Towards understanding atrial cardiomyopathy in atrial fibrillation

Artola Arita, Vicente

DOI:
10.33612/diss.942570356

university of groningen

Towards Understanding Atrial Cardiomyopathy in Atrial Fibrillation

The Role of Comorbidities and its Association with Outcome

PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus Prof. J.M.A. Scherpen
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

Wednesday 17 April 2024 at 16.15 hours

by

Vicente Alberto Artola Arita

born on 18 March 1983

Supervisors
Prof. I.C. van Gelder
Prof. M. Rienstra

Assessment Committee
Prof. C.P. Allaart
Prof. P. van der Meer
Prof. R. Vliegenthart

Towards Understanding Atrial Cardiomyopathy in Atrial Fibrillation

The Role of Comorbidities and its Association with Outcome

Vicente Alberto Artola Arita

Promotors

Prof. dr. I.C. Van Gelder

Prof. dr. M. Rienstra

TABLE OF CONTENTS

Chapter 1 **Introduction**

Atrial fibrillation (AF) is the most common arrhythmia and is a growing epidemic.(1-6) The prevalence of AF has increased 3-fold in the last 50 years and recent data shows a lifetime risk of 1 in 3 in people with European ancestry and 1 in 5 people with Afro-American ancestry.(2,5) Available data estimates that AF affects on average 9 million people in Europe and 5 million people in the United States older than 55 years and it is expected that these ciphers double or triple by the year 2050.(2,6) The incidence of AF is increasing due to aging of the population, improved diagnostic tools to detect AF, more awareness of its existence and better survival due to advanced therapies for associated comorbidities. (2,5,6)

AF is a progressive disease that often starts with sporadic self-terminating episodes and may evolve to more frequent, longer and non-self-terminating AF.(7) AF progression has been associated with worse prognosis, such as an increase in development of heart failure (HF) hospitalizations,(8,9) stroke,(10) mortality,(10) and impairment of quality of life.(11) Patients with AF often present with symptoms such as palpitations, fatigue, dizziness, dyspnoea, chest pain and anxiety.(12) However, AF may also be asymptomatic.(13)

AF almost never comes alone and is associated with risk factors and comorbidities, including hypertension, obesity and heart failure(**Figure 1**).(14,15) Risk factors and comorbidities may start already the remodelling processes of the atria (and ventricles) long before the first AF episode is being detected (**Figure 2**).(3,9,18) Established traditional risk factors and comorbidities include ageing, coronary heart disease, heart failure, hypertension, diabetes, obesity, significant valvular diseases, thyroid disease, and excessive as well as deficient physical activity (**Figure 1**).(3,6,15) Progression of AF further contributes to deterioration of the remodelling processes (**Figure 2**).(17) AF also affects ventricular remodelling. (17) The presence of these risk factors and comorbidities cause electrical and structural changes in the atria, nowadays named Atrial Cardiomyopathy (ACM), and sets the stage for AF initiation and its progression. (7,8,17,19-21)

ACM pathophysiology and contributors

ACM is defined as atrial structural, architectural, contractile, or electrophysiological changes with potentially clinical manifestations.(17) AF is not only a risk factor for ACM but often also a marker of it, and a consequence.(17) Classification of ACM is suggested by the EHRAS scheme (for European Heart Rhythm Association; EHRA/Heart Rhythm Society; HRS/Asian Pacific Heart

Rhythm Association; APHRS/Latin American Society of Electrophysiology and Cardiac Stimulation; SOLAECE) which states four main groups: I) principal cardiomyocyte changes; II) principally fibrotic changes; III) combined cardiomyocyte-pathology/fibrosis; and IV) primarily non-collagen infiltration (with or without cardiomyocyte changes).(17) This classification allows describing underlying pathophysiology in various clinical conditions. For clinical practice, however this classification not feasible yet as we have only limited diagnostic abilities of atrial imaging and mapping.(22)

Structural remodelling is the main contributor to the ACM and includes cellular hypertrophy, fibrosis and myolysis amongst others eventually causing atrial contractile dysfunction and atrial enlargement. Available data now suggests that structural remodelling is caused by a convergence of different pathophysiological processes including the activation of the renin angiotensin-aldosterone system, calcium dysregulation, cardiomyocyte stretching, production of natriuretic peptides, inflammation and the production of reactive oxidative species and may vary among patients depending on their underlying comorbidities,(15,23-26) as type and severity of the comorbidities and the number of comorbidities contribute to this remodelling process.

A very prevalent and impactful comorbidity associated with AF and its progression is HF. AF and HF often coexist and their overlapping risk factors and comorbidities suggest at least in part common underlying pathophysiological processes. Individually, the presence of each condition increases the risk of stroke and death,(27,28) and the combination ameliorates prognosis.(29-31) AF is equally common in patients with HF with preserved ejection fraction (pEF) and patients with HF with reduced ejection fraction (rEF), however, may have even worse adverse effects in HFpEF.(32,33)

ACM increases along with age as the result of chronic subclinical inflammation and the production of reactive oxidative species (ROS) resulting in vascular deterioration. (17,38-40) Fibrotic changes in the atria are the most common histological alteration (EHRAS Class II).

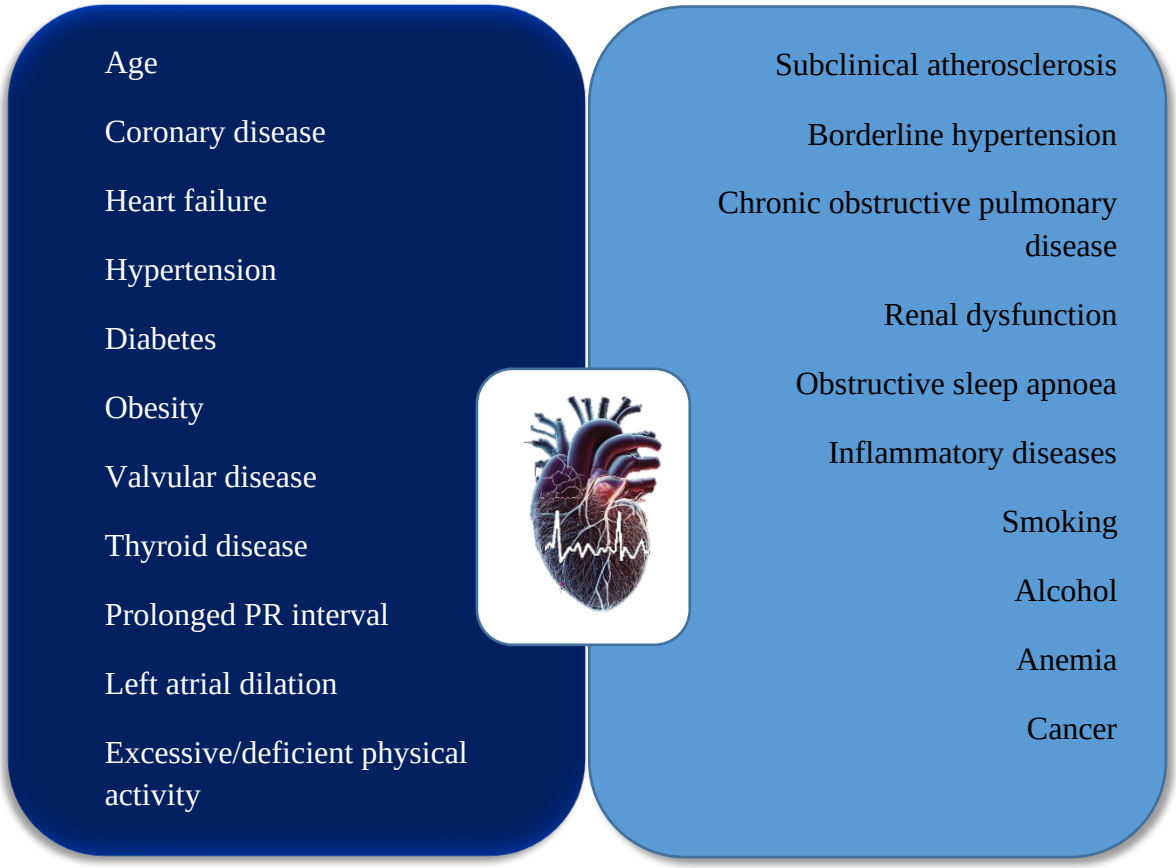


Figure 1 (36)Overview of risk factors associated with the development of ACM in atrial fibrillation (2,5,15,37)

ACM assessment in AF

As stated before the classification for an ACM at present is clinically not useful.(17) Assessment of the severity of the ACM using clinical parameters is relevant as it contributes to optimizing personalized therapy of patients with AF.(17,30) The best validated imaging modality at present to assess ACM is echocardiography, in particular LA size and volume.(17) Newer imaging techniques, however, may provide earlier and more precise detection or ACM.(43,44) Speckle tracking is a 2D echocardiographic technique that measures the percentage of deformation of each

of the cardiac chambers.(45,46) Speckle tracking has the advantage to be less affected by loading conditions in comparison to volumetric methods(47) and has been suggested to be an earlier marker of ACM outperforming atrial volumes. (**Figure 2**)(48-50)

Another option to assess severity of the ACM includes blood biomarkers associated with different underlying pathophysiological processes. Inflammatory biomarkers, such as C-reactive protein, have been associated to incidence of AF in men.(4) N-terminal pro-B-type natriuretic peptide, a marker of myocardial stretch, has also been associated to incidence of AF. (4,11) An important limitation of blood biomarkers is that they are not atrial specific thus also provide information on ventricular remodelling.(51) At present, a way to assess the severity of the ACM has been suggested by the ESC guideline postulation of the 4 S score including Stroke risk, Symptoms, Severity of AF burden and Substrate (30,52) The last S includes the substrate, i.e. ACM but definite tools to assess, as previously stated, are not available.

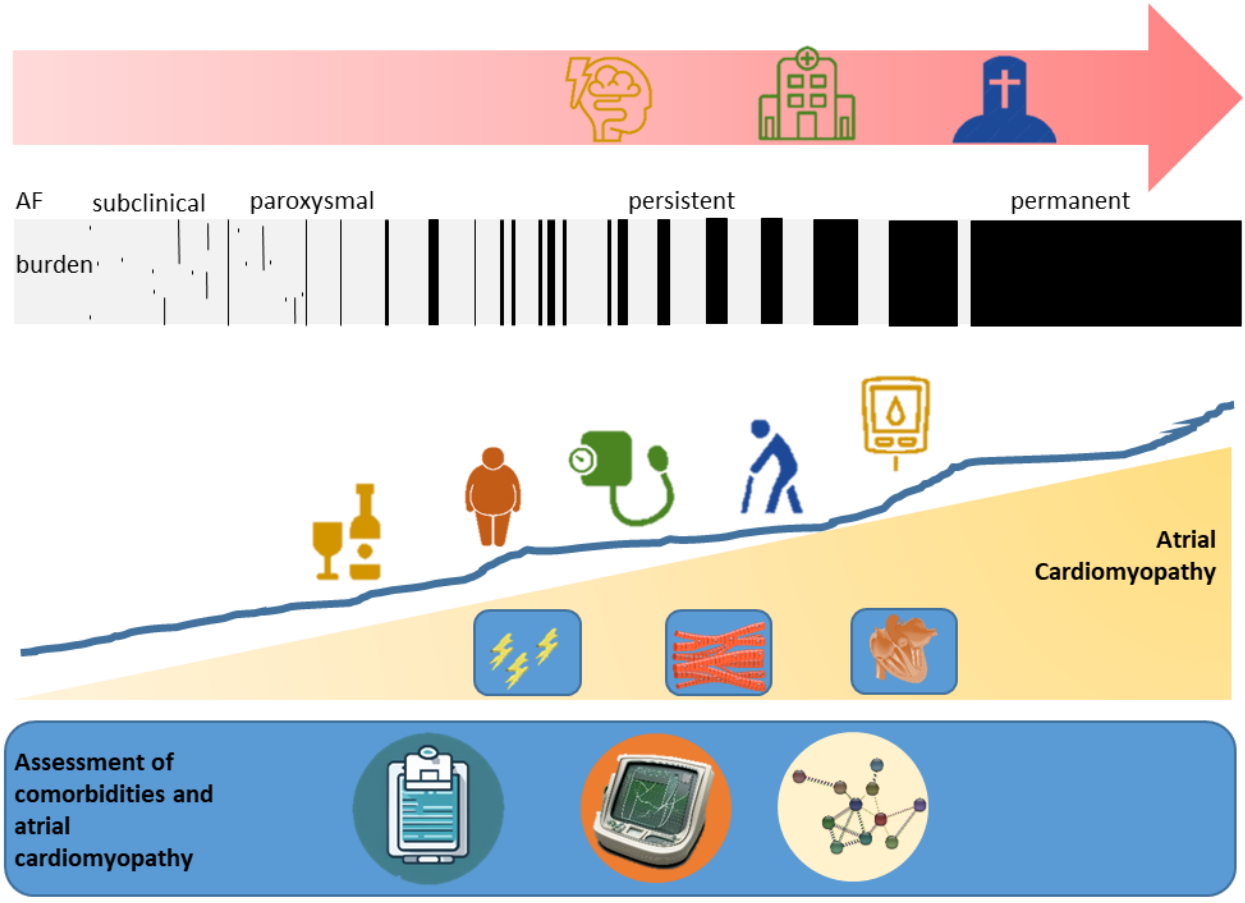


Figure 2. Conceptual figure of the natural history of atrial fibrillation and ACM. In blue section shows tools to detect risk factors and comorbidities and the ACM, including clinical evaluation,

echocardiography and blood biomarkers. The yellow section represents the progression of the remodelling process, i.e. the ACM along the natural history of risk factors and comorbidities and AF itself due to electrophysiological, structural and contractile changes of the atria. The blue line curve represents the accumulation of comorbidities and risk factors among others, alcohol consumption, obesity, hypertension, age, and diabetes. The gray bar represents the total time of a patient with grey indicating sinus rhythm and black episodes of AF, which in the beginning often are very short of duration and self-terminating. The red arrow represents adverse events associated with progression of AF including stroke, hospitalizations and mortality.

AIMS OF THIS THESIS

The comprehension of underlying mechanisms of AF and its progression are still not fully understood. Risk factors and comorbidities underlying ACM play an essential role in the occurrence of AF and AF progression but their relation is complex. Therefore, the aim of this thesis is to provide insights on how some risk factors, comorbidities and sex differences relate to AF and its progression. These insights are provided by exploring underlying mechanisms using clinical, echocardiographic and blood biomarkers. In addition, this thesis aims to explore the role of the ACM in AF and its progression. In **CHAPTER I,** as an introduction, we describe the interrelation between ACM and AF and how risk factors and comorbidities play a role in the origin of both. In order to assess how comorbidities are accompanying ACM in patients with AF, we investigate in **CHAPTER 2** the association of risk factors and comorbidities with the severity of the atrial remodelling, i.e. ACM, in patients with AF. AF and HF often occur together. However, HFpEF is difficult to diagnose in AF patients. With the aim to differentiate between AF patients with and without HFpEF we evaluate the severity of ACM using echocardiographic speckle tracking in both groups of patients in **CHAPTER 3**. Pursuing the understanding of differences in AF mechanisms between men and women, in **CHAPTER 4** we assess differences in pathophysiological biological processes underlying AF and HFrEF using biomarkers and pathway analysis. We also explore new possible understudied comorbidities in patients with AF and HF with reduced ejection fraction in **CHAPTER 5**, with the help of blood biomarkers and pathway analysis. In order to evaluate the clinical utility of the recent proposed 4S-AF (stroke risk, symptoms, severity of AF burden, substrate), in **CHAPTER 6** we use this comprehensive scheme, to characterize patients with self-terminating AF and make a step forward into assessing how this scheme predicts AF progression. In **CHAPTER 7**, we explore how blood biomarkers may help to identify more advanced stages of an ACM and AF. Finally, we summarize and interrelate the main points of this thesis and we put them into perspective for possible research and clinical implications.in **CHAPTER 8**.

References

(1) Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. Int J Stroke 2021 Feb;16(2):217-221.

(2) Kornej J, Börschel CS, Benjamin EJ, Schnabel RB. Epidemiology of Atrial Fibrillation in the 21st Century: Novel Methods and New Insights. Circ Res 2020 Jun 19;127(1):4-20.

(3) Vermond RA, Geelhoed B, Verweij N, Tieleman RG, Van der Harst P, Hillege HL, et al. Incidence of Atrial Fibrillation and Relationship With Cardiovascular Events, Heart Failure, and Mortality: A Community-Based Study From the Netherlands. J Am Coll Cardiol 2015 Sep 1;66(9):1000-1007.

(4) Magnussen C, Niiranen TJ, Ojeda FM, Gianfagna F, Blankenberg S, Njolstad I, et al. Sex Differences and Similarities in Atrial Fibrillation Epidemiology, Risk Factors, and Mortality in Community Cohorts: Results From the BiomarCaRE Consortium (Biomarker for Cardiovascular Risk Assessment in Europe). Circulation 2017 October 24;136(17):1588-1597.

(5) Schnabel RB, Yin X, Gona P, Larson MG, Beiser AS, McManus DD, et al. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. Lancet 2015 Jul 11;386(9989):154-162.

(6) Ball J, Carrington MJ, McMurray JJV, Stewart S. Atrial fibrillation: profile and burden of an evolving epidemic in the 21st century. Int J Cardiol 2013 Sep 1;167(5):1807-1824.

(7) Padfield GJ, Steinberg C, Swampillai J, Qian H, Connolly SJ, Dorian P, et al. Progression of paroxysmal to persistent atrial fibrillation: 10-year follow-up in the Canadian Registry of Atrial Fibrillation. Heart Rhythm 2017 Jun;14(6):801-807.

(8) de Vos CB, Pisters R, Nieuwlaat R, Prins MH, Tieleman RG, Coelen RJ, et al. Progression from paroxysmal to persistent atrial fibrillation clinical correlates and prognosis. J Am Coll Cardiol 2010 Feb 23;55(8):725-731.

(9) B Schnabel R, Pecen L, Engler D, Lucerna M, Sellal JM, Ojeda FM, et al. Atrial fibrillation patterns are associated with arrhythmia progression and clinical outcomes. Heart 2018 Oct;104(19):1608-1614.

(10) Steinberg BA, Hellkamp AS, Lokhnygina Y, Patel MR, Breithardt G, Hankey GJ, et al. Higher risk of death and stroke in patients with persistent vs. paroxysmal atrial fibrillation: results from the ROCKET-AF Trial. Eur Heart J 2015 Feb 1;36(5):288-296.

(11) Dudink EA, Weijs B, Tull S, Luermans JG, Fabritz L, Chua W, et al. The Biomarkers NT-proBNP and CA-125 are Elevated in Patients with Idiopathic Atrial Fibrillation. J Atr Fibrillation 2018 Dec 31;11(4):2058.

(12) Wynn GJ, Todd DM, Webber M, Bonnett L, McShane J, Kirchhof P, et al. The European Heart Rhythm Association symptom classification for atrial fibrillation: validation and improvement through a simple modification. Europace 2014 Jul;16(7):965-972.

(13) Hermans ANL, Gawalko M, Slegers DPJ, Andelfinger N, Pluymaekers, N. A. H. A., Verhaert DVM, et al. Mobile app-based symptom-rhythm correlation assessment in patients with persistent atrial fibrillation. Int J Cardiol 2022 Nov 15;367:29-37.

(14) Heijman J, Luermans, J. G. L. M., Linz D, van Gelder IC, Crijns, H. J. G. M. Risk Factors for Atrial Fibrillation Progression. Card Electrophysiol Clin 2021 Mar;13(1):201-209.

(15) George WD, Van Gelder Isabelle C, Ellinor Patrick T, Go Alan S, Kalman Jonathan M, Narayan Sanjiv M, et al. Lone Atrial Fibrillation. J Am Coll Cardiol 2014;63(17):1715-1723.

(16) Wong JA, Conen D, Van Gelder IC, McIntyre WF, Crijns HJ, Wang J, et al. Progression of Device-Detected Subclinical Atrial Fibrillation and the Risk of Heart Failure. J Am Coll Cardiol 2018 Jun 12;71(23):2603-2611.

(17) Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. Europace 2016 Oct;18(10):1455-1490.

(18) Blum S, Meyre P, Aeschbacher S, Berger S, Auberson C, Briel M, et al. Incidence and predictors of atrial fibrillation progression: A systematic review and meta-analysis. Heart Rhythm 2019 Apr;16(4):502-510.

(19) Nattel S, Guasch E, Savelieva I, Cosio FG, Valverde I, Halperin JL, et al. Early management of atrial fibrillation to prevent cardiovascular complications. Eur Heart J 2014 Jun 7;35(22):1448-1456.

(20) Nguyen BO, Weberndorfer V, Crijns HJ, Geelhoed B, Ten Cate H, Spronk H, et al. Prevalence and determinants of atrial fibrillation progression in paroxysmal atrial fibrillation. Heart 2022 Jul 20.

(21) Schnabel RB, Marinelli EA, Arbelo E, Boriani G, Boveda S, Buckley CM, et al. Early diagnosis and better rhythm management to improve outcomes in patients with atrial fibrillation: the 8th AFNET/EHRA consensus conference. Europace 2022:euac062.

(22) De Jong AM, Maass AH, Oberdorf-Maass SU, Van Veldhuisen DJ, Van Gilst WH, Van Gelder IC. Mechanisms of atrial structural changes caused by stretch occurring before and during early atrial fibrillation. Cardiovasc Res 2011 Mar 1;89(4):754-765.

(23) Savelieva I, Kakouros N, Kourliouros A, Camm AJ. Upstream therapies for management of atrial fibrillation: review of clinical evidence and implications for European Society of Cardiology guidelines. Part I: primary prevention. Europace 2011 Mar;13(3):308-328.

(24) Savelieva I, Kakouros N, Kourliouros A, Camm AJ. Upstream therapies for management of atrial fibrillation: review of clinical evidence and implications for European Society of Cardiology guidelines. Part II: secondary prevention. Europace 2011 May;13(5):610-625.

(25) Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. J Am Coll Cardiol 2014 Jun 10;63(22):2335-2345.

(26) Haeusler KG, Laufs U, Endres M. Chronic heart failure and ischemic stroke. Stroke 2011 Oct;42(10):2977-2982.

(27) Ambrosio G, Camm AJ, Bassand JP, Corbalan R, Kayani G, Carluccio E, et al. Characteristics, treatment, and outcomes of newly diagnosed atrial fibrillation patients with heart failure: GARFIELD-AF. ESC Heart Fail 2021 Apr;8(2):1139-1149.

(28) Kotecha D, Lam CS, Van Veldhuisen DJ, Van Gelder IC, Voors AA, Rienstra M. Heart Failure With Preserved Ejection Fraction and Atrial Fibrillation: Vicious Twins. J Am Coll Cardiol 2016 November 15;68(20):2217-2228.

(29) Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2021 Feb 1;42(5):373-498.

(30) McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2021 Sep 21;42(36):3599-3726.

(31) Linssen GCM, Rienstra M, Jaarsma T, Voors AA, van Gelder IC, Hillege HL, et al. Clinical and prognostic effects of atrial fibrillation in heart failure patients with reduced and preserved left ventricular ejection fraction. Eur J Heart Fail 2011 Oct;13(10):1111-1120.

(32) Olsson LG, Swedberg K, Ducharme A, Granger CB, Michelson EL, McMurray JJV, et al. Atrial fibrillation and risk of clinical events in chronic heart failure with and without left ventricular systolic dysfunction: results from the Candesartan in Heart failure-Assessment of Reduction in Mortality and morbidity (CHARM) program. J Am Coll Cardiol 2006 May 16;47(10):1997-2004.

(33) Kloosterman M, Crijns, H. J. G. M., Mulder BA, Groenveld HF, Van Veldhuisen DJ, Rienstra M, et al. Sex-related differences in risk factors, outcome, and quality of life in patients with permanent atrial fibrillation: results from the RACE II study. Europace 2020 Nov 1;22(11):1619-1627.

(34) Kloosterman M, Oldgren J, Conen D, Wong JA, Connolly SJ, Avezum A, et al. Characteristics and outcomes of atrial fibrillation in patients without traditional risk factors: an RE-LY AF registry analysis. Europace 2020 Jun 1;22(6):870-877.

(35) Wasmer K, Eckardt L, Breithardt G. Predisposing factors for atrial fibrillation in the elderly. J Geriatr Cardiol 2017 Mar;14(3):179-184.

(36) Dzeshka MS, Lip GY, Snezhitskiy V, Shantsila E. Cardiac Fibrosis in Patients With Atrial Fibrillation: Mechanisms and Clinical Implications. J Am Coll Cardiol 2015 Aug 25;66(8):943-959.

(37) Shen MJ, Arora R, Jalife J. Atrial Myopathy. JACC Basic Transl Sci 2019 Sep 23;4(5):640-654.

(38) Todaro MC, Choudhuri I, Belohlavek M, Jahangir A, Carerj S, Oreto L, et al. New echocardiographic techniques for evaluation of left atrial mechanics. Eur Heart J Cardiovasc Imaging 2012 Dec;13(12):973-984.

(39) Tsai W, Lee C, Lin C, Liu Y, Huang Y, Li W, et al. Association of left atrial strain and strain rate assessed by speckle tracking echocardiography with paroxysmal atrial fibrillation. Echocardiography 2009 Nov;26(10):1188-1194.

(40) Voigt JU, Pedrizzetti G, Lysyansky P, Marwick TH, Houle H, Baumann R, et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. J Am Soc Echocardiogr 2015 Feb;28(2):183-193.

(41) Badano LP, Kolias TJ, Muraru D, Abraham TP, Aurigemma G, Edvardsen T, et al. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging 2018 Jun 1;19(6):591-600.

(42) Thomas L, Marwick TH, Popescu BA, Donal E, Badano LP. Left Atrial Structure and Function, and Left Ventricular Diastolic Dysfunction: JACC State-of-the-Art Review. J Am Coll Cardiol 2019 Apr 23;73(15):1961-1977.

(43) Tadic M. The right atrium, a forgotten cardiac chamber: An updated review of multimodality imaging. J Clin Ultrasound 2015;43(6):335-345.

(44) Tomlinson S, Scalia GM, Appadurai V, Edwards N, Savage M, Lam AK, et al. Left atrial reservoir strain provides incremental value to left atrial volume index for evaluation of left ventricular filling pressure. Echocardiography 2021 Sep;38(9):1503-1513.

