growth factor β1

vWF = von Willebrand factor

The TRENDS (A Prospective Study of the Clinical Significance of Atrial Arrhythmias Detected by Implanted Device Diagnostics) and ASSERT (Asymptomatic Atrial Fibrillation and Stroke Evaluation in Pacemaker Patients and the Atrial Fibrillation Reduction Atrial Pacing Trial) investigators demonstrated increased rates of thromboembolism in the presence of device detected atrial high rate episodes, but they also highlighted the temporal dissociation between these atrial tachyarrhythmias and stroke. Only 27.1% and 8% of the patients, in TRENDS and ASSERT, respectively, had an atrial high rate episode preceding their stroke (11,12).

Ischemic stroke is a heterogeneous group with multiple pathophysiological mechanisms for stroke apart from the embolization of atrial thrombus. A potential bias in the device detected subclinical AF and stroke studies has been the inclusion of patients with stroke who never had episodes of subclinical AF; this may have led to the dilution of the thromboembolic effect of AF on stroke. Meta-analysis of these studies after excluding patients that never had subclinical AF did not result in a significant change to the findings. Only 17% of ischemic strokes occurred during episodes of AF, and <30% of patients had an episode of subclinical AF in the 30 days preceding their stroke (13). These findings raise the possibility that transient AF after cryptogenic stroke might not represent causality, but rather an association.

Similarly, on continuous monitoring following a cryptogenic stroke, 30% of patients had AF detected over a 3-year period, but only 12% developed AF within the first year. Ischemic stroke and AF share common risk factors. The delayed presence of AF, identified in studies with prolonged cardiac monitoring following cryptogenic stroke, could represent the natural progression to AF in patients at risk, rather than a causative mechanism (**Central Illustration**).

Furthermore, it is well described that the risk for thrombogenesis is modulated by the presence of vascular risk factors. A CHA_2DS_2VASc (Congestive Heart Failure, Hypertension, Age ≥75 Years, Diabetes Mellitus, Prior Stroke or Transient Ischemic Attack or Thromboembolism, Vascular Disease, Age 65 to 74 Years, Sex) score of 0 confers minimal risk for thrombogenesis even with persistent AF. This indicates that AF by itself is unlikely to be the sole driver for LA thrombogenesis.

Strikingly, NAVIGATE ESUS (Rivaroxaban Versus Aspirin in Secondary Prevention of Stroke and Prevention of Systemic Embolism in Patients With Recent Embolic Stroke of Undetermined Source) and RE-SPECT ESUS (Dabigatran Etexilate for Secondary Stroke Prevention in Patients With Embolic Stroke of Undetermined Source), 2 recently concluded large randomized controlled trials that assessed the utility of oral anticoagulation compared with aspirin for stroke prevention following ESUS, failed to show benefit for oral anticoagulation (14,15). Whereas a broad oral anticoagulation strategy in all-comer patients with ESUS appears futile, a nuanced approach guided by reproducible markers of abnormal atrial substrate and thrombogenesis may yield clinical benefit. An exploratory analysis of the studies revealed a reduction in stroke recurrence with oral anticoagulation in patients with an enlarged left atrium (16). Randomized control trials utilizing markers of cardiac remodeling are currently underway and may provide further insight into the utility of oral anticoagulation (17).

HIGHLIGHTS

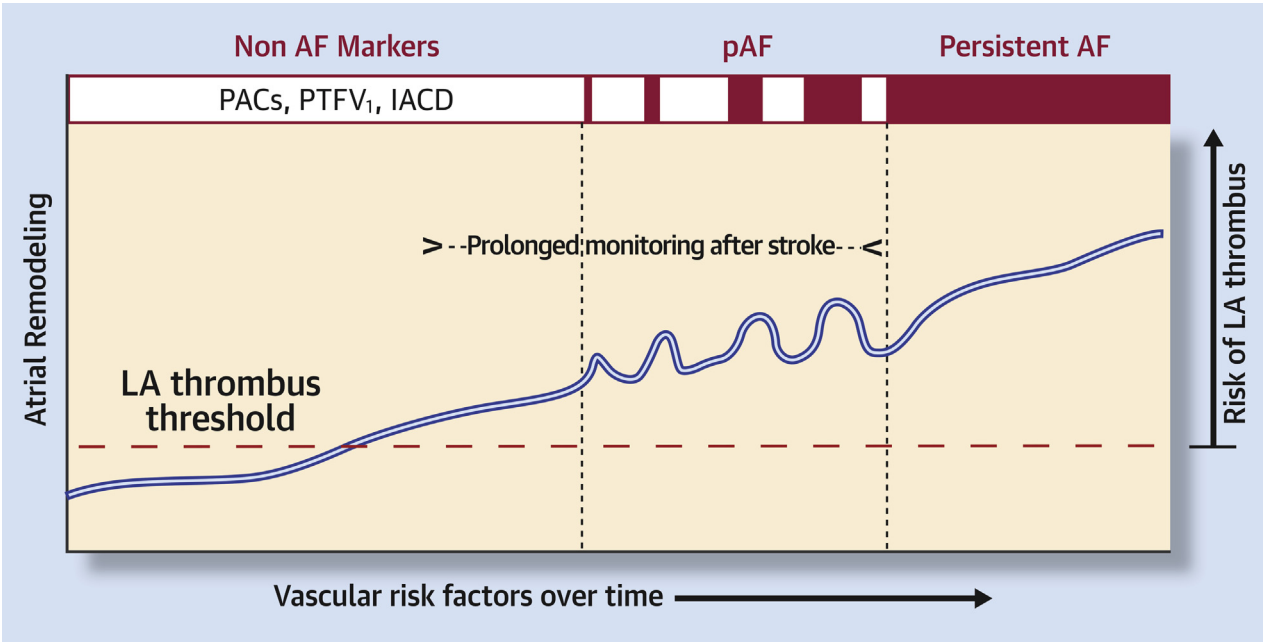
- AF is associated with LA thrombogenesis and cardioembolic stroke.
- Adverse atrial remodeling may lead to cardioembolic stroke even in the absence of AF.
- Novel markers of abnormal atrial substrate are associated with ischemic stroke.
- Robust nonfibrillatory markers of thrombogenesis may allow for early identification of at-risk patients.

There is a reinvigorated search for robust clinical markers to aid in patient selection and stroke prevention. This is based on the premise of an atrial cardiomyopathy that may contribute to thrombogenesis irrespective of atrial rhythm. The consensus working group on atrial cardiomyopathy has recently defined it as a complex of structural, architectural, contractile, or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations (18).

ATRIAL STRUCTURAL REMODELING AND DYSFUNCTION

Atrial structural remodeling and dysfunction often occur in the presence of atrial arrhythmias (**Figure 1**). Animal models have demonstrated that sustained rapid atrial pacing can induce atrial fibrosis, and,

CENTRAL ILLUSTRATION **Complex Interplay Among Vascular Risk Factors, Atrial Remodeling, ECG Markers, and Risk of LA Thrombogenesis**



Sajeev, J.K. et al. J Am Coll Cardiol EP. 2020;■(■):■–■.

Progressive atrial remodeling and vascular risk factors are associated with atrial electrical abnormalities and lead to increasing risk of left atrial (LA) thrombogenesis. Atrial fibrillation (AF) is associated with acceleration of atrial remodeling and LA thrombogenesis. Periods of paroxysmal atrial fibrillation (pAF) are associated with transient rise in risk of thrombogenesis. Longer term cardiac monitoring studies in stroke are likely to represent the pathological progression of atrial disease that results in AF. ECG = electrocardiography; IACD = interatrial conduction delay; PAC = premature atrial complexes; $PTFV_1$ = P-wave terminal force in lead V_1.

conversely, restoration of sinus rhythm following catheter ablation for AF results in the reduction of LA size. This is consistent with reverse atrial remodeling, although the precise mechanism is yet to be elucidated (19).

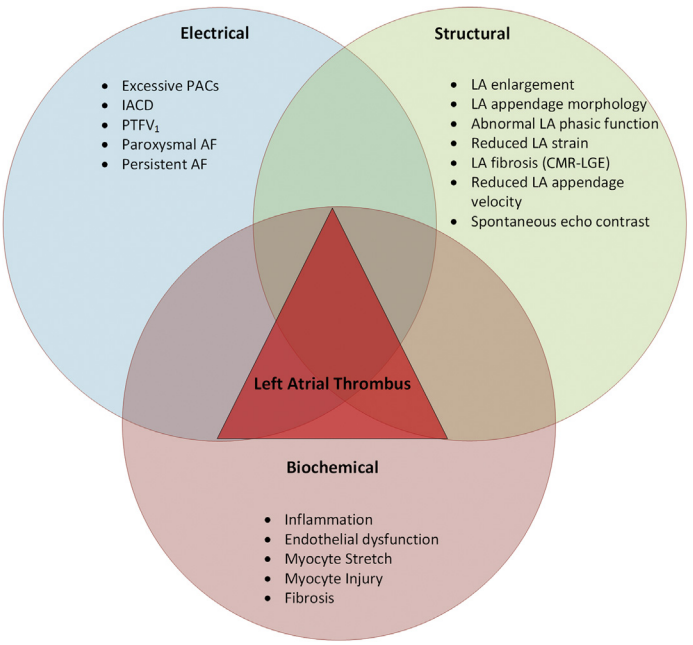
Increased atrial diameter has also been associated with an elevated risk for incident AF and AF recurrence following cardioversion (20,21). Multimodality imaging with cardiac computed tomography and cardiac magnetic resonance (CMR) have corroborated these findings by demonstrating a significant association among LA area, fibrosis, and AF (22,23). Areas of LA fibrosis denoted by late gadolinium enhancement have been associated with reduced atrial conduction velocities in patients with AF. This highlights the relationship between the underlying atrial substrate abnormalities and atrial arrhythmias (24). The relationship between atrial fibrosis and atrial arrhythmias is also evident from the blunted response to catheter ablation in patients with AF and increased fibrosis demonstrated by late gadolinium enhancement (25,26).

Structural abnormalities also occur in patients without demonstrable risk factors for the development of AF, the group designated as "lone AF" (27). Histological analysis in lone AF, demonstrated patchy fibrosis and inflammatory infiltrates that may propagate AF (28). Histological evaluation of atrial tissue also revealed progressive fibrosis in persistent AF compared with in paroxysmal AF (29).

In patients with persistent AF, the extent of fibrosis was not associated with duration of AF (30). However, the lack of vascular risk factors and the relatively young age of the groups, may have led to a reduction in the degree of adverse remodeling in this study.

Atrial structural abnormalities also occur as a result of sustained changes in atrial pressure and are associated with concurrent functional abnormalities. LA function has 3 distinct phases—reservoir, conduit, and contractile function. A reduction in atrial contractile function has been associated with increased incidence of paroxysmal AF (31). Reduced LA reservoir strain predicted subsequent development of AF

FIGURE 1 Markers of Adverse Atrial Remodeling



Markers of adverse atrial remodeling associated with stroke on multimodality investigations. AF = atrial fibrillation; CMR-LGE = cardiac magnetic resonance—late gadolinium enhancement; IACD = interatrial conduction delay; LA = left atrial; PAC = premature atrial complexes.

following cryptogenic stroke (32,33). These studies also revealed an inverse relationship between LA volume and LA strain, highlighting the close association between atrial function and structure.

However, LA functional and structural abnormalities have been described, in the absence of AF. Reversion to sinus rhythm from AF, either following cardioversion or catheter ablation, results in residual LA functional impairment. Spontaneous echocardiographic contrast, indicative of high risk for thromboembolic outcomes, persists for up to 3 months following cardioversion (34). Even in sinus rhythm, spontaneous echocardiographic contrast is associated with an enlarged LA in patients with stroke (35). LA enlargement, in turn, is associated with a graded rise in risk for stroke and an increased risk for recurrence of cryptogenic stroke (36–38).

Reduced reservoir function on atrial strain imaging may represent early atrial fibrosis and may provide incremental discriminatory value in identifying patients with cryptogenic stroke (39). Assessment of atrial fibrosis by late gadolinium enhancement on CMR in patients with AF demonstrated increased rate of major adverse cardiovascular event in those with marked LA fibrosis. Moreover, the increase in major adverse cardiovascular events was primarily driven by higher rates of stroke and transient ischemic attack (40). Even in patients with ESUS and no AF, there were comparable atrial fibrosis rates as for patients with AF (41). Importantly, both these groups had more incidents of atrial fibrosis than did the control group with prior stroke or AF. This may point to a greater propensity for atrial thrombogenesis in ESUS, similar to that for patients with AF. Increasing cardiac fibrosis has been associated with rising age and female sex on regression analysis, independently of AF (23). It is plausible that risk factors such as advanced age and hypertension included in thromboembolic risk prediction models may also be relevant to those without AF (**Central Illustration**).

In patients with no history of cardiovascular disease or AF, the prevalence of atrial fibrosis demonstrated by delayed enhancement on CMR was higher than expected at approximately 9% to 12% (42,43). However, the evidence for an association between specific vascular risk factors and fibrosis remains inconsistent. Cochet et al. (42) demonstrated an association between increasing age and the extent of fibrosis in a non-AF cohort. A similar association was not evident in studies by Marrouche et al. (26) and Siebermair et al. (43), although an elevated body mass index was associated with greater extent of fibrosis.

The histological evidence for an association between vascular risk factors and atrial structural remodeling remains limited. Animal studies have shown histological evidence for fibrosis associated with aging, hypertension, diabetes mellitus, and obesity (18). Conversely, histological analysis did not demonstrate an association between fibrosis and age in humans (29). Whereas there is also evidence for increased collagen deposition within the LA in conjunction with mitral valve disease, demonstrable histological correlation with vascular risk factors is lacking (18).

LEFT ATRIAL APPENDAGE. Apart from generalized LA characteristics, the LAA has separately been implicated in the pathogenesis of thrombus formation and embolic stroke. The LAA is lined by rough trabeculated endocardium; it aids in phasic LA function and secretes atrial natriuretic peptide in response to wall stretch (44,45). Multimodality cardiac imaging has outlined the predominant morphologic variations of the LAA. The "cauliflower" morphology was associated with an 8-fold increase in risk for stroke or transient ischemic attack, independent of the AF (46). Irrespective of the appendage morphology, LAA emptying velocity of <20 cm/s and

TABLE 1 Summary of Biomarkers and Evidence for Association With AF and Stroke

Biomarker	Description of Marker	AF (Ref.#)	Stroke (Ref.#)
Myocardial stretch			
BNP	Natriuretic peptide	↗ (71,72)	↗ (73)
ANP	Natriuretic peptide	↗ (70)	↗ (74)
Myocardial injury			
Cardiac troponin	Troponin subunits that regulate myocyte contraction	↗ (72)	↗ (73)
Inflammation			
IL-6	Proinflammatory cytokine	↗ (75,82)	↗ (83)
CRP	Inflammatory acute phase protein	↗ (76,77,82)	↗ (83,84)
Fibrosis			
TGFβ1	Signaling molecule secreted by various inflammatory cells that promote collagen deposition	↗ (91)	↗ (107)
Gal-3	β-galactoside binding lectin that recruits fibroblasts	↗ (92)	↗ (108)
FGF-23	Phosphate regulating fibroblast growth factor	↗ (109)	X (110,111)
Endothelial dysfunction			
ADMA	Competitive nitric oxide synthase inhibitor	↗ (95)	↗ (95)
vWF	Prothrombotic multimeric glycoprotein	↗ (96)	↗ (99,100)
Hypercoagulability			
D-dimer	Fibrin degradation product	↗ (104)	↗ (100,106)
Fibrinogen	Hemostatic glycoprotein precursor to fibrin	↗ (104)	↗ (83)

ADMA = asymmetric dimethylarginine; AF = atrial fibrillation; ANP = atrial natriuretic peptide; BNP = B-type natriuretic peptide; C-reactive protein; FGF-23 = fibroblast growth factor 23; Gal-3 = galectin-3; IL-6 = interleukin 6; TGF-β1 = transforming growth factor β1; vWF = von Willebrand factor.

reduced contraction are important predictors for ischemic stroke (47).

AF is associated with marked reduction in appendage emptying velocity. This promotes further stasis within a heavily trabeculated structure, leading to accelerated thrombus formation (**Central Illustration**). Similar to other areas of the atrium, electro-anatomical substrate abnormalities are also evident within the LAA in patients with AF, whereas increased fibrosis on CMR also correlates with presence of thrombus and spontaneous echocardiographic contrast (48,49).

Even in the absence of AF, the LAA is associated with a higher incidence of thrombus formation with reduced emptying velocities, which highlights the propensity for thrombus formation within the LAA irrespective of the atrial rhythm (50).

Atrial structural remodeling and functional abnormalities predispose to thromboembolism that occur in the absence of AF in a subset of patients.

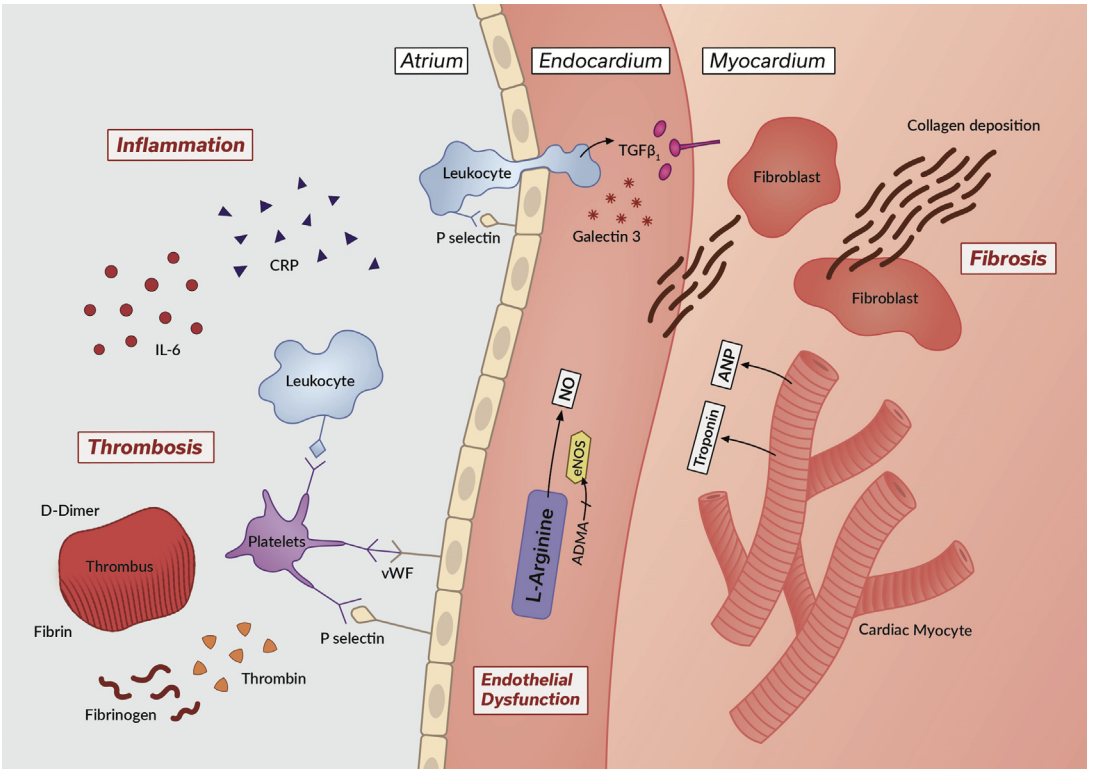
ELECTROCARDIOGRAPHIC MARKERS OF ATRIAL REMODELING

Besides AF, several markers on electrocardiography (ECG) and on continuous monitoring have been associated with stroke. In particular, an excessive burden of premature atrial complexes (PAC) has been shown to increase the risk for stroke (51-53). An excessive burden of PAC has also been associated with increased incidence of AF, and it is plausible that subclinical AF may account for the excessive risk for stroke in patients with excessive PAC burden. However, cohort studies have demonstrated this risk for stroke to be independent of AF (51). Larsen et al. (51) showed a stepwise increase in risk for stroke with increasing CHA_2DS_2VASc score in the presence of excessive PAC. With a CHA_2DS_2VASc score of 2 or more, both excessive PAC and AF conferred similar risk for stroke, again highlighting the importance of vascular risk factors in modulating risk for thromboembolism. Frequent PAC were also associated with adverse atrial structural remodeling and phasic function, with increased LA volume index and a reduced LA peak contractile strain (54).

