Pathophysiology and Risk of Atrial Fibrillation Detected after Ischemic Stroke (PARADISE): A Translational, Integrated, and Transdisciplinary Approach

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Received August 2, 2017; revision received September 22, 2017; accepted September 24, 2017.

Grant support: This study is funded by the Kathleen & Dr Henry Barnett Research Chair in Stroke Research (Western University, London, Ontario, Canada), the Western University Medical and Health Sciences Research Board Seed Research Grant, and the Edward and Alma Saraydar Neurosciences Fund (London Health Sciences Foundation). V. Thorburn is a Lawson Research Fund Scholar. B. Balint is supported by a Boehringer Ingelheim stroke research fellowship. C.W.J. Melling is supported by the Natural Sciences and Engineering Council Discovery Grant (RGPGP-2015-00059). A.R. Khan is supported by grants from the Natural Sciences and Engineering Research Council (NSERC) (RGPIN-2015-06639). M.L. Koschinsky is supported by grants from Canadian Institutes of Health Research (CIHR MOP-# 126076), Heart and Stroke Foundation of Canada (G-13-0003091 and G-17-0018740), NSERC (RGPIN/5006-2015), and Pfizer (W1207819). M.B. Boffa is supported by a grant from the NSERC (RGPIN 05571-2017). A. Ibanez is partially supported by grants from CONICET, CONICYT/FONDECYT Regular (1170010), FONCyT-PICT 2012-0412, FONCyT-PICT 2012-1309, and the INECO Foundation. S.N. Whitehead is supported by NSERC (418489), CIHR (126126), Canadian Foundation for Innovation (CFI 34213), and the Canadian Consortium for Neurodegeneration and Aging (CCNA). L.A. Sposato is supported by the Kathleen & Dr Henry Barnett Research Chair in Stroke Research, the Edward and Alma Saraydar Neurosciences Fund, and the Opportunities Fund of the Academic Health Sciences Center Alternative Funding Plan of the Academic Medical Organization of Southwestern Ontario (AMOSO).

Author contributions: Maryse Paquet cowrote the first draft of the manuscript, contributed to the design of the 3 arms of the study protocol, critically revised the prefinal version for important intellectual content, and gave final approval of the version to be published.

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Background: It has been hypothesized that ischemic stroke can cause atrial fibrillation. By elucidating the mechanisms of neurogenically mediated paroxysmal atrial fibrillation, novel therapeutic strategies could be developed to prevent atrial fibrillation occurrence and perpetuation after stroke. This could result in fewer recurrent strokes and deaths, a reduction or delay in dementia onset, and in the lessening of the functional, structural, and metabolic consequences of atrial fibrillation on the heart. *Methods:* The *Pa*thophysiology and *R*isk of *A*trial Fibrillation *D*etected after *I*schemic *S*trok*e* (*PARADISE*) study is an investigator-driven, translational, integrated, and transdisciplinary initiative. It comprises 3 complementary research streams that focus on atrial fibrillation detected after stroke: experimental, clinical, and epidemiological. The experimental stream will assess pre- and poststroke electrocardiographic, autonomic, anatomic (brain and heart pathology), and inflammatory trajectories in an animal model of selective insular cortex ischemic stroke. The clinical stream will prospectively investigate autonomic, inflammatory, and neurocognitive changes among patients diagnosed with atrial fibrillation detected after stroke by employing comprehensive and validated instruments. The epidemiological stream will focus on the demographics, clinical characteristics, and outcomes of atrial fibrillation detected after stroke at the population level by means of the Ontario Stroke Registry, a prospective clinical database that comprises over 23,000 patients with ischemic stroke. *Conclusions: PARADISE* is a translational research initiative comprising experimental, clinical, and epidemiological research aimed at characterizing clinical features, the pathophysiology, and outcomes of neurogenic atrial fibrillation detected after stroke. **Key Words:** Ischemic stroke—atrial fibrillation—prognosis—outcome—recurrence—pathophysiology.

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Financial disclosures: All authors have approved the final version of the article. R.D. Lopes received institutional research grants from Bristol-Myers Squibb and GlaxoSmithKline and consulting fees from Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Merck, Pfizer, and Portola. M. Koschinsky has held research contracts with Sanofi/Regeneron and Ionis Pharmaceuticals, Inc., has been a member of advisory boards to Sanofi S.A. and Ionis Pharmaceuticals, Inc., and has received speaker's honoraria from Amgen, Regeneron Pharmaceuticals, Inc., and Eli Lilly & Co. M. Boffa has held a research contract with Ionis Pharmaceuticals, Inc. L.A. Sposato has received research funds from Boehringer Ingelheim and Medtronic, and speaker honoraria from Boehringer Ingelheim and Pfizer.

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1052-3057/$ - see front matter

https://doi.org/10.1016/j.jstrokecerebrovasdis.2017.09.038

Introduction

Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia encountered in clinical practice and is associated with increased risk of stroke, dementia, falls, and death, among other outcomes.[1,2] AF diagnosed in patients with stroke can be classified as (1) previously known AF (KAF), which is AF detected before stroke or transient ischemic attack (TIA), or (2) AF newly diagnosed after stroke or TIA (AFDAS).[3] The *Pa*thophysiology and *R*isk of *A*trial *F*ibrillation *D*etected after *I*schemic *S*trok*e* (*PARADISE*) Study is a translational and integrated initiative established by the Stroke, Dementia & Heart Disease Laboratory (Western University, London, Ontario, Canada). It involves a multidisciplinary research consortium (e.g., anatomy and cell biology, immunology, cardiology, cerebrovascular disease, autonomic disorders, molecular biology, physiology, pathology) and comprises 3 complementary research streams conducted simultaneously (Fig 1): (1) experimental: development of a rat model of selective insular ischemic stroke to investigate the consequences of insular ischemic stroke on the heart and brain, autonomic, and inflammatory responses, and heart rhythm trajectories before, during, and after insular stroke[3,4]; (2) clinical: prospective assessment of autonomic function, inflammatory responses, and neurocognitive findings (behavioral measures of cardiac interoception and neuroimaging) in patients with ischemic stroke and TIA with and without AFDAS who will undergo immediate and prolonged electrocardiographic (ECG) monitoring; (3) epidemiological: examination of the prospectively collected, population-based Ontario Stroke Registry, to evaluate and quantify the demographics, clinical characteristics, and prognosis of AFDAS.