(45) Marchandise S, Garnir Q, Scavée C, Varnavas V, le Polain de Waroux, J. B., Wauters A, et al. Prediction of Left Atrial Fibrosis and Success of Catheter Ablation by Speckle Tracking Echocardiography in Patients Imaged in Persistent Atrial Fibrillation. Front Cardiovasc Med 2022 May 25;9:856796.

(46) Hijazi Z, Oldgren J, Siegbahn A, Granger CB, Wallentin L. Biomarkers in atrial fibrillation: a clinical review. Eur Heart J 2013;34(20):1475-1480.

(47) Potpara TS, Lip GYH, Blomstrom-Lundqvist C, Boriani G, Van Gelder IC, Heidbuchel H, et al. The 4S-AF Scheme (Stroke Risk; Symptoms; Severity of Burden; Substrate): A Novel Approach to In-Depth Characterization (Rather than Classification) of Atrial Fibrillation. Thromb Haemost 2021 Mar;121(3):270-278.

Chapter 2

Association between comorbidities and left and right atrial dysfunction in patients with short-lasting paroxysmal atrial fibrillation: analysis of AF-RISK.

Shared first author

Manouk J W van Mourik, Vicente Artola Arita, Aurore Lyon, Joost Lumens, Ruben R. De With, Joost P van Melle, Ulrich Schotten, Sebastiaan C.A.M. Bekkers, Harry J.G.M Crijns, Isabelle C Van Gelder, Michiel Rienstra, Dominik K. Linz

Adapted from International Journal of Cardiology 2022: 1;360:29-35.

Abstract

Aims. To identify the association between comorbidities and left atrial (LA) and right atrial (RA) function in patients with paroxysmal atrial fibrillation (AF).

Methods. This is a cross-sectional study. Speckle-tracking echocardiography was performed in 344 patients with paroxysmal AF at baseline, and available in 298 patients after 1-year follow-up. The number of comorbidities (hypertension, diabetes mellitus, coronary artery disease, body mass index > 25 kg/m^2, age > 65 years, moderate to severe mitral valve regurgitation and kidney dysfunction (estimated glomerular filtration rate < 60 ml/min/1.73 m^2)) was determined and the association with atrial strain was tested.

Results. Mean age of the patients was 58 (SD 12) years and 137 patients were women (40%). Patients with a higher number of comorbidities had larger LA volumes (p for trend <0.001), and had a decrease in all strain phases from the LA and RA, except for the RA contraction phase (p for trend 0.47). A higher number of comorbidities was associated with LA reservoir and conduit strain decrease independently of LA volume ($p < 0.001$, $p < 0.001$ respectively). Patients with 1–2 comorbidities, but not patients with 3 or more comorbidities, showed a further progression of impaired LA and RA function in almost all atrial strain phases at 14 [13–17] months follow-up.

Conclusions. In patients with paroxysmal AF, individual and combined comorbidities are related to lower LA and RA strain. In patients with few comorbidities, impairment in atrial function progresses during one year of follow-up. Whether comorbidity management prevents or reverses decrease in atrial function warrants further study.

Introduction

Atrial fibrillation (AF) is a progressive disease, which is mainly determined by structural atrial remodelling processes, called atrial cardiomyopathy (1), due to long-term exposure to concomitant cardiovascular risk factors and AF itself. One component of the arrhythmogenic atrial substrate is left atrial (LA) dilatation, which is common in patients with AF and has been shown to predict AF occurrence and cardiovascular events (2). In addition to LA dilatation, also LA function is a predictor of stroke risk and cardiovascular outcomes in patients with AF (3,4).

Functional impairment of atrial deformation properties represents an important component of the progressive atrial remodelling and AF substrate (5). During ventricular systole, LA strain (LAS) reflects LA expansibility and stiffness (6). Deformation in the LA reservoir (LASr) strain phase has been related to recurrence rates of AF after catheter ablation (3) and increased propensity for first episode of AF or atrial flutter, independent of LA volume, left ventricular (LV) function, and clinical risk factors (7). Despite the clear correlation between impaired reservoir deformation and AF, determinants of all phases from both the left and, specially, the right atria (RA) in patients with paroxysmal AF are unclear (8).

In this sub-study of the prospective, observational, multicenter "The identification of a risk profile to guide atrial fibrillation therapy (AF-RISK)" study (9), we aimed to accomplish the following two objectives: 1.) to identify the underlying comorbidities associated with reservoir, conduit and contractile phases of both atria, and 2.) to assess strain change after one year follow-up based on underlying comorbidities in patients with paroxysmal AF.

Methods

Study design. This is an ancillary sub-study of "The identification of a risk profile to guide atrial fibrillation therapy (AF-RISK)" study. AF-RISK was a multicenter, prospective study to assess AF progression rate, clinical, echocardiographic factors, and blood biomarkers associated with AF progression in patients with a short history of AF, and the association of AF progression with cardiovascular morbidity and mortality. Details are outlined elsewhere (9). AF-RISK was performed in The Netherlands (University Medical Centre Groningen and the Maastricht University Medical Centre +). The study was performed in concordance with the Declaration of

Helsinki, was approved by the institutional review boards, and was registered on ClinicalTrials.gov (identifier NCT01510197). All patients gave written informed consent.

Study population. Patients with a short history of paroxysmal AF were consecutively and prospectively enrolled for AF-RISK between March 2009 and April 2016 in the University Medical Center Groningen (UMCG) and the Maastricht University Medical Center (MUMC+), the Netherlands. Inclusion criteria for paroxysmal AF were time since diagnosis < 2 years, or < 3 years in case of ≤ 2 AF episodes of ≤ 48 h per month terminating spontaneously. General exclusion criteria were a history of heart failure > 3 years; a history of severe valvular disease; acute coronary syndrome (ACS) within the previous month; AF classified as post-operative; or a contra-indication for oral anticoagulation. All patients received treatment focused on rhythm control according to the AF guidelines (10). This treatment initially included causal treatment of underlying (heart) disease, adequate rate control therapy and initiation of antiarrhythmic drugs (AAD) in case of (frequent) symptomatic AF recurrences. At inclusion, patients' demographics and clinical characteristics were collected. Standard physical examination was performed. Additional examination at baseline consisted of ECG, blood sampling, 24-h Holter-monitoring, and exercise test.

For this sub-study, 344 patients with available transthoracic echocardiography (TTE) during sinus rhythm at baseline were studied. TTE during sinus rhythm at approximately 1-year follow-up was available in 298 patients, of which 225 LA strain (LAS) and 159 RA strain (RAS) analyses were available to assess progression (*Supplementary Figure 1*).

Transthoracic echocardiography. Standard TTE was performed according to the recommendations of the European Association of Cardiovascular Imaging (EACVI), using commercially available ultrasound systems with phased array transducers (Vivid 5, Vidid 7 or Vivid E9, Vivid E95 scanner, GE Vingmed Ultrasound AS, Horten, Norway). Images were acquired in left lateral decubitus position and recorded as ECG-gated digital loops and stored for offline analysis. Because the objective of the main study did not include investigating speckle-tracking echocardiography (STE), image acquisition was not specifically optimized for this purpose (mean frame rate 51 (SD 8) Hz). Atrial and ventricular dimensions, and valvular function were measured according to the EACVI guidelines (11). Systolic left ventricular ejection fraction (LVEF) was measured using the Simpson biplane method of discs.

Speckle-tracking echocardiography. All echocardiography recordings were anonymized and transferred to a core-lab facility for further offline analysis. Longitudinal strain assessment of the LA, RA and LV was performed during one corresponding cardiac cycle in sinus rhythm. Strain analysis was conducted offline by one experienced observer blinded to clinical data, using dedicated vendor-specific software (EchoPAC, GE Healthcare). Strain analysis was performed during sinus rhythm. LV global longitudinal strain (GLS) was analyzed in the apical two-, three- and four-chamber views using the 18-segment model. LAS and RAS were assessed in apical four-chamber view only. The regions of interest were manually outlined by marking the endocardial and epicardial borders in the LV end-systolic frame. End-systole was automatically defined by the software. The software automatically tracks myocardial speckle patterns frame-by-frame during one cardiac cycle (RR-interval). Suboptimal tracking, considered by either visual or automated assessment, was manually adjusted by redrawing the region of interest. If suboptimal tracking persisted despite multiple attempts, the concerning region of interest was eliminated from analysis. For all available strain analysis, the raw data were stored. An example of the LAS analysis is shown in *Supplementary Figure 2*.

LAS was determined during reservoir phase (LASr) and during active contraction phase (LASct). LAS during conduit phase (LAScd) was calculated from LASr minus LASct. RAS was determined in a similar fashion for reservoir phase (RASr), contraction phase (RASct) and conduit phase (RAScd). For the left ventricle, we measured GLS defined as peak negative strain. GLS was measured in the average curve combining all segmental curves, if ≥12 available. If less than 12 segmental strain curves were available, GLS was not thought to be representative and was therefore excluded. All strain parameters were defined conform to the EACVI consensus document (12).

Covariate definitions. Total AF history was defined as time from first documented AF episode till inclusion. Hypertension was defined as a systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg, or by the use of antihypertensive drugs. Diabetes mellitus was defined as history of diabetes or use of anti-diabetic drugs. Clinical presentation of heart failure was defined as left ventricular ejection fraction (LVEF) ≤ 45% at baseline or LVEF > 45% with symptoms associated with heart failure (New York Heart Association functional class II or III) or previous hospitalization for heart failure. Kidney dysfunction was defined as estimated glomerular

filtration rate (eGFR) < 60 ml/min/1.73 m2. The eGFR was calculated using modification of diet in renal disease formula: 175 x (serum creatinine x 0.0113)-1.154 x age-0.203 (x 0.742 if female). The ratio of weight to height squared (kg/m2) was used for calculation of body mass index (BMI).

The number of comorbidities is defined as the sum of the presence of the following comorbidities, awarded each a point: hypertension, coronary artery disease, age > 65 years, diabetes mellitus, BMI > 25 kg/m2, moderate to severe mitral valve regurgitation, or kidney dysfunction.

Values of left atrial volume indexed (LAVI) ≥ 34 ml/m2 were considered abnormal. Values of right atrial volume indexed (RAVI) ≥ 30 ml/m2 were considered abnormal.

Statistical analysis. Continuous variables with normal distribution are expressed as mean and standard deviation (SD), otherwise as median with interquartile range (IQR). Categorical variables are presented as observed number with percentage. Continuous variables were compared using independent Student's t-test or the Mann-Whitney U test, as appropriate. To assess the trend of strain measures over the number of comorbidities, one-way ANOVA was used. For categorical variables Chi-square or Fisher's exact test were used to evaluate differences. Univariate and multivariate logistic regression were performed to establish association between comorbidities echocardiographic parameters. Paired t-test was used to assess changes of strain measures during approximately 1-year follow-up. A two-tailed value of $p < 0.05$ was considered statistically significant. Software R was used to perform analysis.

Results

Mean age of the population was 58 (SD 12) years and 137 patients were women (40%). The median history of AF at baseline was 5 2–18 months, 182 patients had heart failure (52%; 2% heart failure with reduced LVEF) and 272 had hypertension (79%) (**Table 1**).

Table 1. Clinical and echocardiographic baseline characteristics presented for total population and stratified according to number of comorbidities.

	Total population (n = 344)	Number of comorbidities 0 (n = 27)	1 (n = 87)	2 (n = 131)	≥3 (n = 99)	p value
Clinical characteristics						
Age, *years*	58±12	46±13	53±12	57±10	67±8	**<0.001**
Women, *n (%)*	137(40)	11(41)	34(39)	41(31)	51(52)	**0.022**
History of AF, months *(range)*	5(2–18)	3(2−12)	5(2–19)	6(2–19)	6(2–18)	0.573
Follow up time, months	13.9±2.0	15.0 ±3.3	13.9±1.9	13.7±1.8	13.9±1.7	**0.032**
Heart failure, *n (%)*	182 (53)	9 (33)	44 (51)	75 (57)	54 (55)	0.142
HFpEF, *n (%)*	174 (51)	9 (33)	44 (51)	70 (53)	51 (52)	0.299
HFrEF, *n (%)*	8 (2)	0 (0)	0 (0)	5 (4)	3 (3)	0.239
Hypertension, *n (%)*	272 (79)	0 (0)	47 (54)	127 (97)	98 (99)	**<0.001**
Diabetes, *n (%)*	29 (8)	0 (0)	2 (2)	1 (1)	26 (26)	**<0.001**
Coronary artery disease, *n (%)*	18 (5)	0 (0)	1 (1)	0 (0)	17 (17)	**<0.001**
Peripheral artery disease, *n (%)*	8 (2)	0 (0)	0 (0)	3 (2)	5 (5)	0.114
TIA or stroke, *n (%)*	18 (5)	0 (0)	3 (3)	9 (7)	6 (6)	0.408
BMI, *(kg/m^2)*	27.6±5.0	22.6 ±1.7	25.7±4.6	28.5±5.0	29.5±4.3	**<0.001**
Overweight, *n (%)*	223 (65)	0 (0)	31 (36)	102 (78)	90 (91)	**<0.001**
Kidney dysfunction, *n (%)*	35 (10.2)	0 (0.0)	0 (0.0)	5 (3.8)	30 (30.3)	**<0.001**
Mitral valve regurgitation, *n (%)*	3 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	3 (3.0)	0.058
EHRA Score, *n (%)*						0.301
I	94(27)	6(22)	28(32)	28(21)	32(32)	
II	203(59)	18(67)	44(51)	86(65)	54(55)	
III	47(14)	3(11)	15(17)	17(13)	13(13)	
CHA_2DS_2-VASc score[a]	1.5±1.4	0.4±0.5	0.8±0.8	1.3±1.1	2.9±1.3	**<0.001**
Echocardiography						
LAVI, *mL/m^2*	32±9	25±9	30±8	33±10	34±9	**<0.001**

RAVI, *mL/m*^{2}	35±12	33±9	34±12	36±13	35±11	0.507
LV ejection fraction, *%*	57±4	58±2	58±2	57±4	57±4	**0.027**
LV mass index, *g/m*^{2}	81±18	70±19	77±15	84±20	83±17	**0.001**
e’	10±2.8	14±3.6	11±2.6	10±2.4	8±1.9	**<0.001**
E/A ratio, *(range)*	1.1(0.9–1.4)	1.3(1.1–1.6)	1.1(1.0–1.4)	1.0(0.9–1.3)	1.0(0.8–1.2)	**0.001**
E/e’, *(range)*	6.9(5.8–8.8)	5.5(4.7–6.1)	6.6(5.4–7.9)	6.8(5.8–8.1)	8.5(6.7–10.3)	**<0.001**
Speckle-tracking echocardiography						
LA strain, *%*						
Reservoir	34.0±12.6	46.2±10.3	39.0±10.9	33.0±10.6	27.2±10.5	**<0.001**
Conduit	18.2±9.2	27.9±9.6	21.9±9.7	15.3±8.1	13.5±6.3	**<0.001**
Contraction	15.7±7.2	18.3±7.5	17.1±6.7	15.8±7.5	13.6±6.7	**0.004**
RA strain, *%*						
Reservoir	44.5±14.0	53.7±12.5	46.2±13.0	44.4±14.4	39.7±13.4	**<0.001**
Conduit	25.0±10.4	32.3±9.6	26.5±10.3	24.6±10.2	21.2±9.3	**<0.001**
Contraction	19.6±8.2	21.5±8.3	19.7±8.2	19.7±8.2	18.5±8.2	0.470
GLS, *%*	−19.3±2.9	−20.2±1.9	−20.1±2.5	−19.8±3.1	−18.6±2.9	**0.002**

AF = atrial fibrillation; EHRA = European Heart Rhythm Association symptom classification; LAVI = left atrial volume indexed; RAVI = right atrial volume indexed.
a The CHA2DS2-VASc score assesses thromboembolic risk. C, congestive heart failure/LV dysfunction; H, hypertension; A2_, age ≥ 75 years; D, diabetes mellitus; S2_, stroke/transient ischaemic attack/systemic embolism; V, vascular disease; A, age 65–74 years; Sc, sex category (female sex).

Individual and combined comorbidities and atrial strain. In total, echocardiographic studies were available in 344 patients with paroxysmal AF. Strain analysis by STE was feasible in the LA in 309 (90%) patients, in the RA in 253 (74%) patients and in the LV in 321 (93%) patients. The echocardiographic baseline characteristics are presented in **Table 1**.

Assessing the relation between individual comorbidities and atrial strain, BMI > 25 kg/m^2 was associated with lower strain in both atria throughout all phases. Age > 65 years and diabetes were associated with lower strain values of both atria, except for RASct (*Supplementary Table 1*).

Table 2. Comorbidities compared by LAVI for strain parameters of both atria. LAS and RAS cut-off values were determined based on the median in this population.

	Normal LAVI (<34ml/m^2)			**Dilation LAVI (>34ml/m^2)**		
			LA reservoir strain			
	<33.1 (n=65)	>33.1 (n=106)	*p*	<33.1 (n=72)	>33.1 (n=37)	p
Comorbidities	2.1 ±1.0	1.5±1.0	**<0.001**	2.7 ±1.2	1.7 ±1.0	**<0.001**
			LA conduit strain			
	<17.7 (n=71)	>17.7 (n=100)	*p*	<17.7 (n=68)	>17.7 (n=41)	*p*
Comorbidities	2.8 ±0.9	1.4 ±1.0	**<0.001**	2.8 ±1.2	1.7 ±0.9	**<0.001**
			LA contraction strain			
	<15.1 (n=67)	>15.1 (n=104)	*p*	<15.1 (n=73)	>15.1 (n=36)	*p*
Comorbidities	1.8 ±1.2	1.7 ±0.9	0.720	2.5 ±1.2	2.1 ±1.2	0.132
	Normal RAVI (<30ml/m^2)			**Normal RAVI (>30ml/m^2)**		
			RA reservoir strain			
	<44.5 (n=40)	>44.5 (n=50)	*p*	<44.5 (n=80)	>44.5 (n=72)	*p*
Comorbidities	2.3 ±1.3	1.7 ±1.3	**0.031**	2.1 ±1.2	1.6 ±1.0	**0.015**
			RA conduit strain			
	<24.0 (n=41)	>24.0 (n=49)	*p*	<24.0 (n=78)	>24.0 (n=74)	*p*
Comorbidities	2.2 ±1.3	1.7 ±1.3	0.097	2.1 ±1.2	1.6 ±1.0	**0.003**
			RA contraction strain			
	<19.2 (n=39)	>19.2 (n=51)	*p*	<19.2 (n=83)	>19.2 (n=69)	*p*
Comorbidities	2.2 ±1.2	1.8 ±1.4	0.137	2.0 ±1.8	1.7 ±1.0	0.147

LA=left atrium; LAVI=left atrial volume indexed; RA=right atrium; RAVI=right atrial volume indexed. Comorbidities were calculated by awarding a point to each of the following comorbidities, hypertension, age >65 years, diabetes, coronary artery disease, body mass index >25, kidney dysfunction, and moderate or severe mitral valve regurgitation.

Patient characteristics were evaluated by the combined number of comorbidities as 0, 1, 2 and 3 or more (**Table 1**). Patients with more comorbidities had higher LAVI (*p* for trend <0.001) and had lower LAS and RAS, except for the RASct (*p* for trend 0.47).

Table 3. Changes in atrial strain parameters after 1-year follow-up by number of comorbidities.

		n	Baseline	Follow-up 1-year	MD	*p*
				0 Comorbidities		
LA	reservoir	20	46.2 ±10.3	39.3 ±16.4	6.32	0.146
	conduit		27.9 ±9.6	27.0 ±14.3	1.23	0.696
	contraction		18.3 ±7.5	12.3 ±5.4	5.09	**0.003**
RA	reservoir	18	53.7 ±12.5	41.5 ±14.9	10.01	**0.024**
	conduit		32.3 ±9.6	25.6 ±10.7	5.69	0.070
	contraction		21.5 ±8.3	15.9 ±6.9	4.32	0.058
				1 Comorbidities		
LA	reservoir	62	39.0 ±10.9	34.9 ±10.7	4.28	**0.008**
	conduit		21.9 ±9.7	19.7 ±7.6	2.25	**0.041**
	contraction		17.1 ±6.7	15.2 ±7.4	2.04	**0.036**
RA	reservoir	52	46.2 ±13.0	43.4 ±13.5	4.60	**0.027**
	conduit		26.5 ±10.3	26.0 ±9.9	0.85	0.599
	contraction		19.7 ±8.2	17.5 ±7.5	3.74	**0.003**
				2 Comorbidities		
LA	reservoir	88	33.0 ±10.6	31.4 ±10.9	2.44	**0.112**
	conduit		15.3 ±8.1	16.2 ±7.7	1.44	0.142
	contraction		15.8 ±7.5	15.1 ±6.4	0.99	0.223
RA	reservoir	54	44.4 ±14.4	37.5 ±12.0	7.70	**<0.001**
	conduit		24.6 ±10.2	21.6 ±9.6	3.79	**0.005**
	contraction		19.7 ±8.2	16.0 ±6.0	3.91	**<0.001**
				3 or more Comorbidities		
LA	reservoir	55	27.2 ±10.5	26.5 ±8.4	2.58	0.107
	conduit		13.5 ±6.3	13.7 ±6.4	0.38	0.713
	contraction		13.6 ±6.7	12.8 ±5.1	2.20	**0.023**
RA	reservoir	35	39.7 ±13.4	38.2 ±12.9	3.21	0.256
	conduit		21.2 ±9.3	18.1 ±7.8	3.52	**0.016**
	contraction		18.5 ±8.2	20.1 ±8.6	-0.31	0.870

Comorbidities were calculated by awarding a point to each of the following comorbidities, hypertension, heart failure, age >65 years, diabetes mellitus, coronary artery disease, BMI >25 kg/m^2, moderate to severe mitral valve regurgitation and kidney dysfunction (eGFR <60 ml/min/1.73m^2). LA=left atrium; MD=mean difference; RA=right atrium.

Strain decreased proportionally to the number of comorbidities, predominantly in reservoir and conduit phases of both atria (**Table 1** and **Figure 1**). After adjusting for atrial volumes, a higher number of comorbidities was most strongly associated with a decrease in LAScd among all strain parameters, (OR per 1% LAScd decrease 0.92, 95%CI:0.88–0.96) (*Supplementary Table 2*).

Atrial dilation and atrial function. All LAS parameters were correlated with LAVI, and all RAS parameters were correlated with RAVI (*Supplementary Table 3*). However, patients with lower LASr had more comorbidities irrespective of LAVI (LAVI < 34 ml/m^2, 1.9 (SD 1.0) vs 1.3 (SD 1.0), $p = 0.002$; LAVI ≥ 34 ml/m^2, 2.4 (SD 1.2) vs 1.3 (SD 1.0), $p = 0.007$). The same was observed in patients with lower LAScd (**Table 2).** Patients with lower RASr had more comorbidities irrespective of RAVI (**Table 2).**

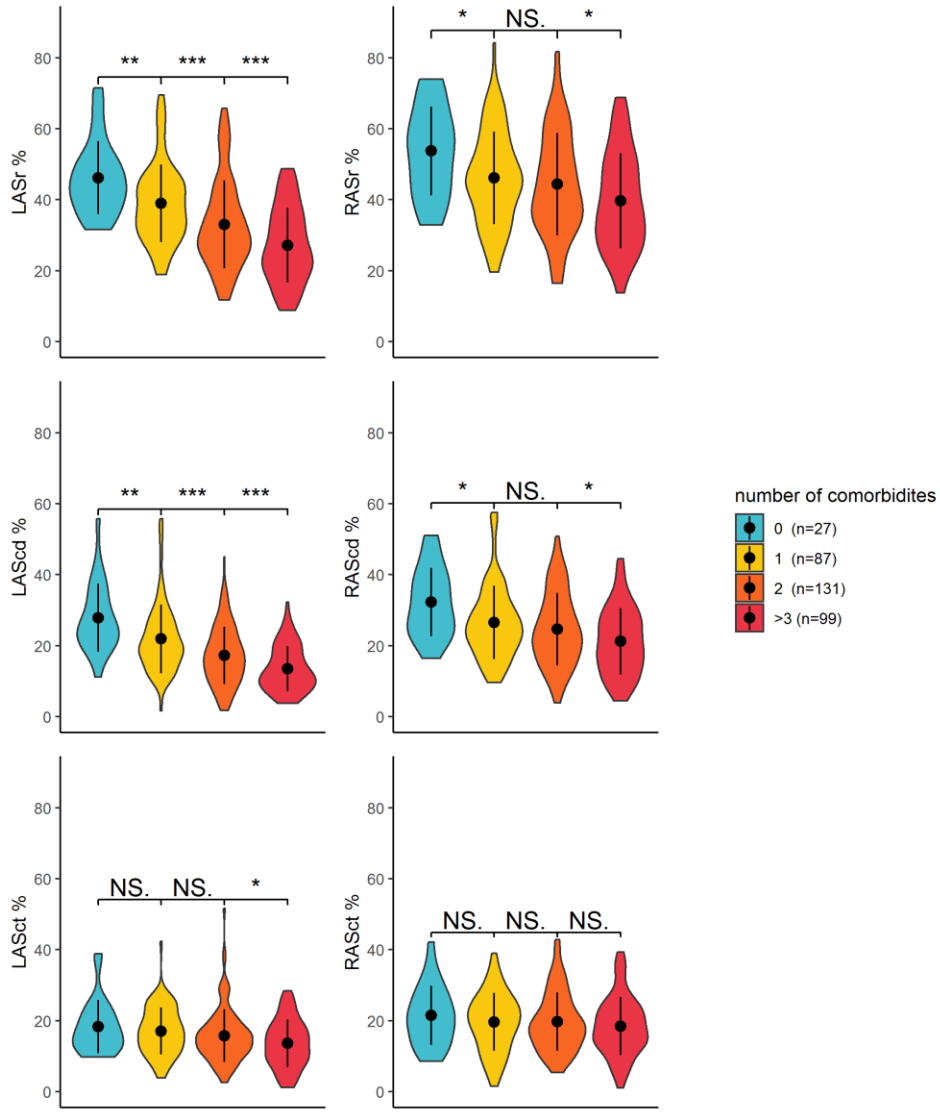
Progression of atrial strain impairment. At follow-up LAS was available in 225 patients and RAS in 159 patients (*Supplementary Figure 1*). Patients with 1 to 2 comorbidities showed a further decrease in LAS and RAS in almost all phases at 14 13–17 months follow-up. Conversely, patients with 3 or more comorbidities had less or no further progressive impairment of LA and RA function within one year follow-up (**Table 3**).