In the absence of AF, various ECG markers, including P-wave terminal force in lead V_1 ($PTFV_1$) and interatrial conduction delay, have been associated with ischemic stroke in large cohort studies (55-59). Similar to PAC, they are also associated with increased LA volume and reduced phasic function (60). However, in patients with AF, there was no increase in LA fibrosis on CMR or a significant gradient effect between worsening $PTFV_1$ measurements and various electrophysiological indices for abnormal LA substrate (61,62). Furthermore, the association among these electrical markers, AF, and stroke appear discordant.

A significant association was noted between an elevated $PTFV_1$ and AF in the ARIC (Atherosclerosis Risk in Communities) study, but this was not evident

FIGURE 2 Proposed Biochemical Process Underpinning Atrial Adverse Remodeling and Thrombus Formation With the Associated Abnormalities



Inflammation-driven leukocyte recruitment and endothelial dysfunction is associated with platelet adhesion and the activation of the coagulation cascade with resultant thrombus formation. Atrial stretch and myocardial injury with resultant release of atrial natriuretic peptide (ANP) and troponin are associated with fibroblast-mediated collagen deposition, which promotes subendocardial and myocardial fibrosis.
ADMA = asymmetric dimethylarginine; CRP = C-reactive protein; eNOS = nitric oxide synthase; IL-6 = interleukin 6; NO = nitric oxide; TGF-β1 = transforming growth factor β1; vWF = von Willebrand factor.

in the Framingham Heart Study (63). Similarly, 2 large cohort studies, NOMAS (Northern Manhattan Study) (55) and MESA (Multi-Ethnic Study of Atherosclerosis) (56), demonstrated an association between $PTFV_1$ and ischemic stroke, but this was absent in a large Finnish cohort study (64). These discordant findings could partly stem from the lack of a standardized methodology to measure $PTFV_1$, thereby reducing the reproducibility of these markers (59,65). Electrocardiographic parameters such as an elevated PAC burden and $PTFV_1$ may serve as markers to identify patients at risk for stroke, but their clinical utility is currently limited by insufficient reliability and the discordant outcomes data. Pre-fibrillatory ECG markers are likely early indicators of such adverse atrial electrical remodeling. However, manifestation of AF may lead to further acceleration of atrial remodeling with resultant increase in risk for thrombogenesis.

ELECTROANATOMIC MARKERS OF ATRIAL REMODELING

Vascular risk factors that promote cardioembolic stroke likely lead to progressive atrial remodeling with multiple electrical abnormalities that ultimately progress to AF. Comparison of atrial electroanatomic properties between patients in sinus rhythm and AF demonstrated increased proportion of low voltage and slowed conduction in AF (66). These changes were more pronounced in persistent AF than in paroxysmal AF, highlighting the progressive nature of atrial remodeling.

Similarly to AF, patients with conventional thromboembolic risk factors in sinus rhythm show evidence of adverse atrial remodeling. Increasing age and hypertension has been associated with prolonged atrial conduction times and diffuse areas of low voltage with regional conduction slowing as

demonstrated by electrophysiological studies (67,68). These findings highlight the effects of vascular risk factors on adverse atrial remodeling beyond AF and allude to the presence of an atrial myopathy as illustrated in the **Central Illustration**. It is possible these changes alone may provide the substrate for the propagation of atrial thrombogenesis in the absence of AF.

However, atrial structural and electrophysiological remodeling is unlikely to promote thromboembolism without a concurrent biochemical prothrombotic milieu (**Figure 1**).

ATRIAL BIOCHEMICAL CHANGES

Numerous biomarkers have been described in relation to altered myocyte stress and injury, inflammation, fibrosis, endothelial dysfunction, and hypercoagulability in both AF and stroke as described in **Table 1**. The cardiac effects of these biomarkers are depicted in **Figure 2**.

MYOCYTE STRESS AND INJURY. Cardiac myocytes secrete various natriuretic peptides with both autocrine and paracrine function in response to altered hemodynamic states with resultant myocardial stretch. Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are secreted predominantly by the atrial and ventricular myocytes, respectively. However, increased ANP has also been associated with electrophysiological changes with reduced intracellular calcium and a shortened action potential duration, which may predispose to AF (69). The significantly higher incidence of AF in patients with an elevated N-terminal-pro-BNP and ANP at baseline appear to corroborate these biochemical observations (70,71). Similarly, troponin, a marker of myocyte injury was detectable in up to 73% of patients with AF in the ARISTOTLE (Apixaban for the Prevention of Stroke in Subjects With Atrial Fibrillation) biomarker study.

The elevation of natriuretic peptides and troponin in patients with AF has also been associated with increased risk for ischemic stroke. BNP and troponin, respectively, conferred a 12-fold and 3-fold increase in hazard ratio for cardioembolic stroke subtype (72,73). Similarly, midregional pro-ANP has also been independently associated with the cardioembolic stroke subtype (74).

Importantly, even after adjusting for the presence of AF, natriuretic peptides and troponin were significantly associated with ischemic stroke (73). Therefore, the association between ischemic stroke and these biomarkers may go beyond mere response to atrial dysrhythmia and represent a diffuse myopathic process within the atria.

INFLAMMATION AND FIBROSIS. There is substantial evidence that implicates inflammation in both the development and perpetuation of atrial arrhythmias and stroke. C-reactive protein (CRP) and interleukin 6 (IL-6) are well-recognized markers of systemic oxidative stress and inflammation and have been associated with both AF and ischemic stroke (75,76). An exploratory analysis of the JUPITER (Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin) primary prevention study in the use of rosuvastatin demonstrated increasing levels of high-sensitivity CRP was associated with increasing incidence of AF (77). Intracardiac blood samples from patients with AF also demonstrate sequestration of inflammatory cytokines within the cardiac chambers, with a peripheral blood to coronary sinus gradient (78).

Furthermore, electro-anatomical mapping studies in patients with AF have shown reduced mean LA voltages in association with an elevated CRP. Patients with both elevated CRP and IL-6 also have a significantly larger left atrium (79). Following catheter ablation of AF, there was a drop in both CRP and IL-6; however, these levels returned to baseline over a 12-month period and were associated with increased recurrence (80). This is reflective of the potentiating effect of inflammation on AF. Colchicine, a potent anti-inflammatory agent, has been used successfully to reduce AF recurrence following pulmonary vein isolation and cardiac surgery (81). Importantly, this effect occurred in conjunction with reduction in the levels of both CRP and IL-6 (81). Meta-analysis of the available published reports has consolidated these findings and shown that both CRP and IL-6 are associated with greater risk for incident AF and AF recurrence (82).

Inflammation has also been associated with both ischemic stroke and recurrence of ischemic stroke (83). A meta-analysis of 9 prospective studies showed a linear association between CRP levels and the incidence of ischemic stroke (84). The association between inflammation and incidence of ischemic stroke remains significant even after accounting for history of AF and conventional vascular risk factors (85,86).

Inflammation and markers of myocyte stretch have been associated with changes to the extracellular matrix and fibrosis in patients with AF (80,87). Atrial fibrosis contributes to heterogeneous atrial impulse propagation and provides the substrate for re-entrant arrhythmia and perpetuation of AF. Histological samples from patients undergoing surgical maze

procedures for AF have revealed reduced collagen volume in patients that maintained sinus rhythm following the procedure (87). An increase in total collagen volume results from abnormal deposition of extracellular matrix by cardiac fibroblasts. Several serological biomarkers have been implicated with cardiac fibrosis.

Transforming growth factor β1 (TGFβ1), is a signaling molecule that initiates a cascade that results in increased fibrogenesis and collagen deposition and is potentiated by inflammation (88). A transgenic goat model with overexpression of TGFβ1 demonstrated a significant increase in atrial fibrosis and myocyte diameter with concurrent increase in P-wave duration, indicating conduction slowing within the atria and susceptibility to AF (89). TFGβ1 was also associated with stroke in the presence of inflammation (90). Histological studies in humans have shown a stepwise increase in TGFβ1 messenger ribonucleic acid and collagen content from right atrial appendage biopsies in patients with sinus rhythm, paroxysmal AF, and chronic permanent AF, respectively (91).

Similarly, Galectin 3 (Gal-3) a β-galactoside binding lectin, has also been implicated in cardiac fibrosis through its role in recruitment of macrophages and fibroblasts. Gal-3 is an independent predictor of AF, conversely an increased LA volume index was independently predictive of Gal-3 (92). Both TGFβ1 and Gal-3 are independently associated with LA fibrosis, and delayed enhancement CMR in patients with AF appear to support these findings (93).

It is unclear whether the association between ischemic stroke and abnormal levels of inflammatory and fibrotic markers are confounded by unrecognized associations with noncardioembolic stroke subtypes. Given the shared risk factor profile between varying stroke subtypes, it is certainly plausible. Alternatively, these markers may highlight or even directly promote underlying atrial dysfunction and arrhythmias, with subsequent atrial thrombogenesis and vascular embolization.

ENDOTHELIAL DYSFUNCTION AND HYPERCOAGULABILITY. Endothelial dysfunction and hypercoagulability are associated with stroke in patients with and without AF. Asymmetric dimethylarginine is a competitive inhibitor of nitric oxide synthase and is associated with oxidative stress and endothelial dysfunction (94). Asymmetric dimethylarginine is associated with a significant risk for AF and ischemic stroke (95). Similarly, von Willebrand factor (vWF), a prothrombotic multimeric glycoprotein, is secreted from endothelium and platelets. vWF is significantly higher in patients with both paroxysmal and persistent AF compared with a control group and correlate with a larger LA area (96). vWF is also overexpressed in overloaded atrial appendices, irrespective of the presence of AF and is an independent predictor for LAA thrombus (97,98). Two large prospective studies that monitored patients for subsequent stroke showed a gradient effect with increasing risk of ischemic stroke with increasing vWF levels, independent of AF (99,100). Importantly, these biochemical changes are also present in patients in sinus rhythm with conventional risk factors for thromboembolism. Advancing age, prior cerebral ischemia, heart failure, and diabetes are all independently associated with raised plasma vWF. This iterates the impact of vascular risk factors on endothelial dysfunction and provides the bridge to thrombogenesis (101).

Hemostatic markers that signal the presence of a prothrombotic state, such as D-dimer, fibrinogen, and thrombin-antithrombin complexes, have been associated with both AF and ischemic stroke (102,103). D-dimer appears to show a gradient effect in relation to the burden of AF, whereby patients with persistent AF have higher levels of D-dimer compared with paroxysmal AF, which in turn is higher than in patients without AF (104). During AF, oral anticoagulation reduces the levels of thrombogenic biomarkers such as D-dimer; however, the underlying inflammatory states persists. Furthermore, the presence of these markers is unlikely to be driven solely by a high atrial rate. Whereas markers of endothelial dysfunction and inflammation rise significantly with the onset of AF, a similar rise is not evident following rapid atrial pacing. These findings support the presence of a proinflammatory state in AF with subsequent endothelial dysfunction and thrombogenesis (105). However, even after adjusting for AF, elevated prothrombotic markers remain a significant risk factor for major stroke (100,106).

SUMMARY

The risk of atrial thrombogenesis during AF is well-characterized. It is associated with adverse atrial substrate and biochemical abnormalities. Conversely, even in the absence of AF, atrial remodeling may lead to LA thrombogenesis. Abnormal electrocardiographic and echocardiographic indices, along with biomarkers that reflect underlying inflammation, fibrosis, and endothelial dysfunction, confer an elevated risk for ischemic stroke in patients with and without AF. The evidence is increasingly suggestive of a complex relationship among vascular risk factors, atrial remodeling, and a biochemical prothrombotic milieu that contributes to cardioembolic stroke.

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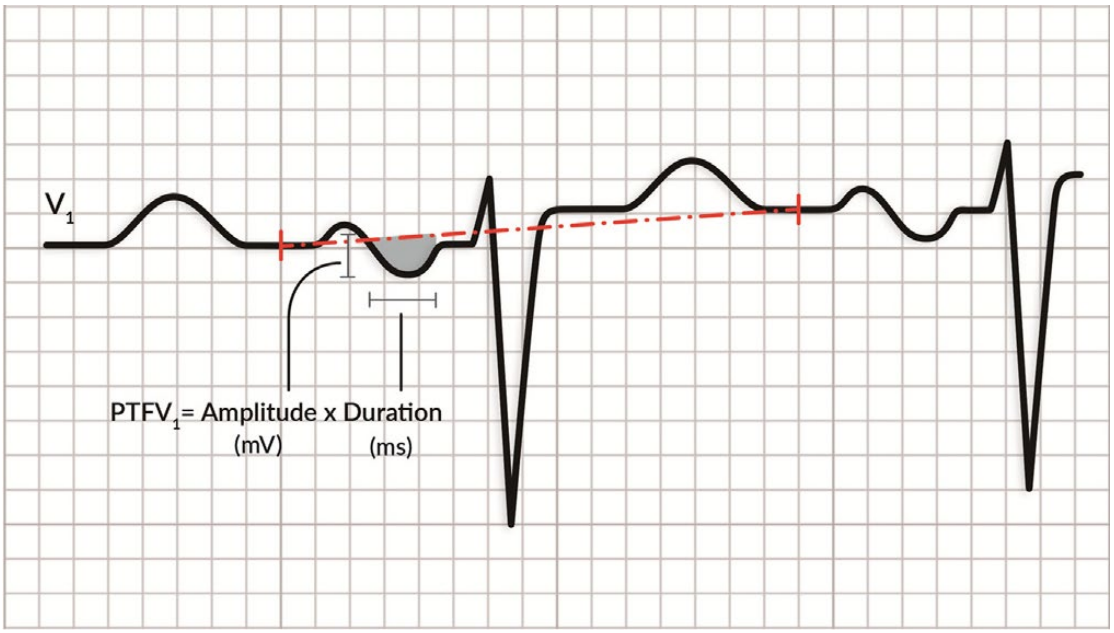
KEY WORDS **atrium, ESUS, myopathy, stroke, thrombogenesis**

P Wave Terminal Force V_1

A 12 lead ECG is readily available and is ubiquitous for cardiac rhythm analysis and assessment. In relation to atrial thrombogenesis and embolic stroke, the focus has primarily been on detecting AF. A 12 lead ECG provides a point in time assessment of the cardiac rhythm. Its utility in detecting intermitted arrhythmias such as paroxysmal AF is limited. Therefore, markers that can be measured from a 12 lead ECG maybe more reproducible and readily measured compared with identifying occult AF. These ECG markers could significantly improve risk assessment for patients at risk for cardioembolic strokes.

Several ECG markers of an atrial myopathy have been described and are thought to identify patients at risk for stroke and atrial fibrillation (1-3). The $PTFV_1$ is such a marker measured from the P wave in lead V_1. It is calculated by identifying the baseline represented by the straight line across the TP segment. The amplitude was measured from the nadir of the P wave till the point of intersection with the baseline as shown in the figure.



It has been associated with concurrent atrial structural and functional changes (4). Left atrial pressure during cardiac catheterisation was demonstrated to be associated with an elevated $PTFV_1$ (4). Similarly, it was associated with left atrial enlargement on echocardiography. Studies have also explored the relationship between $PTFV_1$ and clinical outcomes such as atrial fibrillation and ischaemic stroke. However there remains a clinical equipoise, as an elevated $PTFV_1$ was associated with cerebrovascular events in some large longitudinal studies, but not others (1, 2, 7).

Despite the clinical associations described previously, the reliability of the $PTFV_1$ measurement is not well characterised. P wave morphological changes and significant dispersion has been previously described on ECGs done at different time points and could affect the reliability of a marker such as $PTFV_1$ (6). Given the association between elevated $PTFV_1$ and echocardiographic measures of left atrial pressure and left atrial enlargement, it is plausible that day to day changes in circulating volume, autonomic tone and the individual patient's susceptibility to these changes may also contribute to varying $PTFV_1$ measurements in an individual. Further, measurement of the terminal portion of a P wave can often be difficult due to the low amplitude of the signal, which can also lead to reduced reliability. The validity and clinical reliability of $PTFV_1$ requires further characterisation, as there are current multicentre trials underway assessing the utility of oral anticoagulation in ESUS patients with markers of atrial myopathy, that includes $PTFV_1$ (5).

Despite the association with ischaemic stroke and atrial size, it is unclear if $PTFV_1$ is associated with abnormal atrial substrate. A prior study failed to show an association

between $PTFV_1$, and atrial fibrosis as assessed by late gadolinium enhancement on cardiac MRI in patients with AF (7). Despite $PTFV_1$ being an electrical marker, its association with abnormal electroanatomic substrate is also unknown.

The validity and reliability of $PTFV_1$ measurements, its electroanatomic pathophysiologic basis and association with ischaemic stroke was assessed in the next two chapters.

In chapter 2, we evaluated the reliability and reproducibility of measuring $PTFV_1$ and its association with ischaemic stroke using an age matched control group.

In chapter 3, the association between an elevated $PTFV_1$ and left atrial electroanatomic substrate was assessed. Patients in sinus rhythm but with a history of AF undergoing pulmonary vein isolation were included. Electrophysiological indices of atrial substrate were assessed prior to ablation and correlated with $PTFV_1$.

Chapter 2

Poor reliability of P-wave terminal force V1 in ischaemic stroke.

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Poor reliability of P-wave terminal force V_1 in ischemic stroke

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ABSTRACT

Introduction: Several ECG markers are postulated to represent underlying atrial remodelling and have been associated with ischemic stroke. P-wave terminal force in lead V_1 ($PTFV_1$) is one such marker. We examined the factors that contribute to the reliability of $PTFV_1$ and its association with ischemic stroke.

