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Original article

PREventive left atrial appenDage resection for the predICtion of fuTure atrial fibrillation: design of the PREDICT AF study

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Background **Atrial fibrillation is the most common cardiac arrhythmia, posing a heavy burden on patients' wellbeing and healthcare budgets. Patients undergoing cardiac surgery are at risk of developing postoperative atrial fibrillation (POAF), new-onset atrial fibrillation and subsequent atrial fibrillation-related complications, including stroke. Sufficient clinical identification of patients at risk fails while the pathological substrate changes that precede atrial fibrillation remain unknown. Here, we describe the PREDICT AF study design, which will be the first study to associate tissue pathophysiology and blood biomarkers with clinical profiling and follow-up of cardiothoracic surgery patients for the prediction of future atrial fibrillation.**

Methods **PREDICT AF will include 150 patients without atrial fibrillation and a CHA_2DS_2–VASc score of at least 2 undergoing cardiac surgery. The left atrial appendage will be excised during surgery and blood samples will be collected before surgery and at 6 and 12 months' follow-up. Tissue and blood analysis will be used for the discovery of biomarkers including microRNAs and protein biomarkers. The primary study endpoint is atrial fibrillation, which will be objectified by 24 h Holters and ECGs after 30 days for POAF and after 6, 12 and 24 months for new-onset atrial fibrillation. Secondary endpoints include the dynamic changes of blood biomarkers over time and other atrial arrhythmias. PREDICT AF participants may benefit from extensive postoperative care with clinical phenotyping, rhythm monitoring and primary prevention of stroke.**

Conclusion **We here describe the PREDICT AF trial design, which will enable the discovery of biomarkers that truly predict POAF and new-onset atrial fibrillation by combining tissue and plasma-derived biomarkers with comprehensive clinical follow-up data.**

Trial registration **Retrospectively registered NCT03130985 27 April 2017.**

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Keywords: atrial appendage, atrial fibrillation, biomarker, clinical trial, preventive therapy

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Background

Atrial fibrillation is the most common cardiac arrhythmia.[1] It is accompanied by prolonged hospitalizations and doubled mortality, but its most devastating complication is ischaemic stroke.[2] The burden of atrial fibrillation on patients' wellbeing and healthcare budgets is projected to increase at least two-fold by 2050, emphasizing the need for studies designed to improve early disease recognition and preventive therapies.[3]

Patients undergoing cardiothoracic surgery have a clinical profile that puts them at up to 45–55% risk of postoperative atrial fibrillation (POAF).[4] Moreover, an estimated 15–20% of cardiac surgery patients develop new-onset atrial fibrillation within 2 years after surgery, though the association between POAF and new-onset atrial fibrillation remains unclear.[5,6] Currently, we are insufficiently able to identify cardiac surgery patients at risk of developing new-onset atrial fibrillation. The identification of patients at risk is hampered by the complexity of atrial fibrillation pathophysiology and our lack of knowledge of the relationship between the clinical presentation and underlying atrial substrate.[7] At the same time, no studies have been designed that investigate the atrial substrate in relation to plasma biomarkers, clinical characteristics and new onset of the disease.

We here describe the design of the PREDICT AF study, which aims to discover biomarkers in both blood and myocardial tissue from nonatrial fibrillation patients that can predict POAF and/or new-onset atrial fibrillation.

* A complete list of investigators of PREDICT AF: *Supplementary A*, http://links.lww.com/JCM/A195.

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PREDICT AF includes patients without atrial fibrillation, with a CHA_2DS_2–VASc score of at least 2, undergoing cardiac surgery and follows them for 2 years to detect new-onset atrial fibrillation. The left atrial appendage (LAA) is preventively excluded and used for biomarker discovery. The PREDICT AF study not only investigates atrial tissue, but combines this with the study of plasma biomarkers to enable an in depth analysis of biomarker origin and function. The unique prospective design permits a true prediction of POAF and/or new-onset atrial fibrillation.

PREDICT AF participants, who have an increased risk of developing atrial fibrillation, may benefit from the regular rhythm monitoring that is part of the PREDICT AF study. Early atrial fibrillation detection may have direct clinical implications for stroke prevention with anticoagulant therapy or for the administration of antiarrhythmic drugs. PREDICT AF participants have furthermore been selected based on an increased risk of thromboembolism and may therefore also benefit from removal of the LAA since this is an important source of thromboembolism and can be removed safely.[4,8–10]

We hypothesize that the study of biomarkers in a prospective study will designate true predictive biomarkers of new-onset atrial fibrillation, will enhance our understanding of patient-specific pathophysiology and will increase our ability to select patients for targeted preventive therapies. This study design will provide new principles for the development of therapies targeted at the remodelled substrate. Biomarker discovery in cardiac surgery patients is by all means relevant for a large contingent of at risk patients, whereas findings are also potentially relevant for a more general population at lower risk of atrial fibrillation.

PREDICT AF clinical study design

PREDICT AF is an investigator-initiated, prospective, observational study, in two large cardiothoracic surgery centres: the Amsterdam University Medical Centers, University of Amsterdam and Antonius Hospital, Nieuwegein, The Netherlands. Patients undergo cardiac surgery with additional LAA amputation and will thereafter be followed for 2 years to detect new-onset atrial fibrillation. The study flow chart can be found in Fig. 1.