Discussion. Our study shows that the presence of individual and combined comorbidities is associated with a decrease in atrial function measured by STE in patients with paroxysmal AF. Importantly, these findings were independent of indexed atrial volumes, suggesting that LAS and RAS assessed by STE may be early markers of the atrial remodelling process as a result of concomitant comorbidities even before the atria start to dilate. Furthermore, follow-up data showed that impairment in atrial function progresses after one year in patients with few comorbidities.

Atrial cardiomyopathy. Exposure to comorbidities has been shown to contribute to a progressive atrial remodelling process, which is considered to be an interplay of structural, electrical and functional alterations of the atria (4). Structural remodelling is characterized by atrial dilatation, cardiomyocyte hypertrophy and increased extracellular matrix formation, which influences electrical remodelling by increasing local conduction disturbances and maintenance of AF (11,13). LAVI is well-embedded in the routine echocardiographic examination as a surrogate for structural atrial remodelling (14) and has been shown to be associated with adverse cardiovascular outcomes

in various cardiac diseases. However, functional atrial remodelling is gaining more interest as atrial strain analysis using STE, which is a feasible and reproducible technique (15).

Figure 1. Left and right atrial strain parameters grouped by the number of comorbidities.



Number of comorbidities was determined by awarding a point to each of the following, hypertension, heart failure, age > 65 years, diabetes mellitus, coronary artery disease, BMI > 25 kg/m2, moderate to severe mitral valve regurgitation and kidney dysfunction (eGFR < 60 ml/min/1.73m2). Y-axis expresses percentage of deformation measure by 2D speckle-tracking echocardiography. Point within the graph expresses mean and lines determine standard deviation.

LASr = left atrial reservoir strain; LAScd = left atrial conduit strain; LASct = left atrial contraction strain; RASr = right atrial reservoir strain; RAScd = right atrial conduit strain;

In this study, we demonstrated that atrial dysfunction is associated with atrial dilatation and both parameters are correlated with the cardiovascular risk profile, underlining the interplay of structural and functional alterations within the atrial remodelling. However, within patients with normal sized atria, reservoir and conduit strain were able to further differentiate between the number of comorbidities. These findings suggest that functional deterioration in patients with normal sized atria may represent an early alteration in the remodelling process due to concomitant comorbidities, before the atria start to dilate.

Cardiovascular risk profile. In this study we focused on functional atrial remodelling within the concept of atrial cardiomyopathy. Atrial function comprises the reservoir, conduit and contractile phase, all together contributing to ventricular filling and function. In the current literature, there is no consensus about the best atrial strain parameter for clinical use. Although atrial function and ventricular performance are interdependent, the interaction differs throughout the atrial phases and this may influence the usability of the individual parameters for different purposes (16). Until now, LASr is the best studied parameter in patients with declined atrial function, incidence of AF and outcomes (6,7). Even less is known about RAS, although RA function has previously been introduced as an important early marker for cardiac impairment, especially in pressure or volume overload of the right ventricle, including heart failure, coronary artery disease and AF itself (8).

We demonstrated that all three LA and RA phasic strain functions are affected by the presence of both individual and combined comorbidities. Increased BMI is associated with deteriorating function throughout the entire LA and RA cycle. Additionally, history of diabetes and increasing age are determinants associated with deteriorating function throughout almost the entire LA and RA cycle, except for RASct. On the other hand, presence of coronary artery disease and kidney dysfunction share common associations with decreased LASr and LAScd. The greater influence of comorbidities on LAS reservoir and conduit function may be explained by the more prominent influence of cardiac loading conditions and LV performance, whereas LA contractile function is determined by intrinsic atrial function (17). LA contractile function has potential to compensate for early LA conduit failure, which could explain the lack of association of decreased strain during

the contractile phase with the number of comorbidities in this population with a relatively short AF history (17,18).

Recently, normal reference values were demonstrated for LA strain based on measurements in healthy subjects (19). Interestingly, the mean LA strain values observed in the subgroup of patients with paroxysmal AF without any comorbidities or with one comorbidity in our cohort correspond with the normal reference values for LA strain observed in healthy subjects suggesting that the cardiovascular risk profile is an important factor in the development of atrial dysfunction and maybe even atrial cardiomyopathy. Normal values from RA strain in patients without comorbidities were consistent with a previous study (20), however, these values were obtained using 3D techniques.

Progression of atrial dysfunction. In patients with paroxysmal AF with a low number of comorbidities, our results show that atrial function progressively decreases after 1-year follow-up. This observation supports the concept, that AF itself contributes to its own perpetuation ("AF begets AF"), particularly if no other comorbidities and preexisting remodelling processes are present (21). In contrast, in patients with a higher number of comorbidities, atrial function did not further decline or to a lesser extent, within one year of follow-up, suggesting that patients with a higher number of comorbidities already show a preexisting significant atrial remodelling at baseline. Theoretically, early treatment of concomitant comorbidities with a proactive approach may be required as an important component of the early management of patients paroxysmal AF (22). Additionally, patients with paroxysmal AF with few obvious comorbidities may profit from early rhythm control to prevent the early progression in atrial function impairment observed in this study (14).

Clinical implications. Atrial strain is an emerging topic within world-wide research setting. Atrial strain has been shown prognostic value in both patients with AF (23) and the general population, predicting cardiovascular mortality, morbidity, and for example development of dementia (24,25). In this observational study we focused on the determinants of atrial strain and showed an association of atrial strain with individual and combined comorbidities, irrespective of atrial dilatation. As determination of atrial strain provides additional information about the stage of atrial remodelling in patients with paroxysmal AF beyond atrial volume index, this technique may have potential to be incorporated in routine echocardiographic assessment. Impaired atrial function

should trigger a structured assessment of comorbidities and may represent an interesting measure to guide future risk factor and comorbidity management programs in AF patients. Whether atrial function improves and patients benefit from combined risk factor modification programs or early rhythm control interventions warrants further research.

Limitations. In this study, we focused solely on patients with paroxysmal AF, therefore we cannot translate these results directly to patients with an increased number of comorbidities in absence of AF nor in patients with advanced AF stage. AF is a heterogeneous disease and unidentified phenotypes may dilute specific differences among patients. The number of comorbidities is based on previous studies, other combination of comorbidities could possibly lead to other results. Because there is no long-term follow-up available at this moment, we are not able to study the changes of LA and RA function over time, nor clinical progression or outcome. Due to the observational nature of AF-RISK, we cannot determine causal effects.

Conclusions

In patients with paroxysmal AF, individual and combined comorbidities are related to lower LA and RA strain. In patients with no or few comorbidities, impairment in atrial function progresses during one year of follow-up. Whether comorbidity management and early rhythm control prevents or even reverses decreases in atrial strain function warrants further study.

References

1. A. Goette, J.M. Kalman, L. Aguinaga, et al., EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication, Europace 18 (2016) 1455–1490.

2. M. Thamilarasan, A.L. Klein, Factors relating to left atrial enlargement in atrial fibrillation: "chicken or the egg" hypothesis, Am. Heart J. 137 (1999) 381–383.

3. C. Schneider, R. Malisius, K. Krause, et al., Strain rate imaging for functional quantification of the left atrium: atrial deformation predicts the maintenance of sinus rhythm after catheter ablation of atrial fibrillation, Eur. Heart J. 29 (2008) 1397–1409.

4. B.W. Calenda, V. Fuster, J.L. Halperin, C.B. Granger, Stroke risk assessment in atrial fibrillation: risk factors and markers of atrial myopathy, Nat. Rev. Cardiol. 13 (2016) 549–559.

5. S. Blum, P. Meyre, S. Aeschbacher, et al., Incidence and predictors of atrial fibrillation progression: a systematic review and meta-analysis, Heart Rhythm. 16 (2019) 502–510.

6. Z. Wang, H. Tan, M. Zhong, G. Jiang, Y. Zhang, W. Zhang, Strain rate imaging for noninvasive functional quantification of the left atrium in hypertensive patients with paroxysmal atrial fibrillation, Cardiology 109 (2008) 15–24.

7. W.P. Abhayaratna, K. Fatema, M.E. Barnes, et al., Left atrial reservoir function as a potent marker for first atrial fibrillation or flutter in persons > or = 65 years of age, Am. J. Cardiol. 101 (2008) 1626–1629.

8. M. Tadic, The right atrium, a forgotten cardiac chamber: an updated review of multimodality imaging, J. Clin. Ultrasound 43 (2015) 335–345.

9. R.R. De With, E.G. Marcos, E.A.M.P. Dudink, et al., Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: data from the AF-RISK study, Europace 22 (2020) 352–360.

10. G. Hindricks, T. Potpara, N. Dagres, et al., 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS), Eur. Heart J. 42 (2021) 373–498.

11. R.M. Lang, L.P. Badano, V. Mor-Avi, et al., Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging, Eur. Heart J. Cardiovasc. Imaging 16 (2015) 233–270.

12. L.P. Badano, T.J. Kolias, D. Muraru, et al., Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/industry task force to standardize deformation imaging, Eur. Heart J. Cardiovasc. Imaging 19 (2018) 591–600.

13. J. Heijman, D. Linz, U. Schotten, Dynamics of atrial fibrillation mechanisms and comorbidities, Annu. Rev. Physiol. 83 (2021) 83–106.

14. P. Kirchhof, A.J. Camm, A. Goette, et al., Early rhythm-control therapy in patients with atrial fibrillation, N. Engl. J. Med. 383 (2020) 1305–1316.

15. M.J. Vieira, R. Teixeira, L. Gonçalves, B.J. Gersh, Left atrial mechanics: echocardiographic assessment and clinical implications, J. Am. Soc. Echocardiogr. 27 (2014) 463–478.

16. S. Ramkumar, H. Yang, Y. Wang, et al., Association of the Active and Passive Components of left atrial deformation with left ventricular function, J. Am. Soc. Echocardiogr. 30 (2017) 659–666.

17. M. Greiser, H.R. Neuberger, E. Harks, et al., Distinct contractile and molecular differences between two goat models of atrial dysfunction: AV block-induced atrial dilatation and atrial fibrillation, J. Mol. Cell. Cardiol. 46 (2009) 385–394.

18. R. Mehrzad, M. Rajab, D.H. Spodick, The three integrated phases of left atrial macrophysiology and their interactions, Int. J. Mol. Sci. 15 (2014) 15146–15160.

19. F. Pathan, N. D'Elia, M.T. Nolan, T.H. Marwick, K. Negishi, Normal ranges of left atrial strain by speckle-tracking echocardiography: a systematic review and Metaanalysis, J. Am. Soc. Echocardiogr. 30 (2017) 59–70.e8.

20. A. Nemes, A. ´ Korm´ anyos, P. Domsik, et al., Normal reference values of right atrial strain parameters using three-dimensional speckle-tracking echocardiography (results from the MAGYAR-healthy study), Int. J. Card. Imaging 35 (2019) 2009–2018.

21. MC Wijffels, CJ Kirchhof, R Dorland, MA Allessie, Atrial fibrillation begets atrial fibrillation A study in awake chronically instrumented goats, Circulation 92 (1995) 1954–1968

22. A Brandes, MD Smit, BO Nguyen, M Rienstra, IC Van Gelder, Risk factor management in atrial fibrillation, Arrhythmia Electrophysiol Rev 7 (2018) 118–127

23. M Obokata, K Negishi, K Kurosawa, et al, Left atrial strain provides incremental value for embolism risk stratification over CHA_2DS_2-VASc score and indicates prognostic impact in patients with atrial fibrillation, J Am Soc Echocardiogr 27 (2014) 709–716e4

24. D Modin, SR Biering-Sørensen, R Møgelvang, AS Alhakak, JS Jensen, T Biering-Sørensen, Prognostic value of left atrial strain in predicting cardiovascular morbidity and mortality in the general population, Eur Heart J Cardiovasc Imaging 20 (2019) 804–815

25. W Wang, MJ Zhang, RM Inciardi, et al, Association of echocardiographic measures of left atrial function and size with incident dementia, JAMA 327 (2022) 1138–1148

Chapter 3

Atrial function in paroxysmal AF patients with and without heart failure with preserved ejection fraction: data from the AF-RISK study.

Vicente Artola Arita, Bernadet T. Santema, Ruben R. De With, Bao-Oanh Nguyen, Dominik Linz, Ulrich Schotten, Isabelle C. Van Gelder, Harry JGM Crijns, Adriaan A Voors, Michiel Rienstra

Adapted from American Heart Journal 2022: 244:36-41.

Abstract:

Atrial fibrillation (AF) and heart failure with preserved ejection fraction (HFpEF) are two cardiovascular conditions that often coexist. Strain phases of both the left and right atria are more impaired in paroxysmal AF patients with HFpEF than those without HFpEF in spite of comparable global longitudinal strain of the left ventricle. Atrial function may differentiate paroxysmal AF patients with HFpEF from those without HFpEF.

Heart failure with preserved ejection fraction (HFpEF) and atrial fibrillation (AF) are two cardiovascular conditions that often coexist. Both conditions are associated with aging, hypertension, obesity, and sleep apnea.(1, 2) The presence of one of both conditions leads to an increased risk of the other. However, the overlap in symptoms, biomarker profile, and echocardiographic changes hinder the diagnosis of underlying HFpEF in patients with AF and suggests that both conditions might reflect similar remodeling processes in the heart.(1, 2) The aim of this study was therefore to assess cardiac remodeling in AF patients with versus without concomitant HFpEF by transthoracic echocardiography, focusing on atrial dimension and speckle tracking of the left and right atria.

We included patients from the identification of a risk profile to guide atrial fibrillation therapy study (AF-RISK), a prospective, observational, multicenter study (NCT01510210).(3) In brief, inclusion criteria were patients aged ≥18 years, with paroxysmal AF (total AF history <2 years, or total AF history <3 years in case of ≤2 AF episodes of ≤48 hours per month terminating spontaneously) or persistent AF (total AF history <2 years, and total persistent AF duration >7 days and <1 year). Exclusion criteria were patients with history of heart failure >3 years, severe valvular disease, acute coronary syndrome <1 month, post-operative AF or history of pulmonary vein isolation. For this subanalysis, 287 patients had paroxysmal AF, had a left ventricular ejection fraction (LVEF) ≥50%, were in SR (sinus rhythm) at the moment of performing echocardiography and blood sampling (*Supplementary figure S-1*). The diagnosis of HFpEF was based on the 2016 ESC heart failure guidelines, including symptoms and signs heart failure (dyspnea and fatigue, equivalent to NYHA ≥ II) or history of HF hospitalization and N-terminal pro-B-type natriuretic peptide (NT-proBNP) ≥125pg/ml, and one of the following echocardiographic measures: left atrium volume index (LAVI) >34ml/m^2, left ventricular mass index ≥115g/m^2 for men and ≥95g/m^2 for women, average E/e' ≥13cm/s and average e' <9cm/s.(4) Due to imbalances in age and sex between AF patients with HFpEF in comparison to those without HFpEF, patients were selected using propensity score matching by nearest neighbor. A 1:1 ratio created balanced differences in age and sex resulting in two groups: 1) AF with HFpEF (n=60) and 2) AF without HFpEF (n=60). A sensitivity analysis was performed in patients with cutoff of NT-proBNP≥400pg/ml into the HFpEF definition.(5)

Atrial function was determined by speckle tracking as it is less affected by loading conditions in comparison to volumetric methods .(6) Reservoir, conduit and contraction strain of both atria was performed in apical four-chamber view by manually selecting endocardial borders from one cardiac cycle R-R gated (GE, EchoPac BT12). Tracking was manually adjusted for accuracy if needed. An independent observer, blinded to results, measured 10% of the strains to calculate inter-observer reliability. The average intraclass correlation coefficient of strain measures was 0.831, using average consistency two way random effects method. Associations of clinical and echocardiographic characteristics were tested for collinearity and by multivariable logistic regression analyses. LAVI, LV mass index and NT-proBNP were excluded from multivariable analysis since these markers were part of the HFpEF diagnostic criteria. A two-tailed p-value of <0.05 was considered statistically significant. The study was performed in accordance with the Declaration of Helsinki and after local research ethics committee approval.

Patients with paroxysmal AF and concomitant HFpEF had more often beta-blocker and angiotensin-converting enzyme (ACE) inhibitors medication, had more often impaired strain phases of both the left and right atria as compared to those without HFpEF, and comparable time of AF diagnosis, AF burden, global longitudinal strain (GLS) of the left ventricle and LVEF (**table 1**) (**Figure 1**). In multivariable analyses including age, sex, use of ACE inhibitor and beta-blockers, LA reservoir and contraction decrease was associated with presence of HFpEF (OR per 5% decrease in LA reservoir 1.39, 95% CI 1.11 t– 1.81, P=0.008; OR per 5% decrease in LA contraction 1.96, 95% CI 1.26 – 3.33, P=0.006) (**table 2**). LA reservoir and contraction phases were not placed in the same model due to collinearity (*Supplementary figure S-2*). LA reservoir and contraction were not correlated with LAVI in AF patients with concomitant HFpEF and moderately correlated in patients with AF (*Supplementary table S-1*). Similar trends were observed in a sensitivity analysis of AF patients with HFpEF patients classified by a cutoff of NT-proBNP≥400pg/ml (*Supplementary table S-3 and figure S-2*)

Our results show that atrial function discriminates AF patients with HFpEF from those without HFpEF. In patients with AF, a more impaired structure and function of the left and right atria were associated with concomitant HFpEF, whereas ventricular function, reflected by GLS and LVEF, was comparable. LAVI was a criteria to classify patients with HFpEF and could therefore explain decrease in LA strain (7); however, LA strains were not correlated with LAVI in our study.

Table I. Clinical characteristics and echocardiographic measurements

Characteristic	Total population (N=120)	AF with HFpEF (N=60)	AF without HFpEF (N=60)	*P*-value
Age*(years)*	65±8	66±8	65±8	0.807
Women *%*	58 (48)	31 (52)	27 (45)	0.584
Diagnosis of AF*(months)*	7 (2-20)	10 (2-23)	4 (2-15)	0.142
Percentage AF *%*				0.293
<5	97 (87)	46 (82)	51 (91)	
5-50	10 (9)	6 (11)	4 (7)	
>50	5 (4)	4 (7.1)	1 (2)	
Hypertension *%*	107 (90)	57 (95)	50 (83)	0.078
Diabetes mellitus *%*	17 (14)	7 (12)	10 (17)	0.601
Coronary artery disease *%*	8 (7)	6 (10)	2 (3)	0.272
Peripheral artery disease *%*	7 (6)	2 (3)	5 (8)	0.436
Stroke or TIA *%*	6 (5)	3 (5)	3 (5)	1.000
COPD *%*	9 (8)	5 (8)	4 (7)	1.000
BMI*(kg/m^2)*	27±4	28±4	26±4	0.129
Obesity*(BMI>30) %*	30 (25)	18 (30)	12 (20)	0.292
CHA_2DS_2-VASc score[a]	2.2±1.4	2.5±1.4	2.0±1.3	0.061
Comorbidities[b]	2.5±1.2	2.7±1.2	2.4±1.2	0.150
Fatigue	110 (92)	57 (95)	53 (88)	0.322
Dyspnea	63 (53)	29 (48)	34 (58)	0.405
EHRA class *%*				0.681
I	30 (25)	13 (22)	17 (28)	
II	68 (57)	36 (60)	32 (53)	
III	22 (18)	11 (18)	11 (18)	
Medications				
β-Blocker *%*	82 (68)	48 (80)	34 (57)	**0.011**
Verapamil/Diltiazem *%*	10 (8)	6 (10)	4 (7)	0.741
Digoxin *%*	4 (3)	4 (7)	0 (0)	0.127
ACE-inhibitor *%*	40 (33)	27 (45)	13 (22)	**0.012**
Anticoagulant *%*	81 (68)	45 (75)	36 (60)	0.119
Class Ic AAD *%*	7 (6)	4 (7)	3 (5)	1.000
Class III AAD *%*	8 (7)	5 (8)	3 (5)	0.714
Previous ECV *%*	29 (24)	18 (30)	11 (18)	0.201
Biomarkers				
NTproBNP *(pg/ml)*	189 (93-324)	268 (190-464)	87 (59-159)	**<0.001**
GDF15 pg/ml	1033 (821-1422)	1141 (854-1480)	1002 (780-1339)	0.287
Troponin-T pg/ml	6.4 (4.3-9.9)	6.9 (3.7-11.2)	6.1 (4.7-7.7)	0.271
Echocardiography				
LAVI*(mL/m^2)*	35±10	40±8	29±8	**<0.001**
RAVI*(mL/m^2)*	36±12	39±12	33±12	**0.013**
LV ejection fraction*(%)*	58±3	57±3	58±3	0.168

LV mass index*(g/m^2)*	86±21	89±22	82±21	0.087
e'	8.8±1.9	8.8±1.9	8.8 ±2.0	0.879
E/A ratio	1.0 (0.9-1.3)	1.1(0.9-1.3)	1.0 (0.8-1.2)	0.078
E/e'	7.7 (6.6-9.8)	8.8(6.9-10.3)	7.2 (6.2-8.6)	**0.004**
Left atrial strain*(%)*				
Reservoir	30.1±12.5	26.8±11.1	33.8±12.9	**0.003**
Conduit	16.0± 7.7	14.7±6.7	17.4±8.6	0.066
Contraction	14.1± 7.0	12.1±6.5	16.3±7.0	**0.001**
Right atrial strain*(%)*				
Reservoir	41.6±13.5	39.4±12.5	43.7±14.2	0.136
Conduit	22.5±9.3	22.2±7.8	22.9±10.6	0.718
Contraction	19.1±7.8	17.3±7.6	20.9±7.7	**0.031**
GLS *(%)*	-19.3±3.2	-18.9±3.4	-19.7±3.1	0.212

ACE=angiotensin-converting enzyme; AF=atrial fibrillation; BMI=body mass index; COPD=chronic obstructive pulmonary disease; ECV = electrical cardioversion; GDF-15=growth differentiation factor 15; EHRA=European Heart Rhythm Association symptom classification; HFpEF=heart failure with preserved ejection fraction; LAVI=left atrial volume index; NT-proBNP= N-terminal pro-B-type natriuretic peptide; TIA=transient ischemic attack.

[a]The CHA2DS2-VASc score assesses thromboembolic risk. C, congestive heart failure/LV dysfunction; H, hypertension; A2, age≥75 years; D, diabetes mellitus; S2, stroke/transient ischaemic attack/systemic embolism; V, vascular disease; A, age 65–74 years; Sc, sex category (female sex).

[b]The number of comorbidities was calculated by awarding a point for hypertension, age >65 years, diabetes, coronary artery disease, body mass index >25, kidney dysfunction, and moderate or severe mitral valve regurgitation.

Our results are in accordance to previous studies suggesting LA strains as markers for improving the diagnosis of HFpEF.(8) Katbeh et. al found that LA reservoir and, followed by, LA contraction were associated with the probability of HFpEF in patients with paroxysmal AF and dyspnea, using two scores, H_2FPEF and HFA-PEFF.(9) It has been shown that contraction function is reduced in advanced stages of AF,(10) however in this study AF burden and time of AF history in both groups were comparable, possibly attributing the differences to having concomitant HFpEF. Even though we could only speculate about the explanations for these results, HFpEF plausible mechanisms imply higher cardiac energy consumption and production of reactive oxygen species (11); the latter have been associated with decreased atrial myocardial energetics more than in the ventricles, predominantly in the LA.(12) This could increase remodeling of the atria in AF patients with concomitant HFpEF.