AFDAS can be identified in up to 23.7% of patients with ischemic stroke without KAF.[5] Up to 95% of AFDAS episodes are asymptomatic[6] and half of these episodes last less than 30 seconds.[7] Hence, detecting this specific type of AF is challenging without continuous and early initiation of cardiac monitoring.[3] Very little is known about the pathophysiology of AFDAS due to the paucity of research on this topic. Therefore, we have developed a pathophysiological model of neurogenic AFDAS based on evidence that suggests that neurogenic AFDAS can be triggered by acute autonomic imbalance elicited by transient (in patients with TIA) or permanent (in patients with stroke) damage to specific brain regions.[3,8] Involvement of the insular cortex seems to play a vital

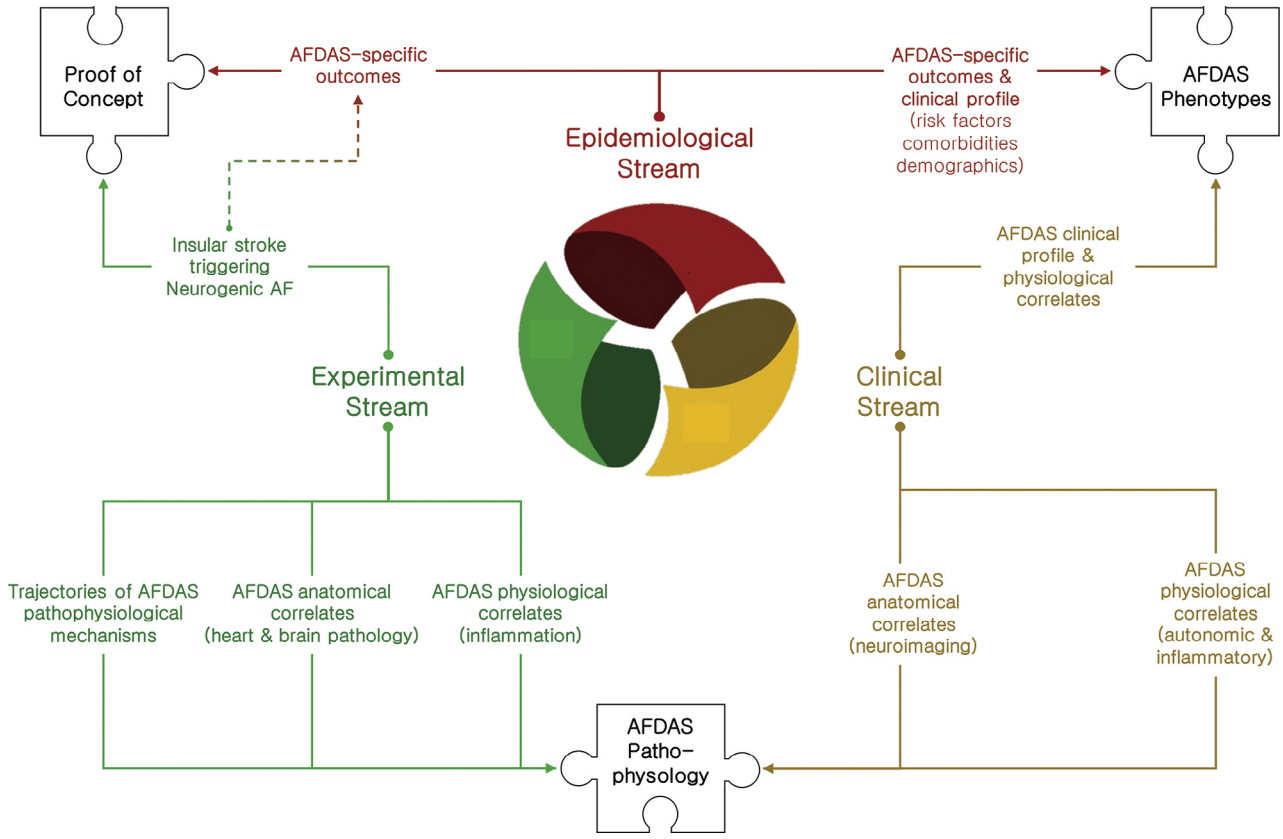

Figure 1. *Translational approach of PARADISE. PARADISE comprises 3 complementary research streams. The experimental, clinical, and epidemiological streams are represented in green, yellow, and red, respectively. Abbreviations: AF, atrial fibrillation; AFDAS, atrial fibrillation detected after stroke or transient ischemic attack. (Color version of figure is available online.)*