The study was approved by the Medical Ethics Committee of the Amsterdam University Medical Center and conforms to the Declaration of Helsinki (NL50754.018.14). Funding was provided by a grant from the Netherlands Organisation for Scientific Research (VIDI 016.146.310). The study is registered under ClinicalTrials.gov: NCT03130985.

Clinical study endpoints

The primary clinical study endpoint is new-onset atrial fibrillation during 2 years of follow-up, defined according to current guidelines (30 s continuous rhythm registration of atrial fibrillation with Holter or device registration, or ECG recording of an atrial fibrillation episode).[1,11] POAF will be discriminated from new-onset atrial fibrillation and is defined as new-onset atrial fibrillation within the first 30 days postoperatively. POAF will be analysed independently from new-onset atrial fibrillation as well as for its association with new-onset atrial fibrillation.

Secondary endpoints include the dynamic change of biomarker levels over time in relation to the time to new-onset atrial fibrillation, changes in quality of life (QoL) and other atrial arrhythmias. *Study definitions are described in Supplementary B*, http://links.lww.com/JCM/A195.

Patient selection

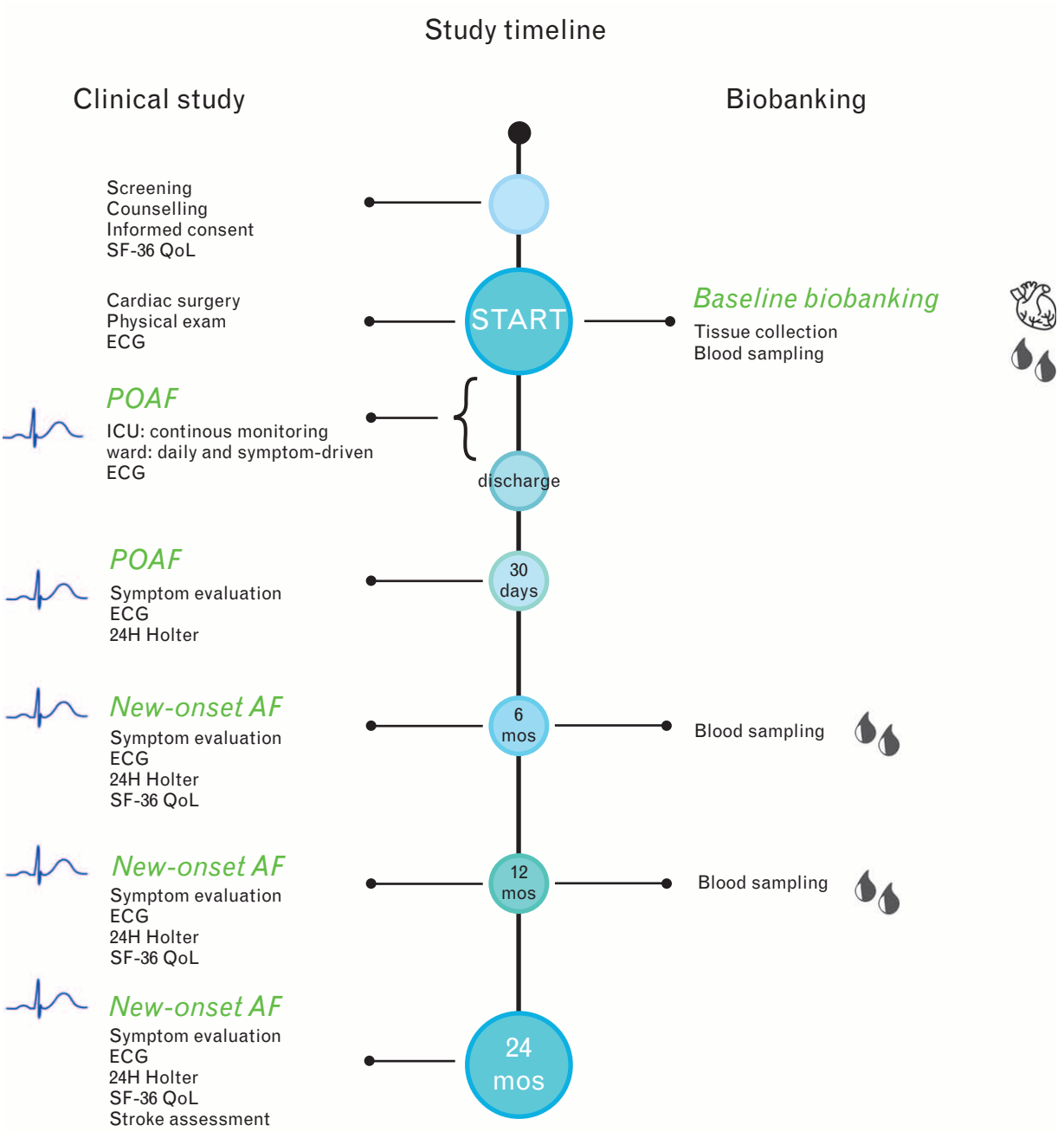
Patients without prior atrial fibrillation and with a CHA_2DS_2–VASc score of at least 2 planned for coronary artery bypass grafting (CABG), aortic(valve) surgery or mitral valve surgery are eligible. All consecutive, eligible patients will receive study information directly upon screening for eligibility and will be asked for written informed consent prior to surgery. The absence of prior atrial fibrillation will be assessed by medical history taking and screening of the patient's referral and rhythm recordings. All inclusion and exclusion criteria are listed in Table 1.