Table 2. Predictors of heart failure with preserved ejection fraction in patients with atrial fibrillation

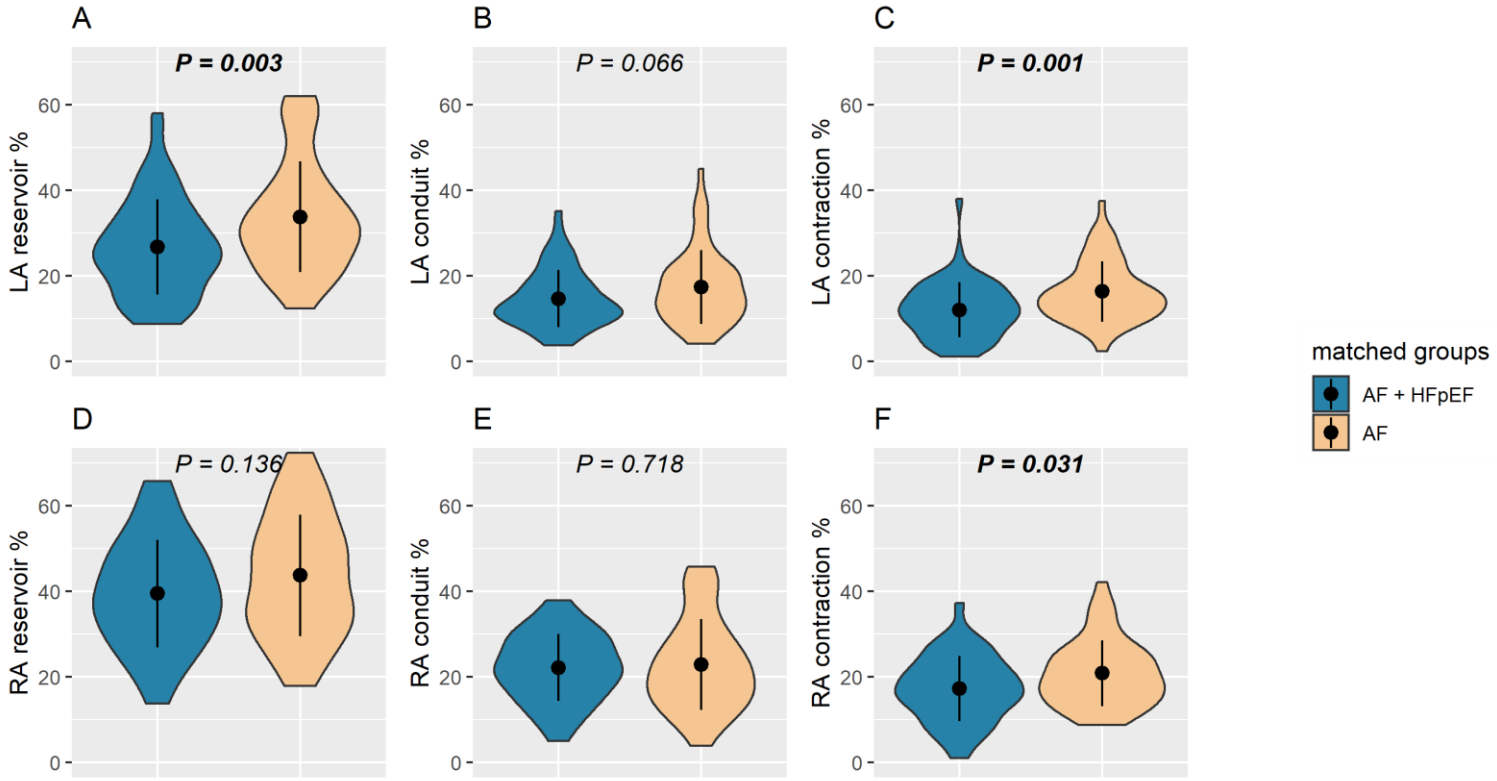
	OR	CI 95%	p
LA reservoir	1.38	1.14 – 1.71	**0.002**
LA contraction	1.89	1.33 – 2.85	**<0.001**
RA contraction	1.57	1.13 – 2.30	**0.012**
LA reservoir +	1.39	1.11 – 1.81	**0.008**
RA contraction	1.37	0.95 – 2.05	0.108
LA contraction +	1.96	1.26 – 3.33	**0.006**
RA contraction	1.36	0.94 – 2.06	0.122

All models included age, sex, use of ACE inhibitor, use of beta-blockers
Results are shown as 5% reduction per strain phase
LA=Left atria; RA = right atria

This analysis has limitations since the results are based on *post hoc* analyses and classification of HFpEF was performed after inclusion. The AF-RISK study had already small inclusion of patients and propensity score matching generated even smaller comparison groups. These results were performed in a population with paroxysmal AF and cannot be generalized to other forms of AF. Given the transversal analysis of the data, it cannot be determined whether HFpEF set the stage for AF nor inversely. Furthermore, the associations found might be affected by residual cofounding. Echocardiographic B-line were not measured which could have provided insights into the congestive state in AF patients with HFpEF. These findings encourage the need for well phenotyped AF with and without HFpEF cohorts to further understand atrial remodeling processes and underlying AF substrate.

In conclusion, in patients with paroxysmal AF, more impaired strain phases of the left and right atria were associated with concomitant HFpEF, whereas ventricular function, reflected by LVEF and GLS, did not differ.

Figure 1. Strain phases distribution of both left and right atria in patients with paroxysmal AF with HFpEF in comparison to those without HFpEF



Y axis expresses percentage of deformation measure by 2D speckle tracking by transthoracic echocardiography. Point within the graph expresses mean and lines determine standard deviation.

References

1. Hindricks G, Potpara T, Dagres N, et al. 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the 39xclude39 association for cardio-thoracic surgery (EACTS). Eur Heart J. 2021;42:373-498.

2. Kotecha D, Lam CS, Van Veldhuisen DJ, et al. Heart failure with preserved ejection fraction and atrial fibrillation: Vicious twins. J Am Coll Cardiol. 2016;68:2217-28.

3. De With RR, Marcos EG, Dudink, E. A. M. P., et al. Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: Data from the AF-RISK study. Europace. 2020;22:352-60.

4. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: The task force for the diagnosis and treatment of acute and chronic heart failure of the 39xclude39 society of cardiology (ESC)developed with the special contribution of the heart failure association (HFA) of the ESC. Eur Heart J. 2016;37:2129-200.

5. Parkash R, Wells G, Rouleau J, et al. A randomized ablation-based atrial fibrillation rhythm control versus rate control trial in patients with heart failure and high burden atrial fibrillation: The RAFT-AF trial rationale and design. Am Heart J. 2021;234:90-100.

6. Thomas L, Marwick TH, Popescu BA, et al. Left atrial structure and function, and left ventricular diastolic dysfunction: JACC state-of-the-art review. J Am Coll Cardiol. 2019;73:1961-77.

7. Gan GCH, Ferkh A, Boyd A, et al. Left atrial function: Evaluation by strain analysis. Cardiovasc Diagn Ther. 2018;8:29-46.

8. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the 39xclude39 society of echocardiography and the 39xclude39 association of cardiovascular imaging. Eur Heart J Cardiovasc Imaging. 2016;17:1321-60.

9. Katbeh A, De Potter T, Geelen P, et al. Heart failure with preserved ejection fraction or non-cardiac dyspnea in paroxysmal atrial fibrillation: The role of left atrial strain. Int J Cardiol. 2021;323:161-7.

10. Schotten U, Ausma J, Stellbrink C, et al. Cellular mechanisms of depressed atrial contractility in patients with chronic atrial fibrillation. Circulation. 2001;103:691-8.

11. Lam CSP, Voors AA, de Boer RA, et al. Heart failure with preserved ejection fraction: From mechanisms to therapies. Eur Heart J. 2018;39:2780-92.

12. Ozcan C, Li Z, Kim G, et al. Molecular mechanism of the association between atrial fibrillation and heart failure includes energy metabolic dysregulation due to mitochondrial dysfunction. J Card Fail. 2019;25:911-20.

Chapter 4

Different Circulating Biomarkers in Women and Men with Paroxysmal Atrial Fibrillation: results from the AF-RISK and RACE V studies.

Shared first author

Ruben R. De With, Vicente Artola Arita, Bao-Oanh Nguyen, Dominik Linz, Hugo Ten Cate, Henri Spronk, Ulrich Schotten, Anton Jan van Zonneveld, Ömer Erküner, M Agustina Bayón, Anders S Schmidt, Justin G L M Luermans, Harry J G M Crijns, Isabelle C Van Gelder, Michiel Rienstra

Adapted from Europace. 2022: 2;24(2):193-201.

Abstract

Aims. The clinical risk profile of AF patients is different in men and women. Our aim was to identify sex differences in blood biomarkers in patients with paroxysmal AF.

Methods. Sex differences in 92 blood biomarkers were measured in 364 patients included in our discovery cohort, the identification of a risk profile to guide atrial fibrillation therapy (AF-RISK) study, assessed by multivariable logistic regression and enrichment pathway analysis. Findings were subsequently confirmed in 213 patients included in our validation cohort, the Reappraisal of Atrial Fibrillation: Interaction between HyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) study.

Results. In the discovery cohort, mean age was 59±12 years, 41% were women. CHA_2DS_2-VASc-score was 1.6±1.4. A total of 46% had hypertension, 10% diabetes and 50% had heart failure, predominantly with preserved ejection fraction (47%). In women, activated leukocyte cell adhesion molecule (ALCAM) and fatty acid binding protein-4 (FABP-4) were higher. In men, matrix metalloproteinase-3 (MMP-3), C-C motif chemokine-16 (CCL-16) and myoglobin were higher. In the validation cohort, four out of five biomarkers could be confirmed: levels of ALCAM ($P=1.73*10^{-4}$) and FABP-4 ($P=2.46*10^{-7}$) and adhesion biological pathways ($FDR=1.23*10^{-8}$) were higher in women. In men, levels of MMP-3 ($P=4.31*10^{-8}$) and myoglobin ($P=2.10*10^{-4}$) and markers for extracellular matrix degradation biological pathways ($FDR=3.59*10^{-9}$) were higher.

Conclusion. In women with paroxysmal AF, inflammatory biomarkers were more often higher, while in men with paroxysmal AF, biomarkers for vascular remodelling were higher.

Our data support the clinical notion that pathophysiological mechanisms in women and men with AF may differ.

Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia around the world and the number will increase due to aging populations and active search for early diagnosing.(1,2) The development of AF is driven by risk factors including, but not limited to, aging, obesity and underlying cardiovascular risk factors and diseases.(2) The cumulative prevalence of AF over the years is high and comparable in both sexes. However, women and men with AF differ regarding age and presence of comorbidities; women with AF are older, have more hypertension, valvular heart disease and heart failure with preserved ejection fraction, and men with AF present more often coronary artery disease. (2) Whether the age difference or clustering of comorbidities are causative of the difference in clinical risk profile of AF between women and men is yet to be determined. Other factors such as sex hormones or differentially expressed blood biomarkers representing distinct biological pathways may also play a role.(1,2)

Blood biomarkers can be seen as representation of distinct biological pathways and may differ between men and women with AF. C-reactive protein (CRP), an inflammatory biomarker, and B-type natriuretic peptide (NT-proBNP), a biomarker indicating cardiac stretch, have been shown to differ in women and men. CRP has been associated with AF incidence in men, whereas NT-proBNP has been associated with incident AF in women.(1) NT-proBNP and fibroblast growth factor 23, a hormone regulating biomarker associated with AF, have been suggested to help to identify those at risk for AF(3); NT-proBNP and Cancer Antigen 125 (CA-125) have been associated with AF in patients without any concomitant disease.(4) Therefore biomarkers may help to find guidance for a personalized approach to patients with AF.(2-4)

Our aim was to identify sex differences in blood biomarkers in patients with paroxysmal AF, to provide an insight into potential sex-specific pathophysiological mechanisms in a well-phenotyped AF population.

Methods

Study Population. Patients included in the identification of a risk profile to guide atrial fibrillation therapy (AF-RISK) study were used as discovery cohort. The methods of the AF-RISK study have previously been described.(5) In short, AF-RISK was a prospective, multicentre, observational study including patients with history of AF, performed in The Netherlands between May 2011 and

March 2016. Inclusion criteria were patients aged ≥18 years who presented at either the inpatient or outpatient cardiology clinic with paroxysmal AF (total AF history <2 years, or total AF history <3 years in case of ≤2 AF episodes of ≤48 hours per month terminating spontaneously) or persistent AF (total AF history <2 years, and total persistent AF duration >7 days and <1 year) in whom a rhythm control strategy was preferred. Exclusion criteria were patients with history of heart failure >3 years, severe valvular disease, contra-indication for oral anticoagulation, acute coronary syndrome <1 month, or post-operative AF. In total 386 patients had paroxysmal AF and were in SR at the moment of blood sampling; from this amount, 364 (94%) had blood biomarker results available and were included for the current analysis.

Patients included in the Reappraisal of Atrial Fibrillation: Interaction between HyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) study were used as validation cohort.(6) In short, RACE V is an ongoing investigator-initiated, prospective, multicentre registry aiming to include 750 patients in multiple centres in The Netherlands. Inclusion criteria were patients aged ≥18 years with paroxysmal AF, a maximum AF history of 10 years since diagnosis at the moment of inclusion, a maximum CHA_2DS_2-VASc score of 5, and no other indication for anticoagulation drugs (e.g. mechanical valve prosthesis). Patients had to have at least two documented episodes of paroxysmal AF in the past year or one documented episode with at least two symptomatic episodes in the past year suspected to be AF without documentation. In patients with a Medtronic pacemaker, atrial high rate episodes (AHRE) >190 beats per minute lasting >6 minutes were qualified as AF episodes. Patients with other types of pacemakers, defibrillators or cardiac resynchronization therapy could not participate due to differences in AHRE algorithm and/or incompatibility with the type of home-monitoring. Further exclusion criteria were patients with a history of persistent AF, currently on amiodarone, current pregnancy or a life expectancy <2.5 years, patients with AF caused exclusively due to transient triggers (e.g. postoperative, due to infection), patients with a previous pulmonary vein isolation (PVI), or intention to undergo PVI, or diagnosed congenital heart disease. In total, 247 patients had available blood samples; from this amount, a total of 34 (14%) 43xcludeed because of AF at the moment of sampling. Samples from the remaining 213 patients were used for the current analyses.

Both AF-RISK and RACE V were performed in concordance with the Declaration of Helsinki. The Institutional Review Board approved both protocols. AF-RISK was registered at Clinicaltrials.gov (Clinicaltrials.gov identifier NCT01510210), as well as RACE V (Clinicaltrials.gov identifier NCT02726698) and all patients gave written informed consent.

Blood biomarkers. An electrocardiogram was performed to assess the heart rhythm prior to blood sampling. Blood sampling was performed in a similar fashion at baseline in both cohorts. EDTA anticoagulated plasma was obtained from ethylenediaminetetraacetic acid tubes and was stored at -80°C. Multiplex immunoassay by proximity extension assay (PEA) technology (Olink Bioscience, Uppsala, Sweden) was used to measure 92 biomarkers from the Olink cardiovascular panel III (full list shown in *Supplementary table S1*). The PEA technology uses a homogeneous assay that uses pairs of antibodies equipped with DNA reporter molecules. In the kits, 92 oligonucleotide-labeled antibody probe pairs are allowed to bind to their respective target if present in the sample. A PCR reporter sequence is formed by a proximity-dependent DNA polymerization event. This is then amplified, and subsequently detected and quantified using real-time PCR. The assay was performed in a homogeneous 96-well format without any need for washing steps. Internal controls were added to each sample and include two immunoassay controls, one extension control and one detection control. Samples for which one or more of the internal control values deviate from a pre-determined range were flagged and removed before statistical analysis. PEA results do not provide absolute concentration of the proteins; instead, proteins are expressed as normalized protein expression on a log2-transformed concentrations where a larger number represents a higher protein level in the sample, typically with the background level at around zero.

Limit of detection (LOD) was defined as 3 standard deviations above background and reported in pg/mL for all assays for which recombinant protein antigen was available. Four biomarkers (bleomycin hydrolase, spondin-1, elafin and cathepsin D) had ≥10% of the values below LOD and were therefore excluded. The remaining 88 biomarkers were used for the analyses.

Comorbidities. Heart failure was defined as one of the following: (i) history of heart failure admission, regardless of the left ventricular ejection fraction (LVEF); (ii) LVEF <45%; (iii) LVEF >45%, an elevated NT-proBNP (> 400 ng/l) with either structural heart disease (history of left ventricular hypertrophy or wall diameter ≥ 11 mm or septum diameter ≥ 11 mm) or diastolic

dysfunction (average annular e' < 8 cm/s, and deceleration time > 220 ms, and average E/e' > 8) on echocardiography.(7) Hypertension was defined by a systolic blood pressure ≥140mmHg or diastolic blood pressure ≥90mmHg, or use of antihypertensive medication. Diabetes mellitus was defined by use of antidiabetic drugs. Coronary artery disease was defined as history of myocardial infarction, percutaneous coronary intervention or coronary artery bypass grafting.

Statistical analysis. Sex differences in blood biomarkers were tested by univariable and multivariable logistic regression. In the multivariable logistic regression model additional adjustment for obesity, age, heart failure and coronary artery disease was performed based on differences found between women and men at baseline and knowledge from previous literature.(2) The final model was tested for significant interactions. Odds ratios (OR) per standard deviation with 95% confidence intervals (CI) were given. Biomarkers with higher values in men were expressed as OR versus women, biomarkers higher in women were presented as OR versus men. Biomarkers found in the discovery cohort were subsequently tested by univariable and multivariable logistic regression in the validation cohort.

Enrichment pathway analyses were performed for blood biomarkers with higher values in women in comparison to men. The median value of each biomarker in women was divided by the median value of the same biomarker in men to produce a sex difference ratio per biomarker. This ratio was then transformed into percentage (*Supplementary figure S1*). Biomarkers found in the discovery cohort were subsequently tested in the validation cohort.

Confirmed biomarkers in the validation cohort were additionally enriched in a network analysis using STRING to identify relevant biological pathways in which the biomarkers are involved.(8) STRING is a database that provides assessment of physical and functional protein interactions which contribute to common biological processes. This knowledge derives from databases and text-mining highly calibrated, such as Gene Ontology (GO) Resource using high level groupings established by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway maps. Biomarkers that entered the pathway analysis were two layer enriched.

In both multivariate logistic regression and pathway analyses, a multiple testing correction was performed using a Bonferroni correction for the 88 biomarkers that were tested. A *P*-value < 5.68×10^{-4} ($\frac{0.05}{88}$) was considered statistically significant. Additionally, pathway enrichment underwent

false discovery rate (FDR) correction for multiple testing. Pathways with higher FDR were selected as main representative processes.

Results

Patient characteristics. Clinical characteristics of patients from the discovery cohort are shown in **Table 1**. Patient characteristics were comparable to the entire AF-RISK cohort (data not shown).(5) In short, mean age was 59±12 years, 150 (41%) were women. CHA_2DS_2-VASc-score was 1.6±1.4. A total of 182 (50%) had heart failure [171 (47%) with preserved and 11 (3%) with reduced ejection fraction] and 167 (46%) hypertension. Women compared to men were slightly older (60±12 vs 58±12 years, P=0.03) and had slightly higher LVEF (58±3 versus 57±5, P=0.01).

Baseline characteristics from the validation cohort are shown in **Table 2**. Compared to the discovery cohort, the proportion of women was comparable in the validation cohort (41%). CHA_2DS_2-VASc-score was 1.9±1.3. A total of 62 (29%) had heart failure [60 (28%) with preserved and 2 (1%) with reduced ejection fraction] and 101 (47%) hypertension. Within the validation cohort, women compared to men were older (66±9 vs 63±10 years, P=0.01), had less often heart failure (22 vs 38%, P=0.01) and had more often obesity (39 vs 21%, P=0.008). Patients in the validation were older in comparison to the discovery cohort (64±9 vs 59±12 years, P<0.01) and had longer history of AF (29 vs 6 months, P<0.01).

Biomarker analysis. The multivariable logistic regression in the discovery cohort showed that levels of activated leukocyte cell adhesion molecule (ALCAM, $P=4.03*10^{-4}$) and fatty acid binding protein-4 (FABP-4, $P=4.48*10^{-12}$) were higher in women. While levels of matrix metalloproteinase-3 (MMP-3 $P=6.46*10^{-13}$), C-C motif chemokine-16 (CCL-16 $P=4.17*10^{-5}$) and myoglobin ($P=2.34*10^{-4}$) were higher in men (***Figure 1***).

The five biomarkers found in the discovery cohort were univariably tested in the validation cohort and all but CCL-16 (univariably OR 1.090, 95% CI 0.829-1.432, *P*=0.537) were confirmed to be differently expressed between sexes (**Table 3**). Based on differences on baseline characteristics and knowledge from previous literature (2), it was adjusted for obesity, age and heart failure; after this adjustment, only FABP-4 remained higher in women (OR 7.442, 95% CI 3.680-15.051, $P=2.32 \times 10^{-8}$). MMP-3 (OR 8.403, 95% CI 4.329-16.393, $P=3.12 \times 10^{-10}$) remained higher in men.

Table 1 – Baseline characteristics AF-RISK discovery cohort.

Characteristic	Total population (N=364)	Women (N=150)	Men (N=214)	P-value
Age (years)	59±12	60±12	58±12	0.030
History of AF (months)	6 (2-18)	5 (2-17)	6 (2-20)	0.329
Heart failure	182 (50%)	66 (44%)	116 (54%)	0.070
HFpEF	171 (47%)	65 (43%)	106 (50%)	0.289
HFrEF	11 (3%)	1 (1%)	10 (5%)	0.059
Hypertension	167 (46%)	76 (51%)	91 (43%)	0.164
Diabetes mellitus	35 (10%)	12 (8%)	23 (11%)	0.471
Coronary artery disease	21 (6%)	6 (4%)	15 (7%)	0.260
Peripheral artery disease	9 (3%)	3 (2%)	6 (3%)	0.741
Stroke or TIA	23 (6%)	10 (7%)	13 (6%)	0.830
COPD	23 (6%)	7 (5%)	16 (8%)	0.382
CHA_2DS_2-VASc score *	1.6±1.4	2.3±1.3	1.1±1.2	<0.001
EHRA class**				0.296
I	110 (30%)	34 (23%)	76 (36%)	
II	204 (56%)	94 (63%)	110 (51%)	
III	49 (14%)	22 (15%)	27 (13%)	
Height (cm)	178±10	170±7	184±7	<0.001
Weight (kg)	88±18	81±17	92±17	<0.001
BMI (kg/m^2)	28±5	28±6	27±5	0.129
Obesity (BMI>30)	99 (27%)	43 (29%)	56 (26%)	0.633
Blood pressure (mmHg)				
Systolic	131±18	134±20	128±15	0.004
Diastolic	78±9	78±11	78±8	0.693
PQ time (ms)	165±25	161±24	168±25	0.007
Left atrial volume (mL)	67±21	62±19	69±21	0.002
Left atrial volume index (mL/m^2)	33±10	33±10	32±10	0.696
LV ejection fraction (%)	57±4	58±3	57±5	0.016

Data are mean (standard deviation), number of patients (%), or median (interquartile range). AF=atrial fibrillation; BMI=body mass index; COPD=chronic obstructive pulmonary disease; EHRA= European Heart Rhythm Association class for symptoms; LV=left ventricular; TIA=transient ischemic attack; HFpEF= heart failure preserved ejection fraction; HFrEF= heart failure reserved ejection fraction. *The CHA_2DS_2-VASc score assesses thromboembolic risk. C=congestive heart failure/LV dysfunction, H=hypertension; A_2=age ≥75 years; D=diabetes mellitus; S_2=stroke/transient ischemic attack/systemic embolism; V=vascular disease; A=age 65-74 years; Sc (sex category (female sex). **In 363 patients, EHRA class data was available.

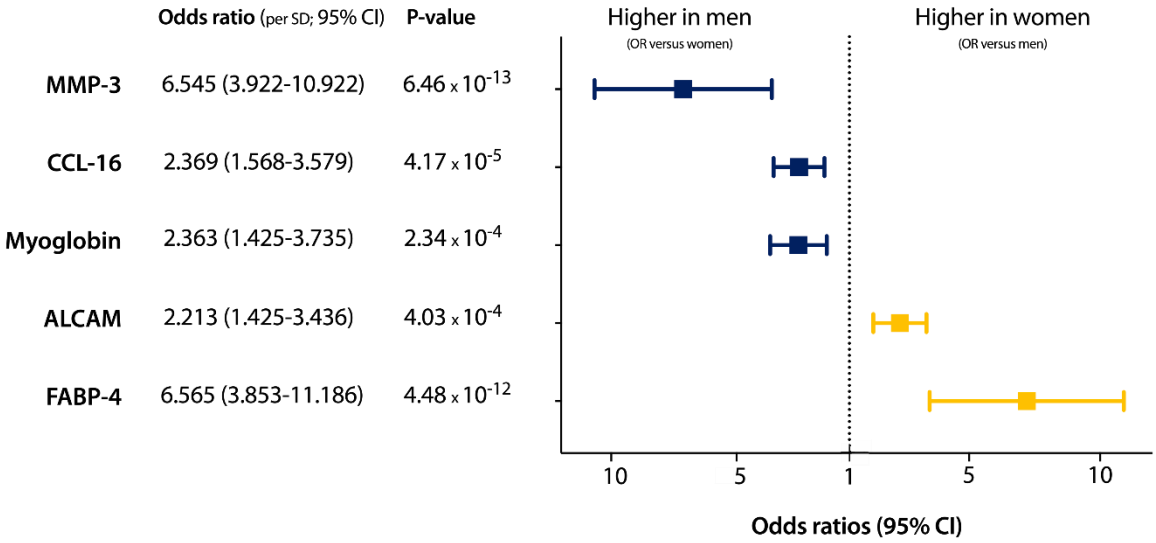
Table 2. Baseline characteristics RACE V validation cohort.

Characteristic	Total population (N=213)	Women (N=88)	Men (N=125)	P-value
Age (years)	64±9	66±9	63±10	0.011
History of AF (months)	29 (8-56)	32 (7-57)	27 (8-56)	0.973
Heart failure	62 (29%)	19 (22%)	48 (38%)	0.014
HFpEF	60 (28%)	17 (19%)	43 (34%)	0.024
HFrEF	2 (1%)	0 (0%)	2 (2%)	0.638
Hypertension	101 (47%)	40 (46%)	61 (49%)	0.732
Diabetes mellitus	21 (10%)	12 (14%)	9 (7%)	0.187
Coronary artery disease	26 (12%)	6 (7%)	20 (16%)	0.071
Peripheral artery disease	2 (1%)	0 (0%)	2 (2%)	0.638
Stroke or TIA	18 (9%)	6 (7%)	12 (10%)	0.639
COPD	16 (8%)	8 (9%)	8 (6%)	0.639
CHA_2DS_2-VASc score *	1.9±1.3	2.6±1.2	1.4±1.2	<0.001
EHRA class				0.023
I	24 (11%)	6 (7%)	18 (14%)	
IIa	89 (42%)	30 (34%)	59 (48%)	
IIb	78 (37%)	40 (46%)	38 (31%)	
III	22 (10%)	12 (14%)	10 (8%)	
Height (cm)	176±10	167±7	183±7	<0.001
Weight (kg)	87±18	80±17	92±17	<0.001
BMI (kg/m^2)	28±5	28±5	28±5	0.159
Obesity (BMI>30)	60 (28%)	34 (39%)	26 (21%)	0.008
Blood pressure (mmHg)				
Systolic	136±18	137±19	136±17	0.559
Diastolic	81±10	80±11	81±9	0.614
PQ time (ms)	172±35	169±38	174±34	0.349
Left atrial volume (mL)	69±23	69±25	69±22	0.923
Left atrial volume index (mL/m^2)	35±11	36±12	34±11	0.094

LV ejection fraction (%)	59±5	60±5	58±5	0.031

Data are mean (standard deviation), number of patients (%), or median (interquartile range). AF=atrial fibrillation; BMI=body mass index; COPD=chronic obstructive pulmonary disease; EHRA= European Heart Rhythm Association class for symptoms; HFpEF= heart failure preserved ejection fraction; HFrEF= heart failure reserved ejection fraction. LV=left ventricular; TIA=transient ischemic attack. *The CHA_2DS_2-VASc score assesses thromboembolic risk. C=congestive heart failure/LV dysfunction, H=hypertension; A_2=age ≥75 years; D=diabetes mellitus; S_2=stroke/transient ischemic attack/systemic embolism; V=vascular disease; A=age 65-74 years; Sc (sex category (female sex).

Figure 1. Blood biomarker sex differences in discovery cohort.