role[9]; it regulates the autonomic control of heart rhythm and has been implicated in the genesis of AF and other cardiac arrhythmias after stroke, potentially causing sudden death. Although, a recent lesion mapping study conducted among patients with ischemic stroke found no association between any specific brain region and AFDAS after adjustment for infarct volume.[10] Inflammation has also been implicated as a potential mechanism of AFDAS generation. Indeed, both acute AF and acute cardioembolic stroke are followed by inflammatory responses sharing common markers, ultimately resulting in abnormal autonomic responses.[11-13] The autonomic and inflammatory mechanisms hypothesized to trigger neurogenic AFDAS are suspected to be self-limited and short-lasting,[14-16] which could possibly lead to low-burden AF paroxysms, ultimately associated with a lower risk of stroke recurrence and death.[8] To date, only one study based on a large commercial claims dataset (http://truvenhealth.com/markets/life-sciences/products/data-tools) has investigated AFDAS-related outcomes.[17] Their results suggest that AFDAS is associated with similar prognoses as those with KAF, and that patients with AFDAS may benefit from oral anticoagulation.[17] More studies are needed to further investigate the prognosis of AFDAS. In a pilot study, we have also demonstrated that patients with AFDAS have less frequent cardiac structural changes (e.g., impaired left ventricular ejection fraction, enlarged left atrium), lower prevalence of cardiovascular comorbidities (e.g., heart failure and coronary artery disease), and more frequent involvement of brain structures in the central nervous system regulation of heart rhythm (e.g., insula, limbic system) compared with patients with KAF.[18]

Among all patients with stroke diagnosed with AFDAS, there may be some preexisting AF most likely caused by baseline cardiovascular disease, but not diagnosed because of insufficient monitoring.[3] We have proposed that this subgroup of AFDAS constitutes one of the extremes of a variety of possible phenotypes and could be regarded as *cardiogenic AFDAS*.[3] On the other extreme of AFDAS phenotypes, there are patients who have never had AF before the stroke, who may have healthier hearts, and whose strokes or TIAs may have involved brain structures implicated in the cerebral autonomic regulation of heart rhythm.[3] These patients are deemed to have neurogenic AFDAS. The whole spectrum of patients in between these 2 phenotypes is considered to have mixed AFDAS.[3]

The overarching aim of PARADISE is to provide the necessary knowledge to plan and successfully execute a randomized controlled trial for the prevention of neurogenic AFDAS occurrence and perpetuation. By preventing neurogenic AFDAS, the risk of stroke recurrence and death could possibly be mitigated, dementia onset could be delayed, and heart disease caused by the perpetuation of AFDAS (e.g., atrial remodeling, endothelial dysfunction, local thrombogenesis) could be minimized.

PARADISE: *The Challenge of Neurogenic AFDAS and the Need for a Stepwise Translational Approach*

Investigating AFDAS is uniquely complex. As such, a translational approach for investigating AFDAS is essential (Fig 1). The most significant challenge in AFDAS research is the difficulty to identify truly neurogenic cases. Neurogenic AFDAS has not been characterized, mainly because knowing which patients had or did not have undiagnosed AF before their stroke is virtually impossible in the vast majority of the cases. In the future, it may be possible to identify specific neurogenic AFDAS biomarkers (e.g., serum markers, clinical features, specific patterns of autonomic dysfunction, or even neuroanatomical topographies of ischemic stroke). A stepwise and translational research approach is a necessary first step toward the identification of these markers and to a better understanding of the pathophysiology and outcomes of AFDAS.

The trajectories of heart rhythm over time (before and after stroke) are a key aspect of AFDAS. Most clinical studies investigating this type of AF have limitations related to the lack of knowledge about prestroke heart rhythm.[3] Heart rhythm data, including the existence of AF before stroke, are only available from studies on patients with implantable cardiac defibrillators and pacemakers, which constitute a highly biased population (e.g., patients with substantial heart disease).[19] Therefore, an animal model of selective insular ischemic stroke constitutes the ideal setting for characterizing autonomic, inflammatory, and electrocardiographic trajectories before, during, and after stroke, as well as the effect of these changes on the generation of cardiac arrhythmias. An experimental approach also offers the opportunity to study temporal trends in molecular and anatomical (micro and macro) changes in both the brain and the heart of animals with neurogenic AFDAS in a more controlled setting.

The experimental pathway of PARADISE complements with the clinical arm. By prospectively and systematically establishing anatomic, autonomic, and inflammatory correlates between animal models and humans, the animal study will establish a solid foundation for future investigation of novel therapeutic agents (e.g., anti-inflammatory drugs for neurogenic AFDAS prevention).[3] In fact, we will measure the same inflammatory markers in our rat model and in patients participating in the clinical stream. We aim to identify specific inflammatory markers associated with purely neurogenic AF in the experimental stream and we will analyze the behavior of those markers in humans with and without AFDAS. We will also aim to identify a specific patient phenotype of AFDAS (possibly neurogenic AFDAS) among those

participants exhibiting a similar inflammatory marker profile as that of rats developing AF after ischemic stroke.

Although the assessment of specific outcomes expressed as continuous measures (e.g., gait, interoception, cognition) requires smaller sample sizes, evaluating hard and dichotomous outcomes, such as stroke recurrence and death, is only feasible among large cohorts such as those of prospective randomized clinical trials.[20] To overcome this limitation, we are implementing an epidemiological approach by using a large population-based clinical database of patients with stroke in Canada's most populous province, the Ontario Stroke Registry.[21] We will use these prospectively collected data to evaluate the demographics and clinical characteristics, risk factor profiles, and prognosis of AFDAS. We hypothesize that the risk of stroke recurrence and death associated with AFDAS in the epidemiological arm is more benign than that of KAF. If proven, this concept would suggest that the underlying pathophysiology of AFDAS is also different from that of KAF. The 3 streams of *PARADISE* will be conducted simultaneously as depicted in the timeline (Fig 2).

Methods and Analysis

We describe the methods of the 3 streams of PARADISE: experimental, clinical, and epidemiological.

Experimental Stream

Hypothesis and Objective

We hypothesize that ischemic strokes selectively induced in the insular cortex will result in the new onset of cardiac arrhythmias, including AF and atrial flutter in the context of specific changes in heart rate variability and the release of inflammatory markers into the bloodstream.

The objective of the experimental stream is to develop a rat model of selective insular stroke and cardiac arrhythmia to assess autonomic and inflammatory changes, as well as structural changes in the heart and brain.