Material and methods: Four hundred and thirty-five patients that presented with an ischemic stroke or transient ischemic attack (TIA) were identified through a prospectively maintained multi-site institutional stroke database. Control group consisted of age matched patients without prior history of an ischemic stroke or TIA. All patients underwent a 12-lead ECG and 24-hour Holter monitoring during the study period to exclude atrial fibrillation.

Results: Morphology consistent with $PTFV_1$ occurred commonly in both the stroke/TIA and control groups. There was no significant difference in the median $PTFV_1$ value between the stroke 3.96 mV ms [Interquartile range (IQR) 2.78–5.58] and control 4.23 mV ms [IQR 2.91–5.57] groups. Measurements of $PTFV_1$ demonstrated excellent intra-observer reliability on assessment of the same P-wave (Intra class correlation (ICC) 0.91, $p < 0.001$) with narrow limits of agreement 2.21 to −2.95 mV ms. A change in the P wave assessed led to a significant reduction in reliability (ICC 0.79, $p < 0.001$). Inter-observer, inter P-wave assessment demonstrated further reduction in reliability (ICC 0.68, $p < 0.002$) with wide limits of agreement 6.17 to −5.78 mV ms, indicating significant under and overestimation of $PTFV_1$.

Conclusion: The utility of $PTFV_1$ as a clinical marker for ischemic stroke is limited by the reduction in reliability associated with inter-observer and inter P-wave measurements.

Introduction

Ischemic strokes result in significant morbidity, loss of function and death. The incidence of ischemic strokes is projected to rise in view of the ageing population [1]. Atrial fibrillation (AF) is widely believed to be the cause for a significant proportion of ischemic strokes [2]. The presence of an atrial myopathy has been postulated in patients with ischemic stroke independent of atrial fibrillation. Electrophysiological and biochemical abnormalities, including elevated fibrotic and inflammatory markers, are preferentially associated with cardioembolic stroke subtype alluding to an underlying myopathic process [3–6].

Several ECG markers thought to represent underlying atrial remodelling have been associated with ischemic stroke and AF [7–9]. They are postulated to be more reliable at identifying patients at risk of stroke than the detection of subclinical AF [10–12]. P-wave terminal force in V_1 ($PTFV_1$) is one such marker derived from a standard 12 lead ECG. $PTFV_1$ has been associated with both elevated left atrial volume and pressure and may represent atrial adverse remodelling [13,14]. However, there is clinical equipoise regarding the utility of this marker in predicting ischemic stroke [13,14].

The inconsistencies in data could be indicative of poor reliability of the measure, as $PTFV_1$ is inherently difficult to measure [15]. Furthermore, previous studies have not provided sufficiently detailed methodology to allow for reproducible measurement of $PTFV_1$, particularly in the presence of subtle baseline and beat to beat P-wave variability that could impact on the accuracy of measurements.

We performed a study to assess the reliability of $PTFV_1$ as a clinical measure and its association with cerebrovascular events. We assessed the reliability of inter-observer and inter P-wave $PTFV_1$ measurements using a standardised technique in an attempt to reduce variability of measurements.

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Material and methods

Study design

A case-control study was conducted among patients that presented to three metropolitan hospitals with an ischemic cerebrovascular event, stroke or transient ischemic attack (TIA), between May 2011 and December 2015.

Patients within the stroke and TIA group were identified through a prospectively maintained stroke unit database. Inclusion criteria were: 1) age ≥ 18 years; 2) adjudicated to have had an ischemic stroke or TIA by a stroke physician; 3) underwent 24-hour Holter monitoring following their index stroke or TIA; and 4) had a 12 lead ECG available for analysis. Exclusion criteria were: 1) haemorrhagic stroke; 2) history of atrial fibrillation or atrial flutter; 3) AF diagnosed on post stroke Holter monitoring or during follow up; 4) underlying severe cardiomyopathy; 5) previous coronary artery bypass grafting or 6) severe chronic obstructive airways disease.

Eligible patients were compared to a group of age-matched controls, without prior history of stroke, TIA or AF, and underwent Holter monitoring to exclude subclinical AF. The stroke/TIA and control groups were matched in a 2:1 ratio with the same exclusion criteria applied to both groups.

The research protocol was approved by the institutional Human Research Ethics Committee.

Clinical assessment and outcome measures

All patients adjudicated as having a stroke or TIA underwent investigation and treatment as per the national stroke guidelines recommendation for standard of care [16]. This included physical examination, blood measurements, 12 lead ECG, pulse oximetry, cerebrovascular imaging and vascular assessment.

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 details of repeat presentations with recurrent stroke or TIA.

All patients underwent Holter monitoring with 24 h of continuous rhythm capture utilising the SEER Light Holter Monitor (GE Healthcare, Milwaukee, USA). Data were analyzed offline upon completion of the monitoring period by cardiac technicians and subsequently reviewed by a cardiac electrophysiologist. Rhythm analysis was conducted using MARS Ambulatory ECG Analysis System (GE Healthcare, Milwaukee, USA). The ECGs were obtained on a MAC 5500 HD resting ECG system (GE Healthcare, Milwaukee, USA).

P wave measurements

A 12 lead ECG was recorded at 10 mm/mV with a paper speed of 25 mm/s after standard lead placement. The ECGs were analyzed in a digital format and all measurements obtained at a minimum five times digital zoom. The digital callipers were calibrated against the reference pulse, with measurements obtained in millimetres and converted to a unit of mV ms.

$PTFV_1$ was defined as the amplitude of the downward deflection of the terminal portion of the P-wave in lead V1 multiplied by its duration [17,18]. The baseline was defined by a straight line that extends from the middle of the TP segment immediately prior to the P-wave of interest, to the following TP segment. The amplitude was measured from the nadir of the P-wave till the point of intersection with the baseline (Fig. 1).

As described in previous studies, $PTFV_1$ was only measured if the P-wave morphology in lead V_1 had a negative or biphasic component [15]. An elevated $PTFV_1$ was defined as a value >4 mV ms as described in previous studies [17,19–22].

$PTFV_1$ was measured by a single observer blinded to the patient group. A subset of 30 ECGs were analyzed by the same observer on a different P-wave and by second observer on the same P-wave to assess inter P-wave and inter-observer variability.

Interatrial conduction block was identified based on a P-wave duration ≥120 ms. P wave morphology as previously defined with a biphasic morphology in leads II, III, and aVF or biphasic morphology in leads III and aVF in association with a notched P in lead II [12].

Statistics

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, with mean ± standard deviation for parametric data and median with interquartile ranges for non-parametric data. Differences between groups with a parametric distribution were tested by an unpaired *t*-test for continuous variables and Chi-square test for categorical variables. Non-parametric data were analyzed using the Mann Whitney *U* test and Kruskal-Wallis test. Correlation trends were analyzed using Spearman's rho for non-parametric data or Cochran-Armitage test for ordinal data. A p-value < 0.05 was deemed statistically significant. $PTFV_1$ values that lay outside 99.9 percentile were categorised as clinical implausible outliers and excluded from analysis. Markers associated with stroke and an elevated $PTFV_1$ were identified by univariate and multivariate logistical regression.

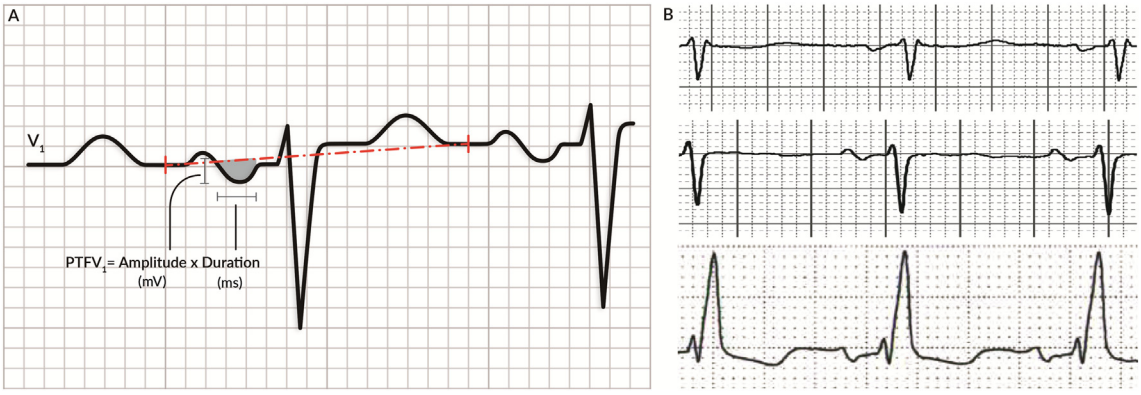

Fig. 1. (A) $PTFV_1$ was calculated by identifying the baseline represented by the straight line across the TP segments. The amplitude was measured from the nadir of the P-wave till the point of intersection with the baseline. (B) Variations in P-wave morphology and baseline wander can affect measurements and calculation of $PTFV_1$.

Intra and inter-observer reliability was assessed using Bland-Altman plots and Intraclass correlation with two-way random effects model. All statistical analyzes were performed with SPSS Statistics 24.0 (IBM, Chicago, IL, USA).

Results

In total, 3235 patients presented with a stroke or TIA during the study inclusion period; 431 were excluded for intracranial haemorrhage identified on computed tomography. Only patients that underwent Holter monitoring and a 12 lead ECG at the study site were included. In the final analysis, 435 patients with a stroke or TIA were compared against 226 age-matched patients that underwent Holter monitoring and ECG recording during the same time period (Fig. 2). The control group was composed of patients that underwent Holter monitoring for investigation of chest pain, syncope, pre-syncope and palpitations.

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. Patients with a stroke or TIA were significantly more likely to have comorbidities of diabetes mellitus, dyslipidaemia, and peripheral vascular disease. Seventy-six (17.5%) patients with a stroke or TIA had a prior ischemic cerebrovascular event. Despite a higher prevalence of hypertension and dyslipidaemia within the stroke or TIA cohort there were no difference in the use of statins or antihypertensive therapy.

The prevalence of interatrial conduction block was not significantly different between the stroke and control group (3.9% vs 2.7%, p 0.40). There was no difference in the occurrence of a negative or biphasic P-wave in lead V1 between the stroke/TIA and control group (90.7% vs 89.2%, *p* 0.54). Four patients from the stroke and 2 from the control group, had a calculated $PTFV_1$ value above the 99.9 percentile and were excluded from analysis involving $PTFV_1$. There were no significant differences in the median $PTFV_1$ values between the control 4.23 mV ms (IQR 2.91–5.57) and stroke/TIA 3.96 mV ms (2.78–5.58) groups. Similarly, there were no significant differences in the median $PTFV_1$ values between the 240 patients with cryptogenic stroke subtype and control group, 4.01 mV ms (IQR 2.86–5.69) and 4.23 mV ms (IQR 2.91–5.57) respectively.

An elevated $PTFV_1$ was defined by the presence of a P-wave morphology consistent with $PTFV_1$ and a $PTFV_1$ value above 4.0 mV ms as described previously. The prevalence of an elevated $PTFV_1$ in the

Table 1
Characteristics of study participants.

	Control n = 226 n (%)	Stroke/TIA n = 435 n (%)	*p* value
Age, y (SD)	70.7 (11.5)	70.0 (12.4)	0.47
Sex, female	79 (35.0)	187 (43.0)	<**0.05**
Vascular risk factors			
Hypertension	121 (53.5)	276 (63.4)	0.14
Dyslipidaemia	79 (35.0)	197 (45.3)	<**0.05**
Diabetes mellitus	36 (15.9)	109 (25.1)	<**0.01**
Any smoking	40 (17.7)	113 (26.0)	0.17
Previous stroke/TIA	0	76 (17.5)	–
Myocardial infarction	40 (17.7)	79 (18.2)	0.83
Peripheral vascular disease	5 (2.2)	25 (5.7)	<**0.05 (f)**
Heart failure	9 (4.0)	24 (5.5)	0.39
CHA_2DS_2VASc score (median)	3.0	5.0	<**0.01**

SD indicates standard deviation; f, Fishers exact test; TIA, transient ischemic attack.
Bold highlights statistically significant values.

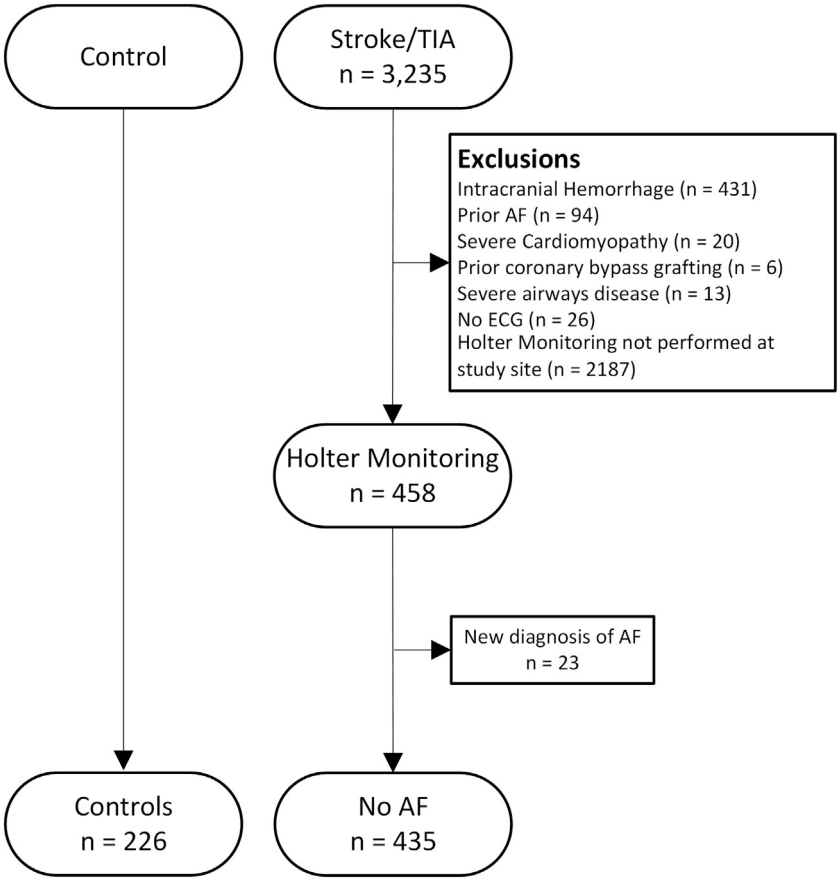

Fig. 2. Patient selection: inclusions and exclusions.

Table 2
Baseline characteristics and $PTFV_1$.

	Normal $PTFV_1$ n = 291 n (%)	Elevated $PTFV_1$ n = 296 n (%)	*p* value
Age, y (SD)	69.8 (11.4)	71.1 (12.3)	0.11
Sex, female	117 (40.2)	123 (41.6)	0.74
Stroke/TIA	196 (67.4)	188 (63.5)	0.33
Vascular risk factors			
Hypertension	171 (58.8)	182 (61.5)	0.50
Dyslipidaemia	128 (44.0)	115 (38.9)	0.21
Diabetes mellitus	98 (24.7)	35 (20.3)	0.19
Smoking	70 (24.1)	68 (23.0)	0.76
Peripheral vascular disease	12 (4.1)	12 (4.1)	0.97
History of heart failure	11 (3.8)	18 (6.1)	0.20
CHA_2DS_2VASc score-median (IQR)	4 (2–6)	4 (2–6)	0.45

SD indicates standard deviation; TIA, transient ischemic attack.

stroke/TIA and control group was, 49.0% and 53.2% (*p* 0.33), respectively. Conventional vascular risk factors were not associated with an elevated $PTFV_1$ on univariate analysis (Table 2).

$PTFV_1$ had excellent reliability for intra-observer, intra P-wave measurements (ICC 0.91, $p < 0.001$) with narrow 95% limits of agreement (LoA), −2.95 to 2.21 mV ms, on Bland-Altman analysis. There was a sequential reduction in reliability for inter-observer, intra P-wave measurements (ICC 0.85, $p < 0.001$) and intra-observer, inter P-wave measurements (ICC 0.79, $p < 0.001$). Inter-observer, inter P-wave measurements were the least reproducible with only moderate reliability (ICC 0.68, $p < 0.01$) and the widest 95% LoA, −5.78 to 6.17 mV ms, on Bland-Altman analysis (Fig. 3).

Discussion

In this cohort of patients, an increased $PTFV_1$ was not associated with ischemic stroke and this remained consistent for the cryptogenic stroke subtype. Further analysis exploring predictors for an elevated $PTFV_1$ defined as a value >4.0 mV ms failed to demonstrate an association with stroke or traditional vascular risk factors. Most importantly, while $PTFV_1$ could be measured reliably by the same assessor using the same P-wave utilising this rigorous methodology, reliability reduced significantly with both inter P-wave and inter-observer measurements.

Research into the pathophysiological basis of $PTFV_1$ dates back to the 1970s when an association between an elevated $PTFV_1$ and left atrial pressure during cardiac catheterisation was demonstrated. Investigators have subsequently reported excellent specificity for an elevated $PTFV_1$ in predicting left atrial enlargement on echocardiography, conversely others have shown that increased left atrial abnormalities correlated with electrocardiographic markers only in the context of a cardiomyopathy [14,21,23,24].

More recently, several studies have explored the relationship between $PTFV_1$ and clinical outcomes such as atrial fibrillation, silent vascular brain injuries and ischemic stroke [25]. Similar to the pathophysiological studies, the data remains inconsistent. A subanalysis of the Northern Manhattan Study and the Multi-Ethnic Study of Atherosclerosis study, that longitudinally followed patients for subsequent cerebrovascular or cardiovascular events demonstrated a significantly higher mean $PTFV_1$ value in the stroke group compared with the control group [18,26]. However, Social Insurance Institution's Coronary Heart Disease Study did not demonstrate a significant association between elevated $PTFV_1$ and ischemic stroke [27].