CI=confidence interval; SD=standard deviation; MMP-3=matrix metalloproteinase-3; CCL-16=C-C motif chemokine-16; ALCAM=activated leukocyte cell adhesion molecule; FABP-4=fatty acid binding protein-4; OR=odds ratio.

In the pathway analysis, six biomarkers in women and eight in men were statistically significant (*Supplementary figure* S2), which included the biomarkers from the multivariate logistic regression analysis in the discovery cohort. These biomarkers were subsequently tested in the validation cohort; six remained statistically significant in women, FABP-4, ALCAM, NTproBNP, contactin-1 (CNTN1), metalloproteinase inhibitor 4 (TIMP4), and integrin beta-2 (ITGB2); three

remained statistically significant in men matrix, extracellular phosphoglycoprotein (MEPE), myoglobin and MMP3 (*Supplementary table S2 and figure S3*).

Table 3. Multivariate logistic regression results of biomarkers in validation cohort

	OR	95%CI	*P*-value
MMP-3	6.289*	3.257-12.195	4.31×10^{-8}
CCL-16			Ns
Myoglobin	3.135*	1.712-5.747	2.10×10^{-4}
ALCAM	3.165	1.735-5.774	1.73×10^{-4}
FABP-4	5.975	3.030-11.78	2.46×10^{-7}

* Odds ratios are expressed versus women.

Abbreviations: CI=confidence interval; SD=standard deviation; MMP-3=matrix metalloproteinase-3; CCL-16=C-C motif chemokine-16; ALCAM=activated leukocyte cell adhesion molecule; FABP-4=fatty acid binding protein-4; OR=odds ratio.

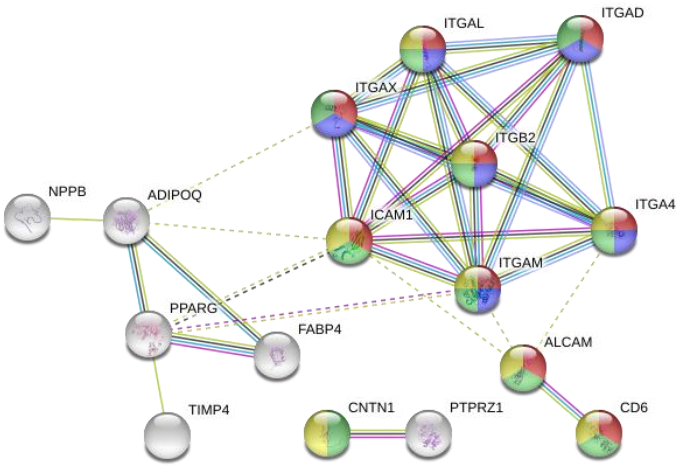
After a two-layer protein enrichment, in women compared to men, pathways with higher FDR under GO analysis showed cell-cell adhesion (FDR=$1.23*10^{-8}$), integrin-mediated signalling pathway (FDR=$3.83*10^{-8}$) and cell adhesion ($6.13*10^{-8}$); moreover, cell adhesion molecules (FDR=$5.19*10^{-12}$) pathways resulted under KEGG analysis. In men, extracellular matrix organization (FDR $3.59*10^{-9}$) pathway resulted from GO analysis without any results under KEGG analysis (**Figure 2**).

Discussion

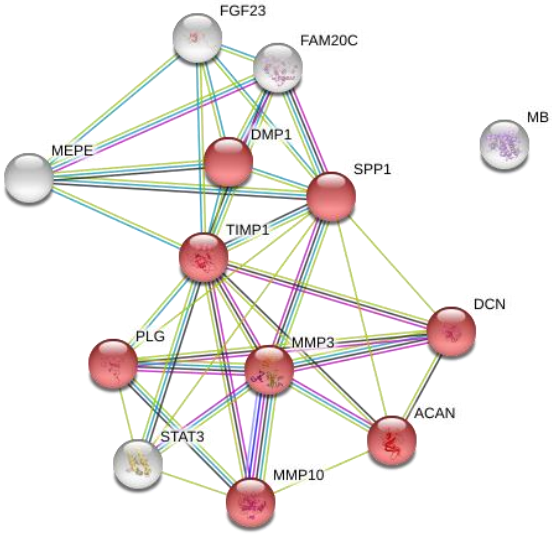
The aim of this study was to identify sex differences in blood biomarkers in patients with AF. We identified five biomarkers that were differently expressed between sexes with paroxysmal AF. In a validation cohort, four out of five markers were confirmed to be differently expressed between sexes.

Figure 2. Pathway enrichment analysis of biomarkers confirmed in validation cohort.

A



B



C

Database	Biological Process	Proteins/set	FDR
GO	cell-cell adhesion	9/416	1.2310^{*-8}
	integrin-mediated signalling pathway	6/84	$3.83*10^{-8}$
	cell adhesion	10/843	$6.13*10^{-8}$
KEGG	cell adhesion molecules	8/139	$5.19*10^{-12}$

D

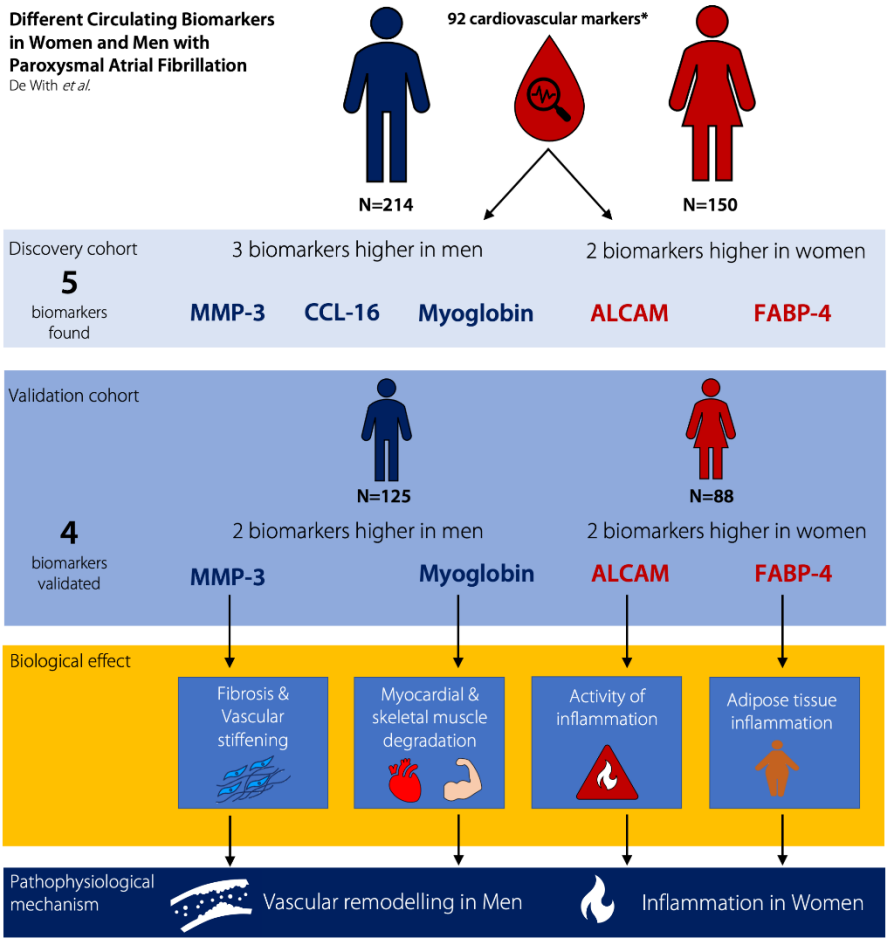
Database	Biological Process	Proteins/set	FDR
GO	Extracellular matrix degradation	8/296	$3.59*10^{-9}$

A. Pathway enrichment analysis with layers of proteins on validated biomarkers in women in comparison to men from both cohorts.
B. Pathway enrichment analysis with layers of proteins on validated biomarkers in men in comparison to women from both cohorts.
C. Biological processes with higher FDR in women in comparison to men. Colours represent biological processes in which the proteins (nodes) are involved as depicted in A.
D. Biological processes with higher FDR in women in comparison to men. Colours represent biological processes in which the proteins (nodes) are involved as depicted in B.

GO=Gene Ontology; KEGG= Kyoto Encyclopedia of Genes and Genomes; FDR=false discovery rate

ACAN=aggrecan; ALCAM=activated leukocyte cell adhesion molecule; CD6= T-cell differentiation antigen CD6; CNTN1=contactin-1; DCN=decorin; DMP1=dentin matrix acidic phosphoprotein 1; ICAM1=intercellular adhesion molecule 1; ITGA4=integrin subunit alpha 4; ITGAD=integrin subunit alpha D; ITGAL=integrin subunit alpha L; ITGAM=integrin subunit alpha M; ITGAX=integrin subunit alpha X; ITGB2=integrin subunit beta 2; MMP10=integrin subunit beta 2; MMP3=matrix metalloproteinase-3; PLG=plasminogen; SPP1=secreted phosphoprotein 1; TIMP1=metallopeptidase inhibitor 1.

Central Figure. Sex differences in blood biomarkers in patients with paroxysmal AF.



Abbreviations: MMP-3=matrix metalloproteinase-3; CCL-16=C-C motif chemokine-16; ALCAM=activated leukocyte cell adhesion molecule; FABP-4=fatty acid binding protein-4.

Blood biomarkers in women. In women, ALCAM and FABP-4 were higher. Cell adhesion molecules, like ALCAM, are involved in leukocyte recruitment in case of tissue damage. In patients with stroke, ALCAM has been associated with long-term mortality.(9) Also, Lim *et al.* previously showed that higher levels of ALCAM were associated with early recurrence after AF ablation.(10) Moreover, cell adhesion mechanisms increase the adhesiveness of platelets and leucocytes incrementing the risk of thrombogenesis even when in sinus rhythm .(11) FABP-4 is mainly expressed in adipose tissue and represents around 6% of the total protein adipocytes. it has been associated a systemic pro-inflammatory state, development of atherosclerosis and metabolic syndrome. In the presence of coronary artery disease, FABP-4 has been associated with left ventricular hypertrophy, systolic dysfunction, clinical heart failure. Increasing levels of FABP-4 have been associated with increased cardiovascular mortality in both women and men, even after adjusting for potential cofounders.(12) Higher FABP-4 levels have also been linked to postoperative AF. (13) *López-Canoa et al.* have shown an association between higher levels of FABP-4 in women with AF in comparison to men, as well as recurrence of AF after ablation in both sexes. (14) In contrast to what we found, Magnussen *et al.* previously found that CRP was associated with incident AF in men but not in women, but with very low hazards ratio; this may be explained by the lack of specificity of C-reactive protein, or use of a relatively healthy population; the latter is supported by the low values that were found.(1) CRP levels were not routinely performed in our population and a direct comparison cannot be made. Despite the fact that our biomarker panel consists of 32 inflammatory markers, only two were found to be higher in women. This can be explained by the fact that most inflammatory markers are derived from similar biological pathways and are therefore not all included in the final multivariable model. Therefore, these data suggest that the contribution of inflammation seems to be more critical in AF substrate formation in women (**Central Figure**). Of interest, in our study women had less HFpEF in both cohorts as compared to men, reaching statistical significance in the validation cohort. This is in contrast to previous data showing that women more often suffer from HFpEF.(2) This might be due to the fact that we only included paroxysmal self-terminating AF, implying less severe remodelling. Also, the fact that hypertension occurred in comparable percentage in men and women may have contributed to this observation. This clearly highlights that there still is a gap of knowledge in sex differences in patients with all types of AF warranting further research.

Blood biomarkers in men. In men, levels of MMP-3 and myoglobin were higher. MMP-3 is part of the family of matrix metalloproteinases that are involved in extracellular matrix degradation and deposition. MMP-3 has been associated with vascular remodelling, including atrial stiffening and coronary artery disease (15), and has also been suggested as potential therapeutic target in atherosclerosis.(16) Moreover, Yue *et al* concluded that the excess of proteins involved in extracellular matrix biological pathways may lead to tissue fibrosis, contributing to vascular remodelling; this affects mechanical and electrical function, and therefore can promote AF.(17) Different studies have reported contrasting results between the association of MMP-3 and LAVI (18). LAVI is comparable between men and women in the current analysis; we could speculate that the association between MMP-3 and LAVI is not present in this population. However, a conclusive statement of this association cannot be drawn since levels of biomarkers are relative measures from the population, making them not comparable to absolute measures. Myoglobin can be detected in case of muscle degradation. Recurrent episodes of silent ischemia, also in patients with subclinical coronary artery disease may be the underlying substrate for myocardial myoglobin release.(19) In addition, higher muscle mass in men could contribute to the observed outcome. The combination of MMP-3 and myoglobin may indicate that in men, vascular remodelling plays an important role in AF substrate formation. Prevalence of clinical coronary artery disease was, however, not different between sexes in our discovery nor validation cohorts. When corrected for differences in underlying disease, MMP-3 remained associated with higher values in men. This could indicate that subclinical vascular disease is more prominent in men (**Central figure**). This in accordance with findings from the Rotterdam study which described subclinical atherosclerosis as an independent risk factor for new onset AF but not only in men.(20) Subclinical atherosclerosis, which may be present in many patients with AF, was, however, not routinely assessed in our discovery cohort. Since the biomarker panel used in this analysis did not include CA-125, our results cannot be compared to previous findings of this biomarker.(4)

Strengths and limitations. Limitations of the current analysis include the use of a biomarker panel with relative values, which impairs comparison with absolute values of other cohorts. Also, this was a cross-sectional study which precludes definite conclusions regarding cause-effect relations. In addition, the AF duration of the validation cohort is longer than in the discovery cohort, implying greater atrial remodelling substrate and differentiated expression of blood biomarkers. Furthermore, information regarding frequency of menstrual cycles in women was not collected,

which could have provided an insight on the association of hormones and biomarker expression in women in pre- and post-menopausal periods. Lastly, residual confounding may have affected results, despite adjustment-analysis. Strengths of the current analysis are the well-phenotyped cohorts and the availability of a large number of biomarkers representative of multiple biological pathways. Furthermore, the use of 2 analytical approaches and 2 independent cohorts yielded synergic results.

Conclusion

In conclusion, in this exploratory analysis, we identified biomarkers differentially expressed in women and men with paroxysmal AF. In a validation cohort, four out of five biomarkers were confirmed. In women with paroxysmal AF, inflammatory biomarkers were higher, while in men with paroxysmal AF biomarkers for vascular remodelling were higher. Our data suggest that pathophysiological mechanisms in women and men with AF may differ. This advocates more research on sex differences in AF and endorses a personalized medicine approach, taking sex differences into account.

References

(1) Magnussen C, Niiranen TJ, Ojeda FM, Gianfagna F, Blankenberg S, Njolstad I, et al. Sex Differences and Similarities in Atrial Fibrillation Epidemiology, Risk Factors, and Mortality in Community Cohorts: Results From the BiomarCaRE Consortium (Biomarker for Cardiovascular Risk Assessment in Europe). Circulation 2017 October 24;136(17):1588-1597.

(2) Linde C, Bongiorni MG, Birgersdotter-Green U, Curtis AB, Deisenhofer I, Furokawa T, et al. Sex differences in cardiac arrhythmia: a consensus document of the European Heart Rhythm Association, endorsed by the Heart Rhythm Society and Asia Pacific Heart Rhythm Society. Europace 2018 October 01;20(10):1565-1565ao.

(3) Chua W, Purmah Y, Cardoso VR, Gkoutos GV, Tull SP, Neculau G, et al. Data-driven discovery and validation of circulating blood-based biomarkers associated with prevalent atrial fibrillation. Eur Heart J 2019 Apr 21;40(16):1268-1276.

(4) Dudink EA, Weijs B, Tull S, Luermans JG, Fabritz L, Chua W, et al. The Biomarkers NT-proBNP and CA-125 are Elevated in Patients with Idiopathic Atrial Fibrillation. J Atr Fibrillation 2018 Dec 31;11(4):2058.

(5) De With RR, Marcos EG, Dudink, E. A. M. P., Spronk HM, Crijns, H. J. G. M., Rienstra M, et al. Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: data from the AF-RISK study. Europace 2020 Mar 1;22(3):352-360.

(6) De With RR, Erkuner O, Rienstra M, Nguyen BO, Korver FWJ, Linz D, et al. Temporal patterns and short-term progression of paroxysmal atrial fibrillation: data from RACE V. Europace 2020 July 08.

(7) Rienstra M, Hobbelt AH, Alings M, Tijssen JGP, Smit MD, Brügemann J, et al. Targeted therapy of underlying conditions improves sinus rhythm maintenance in patients with persistent atrial fibrillation: results of the RACE 3 trial. Eur Heart J 2018 Aug 21;39(32):2987-2996.

(8) Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015 Jan;43(Database issue):447.

(9) Smedbakken L, Jensen JK, Hallén J, Atar D, Januzzi JL, Halvorsen B, et al. Activated leukocyte cell adhesion molecule and prognosis in acute ischemic stroke. Stroke 2011 Sep;42(9):2453-2458.

(10) Lim HE, Choi CU, Na JO, Choi JI, Kim SH, Kim JW, et al. Effects of iatrogenic myocardial injury on coronary microvascular function in patients undergoing radiofrequency catheter ablation of atrial fibrillation. Circ Arrhythm Electrophysiol 2013 Apr;6(2):318-326.

(11) Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. Europace 2016 Oct;18(10):1455-1490.

(12) Egbuche O, Biggs ML, Ix JH, Kizer JR, Lyles MF, Siscovick DS, et al. Fatty Acid Binding Protein-4 and Risk of Cardiovascular Disease: The Cardiovascular Health Study. J Am Heart Assoc 2020 Apr 7;9(7):e014070.

(13) Rader F, Pujara AC, Pattakos G, Rajeswaran J, Li L, Castel L, et al. Perioperative heart-type fatty acid binding protein levels in atrial fibrillation after cardiac surgery. Heart Rhythm 2013 Feb;10(2):153-157.

(14) López-Canoa JN, Couselo-Seijas M, Baluja A, González-Melchor L, Rozados A, Llorente-Cortés V, et al. Sex-related differences of fatty acid-binding protein 4 and leptin levels in atrial fibrillation. Europace 2020 Dec 15.

(15) Beyzade S, Zhang S, Wong YK, Day IN, Eriksson P, Ye S. Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. J Am Coll Cardiol 2003 Jun 18;41(12):2130-2137.

(16) Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002 Feb 22;90(3):251-262.

(17) Yue L, Xie J, Nattel S. Molecular determinants of cardiac fibroblast electrical function and therapeutic implications for atrial fibrillation. Cardiovasc Res 2011 Mar 1;89(4):744-753.

(18) Linssen PBC, Brunner-La Rocca HP, Schalkwijk CG, Beulens JWJ, Elders PJM, van der Heijden, A. A., et al. Serum Matrix Metalloproteinases and Left Atrial Remodeling-The Hoorn Study. Int J Mol Sci 2020 Jul 13;21(14):4944. doi: 10.3390/ijms21144944.

(19) Weijs B, Pisters R, Haest RJ, Kragten JA, Joosen IA, Versteylen M, et al. Patients originally diagnosed with idiopathic atrial fibrillation more often suffer from insidious coronary artery disease compared to healthy sinus rhythm controls. Heart Rhythm 2012 Dec;9(12):1923-1929.

(20) Heeringa J, van der Kuip, D. A., Hofman A, Kors JA, van Rooij FJ, Lip GY, et al. Subclinical atherosclerosis and risk of atrial fibrillation: the rotterdam study. Arch Intern Med 2007 Feb 26;167(4):382-387.

Chapter 5

Pathophysiological Pathways in Patients with Heart Failure and Atrial Fibrillation

Bernadet T Santema, Vicente Artola Arita, Iziah E Sama, Mariëlle Kloosterman, Maarten P van den Berg, Hans L A Nienhuis, Isabelle C Van Gelder, Peter van der Meer, Faiez Zannad, Marco Metra, Jozine M Ter Maaten, John G Cleland, Leong L Ng, Stefan D Anker, Chim C Lang, Nilesh J Samani, Kenneth Dickstein, Gerasimos Filippatos, Dirk J van Veldhuisen, Carolyn S P Lam, Michiel Rienstra, Adriaan A Voors

Adapted from Cardiovascular Research 2022: 24;118(11):2478-2487.

Abstract.

Aims. Atrial fibrillation (AF) and heart failure (HF) are two growing epidemics that frequently co-exist. We aimed to gain insights into underlying pathophysiological pathways in HF patients with AF by comparing circulating biomarkers using pathway overrepresentation analyses.

Methods and Results. From a panel of 92 biomarkers from different pathophysiological domains available in 1,620 patients with HF, we first tested which biomarkers were dysregulated in patients with HF and AF (n=648) compared with patients in sinus rhythm (n=972). Secondly, pathway overrepresentation analyses were performed to identify biological pathways linked to higher plasma concentrations of biomarkers in patients who had HF and AF. Findings were validated in an independent HF cohort (n=1,219, 38% with AF). Patient with AF and HF were older, less often women, and less often had a history of coronary artery disease compared with those in sinus rhythm. In the index cohort, 24 biomarkers were upregulated in patients with AF and HF. In the validation cohort, 8 biomarkers were upregulated, which all overlapped with the 24 biomarkers found in the index cohort. The strongest up-regulated biomarkers in patients with AF were spondin-1 (fold change 1.18, $p=1.33x10^{-12}$), insulin-like growth factor-binding protein-1 (fold change 1.32, $p=1.08x10^{-8}$), and insulin-like growth factor-binding protein-7 (fold change 1.33, $p=1.35x10^{-18}$). Pathway overrepresentation analyses revealed that the presence of AF was associated with activation amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein catabolic processes with a remarkable consistency observed in the validation cohort.

Conclusion. In two independent cohorts of patients with HF, the presence of AF was associated with activation of three pathways related to amyloid-beta. These hypothesis-generating results warrant confirmation in future studies.

Introduction

Atrial fibrillation (AF) is the most common arrhythmia in heart failure (HF) with a prevalence between 20-60% depending on the type and severity of HF.(1-3) Both AF and HF are strongly associated with ageing, share many other clinical risk factors such as obesity and hypertension, and can trigger each other.(2, 3) Distinct differences are observed when comparing patients with HF with and without AF. We recently showed that patients with AF and HF are older, less often have an ischemic aetiology of HF, and have a distinct biomarker profile as compared with HF patients in sinus rhythm.(4, 5) Moreover, patients with AF and HF have a poorer quality of life, and worse outcome as compared with those without AF.(4,6) Pooled individual-patient data revealed that in contrast to the beneficial effects observed in patients with heart failure with a reduced ejection fraction (HFrEF) in sinus rhythm, β-blockers did not improve clinical outcomes in patients with AF and HFrEF.(7) The potential lack of β-blocker efficacy suggests differences in pathophysiology between HF patients with and without AF, but the exact mechanisms remain poorly understood and understudied.(7)

Unravelling the underlying pathophysiology of AF in HF is important since this population might respond to different therapies than HF patients without AF.(8,9) Underlying pathophysiological mechanisms can be studied by performing pathway overrepresentation analyses, a method that can identify associated pathways based on circulating biomarker profiles in specific subgroups.(10,11) Therefore, we compared patients who had HF with and without AF, studied their biomarker profiles and associated pathophysiological pathways, which might help discover new treatment targets for patients with HF and AF.

Methods

Patient population. We performed a *post-hoc* study of patients enrolled in A Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF), of which the design and primary results have been published previously.(12,13) In brief, BIOSTAT-CHF was a prospective, observational, multinational, European HF study, in which a total of 2,516 patients were included. Patients were eligible with either a left ventricular ejection fraction (LVEF) ≤40%, or plasma concentrations of N-terminal pro-B-type natriuretic peptide (NT-proBNP) >2000 ng/L. Another 1,738 patients with HF were included in an independent cohort from six centers in

Scotland, which we have used as our validation cohort. Patients were enrolled into the validation cohort when they were diagnosed with HF and had a previous documented admission with HF requiring diuretic treatment.(12) This study complies with the Declaration of Helsinki, and medical ethics committees of participating centers approved the study. All patients provided written informed consent.

Definitions. Patients were classified as AF when they met the following criteria: 1) a documented history of AF, and 2) AF registered on the standard 12-lead electrocardiogram (ECG) at baseline of the study. Patients were classified as having sinus rhythm when they met the following criteria: 1) no documented history of AF, and 2) sinus rhythm on the baseline ECG. Patients with a pacemaker rhythm (n=320) or unknown rhythm (n=58) were excluded from our analyses. Patients with prior episodes of AF but who were in sinus rhythm at baseline (n=197), and those without a history of AF but with AF on the baseline ECG (n=82) were excluded from our analyses, since these patients could interfere with the contrast in underlying pathophysiological pathways between the AF and sinus rhythm groups under study. We did include these patients in a previous biomarker study on AF in BIOSTAT-CHF, but since subsequent analyses revealed that many biomarkers tend to fluctuate with paroxysmal episodes of AF, we chose to include HF patients with 'permanent' AF as compared with those who never had any previously documented episode of AF in the current analyses.4, 14 A flow chart of the selected patients is displayed in *Supplementary Figure 1*.

Biomarkers. The Olink Cardiovascular III (CVD III) panel includes 92 biomarkers from different pathophysiological domains. The Proseek Multiplex 96*96 kit of Olink Bioscience (Uppsala, Sweden) analysis service was used, which measured the 92 biomarkers in 1µl plasma samples. The reagents are based on the Proximity Extension Assay (PEA) technology, which binds 92 oligonucleotide-labeled antibody probe pairs to the target biomarker.(15) For further quantification, real-time PCR was performed. Olink wizard and GenEx software were used for further data analysis. Proseek® data are presented as arbitrary units (AU) on a log2 scale *(Supplementary Table 1 and 2).* Complete biomarker data was available in 87% of the patients under study.

Statistical analyses. Normally distributed continuous variables were displayed as mean ± standard deviation, non-normally distributed variables as median with the first and third quartile (Q1-Q3).