Methods

As a first step, a total of 56 6-month-old male Wistar rats will be randomly assigned to 1 of 4 groups. To date, there are no models of selective insular ischemic stroke. We have chosen Wistar rats, based on our previous work comprising other stroke models, some of them also using endothelin-1 (ET-1).[22-26] The first 3 groups will receive stereotaxic injections on the right (n = 8) and left (n = 8) posterior regions of the agranular insular cortex (AIP): (group 1) 20 pmol of ET-1 diluted in 1 µL of 100 mM phosphate buffered saline (PBS); (group 2) 5 ug/ul ibotenic acid diluted in 1 µL of PBS; (group 3) vehicle – 1 µL of PBS. Group 4 will not receive an injection (sham, n = 8). The injection sites of the AIP have been predetermined based on the coordinates obtained from a stereotaxic atlas.[27] ET-1 is a common vasoconstrictor, frequently used in rodent models to mimic human ischemic stroke.[28] However, this powerful peptide causes extensive damage to all structures passing through the injection site. As ibotenic acid is a neurotoxin, injection into the AIP will only damage neurons directly associated with the AIP. Animals will be sacrificed at day 28 after stroke. This first step will be used to (1) characterize changes in the brain parenchyma and the heart after the injection of each compound; and (2) test the feasibility of the procedure and the precision of the anatomic topography of the stroke. In parallel to the development of this model, we will test the insertion of wireless biopotential telemeters in 2 rats and we will continuously monitor their heart rhythm for 60 days (almost twice the time established in the final protocol). After completion of this step, we will analyze brain and heart tissues.

In the second step, 20 animals will be implanted with wireless biopotential telemetric monitors to continuously monitor heart rhythm for 10 days before inducing an insular ischemic stroke. Strokes will be induced with ET-1 in 10 animals (5 on right and 5 on the left) and with ibotenic acid in the remaining 10 rats (also 5 on right and 5 on the left). Fifteen additional control rats (no injection of ET-1 or ibotenic acid) will be also included in this phase, 10 with PBS injections (5 on the right and 5 on the left) and 5 without injections. To detect cardiac arrhythmias and to evaluate heart rate variability, all 35 animals will undergo cardiac monitoring with implanted telemeters, starting 10 days before the stroke is induced and for additional 28 days, until the rodents are sacrificed. Blood samples will be collected 10 days before the stroke, as well as at 2 and 6 hours, and at 1, 7, 14, and 28 days after stroke to assess inflammatory responses and ET-1 levels by multiplex analysis. Half of the cohort will undergo behavioral and cognitive testing at day 7 after surgery, whereas the remaining half will be tested at 28 days. At day 28 after stroke, rats will be sacrificed and their brain and heart will be extracted for histologic examination. By comparing outcomes of the 2 treatment groups, we will be able to confirm if brain damage, cardiac arrhythmias, autonomic dysfunction, systemic inflammatory responses, and cognitive impairment following an ET-1–induced AIP stroke is a direct result of AIP injury.

Clinical Stream

Hypotheses and Objective

Because our hypothesis suggests that AFDAS comprises cardiogenic forms (preexisting AF not diagnosed before stroke, likely similar to KAF) and neurogenic forms (AF triggered by brain damage, mainly in individuals without preexisting heart disease), we anticipate that AFDAS will show a specific profile in terms of risk factors, autonomic changes, inflammatory responses, biomarkers,