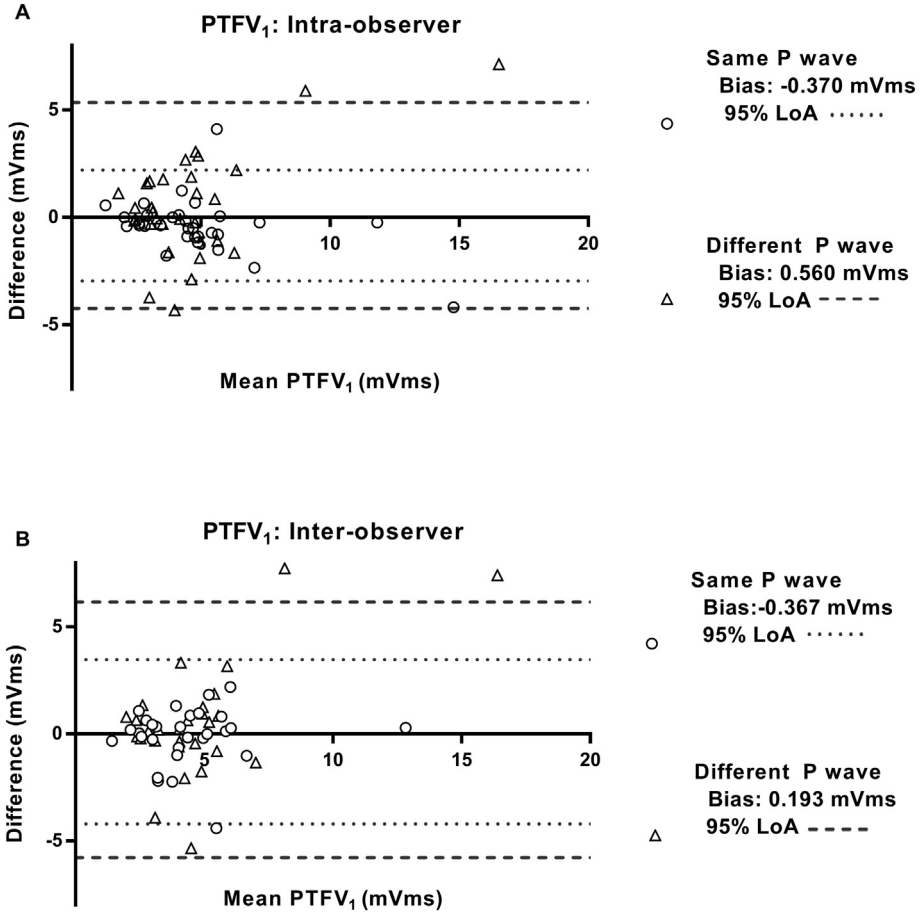

Fig. 3. Bland-Altman analysis with 95% limits of agreement (LoA) and bias for $PTFV_1$ measurements. The small bias highlights the lack of systematic under or over estimation of $PTFV_1$. (A) Demonstrates intra-observer, intra P-wave assessment with narrow LoA, a marked widening of LoA is observed with intra-observer, inter P-wave assessment. (B) Demonstrates inter-observer assessment of P waves, with the widest LoA noted with inter-observer, inter P-wave measurements.

The present study did not demonstrate a significant association between stroke and $PTFV_1$. Furthermore, conventional vascular risk factors were not significantly associated with an elevated $PTFV_1$. It is feasible, that the lack of a significant difference in $PTFV_1$ between the stroke and control group could be a result of selection bias, due to an elevated baseline $PTFV_1$ in patients that presented for Holter monitoring who subsequently formed the control group. AF has been associated with an elevated $PTFV_1$ and despite excluding manifest AF and subclinical AF detected on Holter monitoring, the patients who present for Holter monitoring is likely to have a higher prevalence of occult undiagnosed AF than a community-based patient cohort. However, data from two large longitudinal cohort studies observed discordant results for $PTFV_1$ as a predictor for AF, as a significant association with $PTFV_1$ was only noted in the ARIC study [19].

The conflicting pathophysiological and clinical data raise questions about the clinical reliability and validity of this marker. $PTFV_1$ is an inherently difficult marker to calculate as it relies on the accurate measurement of low amplitude deflections on an ECG, which is subsequently multiplied, resulting in the potential magnification of measurement errors. Previous studies have provided inter-observer reliability that ranged from poor to good, however a robust assessment of factors that affect its reliability has not been outlined [15,18,27]. Our study using a rigorous methodology exposes potential flaws in measuring $PTFV_1$. Even when using a standardised methodology, the specific p-wave measured can markedly affect the result, as can measurements by different observers.

The robust nature of our standardised methodology for $PTFV_1$ measurements was demonstrated by the excellent reliability, ICC of 0.91 with narrow limits of agreement, of intra-observer, intra P-wave $PTFV_1$ measurements. While a change in either the observer or the P wave measured led to a reduction in reliability, the impact of inter-P wave measurements was more pronounced. However, the largest significant reduction in reliability occurred with a change in both the observer and the P-wave measured, with an ICC of 0.68 and a very wide 95% limit of agreement. The wide limits of agreement in particular suggests both significant over and underestimation of $PTFV_1$.

Poor reproducibility of P-wave dispersion and $PTFV_1$ was demonstrated by Snyder et al. with reduction in reliability noted during a single visit and with a further reduction in reliability demonstrated between visits [15]. However, to date, the present study is the first to have analyzed the individual factors that contribute to this reduction in reliability of $PTFV_1$. Our findings raise concerns over the real-world utility of this measure, whereby different assessors will evaluate different P-waves to derive the $PTFV_1$ value.

Automated computerised algorithms for calculating $PTFV_1$ could reduce inter-observer variability and have been shown to correlate well with manual measurements [28]. However, automated algorithms will not overcome differences in measurements that arise from inter P-wave variability. It is likely that the moderate reliability observed in this study will reduce further in a real-world setting when analysing ECGs obtained at differing time points.

The limitations of the present study include: 1) the utilisation of manual measurements to calculate $PTFV_1$, but we improved reliability by adopting a rigorous methodology and by routinely using digital zoom to improve measurement accuracy; 2) a proportion of patients undertook Holter monitoring externally and therefore did not meet inclusion criteria, we cannot exclude a significant difference in $PTFV_1$ within this group of patients; 3) we did not analyze the utility of multiple P-wave measurements to derive an average $PTFV_1$ value, this may reduce the impact of P-wave dispersion on $PTFV_1$. 4) A higher threshold for characterizing $PTFV_1$ may have demonstrated a significant difference between the two groups.

In conclusion, there was no association between $PTFV_1$ and ischemic stroke in this cohort of patients. Despite using a rigorous methodology for measurements, we demonstrated only moderate reliability for inter-observer, inter P-wave measurements of $PTFV_1$. Further refinement of the methodology is required to improve the clinical utility of $PTFV_1$ as a marker of atrial myopathy and ischemic stroke.

Declaration of interests

None.

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Chapter 3

P-wave Terminal Force V_1 is not associated with abnormal electrophysiological substrate.

P-wave Terminal Force V_1 is not associated with abnormal electrophysiological substrate.

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P-wave Terminal Force V_1 is not associated with abnormal electrophysiological substrate.

P wave terminal force in V_1 ($PTFV_1$) is an electrocardiographic marker postulated to represent underlying atrial remodelling. It is a marker derived from a standard 12 lead ECG. An elevated $PTFV_1$ has been associated with increased left atrial size and volume (1,2). This is thought to represent underlying adverse atrial remodelling. The potential utility of $PTFV_1$ as an early marker for patients at risk for atrial fibrillation and cardio-embolic stroke has been described (2). However, recent studies raise questions regarding its clinical utility due to inconsistent association with clinical endpoints and poor reliability of the measurement itself. Further, there is paucity of data on the association between electroanatomic (EA) substrate and $PTFV_1$ measurements. We conducted a cross-sectional study analysing the association between $PTFV_1$ and electroanatomic substrate in patients with AF.

Methods

Patients undergoing index AF ablation for symptomatic drug refractory paroxysmal AF lasting less than 7 days or persistent AF lasting greater than 7 days were prospectively enrolled in the study. Patients were excluded if they were unable to be cardioverted to sinus rhythm, had prior AF ablations, amiodarone use or had a P wave morphology that lacked a negative terminal deflection in lead V1.

$PTFV_1$ was calculated using validated methodology outlined previously (3). Briefly, from the standard 12 lead ECG in sinus rhythm, the amplitude of the downward deflection of the terminal portion of the P-wave in lead V1 was multiplied by its duration. The baseline was defined by a straight line extending from the middle of

the TP segment immediately prior to the P-wave of interest, to the following TP segment. The amplitude was measured from the nadir of the P-wave till the point of intersection with the baseline. Three $PTFV_1$ measurements were obtained and averaged to derive the final measurement.

All antiarrhythmic therapy was ceased at least five half-lives prior to the electrophysiology study. All patients underwent general anaesthesia for the procedure. Bipolar intracardiac electrograms and 12-lead electrocardiography (ECG) were recorded on a digital amplifier system (EPMed Systems, Chicago, IL, USA). Intracardiac electrograms were filtered from 30 to 500 Hz and measured with digital calipers at 200 mm/s sweep speed. Left atrial geometry was constructed with a 20 pole Lasso catheter (Biosense Webster, Irvine, CA, USA, 2-5-2 mm electrode spacing) and merged with a periprocedural CT using the CARTO3 electroanatomical mapping system (figure 1).

Voltage and activation maps were constructed using the Lasso catheter during constant pacing from the distal coronary sinus (CSD) to standardize the direction of wave front propagation at a fixed cycle length of 600ms. Complete coverage of the entire LA geometry was performed and correlated to the CT to ensure smooth coverage across all regions with a minimum of 1000 points using the Confidense™ algorithm to ensure even point distribution (Biosense Webster) (4). Despite the utilisation of the Confidence algorithm to assist automated point acquisition, all acquired points were also manually reviewed. Only points indicative of near-field signals were included. These signals demonstrated at minimum 2 sharp peaks and were consistent with anatomically adjacent signals for quality and electrogram

timing. During offline manual analysis, signals not meeting these criteria were excluded. Point collection was performed by experienced operators aided by assessment of tactile catheter pressure and fluoroscopic motion.

Electrophysiologic indices were measured and analyzed for all patients as previously described (5). Global and regional atrial voltage analysis was performed according to bipolar voltage, which was defined as the peak-to-peak electrogram voltage. The mean voltage was compared between the groups. A low bipolar voltage was defined as <0.5 mV. Conduction velocity was analysed in MATLAB using a polynomial algorithm. This method utilised a fitting window per region with a minimum of 20 points required. These regions were assigned cartesian coordinates in space and activation time and subsequently fitted within a 3-dimentional space using standard least-squares algorithm. The velocity was calculated by calculating the gradient from the fit. The mean LA conduction velocity was calculated. Complex signals were defined as either electrograms with >2 deflections of >50ms duration (fractionated potentials) or 2 separate deflections separated by an isoelectric interval (double potentials). The proportion of these signals was expressed as a proportion of the total number of signals.

Data was analysed using SPSS software version 24 (IBM Corporation, New York). All continuous parameters were assessed for normality using Shapiro-Wilk test. Data are expressed as mean+/-SD. Two-group comparisons were made using chi-square for categorical variables and Student’s t test for continuous variables.

Results

A total of 34 patients that underwent PVI were enrolled; 5 patients did not have morphology consistent with $PTFV_1$ and were excluded. The remaining 29 patients were included in the final analysis. An elevated $PTFV_1$, defined as the upper tertile of $PTFV_1$ values, was compared with the remaining patients forming the control group (table 1). The median $PTFV_1$ in the control group was 4.60 mVms compared with 9.52 mVms in the elevated group. There were no significant differences in baseline indices such as age, gender, hypertension, diabetes mellitus or dyslipidaemia.

Similarly, there were no significant differences in EA substrate properties between the control and elevated $PTFV_1$ groups respectively. Specifically, there was no difference in bipolar mean voltage, percentage of low bipolar voltages, conduction velocity, percentage of double potentials or the percentage of fractionated potentials (table 1).

Discussion

$PTFV_1$ is an electrocardiographic marker, and in a number of studies, showed good specificity for predicting left atrial enlargement and outcome measures such as development of AF, ischaemic stroke and silent vascular injury (1,6,7). Although these studies indicated acceptable predictive capacity of $PTFV_1$ in identifying atrial dysfunction, other large cohort studies failed to consistently demonstrate an association (2,3). The present study characterised the relationship between electrophysiological substrate and $PTFV_1$.

In this well-matched cohort of patients with AF, there were no significant differences amongst any of the measured indices of EA substrate abnormalities in patients with an elevated $PTFV_1$. This is consistent with the findings from the only other study to assess the relationship between $PTFV_1$ and atrial fibrosis. Inoue et al. did not demonstrate a correlation between $PTFV_1$ and atrial late gadolinium enhancement indicative of atrial fibrosis on cardiac MRI in patients with AF(6).

The lack of association between $PTFV_1$ and EA substrate may relate to the poor reliability of the $PTFV_1$ measurement as described previously (3). There is both reduction in reliability as a result of inter-P wave and inter-observer variability. This reduction in reliability could account for the inconsistencies in its association with clinical indices noted in prior studies.

Alternatively, $PTFV_1$ may not be sufficiently sensitive to correlate with the extent of fibrosis after manifestation of AF. In the present study only patients with AF were analysed and onset of AF may have accelerated adverse remodelling. Therefore, after manifestation of AF, $PTFV_1$ may no longer correlate with the extent of adverse atrial remodelling. Both the Northern Manhattan Study and Multi-Ethnic Study of Atherosclerosis study that longitudinally followed patients, demonstrated a significant association between $PTFV_1$ and stroke in patients that did not have underlying AF. The absence of association noted in the current study may merely reflect a lack of sensitivity in this cohort with AF. Therefore, further work is required to determine the association between electro-anatomic substrate and $PTFV_1$ in patients without AF.

The present study is also limited by the small sample size could have led to an underestimation of the true effect size.

In conclusion, elevated $PTFV_1$ may not correlate with underlying atrial electroanatomic substrate abnormalities in patients with AF. Further studies are required to evaluate the pathophysiological basis for an elevated $PTFV_1$ and its clinical utility as a marker of abnormal atrial substrate.

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Table 1

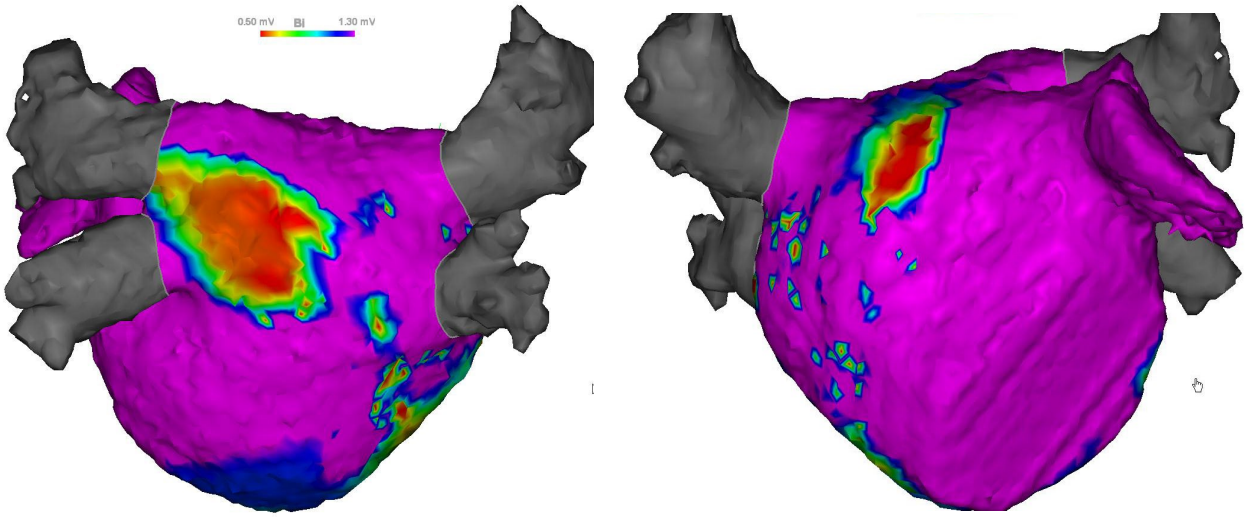
Demographic and electrophysiological parameters according to $PTFV_1$.

	Control N = 19 n(%)	Elevated $PTFV_1$ N = 10 n(%)	P value
Demographics			
Age ± SD	59.6 ±7.3	57.7 ±12.4	0.61
Sex (Female)	7 (37)	4 (40)	0.87
Hypertension	10 (52)	2 (20)	0.13
Diabetes mellitus	2 (10)	2 (22)	0.57
Dyslipidaemia	6 (32)	3 (30)	0.93
AF – Persistent	8 (42)	3 (30)	0.69
EA Substrate			
Bipolar mean voltage (mV) ± SD	1.86 ± 0.60	2.00 ± 0.55	0.54
Low bipolar voltage % ± SD	17.5 ± 7.3	18.1 ± 9.9	0.85
Conduction velocity (cm/s) ± SD	41.7 ± 12.8	38.2 ± 11.1	0.48
Fractionated potentials % ± SD	3.8 ± 2.1	3.1 ± 1.7	0.42
Double potentials % ± SD	0.63 ± 0.65	1.13 ± 1.2	0.16

SD = standard deviation, EA = Electroanatomic substrate; The data is presented as n (%) or mean ±SD. Fisher exact test was used when appropriate.

Figure 1.

Left atrial electroanatomic bipolar voltage map highlighting areas of low voltage



Areas of lower voltage denoted with progressive red shading.

Premature Atrial Complexes

Ambulatory cardiac rhythm monitoring has also provided plausible markers of atrial dysfunction and ischaemic stroke. Excessive premature atrial complexes (PACs) have been associated with increased risk for AF and ischaemic stroke (8-10). The increased risk for AF was thought to contribute to thrombogenesis and therefore ischaemic stroke. However, cohort studies in patients with elevated PAC burden have shown the risk of stroke to be independent of the presence of AF(8). As with AF, there was a stepwise risk in risk for stroke associated with increased vascular risk factors in patients with excessive PACs (8). Frequent PACs were also associated with atrial structural remodelling and function.

Despite the association with ischaemic stroke, it is unclear if elevated PACs confer a risk for the cryptogenic stroke subtype. Atrial structural remodelling occurs in the presence of vascular risk factors, but there is a paucity of data on whether these risk factors also contribute to an increased electrical remodelling. Increased electrical anatomical substrate abnormalities may manifest as abnormal electrical changes such as an excessive PAC burden.

In chapter 4, we conducted a study to evaluate the association between excessive PACs, ischaemic stroke and cryptogenic stroke using an age matched control group. The association between vascular risk factors and PAC burden were also explored.

Chapter 4

Association between excessive premature atrial complexes and cryptogenic stroke: results of a case-control study

BMJ Open

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 multicentre 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% CI 1.29 to 3.02; p<0.01), smoking (OR 1.58; 95% CI 1.06 to 2.36; p<0.05) and hypertension (OR 1.53; 95% CI 1.07 to 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; 95% CI 1.16 to 3.28; p<0.05). Vascular risk factors that promote atrial remodelling, increasing age (≥75 years, OR 3.64; 95% CI 2.08 to 6.36; p<0.01) and hypertension (OR 1.54; 95% CI 1.01 to 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.

Strengths and limitations of this study

- This study employed a case–control design to compare the burden of premature atrial complexes (PACs) 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 PACs.
- This study describes the association between vascular risk factors and PACs 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 UK, 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 atrial fibrillation (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 (PACs) have been thought to be a benign phenomenon with a prevalence in the general population that ranges from 6% to 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 transient ischaemic attack (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 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 AF, 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 with 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 who underwent investigation of chest pain, syncope, presyncope 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, CT of the brain, 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 on 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).

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% 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 V.24.0.

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 raw data will be made available by the corresponding author on reasonable request.