Categorical variables were presented as numbers with percentages. Group comparisons were tested using Student's t tests, Mann-Whitney U tests, or Chi-square tests where appropriate. Differences in expression of the 92 biomarkers between patients with AF versus sinus rhythm were tested using Linear Models for Microarray data analysis (Limma) software (version 3.34.9), using a log2 fold change cutoff of 0.2, and a false discovery rate <0.05 according to the Benjamini-Hochberg method. The biomarkers that were upregulated in patients with AF compared to those in sinus rhythm were further studied by using pathway overrepresentation analysis. Pathway overrepresentation analysis was performed with knowledge from established pathways in publicly available databases: Gene Ontology (GO), Reactome, and the Kyoto Encyclopedia of Genes and Genomes (KEGG), using Cytoscape (version 3.7.1) and plugin ClueGO (version 2.5.4).(10,16,17) Multivariable logistic regression was performed to study the association between the biomarkers within pathways and AF status, adjusting for age, sex, body mass index (BMI), heart rate, a history of coronary artery disease, and renal disease. Since we were interested in underlying pathophysiological differences between patients with heart failure with a reduced/mid-range/preserved ejection fraction HFrEF/HFmrEF/HFpEF, we performed the same analyses in these subgroups. Unfortunately, these subgroups were too small to gain results with pathway overrepresentation analyses. This was still the case when we analyzed only two groups: LVEF <45% versus LVEF ≥45%. We therefore tested for interactions to determine whether the association of biomarkers and AF status was present in patients with HFrEF/HFmrEF/HFpEF by adding the interaction term to the logistic regression model, and also tested for an interaction with LVEF on a continuous scale. A separate network analysis focusing on pathophysiological differences between patients with HFpEF and HFrEF has been published previously.(18) A p-value smaller than 0.1 was considered statistically significant for testing interactions. All other tests were performed two-sided, and a p-value of less than 0.05 was considered statistically significant. Statistical analyses were conducted using R, A Language and Environment for Statistical Computing, version 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria). The data underlying this article will be shared on reasonable request to the corresponding author.

Results

Index cohort

Clinical characteristics. Of 1,620 patients with HF enrolled, 648 (40%) had AF and 972 (60%) were in sinus rhythm. Patients with AF were older, less often women, had a higher BMI and a higher heart rate (**Table 1**). Fewer patients with AF had a history of coronary artery disease, but more often a history of renal disease as compared with those in sinus rhythm. Patients with AF had larger left atrial diameters, and greater interventricular and posterior wall thickness on echocardiography.

Biomarker concentrations. In the index cohort, 24 biomarkers were upregulated and three biomarkers were downregulated in patients with AF as compared to those in sinus rhythm (**Figure 1**). A volcano plot with the up- and downregulated biomarkers is presented in Figure 2A. The three biomarkers that were most significantly differentially expressed in patients with AF as compared with those in sinus rhythm were neurogenic locus notch homolog protein 3 (NOTCH3, fold change 1.30, $p=6.40x10^{-24}$), insulin-like growth factor binding protein-7 (IGFBP7, fold change 1.33, $p=1.35x10^{-18}$), and interleukin-1 receptor-like 1 (IL1RL1, fold change 1.35, $p=1.75x10^{-16}$) *(Supplementary Table 1).*

Pathway overrepresentation analyses of upregulated biomarkers. Pathway overrepresentation analyses of the 24 upregulated biomarkers in the index cohort revealed seven pathways that were dysregulated specifically in patients with AF: 1) amyloid-beta metabolic process, 2) amyloid-beta formation, 3) amyloid precursor protein catabolic process, 4) regulation of insulin-like growth factor (IGF) transport and uptake by IGF binding proteins, 5) embryo implantation, 6) membrane protein ectodomain proteolysis, and 7) regulation of neuroinflammatory response (**Figure 3**).

Table 1. Baseline characteristics of patients included in the index cohort.

	Atrial fibrillation n=648 (40%)	Sinus rhythm n=972 (60%)	P-value
Clinical			
Age (years)	72 ± 10	65 ± 13	<0.001
Women (%)	164 (25)	298 (31)	0.02
BMI (kg/m2)	28 ± 6	27 ± 6	<0.001
NYHA Class I/II/III/IV (%)	9/50/37/4	12/53/32/4	0.07
Systolic blood pressure (mmHg)	125 ± 21	126 ± 23	0.43
Diastolic blood pressure (mmHg)	76 ± 13	75 ± 14	0.23
Heart rate (b.p.m.)	91 ± 24	80 ± 18	<0.001
Smoking			<0.001
Never	270 (42)	322 (33)	
Past	318 (49)	450 (46)	
Current	58 (9)	200 (21)	
History of (%)			
Coronary artery disease*	244 (38)	448 (46)	0.001
Valvular surgery	71 (11)	31 (3)	<0.001
Stroke	72 (11)	71 (7)	0.011
Peripheral artery disease	69 (11)	88 (9)	0.33
Hypertension	419 (65)	597 (61)	0.20
Diabetes	210 (32)	308 (32)	0.80
COPD	116 (18)	140 (14)	0.07
Renal disease	213 (33)	213 (22)	<0.001
Physical examination (%)			
Rales	356 (56)	486 (52)	0.10
Oedema	393 (70)	399 (50)	<0.001
Hepatomegaly	113 (18)	106 (11)	<0.001
KCCQ – Quality of Life			
Functional status score	43 [25, 64]	54 [34, 75]	<0.001
Clinical summary score	41 [24, 61]	50 [32, 71]	<0.001
Overall score	41 [26, 61]	51 [34, 70]	<0.001
Laboratory data			
NT-proBNP (ng/L)	3430 [1872, 6387]	2293 [925, 5347]	<0.001
Haemoglobin (g/L)	13.3 [11.9, 14.6]	13.4 [12.1, 16.6]	0.28
Creatinine (µmol/L)	104 [88, 131]	97 [80, 121]	<0.001
Echocardiographic data			
LVEF, %	33 ± 12	29 ± 10	<0.001
Left atrial diameter, mm	50 ± 8	46 ± 8	<0.001
Interventricular wall thickness, mm	11 ± 2	10 ± 2	<0.001
Posterior wall thickness, mm	11 ± 2	10 ± 2	<0.001

Medication at baseline (%)			
ACE i or ARB	440 (68)	726 (75)	0.003
β blocker	523 (81)	810 (83)	0.20
MRA	318 (49)	516 (53)	0.13
Diuretics	648 (100)	971 (100)	1.00
Amiodarone	81 (13)	110 (11)	0.52
Digoxin	246 (38)	60 (6)	<0.001
Verapamil/diltiazem	22 (3)	7 (1)	<0.001
Class 1c antiarrhythmic drugs	2 (1)	2 (1)	1.00
Ivabradine	0 (0)	23 (2)	<0.001
Vitamin K antagonist	461 (71)	130 (13)	<0.001
Direct oral anticoagulants	7 (1)	0 (0)	0.004

*Coronary artery disease: previous myocardial infarction, percutaneous coronary intervention (PCI) and/or coronary artery bypass graft (CABG). BMI=body mass index, NYHA=New York Heart Association, LVEF=left ventricular ejection fraction, BP = blood pressure, COPD=chronic obstructive pulmonary disease, KCCQ=Kansas City Cardiomyopathy Questionnaire, NT-proBNP=N-terminal pro-B-type natriuretic peptide, ACE i=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker, MRA=mineralocorticoid receptor antagonist. P-values for group comparisons were tested using Student's t tests, Mann-Whitney U tests, or Chi-square tests where appropriate.

Figure 1. Venn diagram displaying the number of significantly upregulated and downregulated biomarkers in patients with atrial fibrillation versus sinus rhythm in the index (n=1,620) and validation cohort (n=1,219) of BIOSTAT-CHF.

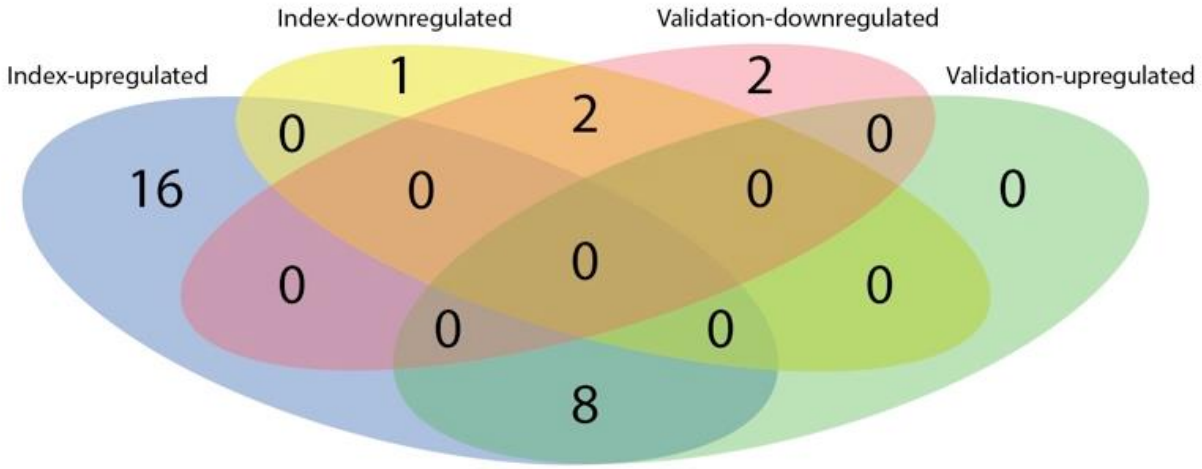
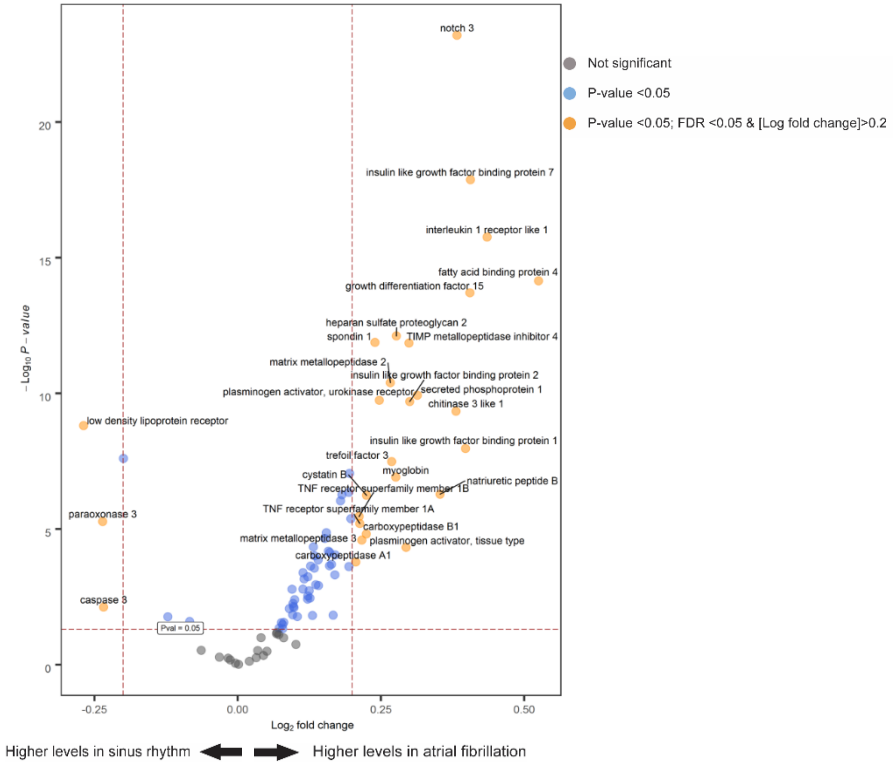


Figure 2. Volcano plots of differential biomarker expression in patients with atrial fibrillation versus sinus rhythm in the index (A, n=1,620) and validation cohort (B, n=1,219) of BIOSTAT-CHF. Y-axis = significance, x-axis = effect size (positive = up-regulated, negative = down-regulated), labelled biomarkers are significantly differentially expressed proteins.

A Index cohort



B Validation cohort

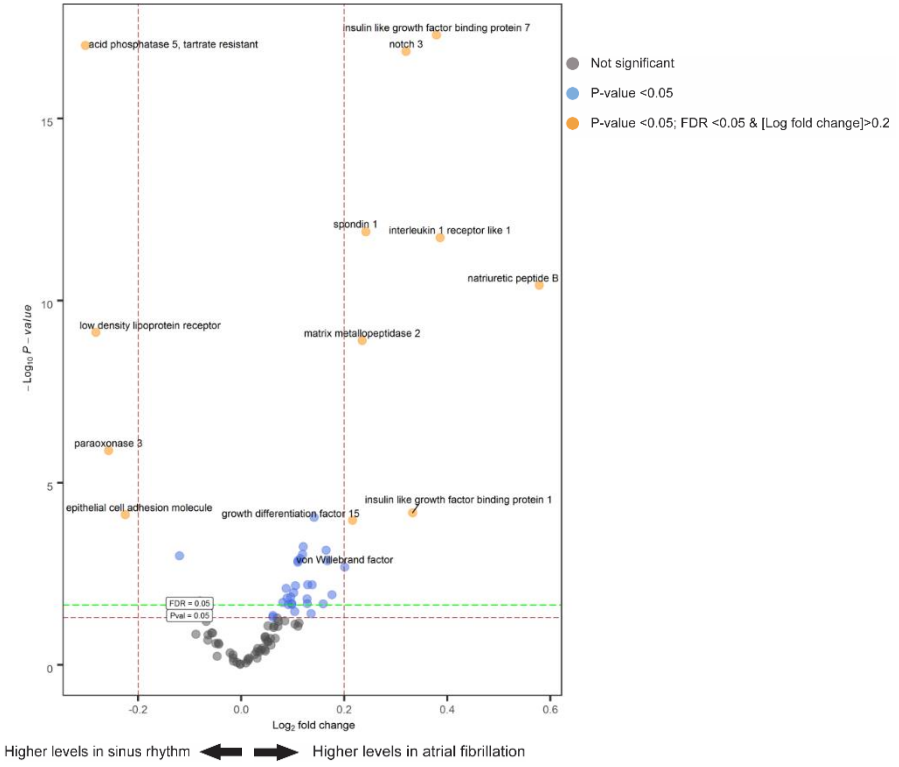
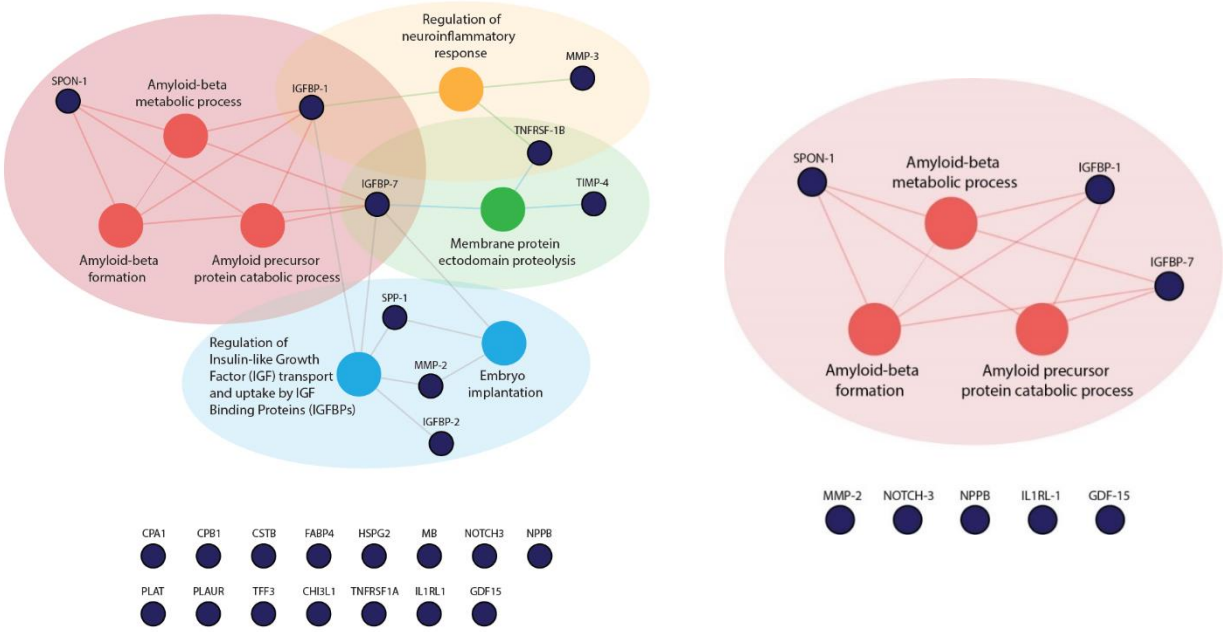


Figure 3. Results of pathway overrepresentation analyses of patients with atrial fibrillation versus sinus rhythm in the index cohort (**A** [n=1,620]) and the validation cohort (**B** [n=1,219]) of BIOSTAT-CHF. The nodes in blue represent the 24 biomarkers that were significantly upregulated in patients with atrial fibrillation as compared with those in sinus rhythm in the index cohort (A), and 8 biomarkers that were significantly upregulated in these patients in the validation cohort (B). The nodes in red reveal the overrepresented pathways of these biomarkers. Based on current knowledge, the blue nodes below the figures are biomarkers that were not found to be overrepresented in a specific biological pathway.

A. **B.**



Abbreviations: SPON-1=spondin 1, IGFBP-1=insulin like growth factor binding protein 1, IGFBP-7=insulin like growth factor binding protein 7, MMP-3=matrix metallopeptidase 3, TNFRSF1B= TNF receptor superfamily member 1B, TIMP-4= Metalloproteinase inhibitor 4, SPP-1= secreted phosphoprotein 1, MMP-2=matrix metallopeptidase 2, IGFBP-2= insulin like growth factor binding protein 2, CPA1= carboxypeptidase A1, CPB1= carboxypeptidase B1, CSTB= cystatin B, FABP4= Fatty acid-binding protein, adipocyte, HSPG2= heparan sulfate proteoglycan 2, MB= Myoglobin, NOTCH3= Neurogenic locus notch homolog protein 3, NPPB=natriuretic peptide B, PLAT= plasminogen activator, tissue type, PLAUR= plasminogen activator, urokinase receptor, TFF3= trefoil factor 3, CHI3L1= chitinase 3 like 1, TNFRSF1A= TNF receptor superfamily member 1A, IL1RL-1=interleukin 1 receptor like 1, GDF-15=growth differentiation factor 15.

Validation cohort

Clinical characteristics. The baseline characteristics of patients included in the smaller validation cohort are presented in **Table 2**. In general, patients enrolled in the validation cohort were older, had a higher LVEF, and lower plasma concentrations of NT-proBNP as compared with patients included in the index cohort. However, similar trends were observed in patients with AF compared to those in sinus rhythm, in which patients with AF were older, less often women, had higher heart rates, and less often a history of coronary artery disease.

Biomarker concentrations. In the validation cohort, eight biomarkers were significantly upregulated in patients with AF, all of which overlapped with the 24 biomarkers that were found to be significantly upregulated in the index cohort (**Figure 1**). The eight biomarkers that were upregulated in AF patients in both HF cohorts included IGFBP7 (fold change 1.30, $p=5.13x10^{-18}$), NOTCH3 (fold change 1.25, $p=1.44x10^{-17}$), spondin 1 (SPON1, fold change 1.18, $p=1.29x10^{-12}$), IL1RL1 (fold change 1.31, $p=3.44x10^{-11}$), natriuretic peptide B (fold change 1.49, $p=3.78x10^{-11}$), matrix metallopeptidase 2 (MMP2, fold change 1.18, $p=1.25x10^{-9}$), IGFBP1 (fold change 1.26, $p=6.68x10^{-5}$), and growth differentiation factor 15 (GDF15, fold change 1.16, $p=1.07x10^{-4}$) *(Supplementary Table 2)*. Low-density lipoprotein receptor (LDLR) and paraoxonase 3 (PON3) were significantly downregulated in patients with AF in both the index and the validation cohort.

Pathway overrepresentation analyses of upregulated biomarkers. Pathway overrepresentation analysis of the eight upregulated biomarkers in patients with AF in the validation cohort revealed three activated pathways: 1) amyloid-beta metabolic process, 2) amyloid-beta formation, and 3) amyloid precursor protein catabolic process **(Figure 3***)*.

Overlap index and validation cohort. The three amyloid-beta-related pathways were found in both the index and validation cohort, and were related to three upregulated biomarkers in patients with AF: SPON1 (fold change 1.18, $p=1.33x10^{-12}$), IGFBP1 (fold change 1.32, $p=1.08x10^{-8}$), and IGFBP7 (fold change 1.33, $p=1.35x10^{-18}$). After adjusting for the clinical covariates age, sex, body mass index, heart rate, a history of coronary artery disease, and renal disease, the concentrations of SPON-1, IGFBP-1, and IGFBP-7 remained associated with the presence of AF (all $p<0.001$), in both the index and the validation cohort. These associations were similar among HF phenotypes

(HFrEF/HFmrEF/HFpEF; p for interaction = 0.42) and across LVEF as a continuous variable (p for interaction = 0.96).

Table 2. Baseline characteristics of patients included in the validation cohort.

	Atrial fibrillation n=468 (38%)	Sinus rhythm n=751 (62%)	P-value
Clinical			
Age (years)	75 ± 10	72 ± 11	<0.001
Women (%)	148 (32)	291 (39)	0.01
BMI (kg/m2)	29 ± 6	29 ± 6	0.13
NYHA Class I/II/III/IV (%)	0/39/46/14	2/42/44/13	0.22
Systolic blood pressure (mmHg)	126 ± 21	127 ± 23	0.38
Diastolic blood pressure (mmHg)	72 ± 15	68 ± 12	<0.001
Heart rate (b.p.m.)	87 ± 27	72 ± 18	<0.001
Smoking			<0.001
Never	275 (59)	358 (48)	
Past	148 (32)	279 (37)	
Current	43 (9)	110 (15)	
History of (%)			
Coronary artery disease*	194 (42)	462 (62)	<0.001
Valvular surgery	39 (8)	39 (5)	0.04
Stroke	105 (23)	105 (14)	<0.001
Peripheral artery disease	96 (21)	160 (22)	0.79
Hypertension	274 (59)	434 (58)	0.80
Diabetes	149 (32)	225 (30)	0.53
COPD	83 (18)	135 (18)	0.99
Renal disease	220 (47)	312 (42)	0.11
Physical examination (%)			
Rales	219 (49)	301 (42)	0.02
Oedema	290 (68)	385 (57)	0.001
Hepatomegaly	20 (5)	23 (3)	0.36
KCCQ – Quality of Life			
Functional status score	45 [25, 65]	46 [27, 71]	0.05
Clinical summary score	41 [25, 65]	45 [26, 70]	0.07
Overall score	42 [30, 60]	45 [30, 68]	0.03
Laboratory data			
NT-proBNP (ng/L)	2105 [1045, 4204]	872 [311, 2807]	<0.001
Haemoglobin (g/L)	13.5 [12.1, 14.7]	13.1 [11.7, 14.3]	0.004
Creatinine (μmol/L)	98 [82, 123]	95 [77, 121]	0.04
Echocardiographic data			
LVEF, %	43 ± 13	41 ± 13	0.03

Left atrial diameter, mm	48 ± 7	43 ± 7	<0.001
Interventricular wall thickness, mm	13 ± 3	12 ± 4	0.29
Posterior wall thickness, mm	12 ± 4	11 ± 5	0.54
Medication at baseline (%)			
ACE i or ARB	322 (69)	540 (72)	0.28
β blocker	339 (72)	540 (72)	0.89
MRA	144 (31)	229 (31)	0.97
Diuretics	457 (98)	746 (99)	0.02
Amiodarone	12 (3)	27 (4)	0.41
Digoxin	193 (41)	12 (2)	<0.001
Verapamil/diltiazem	18 (4)	13 (2)	0.036
Class 1c antiarrhythmic drugs	0 (0)	0 (0)	1.00
Ivabradine	1 (1)	33 (4)	<0.001
Vitamin K antagonist	327 (70)	87 (12)	<0.001
Direct oral anticoagulants	22 (5)	6 (1)	<0.001

*Coronary artery disease: previous myocardial infarction, percutaneous coronary intervention (PCI) and/or coronary artery bypass graft (CABG). BMI=body mass index, NYHA=New York Heart Association, LVEF=left ventricular ejection fraction, BP = blood pressure, COPD=chronic obstructive pulmonary disease, KCCQ=Kansas City Cardiomyopathy Questionnaire, NT-proBNP=N-terminal pro-B-type natriuretic peptide, ACE i=angiotensin converting enzyme inhibitor, ARB=angiotensin receptor blocker, MRA=mineralocorticoid receptor antagonist. P-values for group comparisons were tested using Student's t tests, Mann-Whitney U tests, or Chi-square tests where appropriate.

Discussion

We sought to identify pathophysiological pathways in HF patients with AF using pathway overrepresentation analyses. In two independent HF cohorts we found that the presence of AF was associated with amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein catabolic processes. These three pathophysiological pathways were found based on higher levels of spondin-1, IGFBP1, and IGFBP7 in those with AF. In the larger index cohort, four more pathophysiological pathways were found, which were not observed in the independent validation cohort. Previous studies investigating specific phenotypes or subgroups in HF (e.g. diabetes, ischemic HF, old vs. young, and HFpEF vs. HFrEF), have not revealed any amyloid-beta-related pathways despite using the same methodology, which supports that the current findings might be specific to the presence of AF in patients with HF.(10,11,19)

Individual biomarkers. In the present study, the concentrations of SPON-1, IGFBP-1 and IGFBP-7 were closely linked to the three amyloid-beta-related pathways. Although IGFBP-1 and IGFBP-7 are linked to a wide range of biological processes associated with inhibition and stimulation of cell growth, the current knowledge indicates a more specific role for SPON-1.(20)

SPON-1 is an extracellular matrix cell adhesion glycoprotein that is expressed in multiple organs including the heart and brain.(20) The SPON-1 protein binds to the extracellular domain of amyloid precursor protein and inhibits beta-secretase cleavage of this amyloid precursor protein, a process that is strongly related to the formation of amyloid-beta depositions.(21) In a large genome wide association study (GWAS) investigating the rate of cognitive decline in patients with Alzheimer's disease, the most interesting candidate gene identified was *SPON-1*, since it was strongly associated with the rate of cognitive decline in two independent cohorts.(22) Recent studies showed that increased levels of IGFBP-7, a marker of ageing and cellular senescence, were strongly associated with increased left atrial size, and the presence of AF in patients with HF.(23-25) In Framingham Heart Study participants without HF, increased levels of IGFBP1 were strongly associated with incident AF.(26) In the present study, NOTCH3 was strongly upregulated in patients with AF in both cohorts. The NOTCH system communicates in multiple tissues and systems, including cell proliferation, differentiation, and apoptosis.(27) In the heart specifically, NOTCH signaling has been suggested to be associated with repair of infarcted and overloaded myocardium, but this has only been investigated in a pre-clinical setting.(27) The role of the NOTCH system in patients with AF and HF is yet to be elucidated.