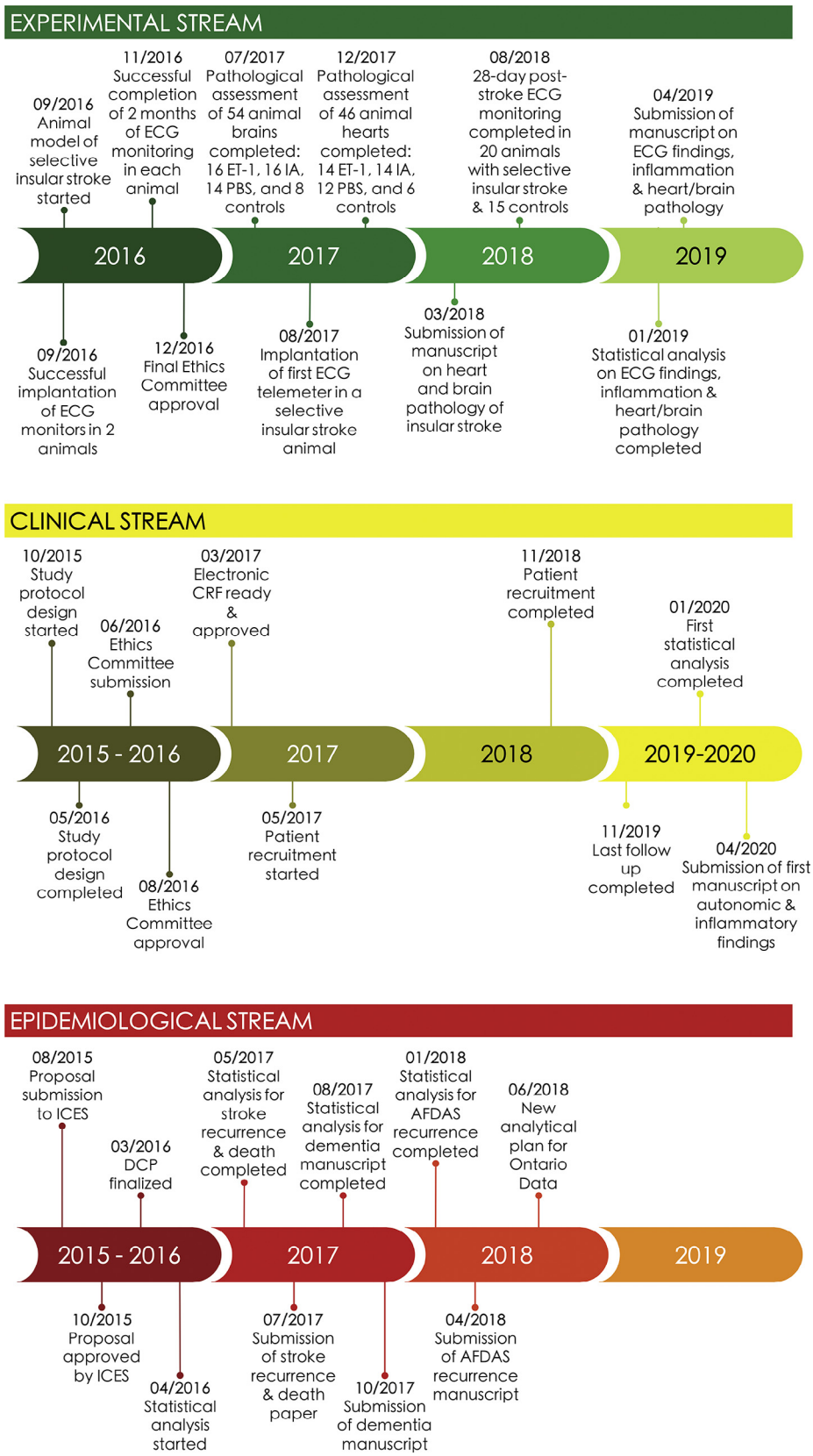

Figure 2. *Timeline of the 3 complementary research streams of PARADISE. The experimental, clinical, and epidemiological streams are represented in green, yellow, and red, respectively. Abbreviations: AFDAS, atrial fibrillation detected after stroke or transient ischemic attack; DCP, dataset creation plan; ECG, electrocardiogram; ET-1, endothelin-1; IA, ibotenic acid; ICES, Institute for Clinical Evaluative Sciences; PBS, phosphate buffered saline. (Color version of figure is available online.)*

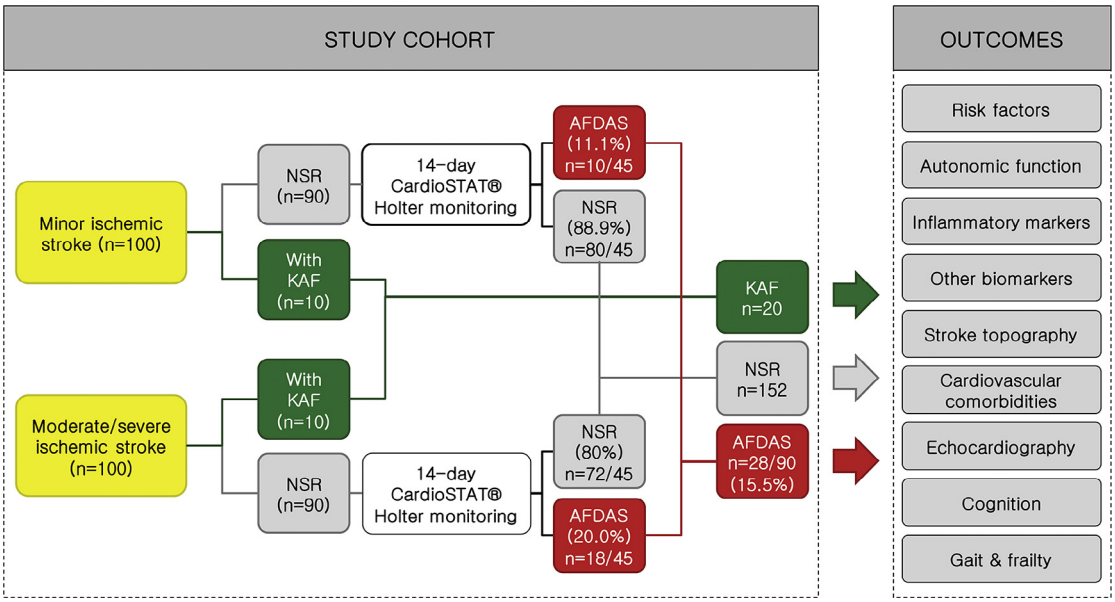

Figure 3. *Study design, clinical stream. The study cohort (left panel) will be composed of 100 patients with minor (NIHSS score ≤ 3) ischemic stroke and 100 patients with moderate or severe (NIHSS > 3) ischemic stroke (yellow rectangles). In each of these 2 groups, 10 patients will have a known history of atrial fibrillation (KAF) before their qualifying ischemic stroke (green squares). The remaining 90 patients in each group with normal sinus rhythm (NSR) at enrollment will undergo continuous cardiac monitoring for 14 days within 72 hours and at 3 months of stroke onset. Based on the results of cardiac monitoring, patients will be classified as having atrial fibrillation detected after stroke (AFDAS, red squares) or sinus rhythm (gray squares). All subjects will be assessed for all outcomes as described in the right panel. Abbreviations: Lp(a), lipoprotein(a); NIHSS, National Institutes of Health Stroke Scale; TAFI, thrombin activatable fibrinolysis inhibitor. (Color version of figure is available online.)*

lesion topography, cardiovascular comorbidities, echocardiographic findings, and cognitive features, and which will be different from that of KAF (almost purely cardiogenic AF).