RESULTS

In total, 537 patients presented with a stroke/TIA during the study inclusion period and underwent Holter monitoring. Twenty-three 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 who 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).

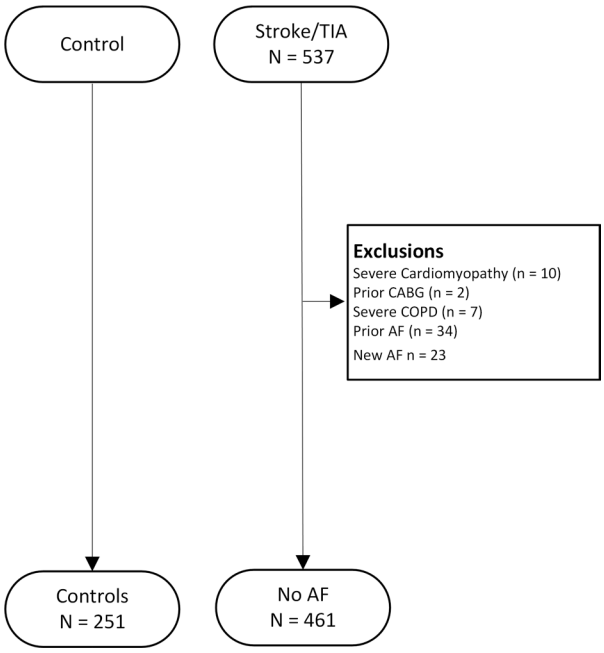

Figure 1 Patient selection: inclusions and exclusions. AF, atrial fibrillation; CABG, coronary artery bypass grafting; COPD, chronic obstructive pulmonary disease; TIA, transient ischaemic attack.

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 OR of 1.97 (95% CI 1.29 to 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 (online supplementary file 1).

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 >3 beats was not significantly different between the two 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; 95% CI 1.16 to 3.28), female sex (OR: 1.78; 95% CI 1.19 to 2.67) and hypertension (OR: 1.67; 95% CI 1.05 to 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 >3 in the control group and two 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 years 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; 95% CI 1.16 to 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 who develop incident AF and ischaemic stroke.[18 20] Existing longitudinal studies from Engström *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 OR of 1.95. This is an important finding and lends further

Table 1 Baseline characteristics of study participants

Characteristics	Control n=251 n (%)	Stroke/TIA n=461 n (%)	P value
Age, years (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

The data are presented as n (%), unless otherwise stated.
PACs, premature atrial complexes; TIA, transient ischaemic attack.

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 PACs, 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 electroanatomical

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

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

Variables adjusted in the multivariate model: age, sex, excessive premature atrial complexes, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease and sleep apnoea.
PACs, premature atrial complexes; TIA, transient ischaemic attack.

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 MRI 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 cardioembolism.

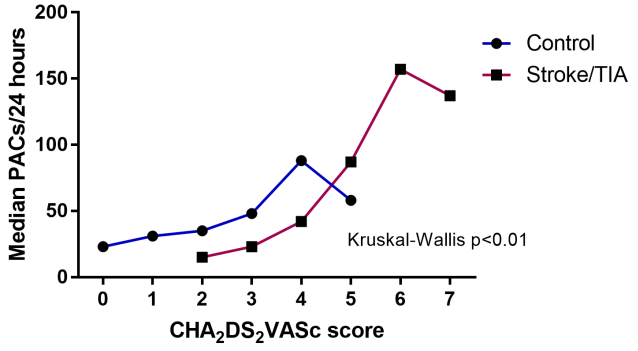

Figure 2 Median PACs by CHA_2DS_2VASc score for control and stroke/TIA groups. CHA_2DS_2VASc, risk score for ischaemic stroke; PACs, premature atrial complexes; TIA, transient ischaemic attack.

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

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

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

Variables adjusted in the multivariate model: age: <65, 65–74, ≥75 years, gender, hypertension, diabetes mellitus, smoking, dyslipidaemia, peripheral vascular disease, sleep apnoea.
PACs, premature atrial complexes.

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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Contributors JKS led and designed the study, collated and analysed data and wrote the manuscript. ANK, KR, MCT and TF collated and analysed the data and revised the manuscript. HD, JMK, LR, JCC and MW contributed to study design and revised the manuscript. MS analysed the data and revised the manuscript. AWT supervised and designed the study revised the manuscript. All authors read and approved the final manuscript.

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Patient consent for publication Not required.

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Provenance and peer review Not commissioned; externally peer reviewed.

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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

Smart Devices for Detection of Atrial Fibrillation and Stroke Prevention

The use of consumer grade wearable technology is growing rapidly around the world (160). These devices are increasingly capable of collecting and analysing biometric data. Furthermore, the gap between consumer devices and medical grade services is narrowing. This is recognised by endorsement of consumer devices for clinical indications by medical regulatory bodies.

Atrial fibrillation is the most common arrhythmia and as described previously in the thesis, is associated with abnormal atrial structure, function and biochemical indices in keeping with a myopathy. A proportion of ischaemic strokes are thought to be due to occult AF. With the proliferation of smart devices, improving cardiovascular outcomes appears to be a prominent goal for smart device manufacturers. At the forefront of this goal is cardiac rhythm analysis with an aim of detecting occult atrial fibrillation to aid with stroke prevention (161,162).

Several devices are currently available, including standalone platforms that can be paired with a smart device, to wrist worn devices that can acquire single lead ECG traces and provide heart rate estimation. However, several questions regarding the utility of these devices remain unanswered. Wearable devices have gone through iterative improvements over the last decade with regards to both the hardware that underpins rhythm assessment and software platforms available with aid of machine learning algorithms.

First generation platforms utilised miniaturised photoplethysmography (PPG) incorporated into smartphones to measure heart rate based on pulsatile blood volume changes with the microvasculature. These were subsequently incorporated into wearable devices such as smart watches (163). These devices were robust for estimating heart rate while patients maintained regular stroke volume as in sinus rhythm or atrial flutter (164, 165). PPG based platforms provided ease of use, however, physical activity, darker skin pigmentation and presence of variable stroke volume as that occurs in atrial fibrillation led to reduced accuracy (164). Further, detection of AF using PPG was solely reliant on variability of the pulsatile blood volume, with no ability to assess the underlying rhythm.

The subsequent platforms provided the ability to acquire single lead ECGs to overcome this limitation. These devices offered both a single lead trace as well as a presumptive diagnosis to aid with detection of subclinical AF, using automated proprietary algorithms. The accuracy of these devices for diagnosing AF is variable and their utility as a community screening tool for detection of AF remains ambiguous (11,12). Positive results with detection of AF from smart devices require confirmation with a clinician overread and a 12 lead ECG. However, there is a paucity of data on the accuracy of both the automated diagnoses and clinician overread of these single lead traces. Importantly, there is the potential for large false positive results from the device algorithms that can lead to health care over-utilisation due to the need for further confirmatory testing. Finally, the optimal treatment strategy of subclinical AF for stroke prevention remains unclear.

In chapter 5, we reviewed various smart device platforms for detection of AF and addressed clinical deficiencies and controversies surrounding these devices. We provided the strengths and limitations of various technologies current available. Constructed from available data, we created a nomogram for AF detection based on single lead ECG platforms to assess the potential utility of these devices for population-based AF screening.

In chapter 6, we evaluated the accuracy of the automated algorithm and clinician overread of single lead ECGs from a smart device platform for the detection of AF. Based on this, a clinical workflow that maintained diagnostic accuracy, while minimising expensive clinical overread was proposed.

Technology	**Strength**	**Weakness**
Photoplethysmography Apple Watch Kardia Band Galaxy Watch Huawei Watch Garmin	-Minimal battery usage and therefore frequent "continuous" sampling possible -Automated use for wearables	-Reliant on R-R variability for rhythm determination. -Inaccurate heart rate estimation in irregular rhythms -No tracing for clinician to review in order to verify rhythm
Single lead ECG *Wearable* Apple Watch Galaxy Watch Withings Move ECG *Non wearable* KardiaMobile WIWE	-Rhythm analysis improves accuracy - Clinician overread of rhythm possible - May function as event monitor	- Rhythm traces prone to artefact - Battery utilisation and need for consumer interaction limits continuous use
Multi-lead ECG *Non wearable* KardiaMobile 6L	-Rhythm analysis improves accuracy - Overread of rhythm possible - May function as event monitor - Rhythm traces may have reduced artefacts and improved ability for rhythm discrimination	- Battery utilisation and need for consumer interaction limits continuous use

Chapter 5

Wearable devices for cardiac arrhythmia detection: a new contender?

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CURRENT CONTROVERSIES

Wearable devices for cardiac arrhythmia detection: a new contender?

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Key words
smart device, smart watch, atrial fibrillation, arrhythmia.

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Abstract

There has been increased consumer uptake of smart devices and wearable technology. They facilitate non-invasive, ambulatory assessment of numerous cardiac indices, including the heart rate and rhythm. Several studies have reported on the utility and deficiencies of these devices in identifying and monitoring cardiac arrhythmias. The rapid uptake of these consumer devices has the potential to generate vast amounts of biometric data. This coupled with gaps in knowledge pertaining to the optimal management of conditions such as sub clinical atrial fibrillation, may result in unnecessary and expensive downstream testing. An improved understanding of this nascent field by the clinician is vital.

Wearable devices are increasingly popular with more than 325 million devices sold in 2016 alone and a projected yearly growth of approximately 18%.[1] Consumer grade devices are rapidly bridging the gap to providing medical grade services, due to progressive improvements in technological capabilities combined with the ability to transfer wirelessly data for remote analysis. This has culminated in the endorsement, by the Food and Drug Administration (FDA), for several wrist worn consumer smart device platforms (SDP) for cardiac rhythm analysis, including the latest Apple Watch.[2,3] The ascension of the 'quantified-self' movement, with continuous acquisition of cardiac physiological data, has potential to be translated into actionable information for the clinician.

Improving cardiovascular health outcomes has recently become a prominent goal for consumer SDP manufacturers. The current generation of SDP have in-built photoplethysmography (PPG), gyroscopes and accelerometers. These can measure heart rate (HR) and encourage physical activity through continuous biofeedback. The miniaturisation and incorporation of PPG facilitates estimation of HR based on pulsatile blood volume changes within the microvasculature. Initial efforts were focused on developing systems for accurately detecting HR in sinus rhythm for the fitness and wellbeing enthusiast. Medical grade PPG systems have demonstrated excellent accuracy in estimating HR in sinus rhythm, with a significant correlation coefficient of 0.96.[4] However, limitations of PPG include the underestimation of HR during sinus tachycardia and reduced accuracy during physical activity.[4] Similar to medical grade PPG systems, two early iterations of SDP with integrated consumer grade PPG, Fit Bit Blaze (Fitbit Inc., San Francisco, CA, USA) and Apple Watch Series 1 (Apple Inc., Cupertino, CA, USA), demonstrated strong agreement with concurrent electrocardiogram (ECG)-derived HR in sinus rhythm.[5]

Utilising PPG-based smart watches for detection or chronotropic assessment of arrhythmias, particularly atrial fibrillation (AF), has garnered interest from both clinicians and patients. However, there was only weak to modest agreement during AF with marked HR underestimation when compared with a criterion standard ECG.[5] This is similar to the pulse deficit identified during manual pulse check in patients with AF. Nevertheless, a HR of ≥100 b.p.m. during atrial arrhythmia closely correlated with an ECG HR ≥ 100 b.p.m. and may warrant consideration of clinical review.[5] The demonstrated

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accuracy is likely to deteriorate further in a real-world setting. Notably, factors such as darker skin pigmentation and ambulation have been shown to impede the accuracy, due to the attenuation of the light wavelength by melanin and a reduction in device to skin contact.[6] Although PPG-based technology ranks highly for ease-of-use, its technical limitations may limit its use in isolation for prolonged HR assessment.

The incorporation of SDP-based automated rhythm analysis systems that acquire single-lead ECG has overcome many of the limitations faced by PPG technology. Although these FDA-approved devices provide both ECG tracings and a presumptive diagnosis, clinician verification is recommended through various paid subscription models. Automated algorithms have demonstrated excellent accuracy in interpreting single-lead ECG when compared with contemporaneous 12-lead ECG as the reference standard (Table 1). However, between 15 and 33% of the traces were deemed unclassified by the automated algorithm, with baseline artefact being the primary reason for this classification.[8,9] Clinicians were able to interpret recordings deemed unclassified by the device, with 100% sensitivity and 80% specificity.[8] A hybrid approach that utilised device proffered automated diagnosis in conjunction with clinician overread limited to the unclassified tracings offered excellent diagnostic accuracy (Table 1).

Opportunistic screening for AF is recommended by the European Society of Cardiology guidelines, by conducting a pulse check or by obtaining an ECG rhythm strip in patient ≥65 years of age.[11] A randomised control trial using a SDP was conducted in patients ≥65 years of age to screen for subclinical AF. The study compared routine care, with participants who acquired weekly single-lead ECG over a 12-month period, overread by an automated algorithm and a cardiologist.[12] Unsurprisingly, more patients were diagnosed with AF in the treatment arm compared with routine care, with a hazard ratio of 3.9 (P = 0.007). However, this study raised several issues. First, there was an unexpectedly low positive predictive value of 5%. The large number of false positives invariably lead to heightened concern for the patient and unnecessary downstream testing. Another potential limitation of SDP includes the significant degradation in the quality of single-lead ECG obtained without medical supervision. This was reflected by the large proportion of unclassified tracings in this study. Last, economic analysis revealed a cost of $10 780 per AF diagnosis, which is significant. However, in this study all tracings were overread by a clinician, rather than limiting this to the unclassified tracings. Regardless, the poor positive predictive value observed in this study necessitates a clinician overread of all positive AF diagnosis, to reduce unnecessary downstream testing.

The prevalence of AF in an unselected adult population is approximately 2%, which rises to 5% in patients aged 65–84 years.[13] In a patient ≥65 years using a SDP for rhythm analysis, we estimate the post-test probability of a positive diagnosis of AF to be 14%, which is only modest (Fig. 1). Non-invasive screening strategies, utilising conventional medical grade systems such as Holter monitors and event monitors, are limited by the intermittent nature of monitoring and by the need to 'return to base' for data download. Utilising SDP as a 'rule out' strategy may be of greater clinical utility, as a negative result demonstrates very low likelihood of

Table 1 Sensitivity and specificity of smart device platforms (SDP) and their underlying technology in analysing cardiac rhythm

Study	SDP and technology	Patients (*n*)	Uninterpretable tracing (%)	Sensitivity (%)	Specificity (%)
Desteghe *et al*.[7]	Kardia Mobile *Single-lead ECG*	265	NA	54.5	97.5
Desteghe *et al*.[7]	MyDiagnostick *Single-lead ECG*	265	NA	81.8	94.2
Bumgarner *et al*.[8]	Kardia Band *Single-lead ECG*	100	33.7	93	84
Koshy *et al*.[9]	Kardia Mobile *Single-lead ECG*	102	15	100	95
Koshy *et al*.[9]	Kardia Mobile *Single-lead ECG + clinician overread of unclassified tracings*	102	2.9	93	92
Tison *et al*.[10]	Cardiogram + Apple Watch *PPG + neural network*	51	—	98	90.2

Kardia Mobile and Kardia Band (AliveCor Inc., Mountain View, CA, USA), MyDiagnostick (Applied Biomedical Systems BV, Maastricht, The Netherlands), Cardiogram (Cardiogram Inc., CA, USA), Apple Watch Series 1 (Apple Inc., Cupertino, CA, USA). ECG, electrocardiogram; NA, not available; PPG, photoplethysmography.

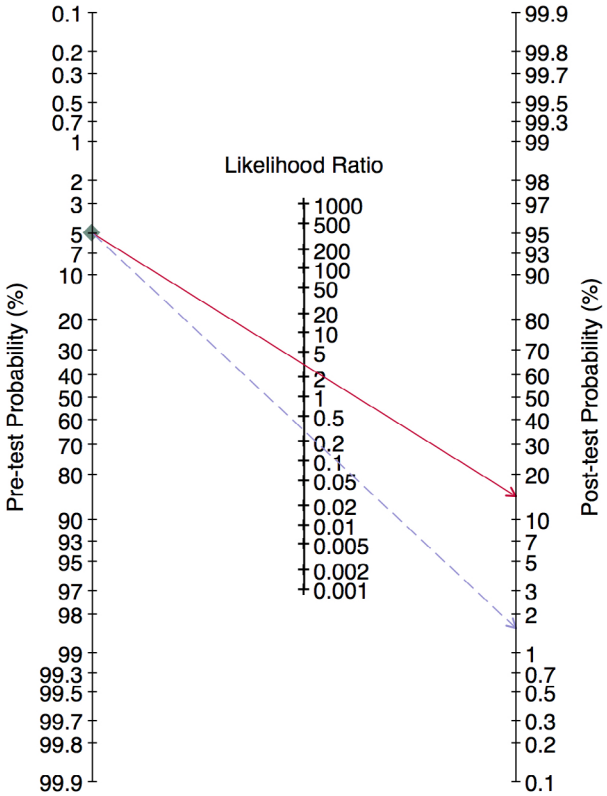

Figure 1 Fagan's nomogram for the confirmation of atrial fibrillation (AF) by the current generation of single-lead ECG platforms. This is a Bayesian graphical tool that estimates how much the result of a diagnostic test changes the probability of a patient having a condition. A line drawn from the pre-test probability through the likelihood ratio of interest intercepts the new post-test probability for the patient. This assumes a 5% population prevalence (pre-test probability) of AF for a patient ≥65 years of age with likelihood ratios used from previously published research. If a patient tests negative, the post-test probability of not having AF would be approximately 2% (- - - -). Alternatively, if the patient tests positive, the post-test probability of AF would be approximately 14% (——). (♦), Prior probe = 5%; (——→), LR_positive = 3, post_Prob_Pos = 14%; (- - - →), LR_negative = 0.30, post_Prob_Neg = 2%. LR_negative, negative likelihood ratio; LR_positive, positive likelihood ratio; Post_Prob_Neg, negative post-test probability; Post_Prob_Pos, positive post-test probability.

underlying AF (Fig. 1). This approach is particularly suited for wrist worn SDP-based screening of paroxysmal arrhythmias, as these devices are designed for almost continuous use with wireless upload of data for remote analysis.

Innovations in big data analysis with machine learning has culminated in development of deep neural networks to identify patients with AF based on PPG guided R–R variability alone.[10] Furthermore, the latest iteration of wearables now uses a hybrid system that prompts the user to acquire a single-lead ECG, when their HR deviates from a personalised R–R variability and physical activity template generated from their PPG data. These systems are likely to compete with conventional medical grade devices, given the ease with which biometric indices can be recorded. However, clinical data pertaining to their use in such a manner are currently lacking.