Inclusion and exclusion criteria aim at the inclusion of a representative cohort of cardiac surgery patients while keeping the potential to extrapolate study findings to the general atrial fibrillation population. Furthermore, the defined criteria will preselect participants who may benefit from study follow-up including frequent rhythm monitoring for early atrial fibrillation detection and from prophylactic LAA resection for the prevention of thromboembolic stroke.[4,9,10] First, patients undergoing cardiac surgery have a 45–55% chance of POAF[4] and the study population has an estimated 15–20% chance of developing new-onset atrial fibrillation in the 2 years after surgery.[5,6] Second, a CHA_2DS_2–VASc score of at least 2 preselects patients at risk of stroke and implies an indication for anticoagulation therapy if atrial fibrillation would develop.[1,12,13] Finally, the CHA_2DS_2–VASc score and its components are associated with an increased risk of developing (subclinical) POAF and new-onset atrial fibrillation.[14–16] Safety of LAA removal is discussed below.

Study start: left atrial appendage amputation

Cardiac surgery constitutes the start of study. Cardiothoracic surgery will be carried out according to institutional guidelines. Transoesophageal echocardiography (TEE) is standardly performed during surgery to assess left atrial (or appendicular) thrombi. The LAA is excised and retrieved directly upon aortic cross-clamping, unless the surgeon deems removal unsafe. Preference of the

Fig. 1



PREDICT AF study timeline. AF, atrial fibrillation; POAF, postoperative atrial fibrillation; SF-36 QoL, short-form 36 quality-of-life questionnaire.

surgeon was considered leading in the choice for stapler, clipping or suture excision of the LAA during extracorporeal circulation. Completeness of LAA excision is confirmed by TEE. This study is not designed to investigate the effects of LAA amputation *per se*, but we will compare all procedural associated complications to those complications occurring in a patient cohort from the same inclusion period matched by inclusion criteria and surgical procedure. Likewise, we will compare POAF and new-onset atrial fibrillation to the reported incidence after thoracic surgery. Furthermore, we will assess the effects on atrial function with pulsed wave Doppler (*E*/*A* ratio) and atrial strain analysis of pre and postoperative echocardiograms. For all PREDICT AF participants, we will assess a stroke-free status at the end of study. *Supplementary C*, http://links.lww.com/JCM/A195, *describes the procedures relating to adverse events and complications.*

Table 1 PREDICT AF inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Elective sternotomy for CABG or aortic (valve) surgery or mitral valve surgery	Documented or reported history of AF, flutter (>5 min) or sustained ventricular tachycardia
On-pump surgery	NYHA class IV heart failure symptoms or LVEF < 35%
CHA_2DS_2–VASc ≥ 2	Surgery for congenital anomalies
Sinus rhythm	Redo-procedure or emergency procedure (<24 h)
Age 18–80 years	Endocarditis or pericarditis (active or in history)
Legally competent and willing to comply	Systemic inflammation or active autoimmune disease or continuous anti-inflammatory drugs
	Active malignancy
	Hyperthyroid or hypothyroid disease or use of thorax
	History of previous radiation of the thorax
	Pregnancy or childbearing potential
	Circumstances that prevent follow-up

AF, atrial fibrillation; CABG, coronary artery bypass grafting; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

Rhythm monitoring

Follow-up for POAF and new-onset atrial fibrillation detection will start directly after cardiac surgery. Extra attention will be paid to POAF onset during the hospital admission period.[17] All follow-up visits will include an ECG, a 24-h Holter and comprehensive history taking to exclude any POAF or new-onset atrial fibrillation in the foregoing period or the existence of rhythm recordings made outside the study centres. Rhythm monitoring includes the study visits and all rhythm recordings made outside the study visits or study centres. Every rhythm recording made during the follow-up period will be collected and study endpoints will be assessed by experienced clinical electrophysiologists, who will be blinded for study outcome.

- POAF detection: patients are postoperatively admitted to the surgical ICU with continuous rhythm monitoring for at least 24 h. Subsequently, they are admitted to the cardiothoracic surgery ward, where symptom-driven and daily ECG recordings are made. In case of POAF, patients are converted to sinus rhythm prior to hospital discharge. An outpatient study visit preceded by a 24-h Holter and ECG is scheduled after 30 days to evaluate the onset of any POAF after discharge until 30 days postoperatively.
- New-onset atrial fibrillation: outpatient study visits are scheduled 6 months, 1 and 2 years after surgery. All visits are preceded by a 24-h Holter registration and ECG.

If patients are physically unable to attend the study visits, by exception, a telephone assessment is performed preceded by a Holter registration and ECG at a centre of preference. Patients are stimulated to contact their physician and the study team for any concerns or complications.