Based on the study hypothesis, the objective of the clinical stream (Fig 3) is to compare the following variables in patients with acute ischemic stroke with AFDAS and KAF:

1) the prevalence of risk factors (e.g., hypertension, diabetes mellitus, hyperlipidemia),
2) results of autonomic testing (e.g., catecholamine plasma levels, autonomic reflex screen, and diurnal variations in heart rate and heart rate variability),
3) levels of inflammatory markers,
4) other biomarkers (e.g., ET-1, thrombin-activatable fibrinolysis inhibitor [TAFI], activated TAFI , lipoprotein(a), and brain natriuretic peptide),
5) infarct topography (e.g., specific regions of the insula or its connections),
6) cardiovascular comorbidities (e.g., congestive heart failure, coronary artery disease, and prior myocardial infarction),
7) echocardiographic findings (e.g., left atrial enlargement, decreased left ventricular ejection fraction, diastolic dysfunction),
8) cognition (e.g., impaired interoceptive processing), and gait and frailty

Design

This is an investigator-initiated, prospective, single-center (University Hospital, London Health Sciences Centre, London, Ontario, Canada), observational, noninterventional cohort study. A total of 200 patients with acute ischemic stroke, 20 with KAF and 180 without KAF, will be enrolled. The cohort of 200 patients will comprise 100 patients with minor stroke defined as a National Institutes of Health Stroke Scale (NIHSS) score less than or equal to 3 and 100 patients with moderate or severe stroke (NIHSS > 3).

Population

Adult (aged ≥18 years) male and female subjects presenting with ischemic stroke involving any vascular territory without a history of prior autonomic dysfunction or inflammatory diseases will be enrolled in this study. Exclusion criteria comprise intake of tricyclic antidepressants and dementia.

Assessments

All study participants without KAF will be monitored for 14 days with a continuous ECG monitor (CardioSTAT, iCentia, Quebec, QC, Canada), initiated within 72 hours after stroke onset. Patients will be monitored for additional 14 days at 3 months after stroke. AF paroxysms of any duration and atrial flutter will be considered

Table 1. *Autonomic reflex screen utilized in* PARADISE *clinical stream*

	Autonomic function assessed				
Test	Integrity of the postganglionic sympathetic sudomotor axon	Cardiovagal	Cardiovascular adrenergic	Heart rate variability	Integrity of the autonomic system
Quantitative sudomotor axon reflex (QSART)	Yes				
Heart rate response to deep breathing (HR_{DB})		Yes		Yes	
Valsalva maneuver		Yes	Yes (blood pressure and heart rate response)		
Head-up tilt			Yes (blood pressure, heart rate response, and plasma catecholamine levels)	Yes	Yes
Handgrip					Yes (blood pressure and heart rate response)
Composite Autonomic Severity Score (CASS) 0-10 points	Sudomotor index 1. Single QSART site reduced (>50% of lower limit) or length-dependent pattern (distal sweat volume <1/3 of proximal value) 2. Single QSART site <50% of lower limit 3. Two or more QSART sites <50% of lower limit Adrenergic index 1. Reduced Phase II (late component) or increased PRT (4-5 s) or absent Phase IV 2. Absent Phase II (late component) or increased PRT (6-9 s) 3. Absent Phase II (late component) and increased PRT (≥10 s) 4. #3 + OH (SBP reduction ≥30 mmHg) Cardiovascular HR index 1. HR_{DB} or VR reduced but above <50% of minimum 2. HR_{DB} or VR reduced to <50% of minimum 3. HR_{DB} and VR reduced to <50% of minimum				

Abbreviations: HR_{DB}, heart rate variability to deep breathing; OH, orthostatic hypotension; PRT, phase recovery time; QSART, quantitative sudomotor axon reflex testing; SBP, systolic blood pressure; VR, valsalva ratio.

as positive for AFDAS.[3,7] We will include AF paroxysms of any duration because it is controversial whether short-lasting AF is related to increased stroke risk. It is clear that AF greater than or equal to 1-hour duration is associated with worse outcomes,[29] but there is only weak evidence supporting that shorter paroxysms are benign,[3] mostly among patients who have already suffered a stroke.[7] Also, rather than evaluating the risk of recurrent ischemic stroke in patients with AFDAS, the aim of this study is to characterize patients with any type of AFDAS. Furthermore, analyzing data from AFDAS of any duration will allow a more comprehensive understanding of the arrhythmia. The autonomic function will be assessed based on catecholamine plasma levels, the autonomic reflex screen (Table 1), and heart rate variability within 72 hours after stroke onset, and at 14, 30, and 90 days. We will also assess diurnal variations in heart rate (daytime versus nighttime and sleep versus awake periods) as a measure of autonomic function based on CardioSTAT recordings. Plasma levels of ET-1, TAFI and activated TAFI, lipoprotein(a), brain natriuretic peptide, and inflammatory plasma markers including C-reactive protein, tumor necrosis factor alpha, interleukin-1 beta, and interleukin 6 will be assessed within 72 hours after stroke onset, and at 14, 30, and 90 days. Two cellular inflammatory ratios, neutrophil-to-lymphocytes and platelet-to-lymphocyte, will be also determined at admission and at the same time points. The samples will be processed as appropriate for complete blood count, quantification of the different plasma markers, and multiplex analysis with BioRad Luminex Assays (inflammatory markers).