With increasing prevalence of AF in the population, the prospect of readily available screening through SDP appears attractive. However, a fundamental question still remains largely unanswered; subclinical AF may not confer the same risk for stroke as manifest AF and several studies have shown an apparent lack of temporal association between cardiac implantable device detected atrial high-rate episodes and subsequent stroke.[14] However, meta-analysis of these studies demonstrate that, while subclinical AF appears to confer a lower risk for ischaemic stroke than manifest AF, it remains higher than in patients without subclinical AF.[15] The absolute annual stroke risk was 1.89 (95% confidence interval (CI) 1.02–3.52) compared with 0.93 (95% CI 0.58–1.49) per 100-person years.[15] However, the overlapping confidence interval makes this comparison problematic. Furthermore, trials have shown significant heterogeneity on what constituted an episode of subclinical AF.[15] As such uncertainties remain regarding the duration of subclinical AF that are required to derive benefit from oral anticoagulation for thromboembolic prevention. Further, a large AF screening study based on single-lead ECG acquisition demonstrated that less than 25% of eligible patients subsequently received oral anticoagulation.[16] At present, there are no trials addressing the net clinical benefit and cost of oral anticoagulation for stroke prevention using these screening techniques.

Technological limitations notwithstanding, potential patient-specific barriers may impede widespread screening using SDP. However, attitudes to SDP-based arrhythmia detection remain favourable compared with conventional Holter monitoring system for symptomatic arrhythmia.[17] In one study, SDP were deemed to be more convenient by 98% of the patients, while 90% were likely to utilise the device to determine cardiac rhythm during symptomatic episodes.[17] Further, patients do not report anxiety and on the contrary appear extremely or very comfortable using SDP and in sharing clinical and personal information they generate for medical purposes.[12] However, these findings may lack generalisability, as participation bias could attribute for the high level of acceptance noted in these studies. Older patients have markedly higher prevalence of atrial arrhythmias and have the potential to derive the most benefit from SDP, but conversely may exhibit reluctance in utilising SDP. The feasibility of SDP in such high-risk patient cohorts, requires further assessment. With the

consolidation of numerous patient biometrics tagged to social and demographic data by commercial entities, privacy remains a central concern. There is a growing need for data privacy laws to keep abreast of the rapid innovations in this nascent field.

Despite the limitations, consumer-grade SDP are increasingly prevalent and are undergoing rapid iterative improvements. The gap between conventional medical grade devices and the SDP continues to narrow. Clinicians should be open to reviewing data generated by these platforms, as they may provide valuable individualised information to aid patient management. However, a regulatory framework for standardising and incorporating these data into routine clinical practice is currently lacking. These devices have the potential to generate vast amounts of biometric data that could lead to unnecessary and expensive downstream diagnostic testing, with significant implications for the individual and the wider healthcare system. The adoption of SDP with incorporated arrhythmia detection, must be carefully balanced against the variable accuracy of these devices and current gaps in evidence pertaining to the optimal management of conditions such as subclinical AF. Therefore, we as clinicians should be wary of turning the person into a patient.

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Chapter 6

Accuracy of blinded clinical interpretation of single-lead smartphone electrocardiograms and a proposed clinical workflow

Accuracy of blinded clinician interpretation of single-lead smartphone electrocardiograms and a proposed clinical workflow

The AliveCor Heart Rate Monitor (AHM; AliveCor Inc, Mountain View, CA) is an FDA-approved smartphone electrocardiogram (ECG) device that can generate a lead I tracing (iECG). Although the AHM application provides both ECG tracings and a presumptive diagnosis, clinician verification is recommended, with a variety of paid subscription models available.[1] In the largest community screening study using the AHM, cost for 1 new diagnosis of AF exceeded $10,000—largely driven by costs of commercial overreads of iECGs.[2] Whether manual interpretation of all tracings is of additive value when compared to the automated device algorithm is uncertain. Furthermore, although studies have screened patients in a primary care setting, they have not evaluated if iECG tracings can be accurately adjudicated by primary care physicians (PCPs).[3-5]

We assessed the accuracy of PCP and cardiologist interpretation of the AHM iECG tracings with contemporaneous 12-lead ECG assessment and aimed to develop a more efficient and cost-effective clinical workflow for integrating iECGs in practice.

Methods

This prospective, blinded, observational cohort study was performed at a tertiary university hospital in Australia. Consecutive patients 18 years and older undergoing electrical cardioversions for AF and atrial flutter were recruited over 12 months. Patients with cardiac implantable electronic devices and those unable to hold the device correctly were excluded. The institutional ethics review board approved the study, and written informed consent was obtained from all subjects (ACTRN: 126169913744459). No extramural funding was used to support this work. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper, and its final contents.

Clinical trial registration: Australian & New Zealand Clinical Trials Registry (ACTRN:12616991374459).

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Declarations of interest: none.

ECG recordings

AHM was paired with an iPhone 6-Plus smartphone (Apple Inc, Cupertino, CA) using the Kardia application (AliveCor Inc, Mountain View, CA; V4.2.0). Three consecutive 30-second lead I recordings (iECG) were obtained with finger placement on the 2 electrodes at the back of the iPhone (where AHM was attached). Following each AHM recording, the automated rhythm analysis recorded the rhythm as (*a*) possible AF, (*b*) normal sinus rhythm, or (*c*) unclassified. A 12-lead ECG was obtained immediately prior to the iECG tracings, both pre- and postcardioversion. This was reviewed by an independent cardiologist (J. S.) and used as the reference standard.

Each patient had 6 iECG recordings (3 prior to and 3 following cardioversion, Figure 1). Each set of 3 iECG tracings that were obtained at each patient encounter was reviewed by 2 electrophysiologists (EPs) (M. W. and A. T.) and 2 PCPs (S. C. and Y. K.) in a randomized order, blinded to the patient details and AHM automated diagnosis. Tracings were then reviewed by the clinicians on an iPhone 6-Plus smartphone (5.5-in screen) and classified as sinus rhythm, AF, atrial flutter, or uninterpretable (Online Figure 1). The final automated iECG diagnosis per patient encounter was based on the majority diagnosis noted in 2 of the 3 readings. Clinicians recorded 1 diagnosis based on the 3 consecutive iECGs recorded in each patient. Each case referred to herein is based on clinician interpretation or AHM adjudicated diagnosis of 3 tracings, obtained at each patient encounter.

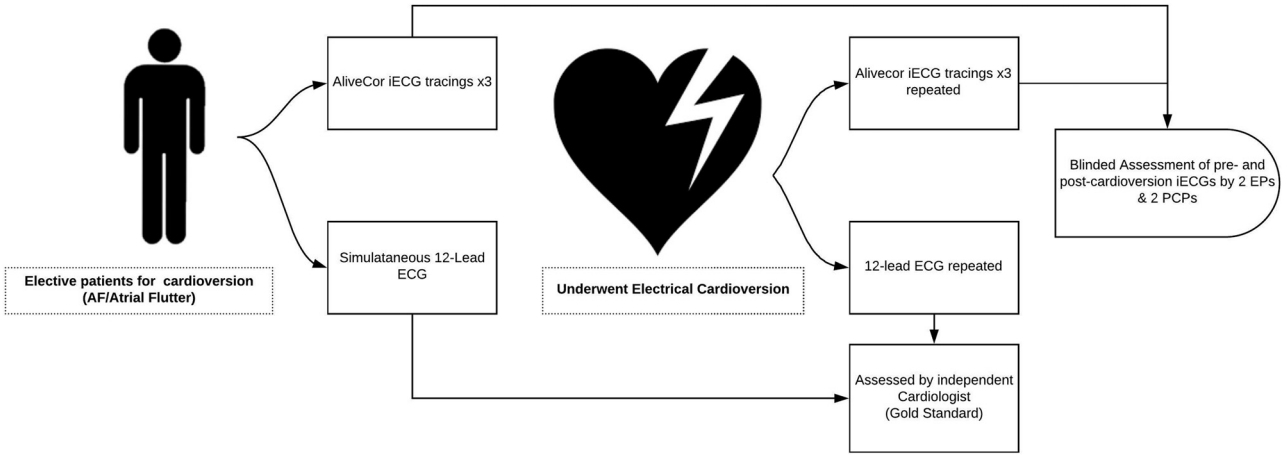
Statistical analysis

Twelve-lead ECG diagnosis was used as the reference standard. Sensitivity, specificity, predictive value, and likelihood ratio were calculated using 2×2 contingency tables. Cohen kappa (κ) coefficient was derived to assess the agreement. Because of the paired nature of the data, only 1 set of 3 iECGs in each patient was selected at random for inclusion in the analysis. A κ of 0.8 was considered excellent agreement.[6]

Results

Data from 102 patient encounters for 51 consecutive patients that underwent elective cardioversions were included (mean age 64 ± 15, 65% male). Clinical characteristics are summarized in Online Table I. There were 408 ECG tracings. This included 306 iECGs that were recorded simultaneously with 102 12-lead ECGs. All of the 12-lead ECGs were interpretable, and only 9 iECG tracings (2.9%) were deemed noninterpretable by both EPs and PCPs. These were subsequently marked as incorrectly identified. This was in contrast to the 46 individual tracings (15%) that the AHM deemed "unclassified." Cardioversion was unsuccessful in 2 cases (4%), where the patients' tracings were classified as AF on both encounters.

Figure 1



Study protocol.*AF*, atrial fibrillation; *iECG*, AliveCor single-lead ECG tracing; *EP*, cardiac electrophysiologist; *PCP*, primary care physician.

Clinician accuracy

EPs demonstrated a mean accuracy of 91% with 2 false-negative diagnoses by EP1 and 5 by EP2 when referenced against the 12-lead ECGs (Table I, *A*). There was satisfactory agreement with κ 0.84 and 0.80 (both $P <$ .001), respectively, for the EPs. The diagnostic yield among PCPs was comparatively lower, with a mean accuracy of 85% with 6 false-negative diagnoses by PCP1 and 4 by PCP2. The κ value did not meet the prespecified criteria for satisfactory agreement with 12-lead ECG diagnosis (κ 0.72 and 0.69; $P <$ .001). Assessment of interobserver variability demonstrated strong agreement between both EPs and PCPs (EPs: κ = 0.80, PCPs κ =0.83, $P <$ .001).

AHM accuracy

On assessing the accuracy of AHM on all tracings, including "unclassified" readings (marked as incorrect), the AHM demonstrated reduced sensitivity (77%) and specificity (76%). However, exclusion of all "unclassified" diagnoses improved overall device algorithm accuracy, demonstrating a sensitivity and specificity of 100% and 95%, respectively. The AHM algorithm demonstrated highest agreement with the 12-lead ECG, on exclusion of "unclassified" tracings (κ = 0.86, $P <$ .001) (Table I, *A*).

Although the total number of "unclassified" tracings amounted to 46, only 12 AHM diagnoses were deemed "unclassified." An "unclassified" diagnosis was based on a majority of 2 out of 3 tracings per encounter. Therefore, there were instances where the AHM recorded an "unclassified" trace that did not amount to an "unclassified" diagnosis. Accurate diagnosis of AHM unclassified tracings was established in 10 of 12 cases (83%) when assessed by EPs and 9 of 12 (75%) on review by PCPs. Further analysis of all "unclassified" tracings was undertaken to elucidate the potential causes of indeterminate tracings (Online Figure 2).

Atrial flutter

Only 2 (29%) of 7 cases of atrial flutter were accurately identified as an arrhythmia by EPs and 1 (14%) by PCPs (Table I, *A*). AHM automated diagnostics identified 2 (28%) of the atrial flutter cases as "Possible AF," 3 (43%) tracings as "unclassified," and 2 (28%) as sinus rhythm. The automated AHM algorithm does not offer atrial flutter as a distinct diagnosis, which limits the ability for a direct comparison with clinician assessment.

Workflow incorporating AHM and clinician interpretation

Incorporating AHM automated diagnosis (unclassified excluded) with clinician assessment of only "unclassified" tracings improved overall diagnostic accuracy and agreement with 12-lead ECG among PCPs. This approach also yielded similar diagnostic accuracy and agreement when benchmarked against EP interpretation of each tracing (Table I, *B*).

Discussion

This study demonstrated 3 key findings: (1) Accuracy of clinician interpretation was variable, with only EPs demonstrating satisfactory agreement with 12-lead ECG. (2) When offered by the AHM, an automated diagnosis yielded comparable diagnostic accuracy to clinician interpretation of tracings. (3) Incorporation of the AHM autodiagnosis with EP interpretation of only "unclassified" tracings resulted in satisfactory diagnostic accuracy which was comparable to EP interpretation of all iECGs.

The accuracy of the AHM machine-learning algorithm in our study was comparable to EP interpretation

Table I. Accuracy of clinician and AHM analysis.

		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR	Accuracy (%)	κ
A: Performance of blinded clinician interpretation and automated device analysis against simultaneous 12-lead ECG									
AF/atrial flutter	EP 1	92	92	92	92	11.1	0.08	92	0.84
	EP 2	81	100	100	83	∞	0.19	90	0.8
	PCP 1	76	96	95	79	18.5	0.24	86	0.72
	PCP 2	85	84	85	84	5.3	0.18	84	0.69
	AHM (unclassified as Incorrect)	77	76	77	76	3.2	0.3	76	0.53
	AHM (unclassified excluded)	100	95	95	100	22	0	98	0.86
Atrial flutter	EP 1	29	97	40	95	8.9	0.7	92	0.35
	EP 2	29	97	40	95	8.8	0.7	92	0.35
	PCP 1	14	99	50	94	13.3	0.9	93	0.22
	PCP 2	14	95	16	94	2.7	0.9	89	0.18
B: Performance incorporating AHM automated analysis and clinician diagnosis of "unclassified" tracings									
	EP1	93	92	93	92	12.1	0.08	92	0.81
	EP2	87	88	87	88	7.6	0.14	88	0.81
	PCP1	88	88	88	88	7.4	0.13	88	0.77
	PCP2	85	88	88	85	7.1	0.17	86	0.73

PPV, Positive predictive value, *NPV*, negative predictive value; *PLR*, positive likelihood ratio; *NLR*, negative likelihood ratio. AF/atrial flutter classified as one diseased state (top set of rows); traces unreadable by clinicians marked as wrong; accuracy calculated as sum of true positives and true negatives divided by total number of tests.

(sensitivity 100%, specificity 95%) when "unclassified" tracings were excluded. This is in keeping with prior reports that estimated the AHM sensitivity and specificity as 67%-95% and ≥94%, respectively.[5,7,8] Incorporating the AHM diagnosis (when it confirmed tracing as AF or sinus rhythm) with clinician interpretation of the "unclassified" tracings led to an improvement in accuracy among PCPs, although this did not meet the prespecified κ threshold of 0.8. Incorporating the EP diagnosis of "unclassified" tracings with the AHM diagnosis however yielded comparable results to manual EP interpretation of each iECG tracing (κ 0.81, $P < .001$). These findings suggest that EP interpretation may be rationalized to the "unclassified" tracings alone. As such, we propose a new AHM workflow (Figure 2) which minimizes the potential costs associated with unnecessary iECG overreads by using the AHM automated diagnosis and limiting clinician interpretation to only a minority of the tracings deemed "unclassified." Given the discrepancy in accuracy noted between clinicians, this strategy may require the iECG interpretation by EPs. Validation of this algorithm using the 306 iECGs in our cohort demonstrated a diagnostic accuracy of 98%. However, in cases of newly diagnosed AF based on AHM diagnosis, we recommend prompt confirmation with a 12-lead ECG, as the risk of false positives will likely be higher in a community population cohort. As such, future studies validating this algorithm in a cross-sectional screening study are essential.

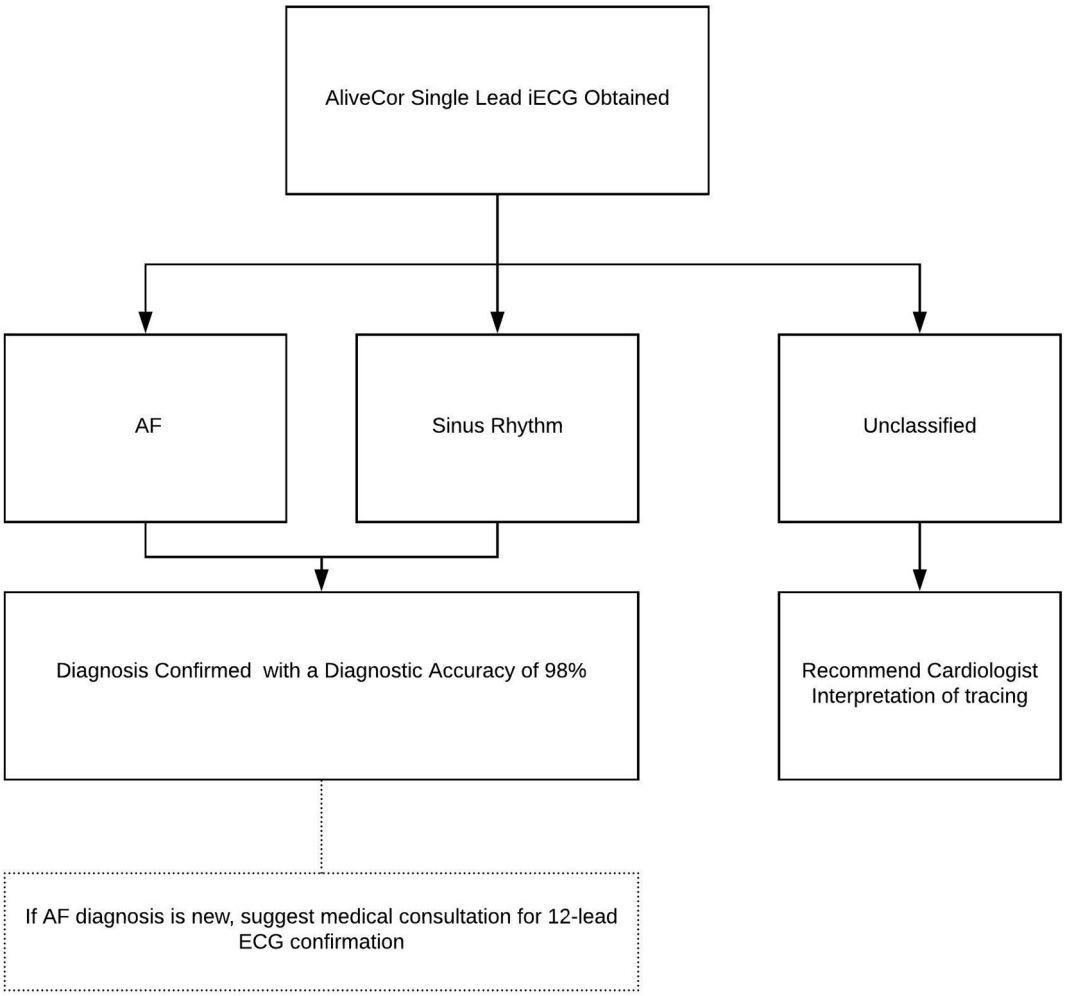
Although numerous studies report on the accuracy of the AHM diagnostic algorithm,[2,6,7] there is a paucity of data on the proficiency of a clinician's interpretation with a corresponding 12-lead ECG. Furthermore, no studies have evaluated PCPs for interpreting single-lead ECGs. In our study, only EPs demonstrated satisfactory agreement when compared to a 12-lead ECG. This may primarily be due to reduced familiarity among PCPs with assessment of cardiac arrhythmias from single-lead tracings.