If POAF or new-onset atrial fibrillation is detected, patients will be treated as per best medical practice. The primary care physician will be informed if atrial fibrillation (or any other abnormality) is detected, who will decide on appropriate treatment. The PREDICT AF protocol recommends anticoagulation despite LAA removal in case of new-onset atrial fibrillation according to the European Society of Cardiology 2016 guidelines for the management of atrial fibrillation (Class IB recommendation).[1] *A schematic overview of all study visits and study procedures is displayed in Supplementary D*, http://links.lww.com/JCM/A195.

Clinical data collection

Data are prospectively collected from electronic medical patient records or from correspondence with referring hospitals and will be stored anonymously.

- Baseline variables encompass demographics, a full physical examination, all rhythm recordings up to 1 year prior to surgery and medication at admission. Collected clinical characteristics relate to a history of cardiovascular disease, surgical history, valvar disease, congestive heart failure, stroke or transient ischemic attack, hypertension, family history, drugs, smoking and alcohol abuse, lung function and exercise test. *A complete list of all variables collected is provided in Supplementary E*, http://links.lww.com/JCM/A195.
- Transthoracic echocardiograms are made as standard care prior to surgery and usually after 1-year follow-up. A transoesophageal echocardiogram (TEE) is made during surgery with recordings prior to and after LAA removal. The original recordings will be collected and used to determine: left and right atrial volume at end systole and end diastole, left atrial emptying fraction, left atrial strain during reservoir, conduit and contraction phase, left ventricular ejection fraction, cardiac output and valvar disease. Additional parameters are *E* wave, *A* wave, *E*/*A* ratio, deceleration time, e' wave, a' wave and E/e'. *A detailed description of the echocardiographic measurements and parameters is provided in Supplementary F*, http://links.lww.com/JCM/A195.
- Procedural and postprocedural variables include the type of surgery, procedure time, timing of LAA removal, complications, reoperations and periprocedural medication such as inotrope or antiarrhythmic drugs, or the need for postoperative antiarrhythmic drugs or electrical cardioversion. *Complications are*

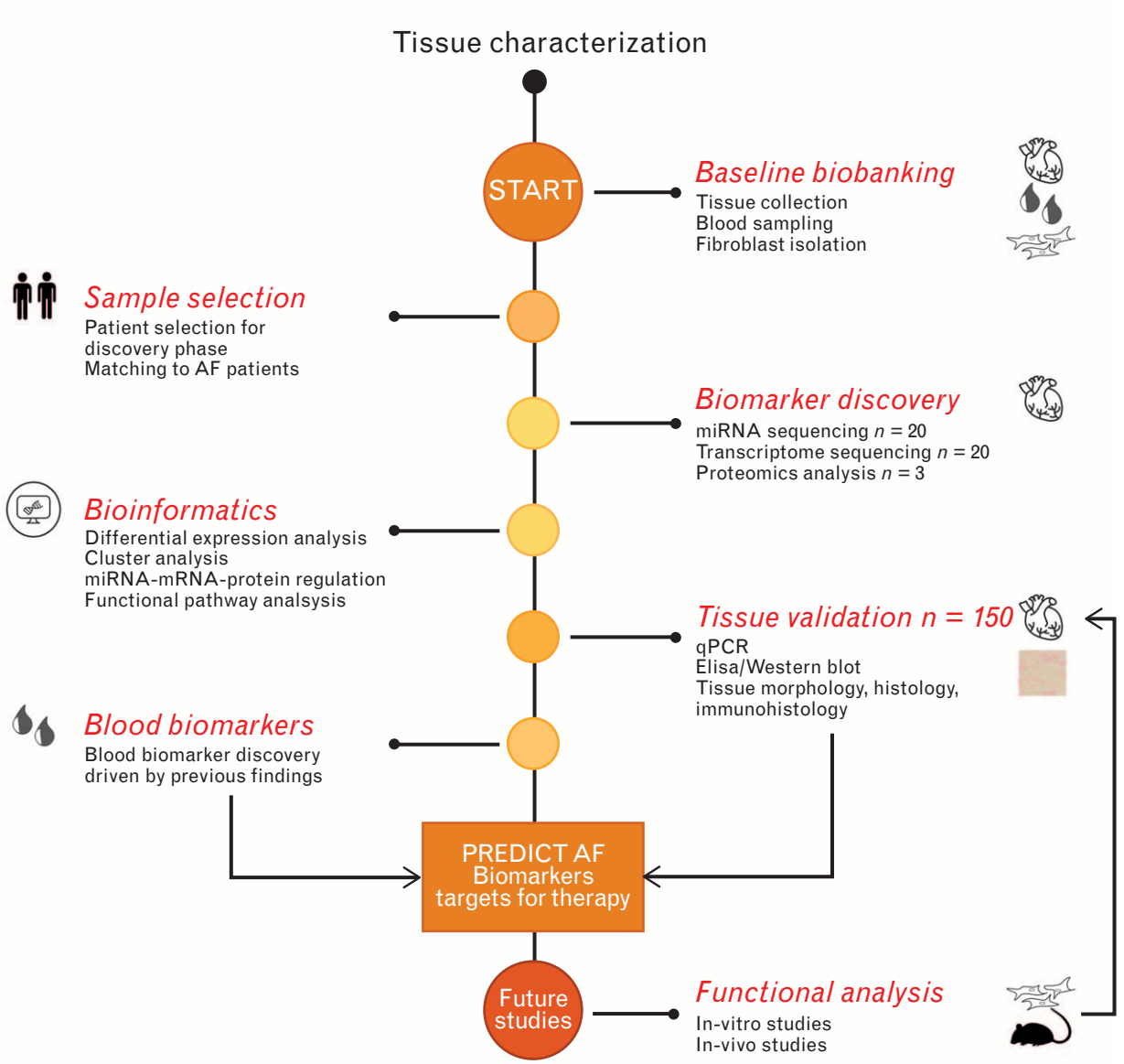
defined in Supplementary B3, http://links.lww.com/JCM/A195.