Cardiac interoceptive processing and related social cognition and screening tasks[30-33] will be assessed at day 14 and 6 months, whereas gait, falls, and frailty will be assessed at 6 months after the qualifying ischemic stroke (Table 2). Data on demographic variables, risk factors, vascular comorbidities, and results of magnetic resonance imaging (MRI) of the brain, and echocardiography from

Table 2. *AFDAS prognosis assessments in* PARADISE *clinical stream*

Clinical outcome	Tests/Questionnaires	Requirements of participants (min/visit)
Balance	Ontario Neurodegenerative Disease Research Initiative (ONDRI)'s Balance Assessment with Wii balance board	15
Cognitive status	INECO Frontal Screening[34]	10
	Theory of Mind in the eyes[35]	10
	Trail Making Tests A & B[36]	6
	Montreal Cognitive Assessment (MoCA)[37]	12
	Emotion Recognition Task[38]	8
	Decision Making Games[39]	10
	State-Trait Anxiety Inventory for Adults (STAI)[40]	12
	Hospital Anxiety and Depression Scale (HADS)[41]	5
	Depression Anxiety Stress Scales (DASS)[42]	15
	Global Physical Activity Questionnaire (GPAQ)[43]	5
Fall	Fall Questionnaire[44]	2
Frailty	Frailty Assessment Questions and Handgrip test[44]	5
Gait	ONDRI's Gait Assessment[45]	40
Interoception	Heartbeat Detection Task[46]	10
	Interoceptive Priming and Emotion Recognition Task	10
	Multiple Assessment of Interoception Awareness (MAIA)[33]	8

routine stroke care will be prospectively collected. As all patients routinely undergo brain MRI scanning at our center, MRI scans will be further processed to assess the correlation between insular or other brain lesions and outcome measures (e.g., AFDAS, autonomic dysfunction, gait, cognitive impairment). For this purpose, we will apply voxel-based lesion symptom mapping consisting of a voxel-by-voxel analysis of overlaid lesion reconstructions of patients with a common outcome variable compared with overlays of patients without that variable.[47,48] Stroke recurrences will be assessed by a structured phone interview at 6 and 12 months.[49]

Analytical Plan

For data analysis, and based on the results of cardiac monitoring, patients will be classified into 3 groups: (1) AFDAS; (2) KAF; and (3) sinus rhythm. As a first step, we will compare all the variables listed under the Hypotheses and Objective section in patients with AFDAS and KAF (Fig 3). Among 180 participants with no history of AF, we estimate a 15.5% detection yield of AFDAS after 14 days of cardiac monitoring,[5] resulting in 28 of 180 screened subjects with newly detected AF. The remaining 20 subjects will have a history of previously known AF. Based on our experience with healthy subjects and patients with autonomic dysfunction assessed as per the autonomic reflex screen, we anticipate that 20 patients with KAF will be enough to obtain a good estimate of their autonomic function and inflammatory states to be compared with patients with AFDAS. As a result, the final cohort will probably result in 28 patients with AFDAS, 20 with KAF, and 152 with sinus rhythm (Fig 3).

A logistic regression analysis to identify variables related to AFDAS would be statistically implausible, given the low number of outcomes (28 cases of AFDAS). Therefore, we will conduct network analyses, which allow establishing correlations between assessed variables and AFDAS.[50]

We will also use cluster-based analyses (partitioning a data set into clusters sharing common or similar features) to group patients into different phenotypes of AFDAS (data clustering software, R, R Foundation for Statistical Computing, Vienna, Austria) to identify cardiogenic and possible neurogenic AFDAS phenotypes for future studies. The cluster-based analysis will be complemented with a univariate comparison of probable and possible cardiogenic versus possible neurogenic AFDAS. AFDAS will be classified as being probable cardiogenic if there is no evidence of any other cardioembolic source, large vessel disease, or high-risk plaque in the aortic arch, if they fulfill any of the following criteria: (1) multiple territory stroke; (2) prior stroke in a different vascular territory; or (3) cortical symptoms (e.g., aphasia, neglect) or cortical topography (e.g., wedge-shaped cortical stroke). AFDAS will be classified as being possible cardiogenic in the absence of the above-mentioned criteria in patients with moderate to severe left atrial enlargement or severely decreased left ventricular ejection fraction (<30%) without evidence of a left ventricular thrombus. Participants with probable or possible cardiogenic AFDAS will be grouped and will be compared against all other

AFDAS, which will be deemed as being possible neurogenic.

Epidemiological Stream

Hypothesis and Objective

Patients with AFDAS will comprise a mix of AFDAS phenotypes: neurogenic with potentially better outcomes and cardiogenic with worse outcomes. Therefore, because of this heterogeneity, we hypothesize that prognosis of patients with AFDAS would be similar to those with sinus rhythm, whereas that of KAF would be worse. Furthermore, we postulate that (1) based on our previous findings in a pilot exploratory study, relative to KAF, patients with AFDAS will have less prevalent heart disease due to the mix of cardiogenic and neurogenic AF in the latter group[18]; (2) relative to AFDAS, frequency of ischemic strokes and TIAs suffered before the qualifying event will be higher among those with KAF, whose AF could have possibly caused strokes and TIAs before the index event; and (3) strokes in patients with AFDAS will be more severe than those of patients with sinus rhythm and KAF. The latter hypothesis is based on data suggesting that damage to the insular cortex and other central nervous system structures involved in the regulation of the autonomic nervous system is more probable among patients with more severe strokes.[48,51]

The main objective of the epidemiological stream is to compare the prognosis of AFDAS and sinus rhythm at 1 year following stroke for the following clinically relevant outcomes: ischemic stroke recurrence (primary outcome), any stroke recurrence, all-cause mortality, and composite outcome of stroke or death (secondary outcomes). We will also investigate whether AFDAS, relative to sinus rhythm, is associated with a lower risk of incident dementia and lower frequency of admissions to the emergency department.

Design

This is a retrospective cohort study of the population-based Ontario Stroke Registry, a prospectively collected clinical database comprising over 23,000 patients with ischemic stroke.