Amyloid-beta in heart failure and atrial fibrillation. Cardiac amyloidosis has been reported to be associated with a high prevalence of AF in several previous studies;(28-33) however prior work focused on isolated atrial amyloidosis (IAA) and transthyretin-derived amyloidosis (ATTR) – the most commonly described forms of cardiac amyloidosis in elderly patients. Our results concerned amyloid-beta depositions, which are generally acknowledged as a hallmark of Alzheimer's disease, in which abnormal cleavage of the amyloid precursor protein leads to pathological amyloid-beta fragments, protein aggregation, and formation of extracellular plaques that can lead to degradation of neurons.(34,35) Even though Alzheimer's disease has been traditionally considered as a brain-specific disease, recent discoveries suggest that other organs might also be involved in the pathophysiology, suggesting that Alzheimer's disease might be a focal manifestation of a systemic disorder.(34,36) The epidemiological link between AF and Alzheimer's disease was first described in 1977, followed by studies showing that younger patients with AF had an increased risk of developing all-cause dementia which could not be explained by the increased incidence of stroke alone.(37-39) Since then, contradictory results have been reported, but neuropathological analyses of autopsies did reveal a higher incidence of amyloid-

beta plaques and amyloid angiopathy in the brains of patients with permanent AF.(40) Suggesting a unifying pathogenesis, Troncone et al. performed a cross-sectional study investigating cardiac involvement of patients with a primary diagnosis of Alzheimer's disease as compared to age-matched controls. Indeed, those with Alzheimer's disease had increased left ventricular septal and inferolateral wall thickness on echocardiography, and expression of amyloid-beta plaques in both the heart and the brain.(36)

Clinical relevance. Even though the current study revealed pathways related to amyloid-beta specifically, considerable overlap can be observed with more commonly recognized protein-misfolding diseases that are known to affect the heart. As recently reviewed, cardiac involvement has predominantly been reported in IAA, light chain (AL) and ATTR amyloidosis, but may occur in other types of amyloidosis, with cardiac arrhythmias, especially atrial fibrillation, as common presenting clinical features.(41) The consistency of cardiac clinical presentations among the various types of amyloidosis, despite differences in involved proteins (e.g. ANP versus AL versus ATTR), suggest common cardiac effects which may also plausibly apply to amyloid-beta deposition.(42) Notably, the emergence of promising new treatment options for ATTR amyloidosis has raised awareness of the importance of screening for amyloidosis in patients with suggestive clinical features.(43-45) Whether similar mechanistic approaches can be of use in patients with cardiac amyloid-beta depositions warrants further study.

Strengths and Limitations. To our knowledge, there have been no previous studies investigating underlying pathophysiological processes using pathway overrepresentation in patients with AF and HF. Therefore, our study adds to the limited understanding of the underlying pathophysiological mechanisms of AF in patients with HF. The greatest strength of the current study is that we were able to validate our results in an independent HF cohort with clear definitions, and that the pathway overrepresentation analysis was based on a large number of measured plasma biomarkers.

A limitation of this study is that the findings are based on *post-hoc* analyses. Unfortunately, we did not have direct biopsy evidence of cardiac/atrial amyloid-beta involvement which are pivotal to confirm the cardiac amyloid-beta hypothesis. Future research in cases (patients with AF+HF) and controls (patients in sinus rhythm with and without HF) with markers derived from atrial tissue will provide more direct insights into our hypothesis.(46) Another limitation concerns the selected

biomarker panel, which did cover many pathophysiological domains, but was primarily a cardiovascular disease-related biomarker panel. The number of significantly upregulated biomarkers was higher in the index cohort than in the validation cohort, which resulted in more associated pathophysiological processes in the index cohort than in the validation cohort. This could be the consequence of the larger number of patients included in the index cohort, the different inclusion criteria of patients that were used for the two independent cohorts, or the different regions of inclusion of the study participants (11 European counties versus six centers in Scotland). The use of amiodarone was higher in the index cohort compared with the validation cohort. However, despite these differences between the two cohorts, all upregulated biomarkers and pathways found in the validation cohort overlapped with those found in the index cohort. The HFrEF, HFmrEF and HFpEF subgroups were unfortunately too small to yield results with pathway overrepresentation analyses. There was, however, no interaction between the amyloid-beta-related biomarkers and the HF subgroups, suggesting a pathophysiological role of amyloid-beta in HF patients across the full LVEF spectrum. Not all (combinations of) biomarkers were annotated by the publicly available databases even though these biomarkers were deemed to be significantly up- or downregulated in our analyses (e.g. NOTCH3 and IL1RL1), since the content of these databases is based on what is currently known about these biomarkers. Therefore, the results of the current pathway overrepresentation analyses might change over time, when the knowledge on the (combination of) biomarkers has increased. The current findings might reflect underlying pathophysiological processes specific to elderly patients with AF and HF, since the mean age of these patients was 72 and 75 years in the two cohorts respectively. The number of women included in the cohorts was limited (n=462 [index] and n=439 [validation], 32%) and mainly comprised postmenopausal women. Even though we have attempted to define the AF and sinus rhythm group mutually exclusive, it is possible that patients with asymptomatic paroxysmal AF were misclassified. Based on the current definition, we may have predominantly included patients with persistent/permanent AF and less patients with paroxysmal AF. Moreover, we do not have data on the duration of AF since no continues rhythm monitoring was incorporated in the study protocol, which might have influenced the biomarker concentrations. Unfortunately, we also do not have information on cognitive function, other neurologic diseases, nor information on systemic or cardiac amyloidosis of patients enrolled in BIOSTAT-CHF, which could have strengthened our

hypothesis linking AF – HF to Alzheimer's disease and/or amyloidosis. As with all cross-sectional studies, we cannot prove causality.

Conclusion

In two independent cohorts of patients with HF, the presence of AF was associated with amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein catabolic processes, based on higher levels of spondin-1, IGFBP1, and IGFBP7 in those with AF. These hypothesis-generating results warrant confirmation in future studies.

References

1. Maisel WH , Stevenson LW. Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *The American Journal of Cardiology* 2003;**91**:2-8.

2. Kotecha D, Lam CS, Van Veldhuisen DJ, Van Gelder IC, Voors AA, Rienstra M. Heart Failure With Preserved Ejection Fraction and Atrial Fibrillation: Vicious Twins. *J Am Coll Cardiol* 2016;**68**:2217-2228.

3. Kloosterman M, Santema BT, Roselli C, Nelson CP, Koekemoer A, Romaine SPR, Van Gelder IC, Lam CSP, Artola VA, Lang CC, Ng LL, Metra M, Anker S, Filippatos G, Dickstein K, Ponikowski P, van der Harst P, van der Meer P, van Veldhuisen DJ, Benjamin EJ, Voors AA, Samani NJ, Rienstra M. Genetic risk and atrial fibrillation in patients with heart failure. *Eur J Heart Fail* 2020;.

4. Santema BT, Kloosterman M, Van Gelder IC, Mordi I, Lang CC, Lam CSP, Anker SD, Cleland JG, Dickstein K, Filippatos G, Van der Harst P, Hillege HL, Ter Maaten JM, Metra M, Ng LL, Ponikowski P, Samani NJ, Van Veldhuisen DJ, Zwinderman AH, Zannad F, Damman K, Van der Meer P, Rienstra M, Voors AA. Comparing biomarker profiles of patients with heart failure: atrial fibrillation vs. sinus rhythm and reduced vs. preserved ejection fraction. *Eur Heart J* 2018;**39**:3867-3875.

5. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal, H H, Emmens JE, Takens J, Samani NJ, Ng LL, Lang CC, van der Meer P, Ter Maaten JM, Damman K, Dickstein K, Cleland JG, Zannad F, Anker SD, Metra M, van der Harst P, de Boer RA, van Veldhuisen DJ, Rienstra M, Lam CSP, Voors AA. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *Clin Res Cardiol* 2019;.

6. Lam CS, Rienstra M, Tay WT, Liu LC, Hummel YM, van der Meer P, de Boer RA, Van Gelder IC, van Veldhuisen DJ, Voors AA, Hoendermis ES. Atrial Fibrillation in Heart Failure With Preserved Ejection Fraction: Association With Exercise Capacity, Left Ventricular Filling Pressures, Natriuretic Peptides, and Left Atrial Volume. *JACC Heart Fail* 2017;**5**:92-98.

7. Kotecha D, Holmes J, Krum H, Altman DG, Manzano L, Cleland JG, Lip GY, Coats AJ, Andersson B, Kirchhof P, von Lueder TG, Wedel H, Rosano G, Shibata MC, Rigby A, Flather MD, Beta-Blockers in Heart Failure Collaborative Group. Efficacy of beta blockers in patients with heart failure plus atrial fibrillation: an individual-patient data meta-analysis. *Lancet* 2014;**384**:2235-2243.

8. Verma A, Kalman JM, Callans DJ. Treatment of Patients With Atrial Fibrillation and Heart Failure With Reduced Ejection Fraction. *Circulation* 2017;**135**:1547-1563.

9. Rienstra M, Hobbelt AH, Alings M, Tijssen JGP, Smit MD, Brugemann J, Geelhoed B, Tieleman RG, Hillege HL, Tukkie R, Van Veldhuisen DJ, Crijns, H J G M, Van Gelder IC, RACE 3 Investigators. Targeted therapy of underlying conditions improves sinus rhythm maintenance in patients with persistent atrial fibrillation: results of the RACE 3 trial. *Eur Heart J* 2018;**39**:2987-2996.

10. Tromp J, Voors AA, Sharma A, Ferreira JP, Ouwerkerk W, Hillege HL, Arevelo Gomez K, Lang CC, Ng LL, van der Harst P, van Veldhuisen DJ, van der Meer P, Lam CSP, Zannad F, Sama IE. Distinct Pathological Pathways in Heart Failure Patients with Diabetes . *JACC Heart Failure* .

11. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, Metra M, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Lang CC, Ng LL, Zannad F, Zwinderman AH, Hillege HL, van der Meer P, Voors AA. Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction. *J Am Coll Cardiol* 2018;**72**:1081-1090.

12. Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng L, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Zwinderman AH, Metra M. A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. *Eur J Heart Fail* 2016;**18**:716-726.

13. Ouwerkerk W, Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Hillege HL, Lang CC, Ter Maaten JM, Ng LL, Ponikowski P, Samani NJ, van Veldhuisen DJ, Zannad F, Metra M, Zwinderman AH. Determinants and clinical outcome of uptitration of ACE-inhibitors and beta-blockers in patients with heart failure: a prospective European study. *Eur Heart J* 2017;**38**:1883-1890.

14. Santema BT, Chan MMY, Tromp J, Dokter M, van der Wal, H H, Emmens JE, Takens J, Samani NJ, Ng LL, Lang CC, van der Meer P, Ter Maaten JM, Damman K, Dickstein K, Cleland JG, Zannad F, Anker SD, Metra M, van der Harst P, de Boer RA, van Veldhuisen DJ, Rienstra M, Lam CSP, Voors AA. The influence of atrial fibrillation on the levels of NT-proBNP versus GDF-15 in patients with heart failure. *Clin Res Cardiol* 2019;.

15. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson A, Lindstedt P, Stenvang J, Gullberg M, Fredriksson S. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PloS One* 2014;**9**:e95192.

16. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498-2504.

17. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pages F, Trajanoski Z, Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics* 2009;**25**:1091-1093.

18. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, Metra M, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Lang CC, Ng LL, Zannad F, Zwinderman AH, Hillege HL, van der Meer P, Voors AA. Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction. *J Am Coll Cardiol* 2018;**72**:1081-1090.

19. Sama IE, Woolley RJ, Nauta JF, Romaine SPR, Tromp J, Ter Maaten JM, van der Meer P, Lam CSP, Samani NJ, Ng LL, Metra M, Dickstein K, Anker SD, Zannad F, Lang CC, Cleland JGF, van Veldhuisen DJ, Hillege HL, Voors AA. A network analysis to identify pathophysiological pathways distinguishing ischaemic from non-ischaemic heart failure. *Eur J Heart Fail* 2020;.

20. UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* 2019;**47**:D506-D515.

21. Ho A , Sudhof TC. Binding of F-spondin to amyloid-beta precursor protein: a candidate amyloid-beta precursor protein ligand that modulates amyloid-beta precursor protein cleavage. *Proc Natl Acad Sci U S A* 2004;**101**:2548-2553.

22. Sherva R, Tripodis Y, Bennett DA, Chibnik LB, Crane PK, de Jager PL, Farrer LA, Saykin AJ, Shulman JM, Naj A, Green RC, GENAROAD Consortium, Alzheimer's Disease Neuroimaging Initiative, Alzheimer's Disease Genetics Consortium. Genome-wide association study of the rate of cognitive decline in Alzheimer's disease. *Alzheimers Dement* 2014;**10**:45-52.

23. Januzzi JL, Packer M, Claggett B, Liu J, Shah AM, Zile MR, Pieske B, Voors A, Gandhi PU, Prescott MF, Shi V, Lefkowitz MP, McMurray JJV, Solomon SD. IGFBP7 (Insulin-Like Growth Factor-Binding Protein-7) and Neprilysin Inhibition in Patients With Heart Failure. *Circ Heart Fail* 2018;**11**:e005133.

24. Gandhi PU, Gaggin HK, Redfield MM, Chen HH, Stevens SR, Anstrom KJ, Semigran MJ, Liu P, Januzzi JL. Insulin-Like Growth Factor-Binding Protein-7 as a Biomarker of Diastolic Dysfunction and Functional Capacity in Heart Failure With Preserved Ejection Fraction: Results From the RELAX Trial. *JACC Heart Fail* 2016;**4**:860-869.

25. Gandhi PU, Chow SL, Rector TS, Krum H, Gaggin HK, McMurray JJ, Zile MR, Komajda M, McKelvie RS, Carson PE, Januzzi JL, Anand IS. Prognostic Value of Insulin-Like Growth Factor-Binding Protein 7 in Patients with Heart Failure and Preserved Ejection Fraction. *J Card Fail* 2017;**23**:20-28.

26. Staerk L, Preis SR, Lin H, Lubitz SA, Ellinor PT, Levy D, Benjamin EJ, Trinquart L. Protein Biomarkers and Risk of Atrial Fibrillation: The FHS. *Circ Arrhythm Electrophysiol* 2020;**13**:e007607.

27. Ferrari R , Rizzo P. The Notch pathway: a novel target for myocardial remodelling therapy? *Eur Heart J* 2014;**35**:2140-2145.

28. van den Berg, M P, Mulder BA, Klaassen SHC, Maass AH, van Veldhuisen DJ, van der Meer P, Nienhuis HLA, Hazenberg BPC, Rienstra M. Heart failure with preserved ejection fraction, atrial fibrillation, and the role of senile amyloidosis. *Eur Heart J* 2019;**40**:1287-1293.

29. Rapezzi C, Merlini G, Quarta CC, Riva L, Longhi S, Leone O, Salvi F, Ciliberti P, Pastorelli F, Biagini E, Coccolo F, Cooke RM, Bacchi-Reggiani L, Sangiorgi D, Ferlini A, Cavo M, Zamagni E, Fonte ML, Palladini G, Salinaro F, Musca F, Obici L, Branzi A, Perlini S. Systemic cardiac

amyloidoses: disease profiles and clinical courses of the 3 main types. *Circulation* 2009;**120**:1203-1212.

30. Longhi S, Quarta CC, Milandri A, Lorenzini M, Gagliardi C, Manuzzi L, Bacchi-Reggiani ML, Leone O, Ferlini A, Russo A, Gallelli I, Rapezzi C. Atrial fibrillation in amyloidotic cardiomyopathy: prevalence, incidence, risk factors and prognostic role. *Amyloid* 2015;**22**:147-155.

31. Pinney JH, Whelan CJ, Petrie A, Dungu J, Banypersad SM, Sattianayagam P, Wechalekar A, Gibbs SD, Venner CP, Wassef N, McCarthy CA, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD, Lachmann HJ. Senile systemic amyloidosis: clinical features at presentation and outcome. *J Am Heart Assoc* 2013;**2**:e000098.

32. Grogan M, Scott CG, Kyle RA, Zeldenrust SR, Gertz MA, Lin G, Klarich KW, Miller WL, Maleszewski JJ, Dispenzieri A. Natural History of Wild-Type Transthyretin Cardiac Amyloidosis and Risk Stratification Using a Novel Staging System. *J Am Coll Cardiol* 2016;**68**:1014-1020.

33. Rocken C, Peters B, Juenemann G, Saeger W, Klein HU, Huth C, Roessner A, Goette A. Atrial amyloidosis: an arrhythmogenic substrate for persistent atrial fibrillation. *Circulation* 2002;**106**:2091-2097.

34. Wang J, Gu BJ, Masters CL, Wang YJ. A systemic view of Alzheimer disease - insights from amyloid-beta metabolism beyond the brain. *Nat Rev Neurol* 2017;**13**:612-623.

35. Murphy MP , LeVine H. Alzheimer's disease and the amyloid-beta peptide. *J Alzheimers Dis* 2010;**19**:311-323.

36. Troncone L, Luciani M, Coggins M, Wilker EH, Ho CY, Codispoti KE, Frosch MP, Kayed R, Del Monte F. Abeta Amyloid Pathology Affects the Hearts of Patients With Alzheimer's Disease: Mind the Heart. *J Am Coll Cardiol* 2016;**68**:2395-2407.

37. Cardiogenic Dementia. *Lancet* 1977;**1**:27-28.

38. Ott A, Breteler MM, de Bruyne MC, van Harskamp F, Grobbee DE, Hofman A. Atrial fibrillation and dementia in a population-based study. The Rotterdam Study. *Stroke* 1997;**28**:316-321.

39. Ihara M , Washida K. Linking Atrial Fibrillation with Alzheimer's Disease: Epidemiological, Pathological, and Mechanistic Evidence. *J Alzheimers Dis* 2018;**62**:61-72.

40. Dublin S, Anderson ML, Heckbert SR, Hubbard RA, Sonnen JA, Crane PK, Montine TJ, Larson EB. Neuropathologic changes associated with atrial fibrillation in a population-based autopsy cohort. *J Gerontol A Biol Sci Med Sci* 2014;**69**:609-615.

41. Ihne S, Morbach C, Obici L, Palladini G, Stork S. Amyloidosis in Heart Failure. *Curr Heart Fail Rep* 2019;**16**:285-303.

42. Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D'Avila A, Dobrev D, Fenelon G, Gonzalez M, Hatem SN, Helm R, Hindricks G, Ho SY, Hoit B, Jalife J, Kim YH, Lip GY, Ma CS, Marcus GM, Murray K, Nogami A, Sanders P, Uribe W, Van Wagoner DR, Nattel S, Document Reviewers:. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Europace* 2016;**18**:1455-1490.

43. Solomon SD, Adams D, Kristen A, Grogan M, Gonzalez-Duarte A, Maurer MS, Merlini G, Damy T, Slama MS, Brannagan TH, Dispenzieri A, Berk JL, Shah AM, Garg P, Vaishnaw A, Karsten V, Chen J, Gollob J, Vest J, Suhr O. Effects of Patisiran, an RNA Interference Therapeutic, on Cardiac Parameters in Patients With Hereditary Transthyretin-Mediated Amyloidosis. *Circulation* 2019;**139**:431-443.

44. Minamisawa M, Claggett B, Adams D, Kristen AV, Merlini G, Slama MS, Dispenzieri A, Shah AM, Falk RH, Karsten V, Sweetser MT, Chen J, Riese R, Vest J, Solomon SD. Association of Patisiran, an RNA Interference Therapeutic, With Regional Left Ventricular Myocardial Strain in Hereditary Transthyretin Amyloidosis: The APOLLO Study. *JAMA Cardiol* 2019;**4**:466-472.

45. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, Kristen AV, Grogan M, Witteles R, Damy T, Drachman BM, Shah SJ, Hanna M, Judge DP, Barsdorf AI, Huber P, Patterson TA, Riley S, Schumacher J, Stewart M, Sultan MB, Rapezzi C, ATTR-ACT Study Investigators. Tafamidis Treatment for Patients with Transthyretin Amyloid Cardiomyopathy. *N Engl J Med* 2018;**379**:1007-1016.

46. Molina CE, Abu-Taha IH, Wang Q, Rosello-Diez E, Kamler M, Nattel S, Ravens U, Wehrens XHT, Hove-Madsen L, Heijman J, Dobrev D. Profibrotic, Electrical, and Calcium-Handling Remodeling of the Atria in Heart Failure Patients With and Without Atrial Fibrillation. *Front Physiol* 2018;**9**:1383.

Chapter 6

Clinical Utility of the 4S-AF Scheme in Predicting Progression of Atrial Fibrillation: Data from the RACE V Study

Vicente Artola Arita, Martijn E Van De Lande, Neda Khalilian Ekrami, Bao-Oanh Nguyen, Joost M Van Melle, Bastiaan Geelhoed, Ruben R De With, Vanessa Weberndörfer, Ömer Erküner, Hans Hillege, Dominik Linz, Hugo Ten Cate, Henri M H Spronk, Tim Koldenhof, Robert G Tieleman, Ulrich Schotten, Harry J G M Crijns, Isabelle C Van Gelder, Michiel Rienstra

Adapted from Europace, 2023: 15;25(4):1323-1331

Abstract

Aims. The recent 4S-AF scheme has been proposed as a structured scheme to characterise patients with atrial fibrillation (AF). We aimed to assess whether the 4S-AF scheme predicts AF progression in patients with self-terminating AF.

Methods. We analysed 341 patients with self-terminating AF included in the well-phenotyped Reappraisal of Atrial Fibrillation: Interaction between HyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) study. Patients had continuous monitoring with implantable loop recorders or pacemakers. AF progression was defined as progression to persistent or permanent AF or progression of self-terminating AF with >3% burden increase. Progression of AF was observed in 42 patients (12.3%, 5.9% per year). Patients were given a score based on the components of the 4S-AF scheme.

Results. Mean age was 65 (IQR 58-71) years, 149 (44%) were women, 103 (49%) had heart failure, 276 (81%) hypertension and 38 (11%) coronary artery disease. Median CHA_2DS_2-VASc score was 2 (IQR 2-3) and median follow-up was 2.1 (1.5-2.6) years. The average score of the 4S-AF scheme was 4.6±1.4. The score points from the 4S-AF scheme did not predict the risk of AF progression (OR 1.1 95% CI 0.88 – 1.41, C-statistic 0.53). However, excluding the symptoms domain, resulting in the 3S-AF scheme, predicted the risk of progression (OR 1.59 95% CI 1.15 – 2.27, C-statistic 0.62) even after adjusting for sex and age.

Conclusions. In self-terminating AF patients, the 4S-AF scheme does not predict AF progression. The 3S-AF scheme, excluding the symptom domain, may be a more appropriate score to predict AF progression.

Background

Atrial fibrillation (AF) is a progressive disease that generally starts with sporadic, short and self-terminating episodes and progresses to more frequent, long-standing and non-self-terminating episodes.(1) AF Progression is the phenotypical representation of continuous atrial remodelling causing atrial cardiomyopathy.(2,3) AF Progression rates vary in different populations ranging from 2% to 20% per year depending on the population included, follow-up duration and type of monitoring of AF progression.(2,4,5) AF Progression is associated with worse prognosis, including more heart failure hospitalisations(2,6), stroke(7), increased mortality(7) and detriment in quality of life.(8)

The HATCH score was proposed more than a decade ago to determine the risk of AF progression in patients with self-terminating AF.(6) Contrasting results of the HATCH score suggest room for improvement.(4,9) The introduction of continuous rhythm monitoring devices, deep phenotyping and new techniques studying early markers of atrial remodelling may contribute to an improved AF progression risk score.(5,10)

The 2020 European Society of Cardiology (ESC) AF guidelines have proposed an integrated frame, the 4S-AF scheme, which addresses stroke risk, symptom severity, severity of AF burden, and substrate of AF to provide a structured phenotyping of AF patients in clinical practice to guide therapy and assess prognosis.(11-13) Its clinical utility, however, in predicting AF progression remains to be validated. We, therefore aimed to pursue the following two objectives (i) to assess the clinical profile of patients with self-terminating AF using the 4S-AF scheme, (ii) to evaluate whether the 4S-AF scheme score predicts AF progression in deeply phenotyped patients with self-terminating AF.

Methods

Study population. The Reappraisal of Atrial Fibrillation: Interaction between HyperCoagulability, Electrical remodelling, and Vascular Destabilisation in the Progression of AF (RACE V) is an investigator-initiated, prospective, multicentre study performed in the Netherlands, and it is part of the translational RACE V consortium aiming to determine mechanisms and predictors of AF progression. The design and methods have previously been described.(10) In brief, the RACE V included patients aged >18 years with a history of less than

10 years of self-terminating AF and a maximum CHA_2DS_2-VASc score of 5. Patients were eligible if they had at least two documented episodes of self-terminating AF or one documented episode in combination with ≥ two symptomatic episodes suspected of being AF and were willing to undergo implantation of a Medtronic (Minneapolis, USA) Reveal LINQ® implantable loop recorder. Patients who already had Medtronic pacemakers were also eligible if atrial high rate episodes (AHRE) > 190 beats per minute lasting > 6 minutes, qualified as AF episodes, were detected. Patients with other types of pacemakers were not included due to incompatibility of algorithms for AF episode detections. Patients with a history of persistent AF, with AF solely due to transient triggers, currently pregnant, treated with amiodarone, on the waiting list for pulmonary vein isolation (PVI), or with a life expectancy <2.5 years were not eligible to participate. Of the 417 patients included in RACE V, 341 (82%) had ≥ 1 year follow-up of continuous rhythm monitoring as of May 1st 2020 and had available echocardiography data. This subset of patients is considered in the current analysis. The study was performed in concordance with the Declaration of Helsinki. The Institutional Review Board approved the protocol, and the study was registered at Clinicaltrials.gov (identifier NCT02726698). All centres approved the protocol and all patients gave written informed consent.