- Variables collected during follow-up include complications, adverse events, hospitalizations, cardiac imaging and medication changes. Special attention will be paid to the use of antiarrhythmic drugs and dosage as well as the use of antiplatelet or anticoagulation therapies. QoL is assessed by the short-form 36 at baseline, 6, 12 and 24 months' follow-up.[18]
- Stroke-free status is assessed with The Questionnaire for Verifying Stroke-Free Status[19] at final follow-up.

Strategies for biomarker discovery

Tissue and blood are collected at baseline as well as during the course of follow-up. PREDICT AF will commence with an unbiased discovery phase to identify new biomarkers in tissue on different levels of atrial fibrillation pathophysiology regulation. The resulting complete biomarker profile will then be used to select biomarkers for discovery in blood samples. In future studies, results may be validated in independent cohorts and studied in functional models. A schematic figure illustrating the biomarker discovery in PREDICT AF can be found in Fig. 2.

Fig. 2



Tissue characterization. The flow chart indicates how the tissue discovery phase is followed by tissue validation and blood biomarker discovery, which form the final results of the PREDICT AF study. Also indicated is that study results may lead to future functional studies. Likewise, additional results from functional studies or even independent functional studies may be validated in the PREDICT AF cohort to test association with new-onset atrial fibrillation.

Table 2 Candidate biomarkers

microRNAs	Oxidative stress	Inflammation	Fibrosis	Hormones	Histopathology
miR-29b	MPO	hs-CRP	TGF-β	ANP	Fibrosis
miR-208b	NADPH ox	IL-1,2,6,8,10	PIIINP	NT-proBNP	Fibroblasts
miR-21	NOS	TNFα	PICP	Renin	Myofibroblasts
miR-31		MCP-1	CITP	Angiotensin	Myocytolysis
miR-328			MMPs		
			TIMPs	Aldosterone	Myocyte hypertrophy
			Galectin-3	Apelin (adipokine)	
			OPN		
			LOX		
			POSTN		

ANP, atrial natriuretic peptide; CITP, carboxyl-terminal telopeptide collagen type-I; hs-CRP, high-sensitive C-reactive protein; LOX, lysyl oxidase; MCP, monocyte chemoattractant protein-1; MMP, matrix metalloproteinases; MPO, myeloperoxidase; NADPH ox, NADPH oxidase; NOS, nitric oxide synthase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPN, osteopontin; PICP, carboxyterminal propeptide of procollagen type-I; PIIINP, type-III procollagen-*N*-peptide; POSTN, periostin; TGF-β, transforming growth factor beta; TIMP, tissue inhibitors of metalloproteinases.

In parallel to this hypothesis-free approach, we will assess biomarkers of interest that have previously been associated with atrial fibrillation or the risk of new-onset atrial fibrillation. A list of candidate biomarkers is displayed in Table 2, but may be subject to change or turn out to be incomplete as a result of advancing insights.

Biomaterials are stored anonymously for 20 years in the *Arrhythmia and conduction Disorders: towArds Pathophysiology based Treatment (ADAPT)* biobank, which allows the use of biomaterials for future studies. The biobank was approved by the Biobank Ethics Committee of the Academic Medical Center University of Amsterdam.

Tissue and blood collection and processing

The LAA will be processed immediately upon excision within the operating room. The LAA is rinsed in PBS or 0.9% NaCl to remove excessive blood clots and dilute potential cardioplegic agents and heparin.

- Part of the LAA is snap frozen in liquid nitrogen and stored at −80 °C.
- The epicardial fat on the LAA is removed and snap frozen separately.
- Part of the LAA is fixed in 4% formalin solution and embedded in paraffin.
- Fresh fibroblast will be isolated from a pilot population and stored in (viable) freeze medium BAMBANKER (Wako, Cat. No. 302-14681).
- Samples may be used for cardiomyocyte isolation and patch clamping.

Blood samples are retrieved prior to surgery, at 6 and 12 months' follow-up and are processed within 1 h after withdrawal. *The collected blood tubes and laboratory processing is described in Supplementary G*, http://links.lww.com/JCM/A195.

Biomarker discovery

The PREDICT AF study will commence with a hypothesis-free discovery phase in a subset of atrial tissues that will target three levels of biological regulation:

- A subset of 20 LAAs including patients without atrial fibrillation, with POAF and with new-onset atrial fibrillation will be used for whole transcriptome RNA sequencing.
- A subset of 20 LAAs including patients without atrial fibrillation, with POAF and with new-onset atrial fibrillation will be used for microRNA (miRNA) sequencing.
- Protein discovery with high-throughput liquid-chromatography online coupled to tandem mass spectrometry (LC-MS/MS) will be performed in a pilot population of three PREDICT AF patients and will be compared with three LAAs from patients with a history of atrial fibrillation.