Reentrant arrhythmias such as atrial flutter are difficult to diagnose on single-lead ECGs. This has led to the recommendation that all traces with demonstrable tachycardia (HR >100) at rest should undergo manual interpretation.[9] However, we report a low sensitivity of atrial flutter detection with manual clinician interpretation, where patients were mislabeled as sinus rhythm in ~80% of cases. Given that atrial flutter and AF share a similar thromboembolic risk profile,[10] it is vital that this discrepancy is recognized and addressed. A formal 12-lead ECG may be necessary in these instances.

Limitations

The recruitment of a selected group of patients undergoing electrical cardioversion may limit the generalizability of the findings. Only 2 EPs and PCPs assessed iECGs in this study. It remains unclear if these findings are generalizable to other physicians and whether prior clinician experience with iECGs and level of training affect accuracy. The frequency of "unclassified" tracings is likely to be higher when the device is used at home by patients who may lack the knowledge and training to amend their technique to improve iECG acquisition. This, in turn, could further increase the number of iECGs that requires clinician review. Lastly, clinician assessment of

Figure 2



Proposed clinical workflow.Diagnostic accuracy estimated as the sum of true positives and true negatives divided by the total number of tests.

the iECGs was undertaken on a smartphone. Although this may affect recognition of subtle QRS irregularities or P waves, this was done intentionally to replicate the real-world point-of-care use of the technology.

Conclusion

In this cohort of patients, EP interpretation of iECG tracings demonstrated satisfactory diagnostic accuracy when compared with 12-lead ECG. The automated device algorithm was comparable to this only when uninterpretable traces were excluded. However, combining the device automated diagnostic algorithm with EP interpretation of only uninterpretable traces yielded excellent results and provides an efficient, cost-effective workflow for the utilization of a smartphone-based ECG in clinical practice.

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Supplement

Table 1

Baseline Characteristics	
Age (years)	64±15
Sex (Male)	65 (33)
BMI	29 (± 4)
Ischemic Heart Disease	16 (31)
Hypertension	31 (61)
Peripheral Vascular Disease	2 (4)
Heart Failure	17 (33)
Diabetes Mellitus	5 (10)
Stroke or Systemic Embolism	6 (12)
CHA_2DS_2-VASC Score	3 ±2
Owns Smartphone	29 (57)
Uses mHealth Smartphone Applications	24 (83)
Owns Wearable Heart Rate Monitor	6 (12)

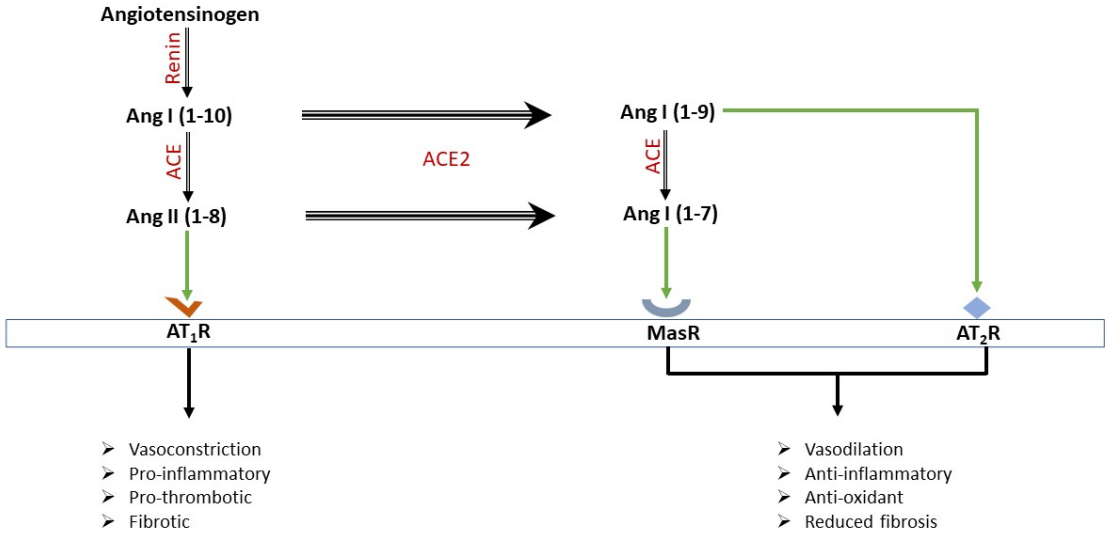
Values are presented as mean±SD or as n (%), mHealth, mobile health

Atrial structural and biochemical remodelling

Atrial electrical remodelling occurs in the presence of underlying left atrial structural and functional remodelling. Increased atrial volume and fibrosis have been associated with increased presence of AF (13,14). Areas of atrial fibrosis in turn have been associated with abnormal electrical properties on electrophysiologic studies (15). Atrial functional impairment has also been associated with increased risk for AF. However, even in the absence of AF, these changes have been associated with ischaemic stroke. Patients with ESUS, and therefore no history of AF, had comparable atrial fibrosis to patients with manifest AF (16). In turn, both these groups have higher rates of fibrosis than a control group. This alludes to the possibility that that patients with ESUS have higher rates of atrial structural abnormality that may promote thrombogenesis independent of AF.

Atrial thrombogenesis is dependent on a thrombotic biochemical milieu. Numerous biomarkers have been associated with myocyte stress, injury, inflammation, endothelial dysfunction and hypercoagulability as outlined in the first chapter (17). These biomarkers are associated with changes to atrial function and structure.

Angiotensin converting enzyme 2 is an integral membrane protein that is found within the endothelium, heart, lungs and kidneys. It counteracts the effect of the renin angiotensin system and its interaction with the respective receptors (18).



Increased ACE2 activity levels within the plasma have been implicated with worsening left atrial electroanatomical substrate on electrophysiologic studies (19). Recently, atrial fibrosis has also been shown to be associated with increased ACE2 activity levels (20).

Despite the association between ACE2 activity and abnormal left atrial histological and electro-anatomical properties, any association with ESUS has not been previously described. Given its previously described associations with atrial remodelling, we hypothesised that ACE2 activity may be associated with ESUS, with the potential to be a novel marker of underlying atrial myopathy that predisposes a subset of ESUS patients to cardioembolic stroke.

In this chapter, we describe the association between ACE2 activity and ESUS compared with a risk factor matched control population without prior stroke or AF. We also evaluated left atrial structural and phasic functional properties on transthoracic echocardiography between the two groups.

Chapter 7

Angiotensin Converting Enzyme 2 Activity is Associated with Embolic Stroke of Undetermined Source

Angiotensin converting enzyme 2 activity is associated with embolic stroke of undetermined source.

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Abstract

Background: Embolic stroke of undetermined source (ESUS) accounts for one in four ischaemic strokes. Early markers of abnormal atrial substrate that confer a risk for stroke are required to guide therapeutic decisions. Angiotensin converting enzyme 2 (ACE2), an integral membrane protein that degrades angiotensin II, has been associated with abnormal atrial substrate and fibrosis. The relationship between ACE2 plasma activity and ischaemic stroke is unknown.

Methods: A total of 104 patients were included; 51 patients with ESUS were compared to 53 vascular risk factor matched control patients without prior stroke or atrial fibrillation (AF). Plasma ACE2 activity was significantly higher in ESUS compared to the control group (9.66 vs 6.61 pmol/min/ml, p 0.04). On regression modelling, adjusting for left atrial volume and vascular risk factors, ACE2 activity remained significantly associated with ESUS (OR: 1.04, 95% CI: 1.01 – 1.08, p 0.04). An elevated ACE2 activity was also associated with reduced left atrial reservoir strain (33.1% vs 37.9%, p 0.01)

Conclusion: ACE2 activity was significantly higher in patients with ESUS. An elevated ACE2 activity was associated with atrial functional impairment. Plasma ACE2 activity maybe an early marker of abnormal atrial substrate that confer a heightened risk for thromboembolic stroke.

Introduction

Embolic stroke of undetermined source (ESUS) accounts for one in four ischaemic strokes. Cardioembolic phenomena due to occult atrial fibrillation (AF) are thought to account for a substantial proportion of these strokes(1,2). However, recent trials of oral anticoagulation in an unselected population with ESUS failed to confer benefit (3,4). This highlights the importance of developing non fibrillatory markers of atrial dysfunction to facilitate early identification of patients at risk for cardioembolic stroke.

Angiotensin converting enzyme 2 (ACE2) is an integral membrane protein that degrades angiotensin II (5). The renin angiotensin system is a well-recognised driver of adverse cardiac remodelling. An association between ACE2 plasma activity and left atrial electroanatomic substrate abnormalities in patients with AF have been highlighted(6). More recently, histological evidence for left atrial fibrosis in conjunction with ACE2 plasma activity has also been described(6,7). Despite its relationship with atrial remodelling and fibrosis, the association between ACE2 activity and ischaemic stroke is unknown.

In this study, we assessed the association between ESUS and plasma ACE2 activity, by comparing a cohort with ESUS against a matched control group. The relationship between ESUS and markers of thrombogenesis, inflammation and cardiac structural remodelling was also assessed.

Methods

One hundred and four patients were prospectively recruited from a tertiary hospital (Melbourne, Australia); including 51 patients with ESUS and 53 control patients with no history of prior stroke or AF. The groups were matched for CHA_2DS_2-VASc Score, excluding points for stroke in the ESUS cohort. The study was approved by the Institutional Human Research Ethics Committee (study ID LR84/2017) and written consent was obtained from all study participants. Australian & New Zealand Clinical Trials Registry (ACTRN12617000771358).

Patients were included if they had an embolic stroke of undetermined source diagnosed following a non-lacunar ischaemic stroke detected by CT or MRI of the brain in the absence of extra and intracranial atherosclerosis causing >50% luminal stenosis in the arteries supplying the area of ischaemia. All patients underwent transthoracic echocardiography and a minimum of 24 hours of cardiac rhythm monitoring to exclude the presence of cardiac abnormalities that could lead to embolic strokes. Patients were adjudicated to have had an ischaemic stroke by the institutional stroke team. Patient were recruited to the control arm if they presented for an outpatient transthoracic echocardiogram and had no prior history of cerebral ischaemia or stroke.

Patients were excluded if age was <55 or >80 years, had a history of atrial fibrillation or atrial flutter, myocardial infarction in the preceding 30 days, previous coronary artery bypass grafting or prosthetic cardiac valves, native valvular dysfunction of greater than mild severity, non-ischaemic cardiomyopathy, left ventricular ejection

fraction < 50%, infective endocarditis or valvular vegetation, systemic inflammatory or thrombotic disorders.

2-Dimensional Echocardiography

Transthoracic echocardiography was performed using GE Vivid E9 cardiac ultrasound (GE Medical Systems, Milwaukee, WI). All images were stored digitally and analysed offline using commercially available software (EchoPAC, GE Healthcare). Standard measurements were obtained in keeping with relevant guidelines and measured over 3 - 5 consecutive cardiac cycles(8). Left atrial volume was calculated by the biplane method of discs. Two-dimensional left atrial speckle tracking was measured from 2 and 4 chamber views with R-R gating. Focussed left atrial views were obtained were feasible. Frame rate was maintained between 60 – 80 Hz and left atrial endocardial border was manually traced and a region of interest automatically generated with 6 segments in each view. The region of interest was visually assessed and adjusted to ensure adequate tracking. If >1 segment was dropped in any view, the study was deemed inadequate for strain analysis.

Blood samples

Blood samples were collected at the time of echocardiography, a minimum of 3 weeks after the index stroke.

Blood for ACE2 activity measurement was collected in lithium heparin tubes and centrifuged immediately and plasma stored at – 80 °C. Plasma ACE2 activity was measured using a sensitive quenched fluorescent substrate [(7-methoxycoumarin-4-yl)-acetyl-Ala-Pro-Lys (2,4-dintirophenyl), Auspep, Victoria, Australia] based assay as previously described(7). Briefly, plasma underwent an anion exchange extraction

process using low-ionic-strength buffer (20 mmol/L Tris–HCl, pH 6.5) and ANXSepharose 4 Fast-Flow-Resin (GE Healthcare Bio-sciences AB, Uppsala, Sweden) that removed a previously characterized endogenous inhibitor of ACE2 activity(9). The resulting eluate was assayed for ACE2 catalytic activity. Duplicate samples were incubated with the ACE2-specific quenched fluorescent substrate, with or without 100 mM ethylenediaminetetraacetic acid. The rate of substrate cleavage was determined by comparison to a standard curve of the free fluorophore, 4-amino-methoxycoumarin (MCA; Sigma Aldrich Inc., MO, USA) and expressed as picomole of substrate cleaved/mL of plasma/min. The intra-assay and inter-assay coefficient of variation in our laboratory was 5.6% and 11.8% respectively.

Remaining samples were collected, centrifuged and processed immediately through the institutional pathology service. D-Dimer assay had a limit of detection of 0.27 mcg/ml and the fibrinogen assay had a measurement range of 0.4 – 12.0 g/L. High sensitivity troponin T assay from Roche Diagnostics (Indianapolis, IN, USA) had a limit of detection of 5 ng/L. High sensitivity CRP assay from Roche Diagnostics (Indianapolis, IN, USA) had a limits of detection of 0.15mg/L.

Statistical methods

Normality of data was assessed using Shapiro-Wilk method. Comparisons between groups with continuous data was performed using 2-sided student's t-test. Categorical variables were compared using Pearson's x2 test. Non-parametric data was assessed using Mann Whitney U test and presented as median with interquartile range (IQR). Correlation trends were analysed using Spearman's rho for non-parametric data. Selection of variables for inclusion in the multivariate logistical

regression model was based on clinical and/or statistical significance with p value <0.2 on univariate analysis. A two-sided p-value <0.05 was deemed statistically significant and a 95% confidence interval (CI) is presented where applicable. An n = 49 in each group provided us with 80% power at a significance level of p = 0.05 to detect a 4% difference in mean reservoir strain between the groups. An elevated ACE2 activity was represented by the upper quartile of ACE2 activity measurements. Statistical analysis was performed with SPSS Statistics 24.0 (IBM, USA).

Results

The mean age was 66 ± 6 years in the control group compared with 67 ± 7 years in the ESUS group. There was no significant difference in age, gender or vascular risk factors between the groups as outlined in Table 1. The groups were well matched for CHA_2DS_2-VASc score, with a median score of 2 (Interquartile range (IQR): 1 – 3) in both groups, after omitting 2 points attributable to the stroke. The median modified Rankin score was 1 (IQR 0 – 2) on discharge within the ESUS group. Blood samples were obtained a median of 36 days (IQR: 29 – 66) following the stroke.

The use of statins, antiplatelet therapy and angiotensin receptor blockade were significantly higher in the ESUS group. However, there were no significant differences in use of ACE inhibitors between the groups. There was no difference in left ventricular parameters between the groups (Table 2). Five patients were excluded from atrial strain analysis due to poor image quality. The intra and inter observer variability for reservoir strain assessed using intraclass correlation coefficient was 0.91 and 0.87 respectively.

Left atrial parameters were significantly different between the group. Both median E/e' 9.71 (IQR 7.66 – 11) vs 10 (IQR: 8.57 – 13.33, p 0.03) and left atrial volume index 36.94 ml/m2 (IQR 32.60 – 42.49) vs 39.08 ml/m2 (IQR: 35.94 – 46.41, p 0.04) were higher within the ESUS group, while left atrial reservoir strain 39.08% (IQR 35.94 – 46.41) vs 38.61% (IQR 32.60 – 42.49, p 0.02) was significantly lower in the ESUS group (Figure 1).

Median plasma ACE2 activity levels were higher in the ESUS group 9.66 pmol/min/ml (IQR: 4.67 – 17.91) compared with the control group 6.61 pmol/min/ml (IQR: 2.26 – 13.62, p 0.04). No significant difference existed between the remaining biomarkers (Table 2). On logistical regression modelling, ACE2 activity remained significantly associated with ESUS after adjusting for LA volume and vascular risk factors (Table 3). Indices associated with an elevated plasma ACE2 activity within the entire cohort was assessed. An elevated plasma ACE2 activity, defined as the upper quartile (>7.32 pmol/min/ml), was associated with increased left ventricular mass 75.7 g/m2 (65.1– 88.3) vs. 83.4 g/m2 (74.6 – 102.3, p 0.04) and a reduced left atrial reservoir strain 37.9% ± 8.1vs. 33.1% ± 8.3 (p 0.01). However, demographic indices, vascular risk factors and left atrial volume index was not associated with an elevated plasma ACE2 activity. The use of an ACE inhibitor was not significantly different between the groups, but statin, ARB, beta blocker use was significantly higher in the ESUS group.

Discussion

This study compared patients with ESUS against a control group without prior stroke or atrial fibrillation. We demonstrated that ESUS was significantly associated with increased ACE2 activity levels, a novel finding. We have previously described the association between increasing plasma ACE2 activity levels with abnormal atrial electroanatomical remodelling and atrial fibrosis (6,7). Therefore, ACE2 activity may indicate the presence of an atrial myopathy in a subset of patients with ESUS. Atrial fibrillation can be difficult to identify following ESUS and oral anticoagulation in an unselected ESUS cohort was not beneficial (4). There is an emergent need to identify novel reproducible markers of abnormal atrial substrate in patients with ESUS to guide therapeutic decisions.

ACE2 is a homologue of angiotensin enzyme (ACE) that degrades Ang I and Ang II(10). It acts as an endogenous inhibitor of the renin angiotensin system (RAS)(11). Degradation of angiotensin II by ACE2 results in the formation of angiotensin 1-7 (Ang 1 – 7) and these peptides oppose the fibrotic effects of angiotensin II. Despite its similarities to ACE, ACE2 is not antagonised by an ACE inhibitor (12). As ACE2 is a homologue of ACE, an angiotensin receptor blocker (ARB) is unlikely to directly interface with the ACE2 membrane protein. Despite one animal study that reported higher tissue ACE2 gene expression after ARB exposure in rats, there are no data on any indirect effect of ARB on plasma ACE2 activity (REF).

As the RAS pathway is a well-known mediator of maladaptive cardiovascular remodelling, ACE2 as an opposing system has also been implicated in hypertension, diabetes and heart failure(5,13,14). More recently, increased ACE2 activity levels

were shown to be associated with atrial fibrillation and adverse electroanatomic remodelling(6). The elevated ACE2 activity in adverse atrial remodelling is postulated to be due to shedding of the enzyme from the cellular architecture. This is supported by recent data with histological characterisation of left atrial tissue that demonstrated reduced atrial ACE2 gene expression and increased fibrosis in association with increased ACE2 plasma activity (7).