Population

All patients with ischemic stroke and TIA enlisted in the Ontario Stroke Registry from July 1, 2003 to March 31, 2013. The study cohort and outcomes will be defined according to validated algorithms from the Institute for Clinical Evaluative Sciences.[21,52,53]

Analytical Plan

We will estimate the crude and adjusted risk of ischemic stroke recurrence at 1 year for KAF, AFDAS, and sinus rhythm. For this purpose, we will use incidence function curves for recurrent ischemic stroke in KAF, AFDAS, and sinus rhythm and we will use subdistribution Cox proportional hazards, which allow estimating the effect of covariates on the absolute risk of a given time-to-event outcome.[54] All-cause mortality following the index ischemic stroke will be adjusted as a competing risk. Death within 1 year of the incident ischemic stroke will be determined as per the Ontario Stroke Registry and the Registered Persons Database.[55] We will generate 3 different models by using variables known to influence the risk of recurrent ischemic stroke. Model 1 will be adjusted for age, sex, stroke severity, and prescription of oral anticoagulants at discharge. Model 2 will be adjusted for the same variables as model 1 plus systemic hypertension, diabetes mellitus, congestive heart failure, coronary artery disease, and prior history of stroke or transient ischemic attack. Model 3 will be adjusted for the same variables as model 2 plus prior history of dementia and modified Rankin scale at discharge.

To estimate the association of each AF subgroup (AFDAS and KAF) with recurrent ischemic stroke at 1 year after discharge, we will employ cause-specific Cox proportional hazards. We will generate 3 different cause-specific Cox models, adjusted for the same variables as those to be used for the subdistribution Cox analyses. Sinus rhythm will be the comparator for both types of AF (e.g., AFDAS and KAF). We will use a sandwich covariance matrix estimate to account for the intracluster dependence of hospitals for both cause-specific and subdistribution models.

To assess the hypothesis of neurogenically mediated versus cardiogenic AFDAS, we will compare the prevalence of available cardiovascular comorbidities (e.g., congestive heart failure, myocardial infarction, and coronary artery disease) between AFDAS and KAF, and between AFDAS and sinus rhythm. We will use the z-test for comparing proportions and we will report two-tailed probabilities. We will estimate the difference in proportions with asymptotic (normal approximation) 95% confidence intervals (based on a significance level of .05).

Similar approaches will be followed for other outcomes such as (1) recurrent ischemic stroke or hemorrhagic stroke; (2) death; and (3) dementia.

Strengths and Limitations of This Study

Conceptually, investigating AFDAS is methodologically complex because of the difficulty in determining the time of onset of AF relative to the stroke. The clinical stream will be conducted in a relatively low number of patients (n = 200), which may result in underpowered estimations if the number of patients diagnosed with AFDAS is lower than expected. This will undermine our ability to perform subgroup analyses. Also, as short paroxysms of atrial tachycardia can mimic AF, the inclusion

of very brief AF episodes may be of questionable clinical significance. However, due to the lack of knowledge about the prognostic implications and pathophysiology of these short paroxysms, we consider important to record them to help disentangle their meaning. The epidemiological stream is based on a retrospective analysis of a large clinical database, inherent with all the limitations of retrospective analyses. In the experimental stream, although highly unexpected, the insular cortex strokes may result in no cardiac arrhythmias. We expect to overcome all these limitations by applying a multidisciplinary and translational approach, which represents the major strength of our study.

Ethical Aspects

The clinical study will be conducted in accordance with the Declaration of Helsinki and local laws and regulations. The study protocol (#108150) has been approved by the Western University Health Sciences Research Ethics Board (IRB 00000940) and has been registered at clinicaltrials.gov (NCT03275155). The experimental study protocol was approved by the local ethics committee (AUP 2016-27).

Potential Implications of This Study

Oral anticoagulants can reduce the risk of stroke by 67% in patients with AF[56] and these oral anticoagulants are used to prevent strokes among a substantial proportion of the 30 million people living with AF globally. However, a considerable proportion of this population remains at a high risk, as oral anticoagulants fail to prevent strokes in over 30% of patients with AF.[57] Moreover, they remain underused worldwide.[2] All this evidence constitutes a largely unattended call to develop innovative strategies to prevent strokes in a substantial proportion of patients with AF. *PARADISE* is a translational research initiative, which aims to fill this gap by setting the grounds for the development of novel therapeutic approaches to prevent neurogenic AFDAS occurrence and perpetuation, as well as its consequences, comprising stroke recurrence, death, and AF-related heart disease (e.g., remodeling). Our results will also serve to advance our general understanding about AF, not restricted to patients with stroke.

Clarifying AFDAS mechanisms could result in the identification of potential therapeutic targets to prevent AFDAS occurrence and perpetuation. These new therapeutic approaches will be tested in our animal model and subsequently in a clinical trial. If these novel therapeutic interventions are proven to successfully prevent AFDAS, the ultimate consequence may be fewer stroke recurrences and deaths, as well as a less AF-related heart disease and a delay in dementia onset, which will benefit a large proportion of the aging global population. More precisely, within the first 24 hours and in the context of endothelial dysfunction, the left atrium undergoes remodeling shortly after paroxysmal AF onset.[58] Short paroxysmal episodes of AF can lead to AF perpetuation if they become recurrent through structural changes that take place in the atrial myocardium (e.g., remodeling).[59] If inflammatory responses persist and AFDAS recurs, then the AF may become chronic, leading to more cardiac structural changes such as left atrial enlargement, and ultimately becoming a cardiogenic AF. The prevention of this "neurogenic to cardiogenic AFDAS shift" is one of the main targets for reducing the consequences of both recurrent AF and AF-related cardiac structural changes. Therefore, interventions such as anti-inflammatory agents,[60-62] aldosterone antagonists,[63] modulators of autonomic[64] and catecholamine[65] surges, and suppressors of the renin-angiotensin system[66] could be tested first in the animal model and then in humans. In terms of economics, identifying patients at higher risk of developing AFDAS immediately after stroke will help allocate diagnostic technologies more efficiently. Additionally, fewer strokes may result in reduced costs for the healthcare system. Quantifying the risk of stroke recurrence, AF recurrence, dementia, and death associated with AFDAS will allow us to estimate whether patients with AFDAS would benefit from oral anticoagulants.

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