A thorough bioinformatics analysis will be performed to determine differential gene expression associated with new-onset atrial fibrillation. The analysis will not only focus on a single gene or miRNA, but aims at identifying gene and miRNA clusters involved in relevant biological processes. Moreover, we aim to reconstruct pathways, highlighting genes and miRNAs involved in new-onset atrial fibrillation pathophysiology. Findings from the discovery phase, either a single miRNA or gene, or comprehensive pathways will be validated in the complete PREDICT AF cohort and may be studied in functional models in future studies.

For blood biomarker discovery a more targeted approach will be employed based on first, the findings from the tissue discovery phase and second, we will test biomarkers that previously demonstrated to be associated with prevalent atrial fibrillation or new-onset atrial fibrillation. Blood biomarkers will be selected for their potential role in biological processes, their excretion pattern or potential diagnostic value. Blood biomarker levels will be associated with LAA tissue expression levels.

Statistical considerations

New-onset atrial fibrillation has a lower expected incidence than POAF. As we estimate a 15–20% risk of developing new-onset atrial fibrillation in the 2 years after surgery,[5,6] we anticipate a sample size of 150 participants

to generate a sufficient number of patients for analysis of new-onset atrial fibrillation. Inherent to the exploratory character of the study design, a detailed power calculation cannot be provided and an alpha reduction for multivariate analysis is not required for this exploratory study. This study design is hypothesis generating and findings must therefore be validated in independent cohorts in future studies. The study is insufficiently powered or designed for analysis of the efficacy of LAA resection on stroke prevention.

Atrial fibrillation will be assessed in two stages. The first analysis will focus on POAF. The second analysis will focus on new-onset atrial fibrillation during the 2-year follow-up. We will determine the similarities and differences between POAF and new-onset atrial fibrillation.

For the discovery of potential biomarkers of POAF and/or new-onset atrial fibrillation, a Cox regression model will be used including the time to POAF or new-onset atrial fibrillation. We will determine the discriminative value of biomarkers for POAF and new-onset atrial fibrillation, by constructing receiver operator curves with calculation of the area under the curve. The optimal sensitivity and specificity of the cut-off value based on the Youden's index will be provided. The analyses will be performed for single biomarkers and may lead to the evaluation of a panel of biomarkers in a similar manner.

We included a large, but relatively heterogeneous population. We do not intend to perform subgroup analyses in our primary exploration, but a post-hoc analysis based on relevant clinical parameters will be performed. Parameters that will be assed are sex, age, BMI, surgery type (CABG, valve or combined CABG/valve), technique of LAA amputation, left atrial volume index, hypertension, diabetes mellitus and the use of antiarrhythmic drugs. Results from the primary analyses may be assessed in detail for their distribution over the subgroups and will be considered for inclusion in the predictive model.

Quantitative continuous data will be displayed as mean with SD or median with interquartile range for nonparametric data. Qualitative comparisons of continuous data will be performed using two-sample unpaired *t* test or the Mann–Whitney *U* test, when appropriate. Categorical data will be described as frequencies with percentages and compared using the Pearson χ^2 test or Fisher exact test. A two-sided *P* value less than 0.05 will be considered as statistically significant. For transcriptome and proteome analysis, a false discovery rate adjustment using the Benjamini–Hochberg procedure will be applied for multiple testing.

Discussion

While the prevalence of atrial fibrillation is rapidly increasing, early identification of patients at risk and treatments targeting the patient-specific substrate are warranted. The PREDICT AF study design enables the identification of clinically relevant tissue and blood biomarkers that can be used for the prediction of POAF and/or new-onset atrial fibrillation.

Pathophysiological association between postoperative atrial fibrillation and new-onset atrial fibrillation

PREDICT AF will increase our understanding of the differences and similarities in pathophysiology between POAF and new-onset atrial fibrillation. POAF and new-onset atrial fibrillation are considered two separate disease entities, but in reality, similar clinical risk factors seem to play a role and patients with POAF have higher chances of developing new-onset atrial fibrillation compared with patients without POAF.[5,20] It therefore seems likely that in specific patients there may be a (partially) shared pathophysiology. For example, POAF can be triggered by inflammation and reactive oxygen species,[21,22] whereas structural remodelling and fibrosis are established pathophysiological processes leading to new-onset atrial fibrillation.[23] However, structural remodelling has also been associated with POAF.[24]

Biomarkers in atrial fibrillation

Molecular biology: microRNAs, mRNA and protein

PREDICT AF will commence with a genome wide miRNA and mRNA sequencing approach, which enables quantification of all miRNAs and mRNAs present. Unbiased sequencing allows the discovery of novel biomarkers; it will enable the reconstruction of complex regulatory pathways and will designate relevant biological processes. Pathway reconstruction will include miRNAs, which are small noncoding RNAs that are present in tissue and plasma and can modify gene expression at the posttranscriptional level. miRNAs are estimated to regulate at least 30% of gene expression.[25] miRNAs are furthermore of interest because they are candidates for in-vivo manipulation with miRNA mimics or antimiRs and are therefore promising new targets for atrial fibrillation therapies.[26] Finally, we will gain insight in atrial fibrillation regulation at the protein level with explorative proteomics by LC-MS/MS in a small subset of patients.