In the present study, demographics and clinical features were comparable to contemporary trials on ESUS. A delayed collection of plasma samples was mandated in the ESUS group, as acute reduction in ACE2 activity levels following an ischaemic stroke have been described previously, with normalisation occurring within a week (15).

Plasma ACE2 activity level was significantly higher in the ESUS group. ACE2 activity was the only biomarker assessed that was significantly associated with ESUS (Figure 2). This is a novel finding and this association persisted even after correcting for left atrial volume on transthoracic echocardiography. This indicates that changes in ACE2 activity levels may occur independent of overt atrial structural remodelling. While it is possible that differences in ACE2 activity levels could be due to other risk factors associated with ischaemic stroke, such as hypertension, diabetes mellitus or age, in the present study, both groups were well matched for CHA_2DS_2-VASc score and therefore vascular risk factors.

Markers of atrial function and structure were significantly different between the ESUS and control groups. Both left atrial pressure and volumes were higher in the ESUS

group. Similarly, LA reservoir strain, a marker of phasic function was also significantly reduced in the ESUS group. These findings are consistent with prior data, whereby left atrial enlargement conferred a graded rise in risk for stroke and reduced reservoir strain predicted development of AF following cryptogenic stroke(16-18). Similarly, ESUS patients have comparable left atrial fibrosis to patients with AF(19). Therefore, a reduced reservoir function in the ESUS group noted on atrial strain imaging, may represent underlying early atrial fibrosis.

A reduced atrial reservoir strain was significantly associated with an elevated ACE2 activity level, but notably, left atrial volume did not demonstrate similar association. This may indicate that ACE2 activity is an early marker of adverse atrial remodelling that occur prior to marked atrial structural changes. These changes are likely to occur in the context of underlying electroanatomic abnormalities. The results of ACE2 activity levels in patients with AF by Walters et al. was informative in this regard. There was a graded rise in ACE2 activity in patients without AF, paroxysmal AF and persistent AF, respectively. Increased ACE2 activity was also significantly associated with low mean left atrial bipolar voltages and fractionated electrograms, indicative of advanced atrial disease(6).

Furthermore, the absolute values of ACE2 activity was significantly higher in patients with AF compared with the ESUS cohort in the present study(6). This alludes to a dose response relationship, with marked elevation of ACE2 activity occurring in patients with advanced atrial remodelling as with AF, compared to lower absolute levels in patients with ESUS with presumably less atrial structural abnormalities.

Conversely, the association between ACE2 activity and ESUS maybe be attenuated. ESUS encompasses a heterogenous group of patients that may also include a cohort with non-cardiac mechanisms of cerebral embolism. This may have led to the apparent lack of benefit for oral anticoagulation within an unselected population with ESUS(4). A sub study of the NAVIGATE-ESUS trial showed reduced stroke with oral anticoagulation in patients with an enlarged atrium(20). This highlights the importance of identifying indices of abnormal atrial substrate that may promote thrombogenesis to guide therapeutic decisions. Similarly, ACE2 activity may identify the subset of ESUS patients with an underlying atrial myopathy.

The present study has limitations. Patients did not have implantable loop recorders and therefore it is unclear if occult AF could be contributing to the difference in ACE2 activity levels. The wide spread of ACE2 activity levels with both the groups may limit its use at an individual level. Larger studies may allow for further refinement of optimal cut-off values. We cannot exclude the possibility that the increased use of beta blockers, statins and ARBs within the stroke cohort may have influenced ACE2 activity levels. The presence of a patent foramen ovale was not formally assessed as patients were over the age of 55 years and it was not deemed to be clinically indicated. ACE2 activity levels were obtained after index stroke and therefore conclusions about causality cannot be drawn from the present data.

Conclusion

We have described a novel association between elevated ACE2 activity and ESUS. It may be a reproducible marker of adverse atrial remodelling with a heightened risk for thromboembolic stroke. Further prospective cohort studies are required to confirm these findings. Novel markers of an atrial myopathy in ESUS may allow for targeted initiation of pharmacotherapy to prevent recurrent strokes.

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Tables

Table 1. Baseline characteristics between control participants and ESUS patients.

	Control n = 53 n (%)	**ESUS** n = 51 n (%)	**P value**
Age (years)	66.15 ± 6.64	67.10 ± 7.12	0.48
Sex – Female	24 (45.3)	19 (37.3)	0.41
BMI (kg/m^2)	27.24 ± 4.29	27.47 ± 4.73	0.20
Hypertension	26 (49.1)	23 (45.1)	0.69
Diabetes mellitus	10 (18.9)	11 (21.6)	0.73
Dyslipidaemia	24 (45.3)	18 (35.3)	0.30
Coronary artery disease	7 (13.2)	3 (5.9)	0.32‡
Smoking	17 (32.1)	16 (31.4)	0.94
Median CHA_2DS_2-VASc score	2 (1 – 3)	2 (1 – 3)	0.94
Systolic BP (mmHg)	137 (126 - 147)	133 (122 - 143)	0.28
Diastolic BP (mmHg)	81 (73.5 - 86)	78 (74 - 84)	0.35
MRS score - discharge	-	1 (0 – 2)	
Time blood sample (days)	-	36 (29 – 66)	
Medications			
Anti-platelets	17 (32.1)	48 (94.1)	**<0.01**
Beta blockers	11(20.8)	1 (2)	**<0.01**
ACEi	13 (24.5)	21 (41.2)	0.70
ARB	6 (11.3)	14 (27.5)	**0.04**
Statin	24 (45.3)	46 (90.2)	**<0.01**

Data expressed as mean ± standard deviation, n(%) or median (interquartile range); ‡, Fishers exact test; BMI, body mass index; MRS, modified Rankin score; ACEi, ace inhibitor, ARB, angiotensin receptor blocker.

Table 2. Echocardiographic parameters and biomarkers

	Control n = 53	**ESUS** n = 51	**P value**
Echocardiography			
LVEF (%) ± SD	61.3 +/- 6.1	61.7 +/- 8.3	0.74
LVMI (g/m2)	78 (66 – 91)	79 (66 - 91)	0.79
LVEDV (mL)	64 (55 – 90)	80 (61 - 96)	0.24
LVESV (mL)	26 (21 – 35.5)	32(22 - 40)	0.07
E (cm/s)	0.60 (0.53 – 0.72)	0.60 (0.54 – 0.79)	0.44
A (cm/s)	0.70 (0.60 – 0.80)	0.70 (0.60 – 0.90)	0.11
E/A	0.89 (0.72 – 1.14)	0.88 (0.69 – 1.04)	0.69
Deceleration time (ms)	208 (182 – 239)	206 (179 – 236)	0.61
Average e' (cm/s)	7 (5.7 – 7.5)	6 (4.5 – 7.5)	0.12
Mean E/e'	9.7 (7.7 - 11)	10 (8.6 – 13.3)	**0.03**
LA volume index (mL/m^2)	36.9 (32.6 – 42.5)	39.1 (35.9 – 46.4)	**0.04**
LA reservoir strain %	38.6 ± 8.5	34.7 ± 7.8	**0.02**
LA contractile strain %	19.3 (16.4 – 22.7)	19.3 (15.8 – 22.7)	0.92
Plasma Biomarkers			
Fibrinogen (g/L)	3.5 (3.1 – 3.9)	3.6 (3.1 – 4.3)	0.39
D-Dimer (mg/L)	0.4 (0.3 – 0.5)	0.4 (0.3 – 0.6)	0.19
hsTroponin (ng/L)	7.0 (5.0 – 11.5)	8 (6.0 – 13.0)	0.15
hsCRP (ng/L)	1.4 (0.8 – 2.9)	1.1 (0.6 – 2.3)	0.09
ACE2 (pmol/min/ml)	6.6 (2.3 – 13.6)	9.7 (4.7 – 17.9)	**0.04**

Data expressed as mean ± standard deviation or median (interquartile range); LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; LVEDV, left ventricular end diastolic volume; LVESV, left ventricular end systolic volume.

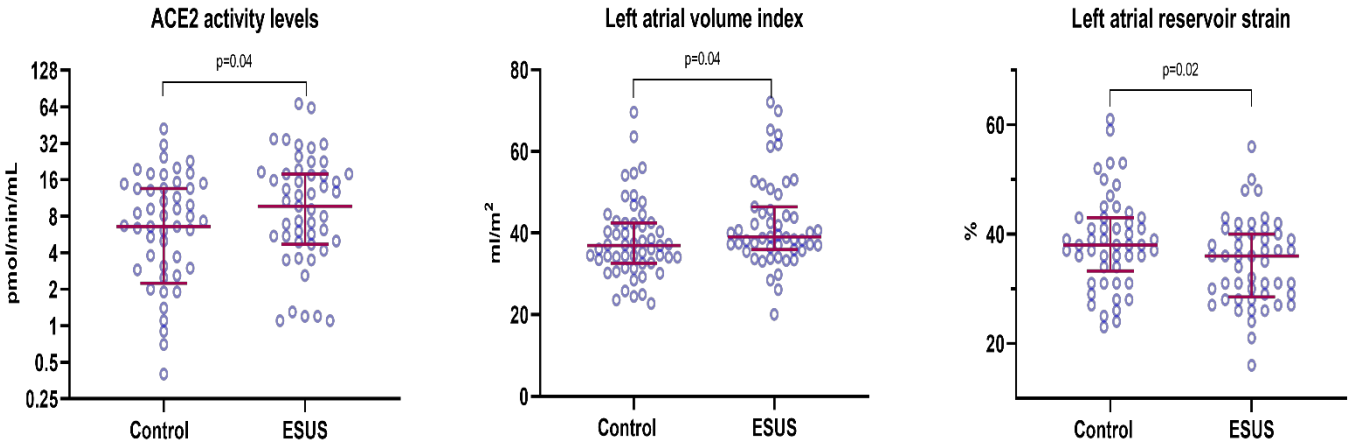
Table 3. Biomarker regression modelling for the individual biomarkers and ESUS

Biomarker	Model 1		Model 2		Model 3	
	OR (95% CI)	*P value*	*OR (95% CI)*	*P value*	*OR (95% CI)*	*P value*
Fibrinogen	1.28 (0.73 – 2.22)	0.39	1.30 (0.73 – 2.29)	0.37	1.37 (0.77 – 2.43)	0.32
D-dimer	2.46 (0.47 – 12.79)	0.28	2.45 (0.46 – 12.95)	0.29	2.63 (0.48 – 14.50)	0.27
hsTroponin	1.04 (0.97 – 1.11)	0.24	1.04 (0.98 – 1.12)	0.21	1.04 (0.98 – 1.11)	0.22
hsCRP	0.84 (0.68 – 1.04)	0.10	0.84 (0.68 – 1.04)	0.11	0.86 (0.70 – 1.05)	0.14
ACE2	1.04 (0.99 – 1.08)	0.05	1.04 (1.00 – 1.08)	0.04	1.04 (1.01 – 1.09)	0.04

Models adjusted for the individual biomarker with demographic indices, vascular risk factors and left atrial volume. Model 1 adjusted for age and sex; Model 2 adjusted for model 1 + hypertension, diabetes mellitus; Model 3 adjusted for model 2 + left atrial volume index.

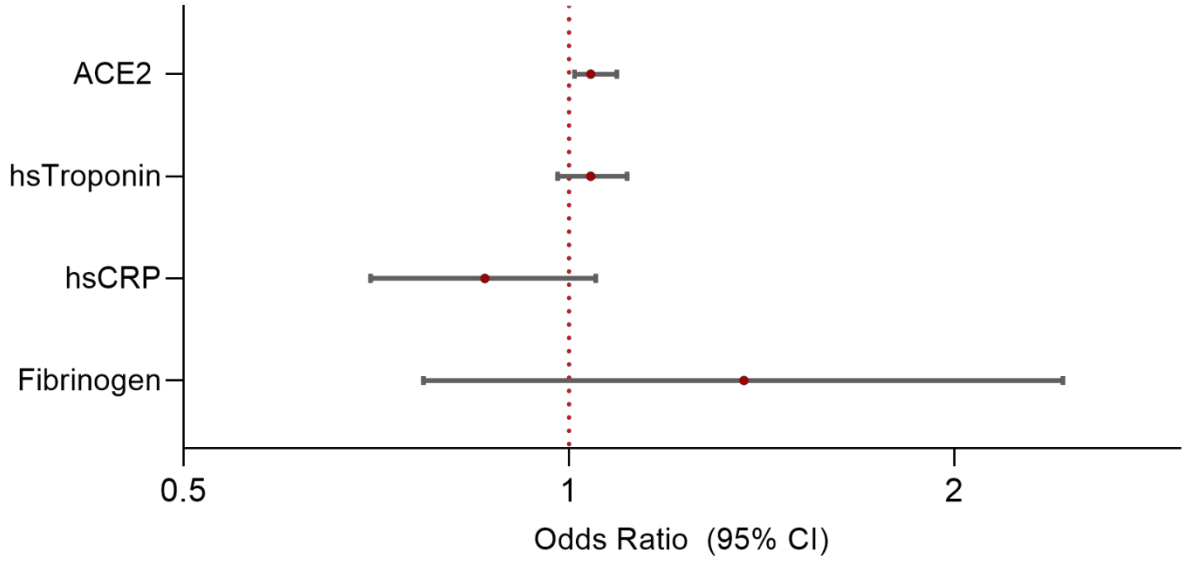
Figures

Figure 1. ACE2 and left atrial indices by study group.



Median and interquartile ranges denoted in red overlying the values for control and ESUS groups. ACE2 activity and LA volume index were significantly higher and LA reservoir strain significantly lower in the ESUS group.

Figure 2. Biomarker regression modelling for ESUS



Individual biomarkers adjusted for age, gender, hypertension, diabetes mellitus and left atrial volume index.

Conclusions and Future Directions

In this thesis we have demonstrated several important findings with clinical implications. We have reviewed and advanced the hypothesis that an atrial myopathy contributes to atrial thrombogenesis independent of atrial rhythm. We evaluated markers indicative of an atrial myopathy utilising various modalities, with assessment of atrial rhythm, structure, function and biochemical changes.

Firstly, we evaluated the association between atrial electrical markers and ischaemic stroke. We evaluated the utility and reliability of these markers with regards to ischaemic stroke. Premature atrial complexes were significantly associated with cryptogenic stroke. We demonstrated the graded association between vascular risk factors and increased burden of premature atrial complexes.

Conversely, despite providing a robust methodology for measuring PTFV1, we demonstrated the inherent limited reliability for P wave terminal force V_1 and a lack of association with electrophysiologic indices of abnormal atrial substrate. Further refinement from large prospective validation studies could focus on the utility of averaged $PTFV_1$ values to reduce inter P wave variability or measurement using an area under the curve method. However, we believe that difficulties in measurement, coupled with various factors that contribute to P wave variability within and between ECGs of the same individual will severely limit the utility of this measure as a marker of atrial dysfunction and ischaemic stroke. This has clinical implications for the utility of $PTFV_1$ as a surrogate for atrial myopathy and thrombogenesis and raises concerns around its use in ongoing clinical trials.

Secondly, we assessed the utility of emerging consumer technology in stroke prevention and AF detection. This rapidly emerging area has raised important clinical considerations around the diagnostic accuracy of these devices and the role of the clinician in caring for patients that use them. We proposed a clinical workflow that may aid in reducing healthcare utilisation from consumer derived single-lead ECG recordings without impacting the accuracy of these devices for detecting AF. We outlined the limitations of these devices in population screening for AF to aid with stroke prevention. The capabilities of consumer wearable platforms continue to improve rapidly, which will lead to evolving use of these platforms. Single lead ECG acquisition has already improved rhythm analysis over photoplethysmography based systems. Wearable hybrid systems that utilise photoplethysmography for continuous monitoring alongside single lead ECG systems have been recently developed. Under this model, photoplethysmography is used to constantly monitor heart rate variability and subsequently trigger the wearer to obtain a single lead ECG acquisition for confirmation of underlying rhythm, if AF is suspected. These advances may improve accuracy and provide an opportunity to identify asymptomatic subclinical arrhythmias such as AF. Furthermore, multi-lead systems are being developed to improve the quality of electrical traces from these platforms. Improvements in automated algorithms and the resultant diagnoses are likely with increased fidelity of the baseline data and further developments in machine-learning algorithms. Despite these advances, it will be important to remember that the clinician will always remain an important part of patient care. Nuanced discussions with patients are required as the benefits of treating individuals with asymptomatic subclinical atrial arrhythmias remains uncertain.

Finally, we assessed a novel biomarker, ACE2, and showed a significant association with embolic stroke of undetermined source. Further, ACE2 activity was also associated with indicators of early atrial functional impairment. This has clinical implications, as there is an emergent need to define novel markers of an atrial myopathy in ESUS to identify the subset of patients that may benefit from initiation of stroke prevention pharmacotherapy, such as oral anticoagulation.

Future research will need to focus on further characterisation of these markers and mechanisms of atrial myopathy. These non-fibrillatory electrical, structural, functional and biochemical markers require evaluation in the context of prospective cohort studies to determine causality. Numerous markers have been independently associated with stroke and AF. Importantly, AF and ischaemic stroke subtypes share common independent risk factors. Therefore, validating individual biomarkers in ischaemic stroke will require long term rhythm analysis to exclude the presence of subclinical AF.

Suitable markers should be reproducible, readily analysed and be economical to undertake. The presence of clinical risk factors such as advancing age, hypertension, diabetes mellitus and vascular disease are readily evaluated as with pre-existing risk scores such the CHA_2DS_2-VASc score. However further refinement with addition of new markers may improve their predictive capacity. Structural markers identified on cardiac echocardiography include left atrial volume index, left atrial strain and phasic function on transthoracic echocardiography. Similar information can be obtained from cardiac MRI, along with features of left atrial

fibrosis. However, difficulty in obtaining routine access to imaging modalities such as cardiac MRI may limits its utility. Electrical markers may include excessive burden of premature atrial complexes or electrocardiographic features indicative of left atrial enlargement. There are numerous biochemical markers associated with atrial dysfunction and stroke, including natriuretic peptides such as ANP and BNP, troponin, CRP, IL-6, ADMA, vWF, D-dimer and Fibrinogen. However, there is paucity of data on the comparative utility of these markers in identifying ischaemic stroke or underlying atrial myopathy. Further trials comparing various markers prospectively with each other are therefore required in order to assign relative weighting of each of them into risk prediction modelling.

Development of risk modelling to predict ESUS will likely employ a combination of markers, including clinical risk factors, structural markers of atrial dysfunction on cardiac imaging, electrical and biochemical markers. A proposed model could include the CHA_2DS_2-VASc score in conjunction with left atrial volume and reservoir function obtained from transthoracic echocardiogram and biochemical markers including natriuretic peptides, high sensitivity troponin, CRP, D-dimer and ACE2 activity. The relative weight of each marker would need further characterisation through a derivation and validation cohort. The development of such a model would allow for the design of much-needed prospective randomised studies of treatments for stroke prevention.

In the future, a nuanced approach incorporating assessment of an individual's degree of atrial myopathy may allow for individually tailored therapeutic strategies to prevent ischaemic stroke.

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