Tissue morphology and structural remodelling

Part of the collected atrial tissue will be embedded in paraffin to allow a comparison of the molecular substrate with tissue morphology and structural remodelling. Atrial structural remodelling is one of the hallmarks of atrial fibrillation pathophysiology and is characterized by atrial fibrosis, but it remains unclear to what extent fibrosis in the atrium predisposes to the first onset of atrial fibrillation or arises as a consequence of atrial fibrillation.[23] In addition, the entire composition and genesis of the extracellular matrix may be relevant for arrhythmogenic remodelling. For example, abundant fibroblasts have been associated with the thickness of collagen strands, increased longitudinal conduction velocity and atrial

fibrillation persistence.[27] Atrial fibrillation has also been associated with increased lysyl oxidase, collagen cross-linking and fibronectin.[28]

Blood biomarkers

Blood biomarkers may have direct clinical applicability for risk stratification. We foresee that future diagnostics and treatment of cardiovascular disease will become increasingly patient- and pathophysiology-specific after an initial screening of blood biomarkers. Anticipating these developments, PREDICT AF aims to associate blood biomarker levels to specific tissue pathophysiology. As such, blood biomarkers of interest will be the result of tissue exploration and can be signals that are themselves involved in atrial remodelling or markers that merely reflect remodelling. A specific group of blood biomarkers of PREDICT AF is formed by circulating miRNAs. Circulating miRNAs have been associated with atrial fibrillation and are promising biomarkers as they are stable, pathology-specific and can be detected with high sensitivity and specificity in blood.[25]

Strengths of PREDICT AF

Several biomarkers have been studied for their association with atrial fibrillation, but their predictive value and clinical applicability often remain undetermined. It is usually uncertain whether a biomarker is causally related to atrial fibrillation or whether the biomarker and atrial fibrillation independently result from the same underlying processes. Proof linking a serological biomarker to alterations in the organ of interest is often lacking or confined to cross-sectional or experimental studies. PREDICT AF overcomes these concerns. The combination of tissue and plasma enables a detailed analysis and interpretation of biomarker origin and function, whereas the prospective design permits a true association with new-onset atrial fibrillation and allows investigation of causality. Moreover, repeated blood sampling enables the investigation of sequential biomarker levels over time in relation to the development and timing of new-onset atrial fibrillation. In addition, the design of the study permits the combination of different biomarkers into a panel of biomarkers to improve sensitivity and specificity. The study design could provide new insights into the sequence of events leading to atrial fibrillation.

To fulfil the need for more personalized medicine including pathophysiology screening with blood biomarkers, we include a sufficient number of patients and deeply profile the clinical features of PREDICT AF participants at baseline and follow-up.

Finally, patients are likely to benefit from study participation. Prophylactic LAA removal potentially lowers stroke risk. Moreover, the described rigorous follow-up of participants with rhythm monitoring enables adequate treatment, if atrial fibrillation develops, with antiarrhythmic drugs and anticoagulation.

Safety of left atrial appendage removal

In this study, the LAA is removed to investigate early tissue remodelling predisposing to atrial fibrillation. We postulate that LAA resection is safe and may be beneficial for stroke prevention in the study population of PREDICT AF with a CHA_2DS_2–VASc score of at least 2.[9,12,13,29,30]

LAA removal is a safe procedure during on-pump cardiac surgery, as a recent meta-analysis found no increased bleeding risk or increased mortality after LAA exclusion.[9] In addition, no effect of LAA exclusion on the rate of 5-year mortality or postoperative complications, such as reoperation for bleeding, pneumonia or acute renal failure was found.[4] In a prospective study including 240 patients performed by our own study group, the LAA was excised in all participants, without any complications occurring.[31] The results of the first trial randomizing atrial fibrillation patients to LAA closure or no closure are expected in 2019 [Left Atrial Appendage Occlusion Study (LAAOS) III].[32,33]

Concerns have been raised regarding an increased risk of stroke after incomplete LAA exclusion.[34] In the current study, the LAA is excised and it can be rationalized that there is no trabeculation or stasis of blood in a remnant LAA, which thus does not pose a risk for thromboembolism.

The LAA is an important source of atrial natriuretic peptide and is as such involved in salt and water homeostasis.[35] Bilateral appendage removal may indeed cause fluid retention, but this has not been reported after lone LAA resection and the LAAOS I study suggested no adverse effects.[31,36]

Finally, the LAA is thought to serve as decompression chamber of the left atrium and removal may thus affect left atrial function or even cause POAF onset.[4] However, a recent echocardiographic study described an increased left atrial transport function, with improved reservoir and contractile function of the left atrium after LAA closure.[37,38]

Atrial fibrillation carries a five-fold increased risk of thromboembolic events and may be responsible for 15% of all ischaemic stroke incidents.[39] In an autopsy study, the LAA was indicated as the source of thrombus formation in approximately 90% of thromboembolic strokes in nonrheumatic atrial fibrillation.[8] Overall, there is accumulating evidence for a beneficial effect of LAA removal on stroke prevention in patients with atrial fibrillation and we may speculate on this for patients at increased risk of atrial fibrillation. In conclusion, it is a safe procedure and therefore justified in the context of this study.

Limitations

The current study includes patients undergoing CABG, aortic surgery or mitral valve surgery with an increased CHA_2DS_2VASc score. The prognostic value of

biomarkers may be restricted to the defined population and may not *per se* be generalizable to all atrial fibrillation patients. However, many patients from the general atrial fibrillation population have similar comorbidities and findings are potentially relevant for a broader population than cardiac surgery patients. The study population will consist of patients with a variety of underlying disease. Although the study is not powered to perform primary subgroup analyses, these will be performed in a post-hoc analysis. Of note, all findings should be validated in independent cohorts to further investigate the effects of comorbidities and the generalizability of the results.

An increased risk of POAF after LAA removal during cardiac surgery has been reported after LAA occlusion[4] but not substantiated in a meta-analysis.[9] LAA removal was suggested to decrease left atrial compliance and increase left atrium and pulmonic vein stretch, which may contribute to POAF.[4] On the other hand, the LAA has been suggested as a source of atrial fibrillation triggers and removal may lower long-term new-onset atrial fibrillation.[40] PREDICT AF is an observational one-arm study and no control group is included to assess the effects of LAA removal *per se*. These effects may be clarified by the results from the ongoing LAAOS III trial. In the PREDICT AF trial, all included patients undergo LAA removal for which the intervention will have only limited effect on biomarkers related to new-onset atrial fibrillation.

Another limitation is the inability to exclude preexisting silent atrial fibrillation as inclusion may be performed up to 1 day prior to surgery.[16,41] Nevertheless, the employed screening for atrial fibrillation conforms to the standard of care in clinical practice and minimizes patient selection. In addition, the rate of new-onset atrial fibrillation may be underestimated despite frequent Holter registrations as the implantation of a loop recorder is not feasible.[16,41] However, the study follow-up is more rigorous than the strict criteria brought forward by the HRS/EHRA/ECAS consensus document for the detection of atrial fibrillation.[11] In addition, patients are explicitly encouraged to seek medical attention whenever symptomatic.

Implications

We designed the PREDICT AF study to identify new, individualized biomarkers that truly predict POAF and new-onset atrial fibrillation and identify new targets of therapies. PREDICT AF is the first study to investigate blood and tissue markers of pathophysiological changes leading to new-onset atrial fibrillation, correlated to the specific clinical phenotype, and may at the same time, by virtue of LAA excision, contribute to primary prevention of the complications associated with this serious disease.

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Ethics, consent and permission: The study was approved by the Medical Ethics Committee of the Amsterdam University Medical Centres, University of Amsterdam and conforms to the Declaration of Helsinki (NL50754.018.14). All patients provide written informed consent prior to study participation. Consent for publication: Not applicable. Competing interests: To the best of our knowledge, no financial or other exists.

Authors' contributions

N.W.E.v.d.B. is the first author of the article and responsible for the study concept and design. N.W.E.v.d.B. designed the complete methodology of the study and wrote the first draft of this article. N.W.E.v.d.B. is responsible for the successful implementation of the study.

J.N. is the second author of the article and as such was involved in the improvement of the study concept as designed by N.W.E.v.d.B. J.N. coauthored several paragraphs in the article and is as a study coordinator also responsible for the successful implementation of the study.

W.R.B. initiated as a study coordinator the storage of biomaterials in the *ADAPT Biobank* and read and improved the first draft of the article and approved the final article.

L.V.A.B. is the principal investigator of the study in the St. Antonius Hospital in Nieuwegein and is responsible for adequate follow-up and rhythm monitoring of patients in the St. Antonius Hospital in Nieuwegein.

W.J.v.B. was involved in the conceptualization of the study and study design. W.J.v.B. read and approved the final article.

B.P.v.P. is as cardiothoracic surgeon responsible for the per protocol removal of LAA during cardiac surgery in the St. Antonius Hospital in Nieuwegein. B.P.v.P. is the principal investigator in the St. Antonius Nieuwegein together with L.V.A.B. B.P.v.P. improved the study design and read and approved the final article.

A.K. is cardiothoracic surgeon who is responsible for the largest proportion of LAAs that will be removed and read and approved the final article.

M.K. is a molecular biologist who was involved in the study design regarding adequate collection and storage of biomaterials and methodology for biomarker discovery. M.K. read and improved the first draft of the article and approved the final article.

A.H.G.D. was involved in the conceptualization of the study and study design. A.H.G.D. is as cardiothoracic surgeon responsible for the per protocol removal of LAA during cardiac surgery in the Academic Medical Center. A.H.G.D. read and improved the first draft of the article and approved the final article.

J.R.d.G. is principal investigator of the study and responsible for the overall design and conduction of the study. As such, J.R.d.G. was involved in the conceptualization

and design of the study. J.R.d.G. read and improved the first draft of the article and approved the final article.

Group authorship of the PREDICT AF investigators: The PREDICT AF investigators are cardiothoracic surgeons involved in removal of the LAA or are study coordinators involved in the inclusion of study participants, the organization of follow-up and the collection and storage of biomaterials. All authors read and approved the final article.

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Conflicts of interest

There are no conflicts of interest.

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