Clinical assessment. Clinical history, physical examination, symptoms, medication use, and a 12-lead electrocardiogram (ECG) were assessed at baseline. In addition, echocardiography was performed and analysed offline in an anonymised format in a central core lab.(5) In addition to the standard echocardiography measurements, speckle tracking was used to analyse strain deformation of the left atrium (LA) and the left ventricle in a vendor-independent software (TOMTEC-ARENA, Imaging Systems, Germany) *Supplementary table S1 and figure S1*.

Follow-up. All patients were treated according to the ESC AF guidelines.(11) Follow-up visits were performed at 1 and 2.5 years. Patients could consent for 2.5 years continuous rhythm monitoring, until the end of battery of Reveal LINQ, or for 4 years in case patients had a pacemaker.

To collect continuous data on arrhythmias all patients received a home monitoring device (Medtronic Carelink®). Both Reveal LINQ and pacemaker were set to AT/AF detection settings. Episodes of AF ≥2 minutes were automatically detected and later independently validated by five physicians. Arrhythmias with ≥ 182 beats per minute and at least for ≥24 beats were automatically

classified as tachycardia. Arrhythmias with ≤ 30 beats per minute for at least 12 beats were automatically classified as bradycardia. Asystole ≥ 4.5 seconds were automatically classified as pauses.(5)

Covariate and outcome definitions. Patients were classified as having heart failure in the presence of a left ventricular ejection fraction (LVEF) ≤ 45% at baseline or LVEF > 45% with symptoms associated with heart failure (New York Heart Association functional class II or III) or previous hospitalisation for heart failure. Hypertension was defined by a systolic blood pressure ≥140mmHg or diastolic blood pressure ≥90mmHg, or use of antihypertensive medication. Chronic kidney dysfunction was defined as estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73m^2. Obesity was defined as body mass index (BMI) > 30 kg/m^2. Left atrial volume index (LAVI) was categorized as normal (< 29 mL/m^2) or increased (mild, 29-33 mL/m^2; moderate, 34-39 mL/m^2; severe, ≥ 40 mL/m^2). LA strain impairment was defined as having at least one low value of any of the strain phases expressed in strain percentage deformation (reservoir strain < 38% or conduit strain < 21% or contractile strain < 16%) *Supplementary table S1*.(14) AF progression was defined as either one of the following verified in the implantable loop recorder or pacemaker in comparison to the first six months, (1) development of persistent or permanent AF during follow-up, or (2) an increase of > 3% AF burden over six months or total follow-up.(5)

4S-AF scheme assessment. Patients were assessed based on the components of the 4S-AF scheme awarding a maximum points per domain as stated in the 2020 ESC AF guidelines (stroke = 1; symptoms = 2; severity of burden = 2; substrate = 5) to a total maximum of 10 points (**Table 1**).(11,12) Stroke risk was assessed using the CHA_2DS_2-VASc score, awarding one point to the risk of stroke with a CHA_2DS_2-VASc score of one or higher for men, and a score of two or higher for women. Symptoms were assessed using the European Heart Rhythm Association symptom classification, awarding zero points to patients in category I or IIa, one point to patients in category IIb and two points to patients in category III or IV. The severity of AF burden was assessed based on the duration and frequency of the AF episodes. Given that the population in this study had self-terminating AF, all patients were given zero points in this category. Substrate was assessed based on three subdomains I) Comorbidity/cardiovascular risk subdomain; by awarding zero points to patients without comorbidities, one point to patients with any comorbidity (hypertension, heart failure, diabetes mellitus, BMI > 25 kg/m^2, moderate to severe mitral valve regurgitation, or kidney

dysfunction) and two points to patients with more than one comorbidity; II) LA enlargement/dysfunction subdomain; points were awarded to patients based on the presence of atrial enlargement assessed by LAVI values (zero points if LAVI < 29 ml/m^2, one point if LAVI ≥ 29 ml/m^2 and LAVI <40 ml/m^2 ; two points if LAVI ≥ 40 ml/m^2) and one extra point if patients presented LA dysfunction in any of the LA phases assessed by 2D speckle tracking strain (reservoir strain < 38% or conduit strain < 21% or contractile strain <16%) (**Figure 1** and *Supplementary Table S1*) adding to a maximum of 2 points and; III) Age subdomain; awarding a point to patients who were 75 years or older. A modified 4S-AF scheme was derived by eliminating the symptom domain, resulting in a 3S-AF scheme.

Statistical analysis. Continuous variables with normal distribution are expressed as mean ± standard deviation (SD); otherwise as median with interquartile range (IQR). Categorical variables are presented as observed number with percentage. Continuous variables are compared using independent Student's t-test or the Mann-Whitney U-test, as appropriate. Logistic regression was performed to assess association with AF progression. Models were adjusted for sex and age. Interactions were examined in the models. C-statistic was performed to assess the prediction of the score, for both the 4S-AF and the modified 3S-AF scheme. The Likelihood ratio test was used to assess the goodness of fit of a model. The analysis was performed using software R v 3.3.3 (R Foundation for Statistical Computing, Vienna, Austria).

Table 1. Domains, description and definition of the 4S-AF scheme.(12)

domain	score	description	definition
Stroke (*St*)			4S-AF 3S-AF
(max 1 point)	0	low risk	CHA_2DS_2-VASc score = 0 (males) or ≤1 (females)
	1	not low risk, OAC indicated	CHA_2DS_2-VASc score ≥1 (males) or ≥2 (females)
Symptoms (*Sy*) (max 2 points)			4S-AF
	0	no or mild symptoms	EHRA I - IIa
	1	moderate symptoms	EHRA IIb
	2	Severe or disabling symptoms	EHRA III - IV
Severity of AF burden (*Sb*)			4S-AF 3S-AF
(max 2 points)	0	short episodes and infrequent episodes	self-terminating AF or first onset
	1	intermediate duration and/or frequent episodes	persistent
	2	long or frequent episodes	long-standing persistent AF or permanent AF
Substrate (*Su*) (max 5 points)			4S-AF 3S-AF
Comorbidity/CV risk factors* (max 2 points)			
	0	no	no comorbidity/CV risk factor
	1	single	at least one comorbidity/risk factor
	2	multiple	more than one comorbidity/risk factor
LA enlargement/dysfunction (max 2 points)			
LA enlargement	0	no	LAVI <29 ml/m^2
	1	mild-moderate	LAVI ≥29 ml/m^2 and LAVI <40 ml/m2
	2	severe	LAVI ≥40 ml/m^2
LA dysfunction	+1	impairment in LA strain phase	one extra point if any of the following: reservoir strain < 38% or

			conduit strain < 21% or contractile strain <16%)
Age >75 (max 1 point)			
	0	no	≤ 75 years
	1	yes	> 75 years

CV = cardiovascular; EHRA = European Heart Rhythm Association; LA = left atrium; LAVI = left trial Volume index; OAC = oral anticoagulation; The CHA2DS2-VASc score assesses thromboembolic risk. C = congestive heart failure/LV dysfunction, H = hypertension; A2 = age ≥ 75 years; D = diabetes mellitus; S2 = stroke/transient ischemic attack/systemic embolism; V = vascular disease; A = age 65-74 years; Sc = sex category (female sex); *Comorbidities and risk factors were considered as any of the following: hypertension, heart failure, diabetes mellitus; coronary artery disease, BMI > 25 kg/m^2, moderate or severe mitral valve regurgitation and kidney dysfunction (eGFR < 60 ml/min/1.73m^2)

Results

Clinical characteristics. We included 341 (82%) from the 417 patients in RACE V. There were no differences in baseline characteristics between the present analysed group and the ones not included in the analysis (*Supplementary Table S2*). Median age was 65 (58-71) years, 149 (44%) were women, 103 (49%) had heart failure, 276 (81%) had hypertension, and 38(11%) had coronary artery disease **(Table 2**). The majority had a CHA_2DS_2-VASc score ≥ 2 (n = 258, 76%) (**Table 2)**. Median number of comorbidities was 2 (2-3) and 16 (5%) patients had no identified comorbidities.

4S-AF and 3S-AF schemes. The average score of the 4S-AF scheme was 4.6 ± 1.4. The majority had less than half of the maximum possible score (4S-AF score <5, n = 243 [71%]). Of the total score, 20% was explained by the stroke domain, 17% by the symptoms domain, and 67% by the substrate domain (**Figure 1**, **Table 3**). Most of the patients did not have a low stroke risk (n=303, 89% [no progression group n = 262, 88%; progression group n = 41, 98%, p = 0.096]). More than half had multiple comorbidities and/or cardiovascular risk factors (n = 232, 68% [no progression group n = 198, 66%; progression group n = 34, 81%, p = 0.141]). Most had LA enlargement or dysfunction, (n = 292, 86% [no progression group n = 258, 86%; progression group n = 34, 81%]) with more severe dysfunction in patients with progression (p = 0.006) (**Table 3)**.

Table 2. Baseline characteristics of the population

	n = 341
Clinical characteristics	
Age *years*	65 (58 - 71)
Female sex *n (%)*	149 (44)
Total history AF *years*	2.7 (0.7 - 5.0)
Heart failure *n (%)*	103 (49)
HFrEF *n (%)*	6 (2)
HFpEF *n (%)*	97 (46)
Hypertension *n (%)*	276 (81)
Diabetes mellitus *n (%)*	30 (9)
Coronary artery disease *n (%)*	38 (11)
Atherosclerosis* *n (%)*	162 (48)
Ischemic Stroke *n (%)*	16 (5)
Pacemaker *n (%)*	17 (5)
Number of Comorbidities**	2 (2 -3)
Patients without identified comorbidity *n (%)*	16 (5)
CHA_2DS_2-VASc score	2 (2 - 3)
CHA_2DS_2-VASc score *n (%)*	
< 2	83 (24)
≥ 2	258 (76)
EHRA class *n (%)*	
I	33 (10)
IIa	110 (32)
IIb	140 (41)
III	56 (16)
IV	2 (1)
Height *cm*	176 (168 - 184)
Weight *kg*	85 (74 - 97)
BMI *kg/m2*	27 (24 - 30)
Obesity *BMI>30 n (%)*	93 (27)
Waist circumference *cm*	100 (93 - 108)
Systolic blood pressure *mmHg*	133 (125 - 145)
Diastolic blood pressure *mmHg*	80 (74 - 85)
Laboratory results	
eGFR *mL/min x 1.73m^2*	81 (70 - 90)
Electrocardiogram	
PR-interval *ms*	165 (150 - 186)
QRS-interval *ms*	96 (88 - 102)
Medications *n (%)*	
β-blocker	172 (51)
Verapamil/Diltiazem	61 (18)
Digoxin	6 (2)
Antiarrhythmic drugs	94 (28)
ACE-inhibitor	64 (19)

Angiotensin Receptor Blocker	68 (20)
Statin	120 (35)
Diuretic	52 (15)
Anticoagulant	235 (69)
Vitamin K antagonist	49 (14)
NOAC	186 (55)
Echocardiographic variables	
Left atrial volume *mL*	58 (48 - 75)
Left atrial volume index (mL/m^2)	29 (23 - 36)
Left atrial reservoir function *%*	36.0 (29.2 - 42.8)
Left atrial contractile function *%*	16.3 (12.7 - 21.6)
Left atrial conduction function *%*	19.3 (13.9 - 24.4)
Left ventricular ejection fraction *%*	50 ± 8
Left ventricular mass *g*	150 (130 - 181)
Left ventricular mass index *g/m2*	76 (67 - 88)
Left ventricle global longitudinal strain *%*	-14.0 ± 2.4

Data are presented as number of patients n (%), mean (standard deviation), or median (interquartile range).

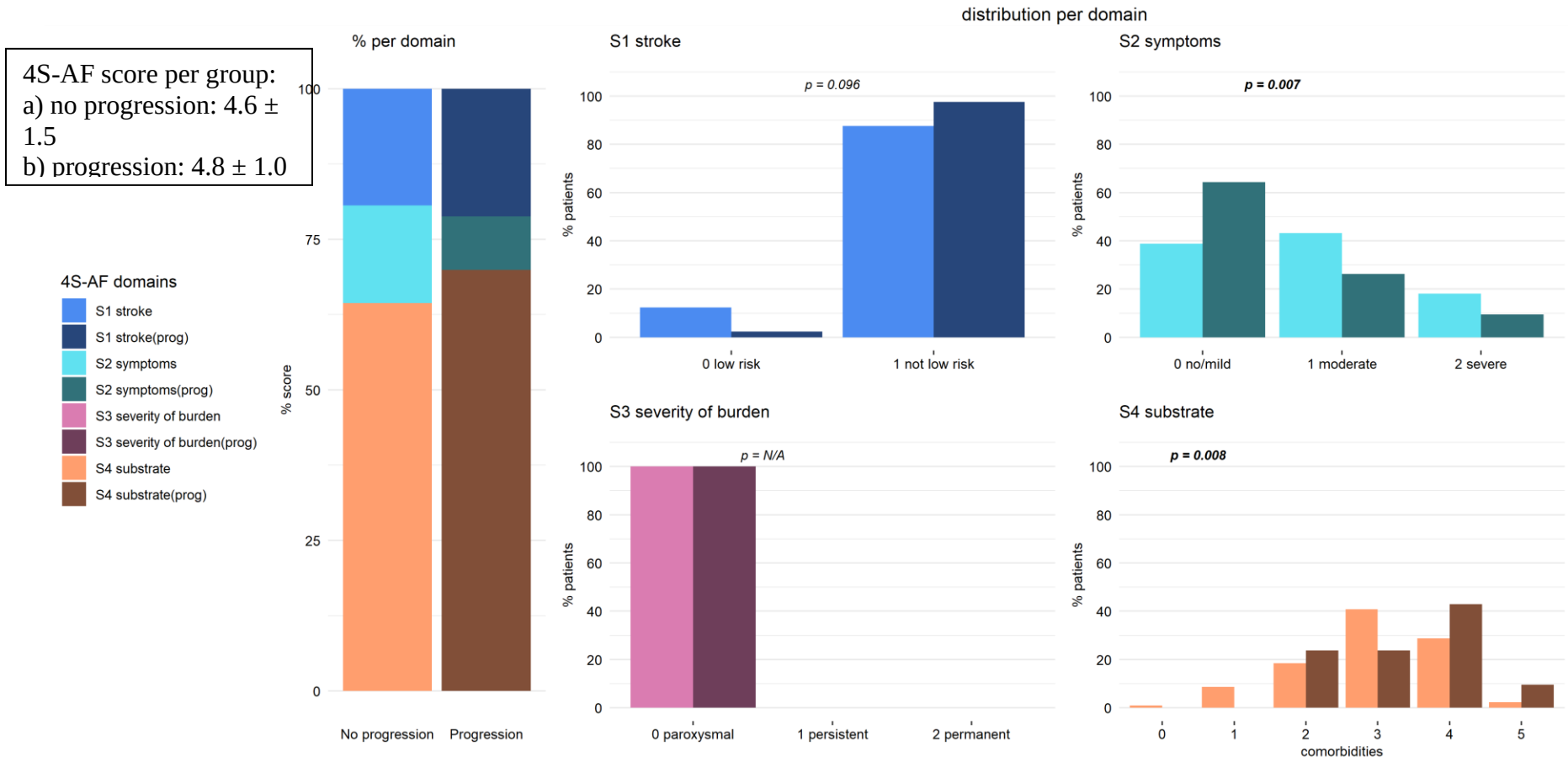
*Atherosclerosis is presence of history of myocardial infarction, percutaneous coronary intervention, coronary artery bypass graft, ischemic cerebral infarction, peripheral vascular disease, Agatston score > 400 or plaque.

**The number of comorbidities was calculated by awarding a point to each of the following comorbidities, hypertension, heart failure, age > 65 years, diabetes mellitus; coronary artery disease, BMI > 25kg/m^2, moderate or severe mitral valve regurgitation and kidney dysfunction (eGFR < 60 ml/min/1.73m^2);

The CHA2DS2-VASc score assesses thromboembolic risk. C = congestive heart failure/LV dysfunction, H = hypertension; A2 =age ≥75 years; D = diabetes mellitus; S2 = stroke/transient ischemic attack/systemic embolism; V = vascular disease; A = age 65 - 74 years; Sc = sex category (female sex);

ACE = Angiotensin-converting enzyme; AF = atrial fibrillation; BMI = body mass index; eGFR = estimated glomerular filtration rate; EHRA=European Heart Rhythm Association symptoms classification; HFpEF = heart failure with preserved ejection fraction ; HFrEF = heart failure with reduced ejection fraction; NOAC = novel oral anticoagulation.

Figure 1. Characterization of patients with self-terminating AF using the 4S-AF scheme grouped by progression status



The bar "% per domain" shows the percentage of score explained by each domain of the 4S-AF scheme. Each of the panels shows the percentage of patients characterized using each of the domains in the 4S-AF scheme grouped by progression status. Darker colours in the bars represent the group of patients with atrial fibrillation progression. P values represent the difference in patient distribution per progression status in each of the 4S-AF scheme domains.

4S-AF scheme = maximum score per domain (stroke = 1; symptoms = 2; severity of AF burden = 2; substrate = 5) to a total maximum of 10 points. AF = atrial fibrillation

Table 3. Characterisation using the 4S-AF scheme in the total patient population and per progression status

	total population n=341	no progression n=299	progression n=42	p value
4S-AF scheme score	4.6 ± 1.4	4.6 ± 1.5	4.8 ± 1.0	0.401
4S-AF scheme score <5 points *n (%)**	243 (71)	121 (71)	31 (74)	0.835
Domain scores				
S1 stroke	1 (1 - 1)	1 (1 - 1)	1 (1 - 1)	0.054
S2 symptoms	1 (0 - 1)	1 (0 - 1)	0 (0 - 1)	**0.003**
S3 severity of AF burden	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)	N/A
S4 substrate	3 (2 - 4)	3 (2 - 4)	4 (3 - 4)	**0.014**
Percentage of score explained by each domain **				
S1 stroke	20 (17-25)	20 (17 – 25)	20 (17 - 25)	0.181
S2 symptoms	17 (0 – 25)	17 (0 – 25)	0 (0 - 17)	**0.002**
S3 severity of AF burden	0	0	0	N/A
S4 substrate	67 (57 – 75)	67 (57 - 75)	75 (67 - 80)	**0.002**
Characterization per each domain				
S1 stroke				0.096
low risk	38 (11)	37 (12)	1 (2)	
not low risk, anticoagulation indicated	303 (89)	262 (88)	41 (98)	
S2 symptoms *n (%)*				**0.007**
no or mild symptoms	143 (42)	116 (39)	27 (64)	
moderate symptoms	140 (41)	129 (43)	11 (26)	
severe or disabling symptoms	58 (17)	54 (18)	4 (10)	
S3 severity of burden *n (%)*				N/A
paroxysmal AF or first onset	341 (100)	299 (100)	42 (100)	
persistent	0 (0)	0 (0)	0 (0)	
long-standing persistent AF or permanent AF	0 (0)	0 (0)	0 (0)	
S4 substrate *n (%)*				**0.008**
0 points	3 (1)	3 (1)	0 (0)	
1 points	26 (7)	26 (8)	0 (0.0)	
2 points	65 (19)	55 (18)	10 (24)	
3 points	132 (39)	122 (41)	10 (24)	
4 points	104 (31)	86 (29)	18 (43)	
5 points	11 (3)	7 (2)	4 (9)	
Comorbidities/CV risk factors n (%)*				0.141
no	23 (6)	22 (7)	1 (2)	
single	86 (25)	79 (27)	7 (17)	
multiple	232 (68)	198 (66)	34 (81)	
LA enlargement/dysfunction *n (%)*				**0.006**
no	49 (14)	41 (13.7)	8 (19.0)	

mild-moderate	143 (42)	135 (45.2)	8 (19.0)	
severe	149 (44)	123 (41.1)	26 (61.9)	
Age >75 *n (%)*				0.148
no	309 (91)	274 (92)	35 (83)	
yes	32 (9)	25 (8)	7 (17)	

* 5 points is the half of scale based on the maximum score of the 3S-AF scheme
** calculated by dividing the points of the domain by total score the scheme
4S-AF scheme = maximum score per domain (stroke = 1; symptoms = 2; severity of AF burden = 2; substrate = 5) to a total maximum of 10 points. AF = atrial fibrillation.
Data are presented as number of patients n (%), mean (standard deviation), or median (interquartile range).
CV=cardiovascular; LA=left atrium

The average score of the 3S-AF scheme, the 4S-AF scheme without the symptom domain, was 3.9 ± 1.2. The majority had less than half of the maximum possible score (3S-AF score < 4, n = 226 [66%]). From the total score, 25% was explained by the stroke domain and 75% by the substrate domain (**Table 4).**

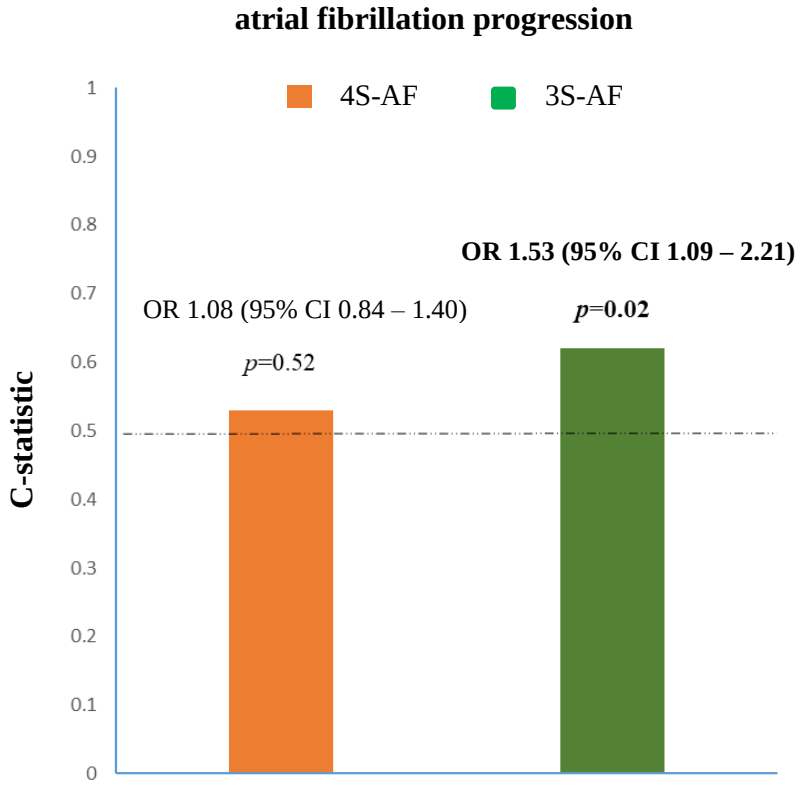
Table 4. Characterization using the 3S-AF scheme in the total patient population and per progression status

	total population n=341	**no progression n=299**	**progression n=42**	**p value**
3S-AF scheme score	3.9 ± 1.2	3.8 ± 1.2	4.4 ± 1.0	**0.006**
3S-AF scheme score <4 points *n (%)**	226 (66)	206 (69)	20 (49)	**0.011**
Percentage of score explained by each domain **				
S1 stroke	25 (20 - 25)	25 (20 - 25)	20 (20 - 25)	0.587
S3 severity of AF burden	0	0	0	N/A
S4 substrate	75 (75 – 80)	75 (75 – 80)	80 (75 – 80)	0.587

* 4 points is the half of scale based on the maximum score of the 3S-AF scheme
** calculated by dividing the points of the domain by total score the scheme
3S-AF scheme = maximum score per domain (stroke = 1; severity of AF burden = 2; substrate = 5) to a total maximum of 8 points. AF = atrial fibrillation.
Data are presented as number of patients n (%), mean (standard deviation), or median (interquartile range).

Progression. Median follow-up of continuous rhythm monitoring was 2.1 (1.5-2.6) years. AF progression was seen in 42 patients (12.3%, 5.9% per year). Two thirds of the patients progressed from self-terminating AF to persistent or permanent AF (n=28, 67%). Few patients had severe AF symptoms (n = 58, 17%) and patients who developed progression had less AF symptoms (p = 0.007) (**Figure 1)**.

Figure 2. Prediction of progression in patients with self-terminating AF using the 4S-AF and the 3S-AF scheme scores



Dotted line represents minimum value for C-statistic to be considered predictive. P-values represent significance of the association between each scheme and the outcome based on logistic regression after adjusting for sex and age. 4S-AF scheme = maximum score per domain (stroke = 1; symptoms = 2; severity of AF burden = 2; substrate = 5) to a total maximum of 10 points
3S-AF scheme = same domains as in 4S-AF scheme without the symptoms domain adding up to a total maximum of 8 points.
AF = atrial fibrillation

There were no differences in the 4S-AF scheme scores between patients without and with progression (p = 0.401) (**Table 3)**. However, when using the 3S-AF scheme, patients without progression were more often in the lower half of maximum possible score (3S-AF score < 4, n =

206 [69%] versus n = 20 [49%], p = 0.011) and had a lower total score (3.8 ± 1.2 versus 4.4 ± 1.0, p=0.006) as compared to those with progression (**Table 4).**

The 4S-AF scheme score was not associated with AF progression (OR 1.11 95% CI 0.88 – 1.41, p = 0.40). The 3S-AF scheme score, without the symptom domain, was associated with AF progression (OR 1.59 95% CI 1.15 – 2.27, p = 0.007); this association persisted after adjusting for sex and age (*Supplementary Table S3).* The 3S-AF scheme showed a significant predictive value of AF progression (C-statistic 0.62, 95% CI 0.53 – 0.71) (**Figure 2** and *Supplementary Table S3*). There were no significant interactions for sex and age for any of 4S-AF or 3S-AF schemes (*Supplementary Table S3*). From the individual domains of the 4S-AF scheme, an increase in the substrate domain score was associated with progression (OR 1.62 95% CI 1.14 – 2.36, p = 0.010). This association persisted after adjusting for sex and age. The association with progression within the substrate domain was mainly driven by comorbidities and/or cardiovascular risk factors (OR 1.48 95% CI 1.09 – 2.02, p = 0.010) *Supplementary Table S3.* When comparing the models associated with progression, the 3S-AF scheme performed better than the substrate domain alone (likelihood ratio test p < 0.001).

Discussion

In a population of well-phenotyped self-terminating AF patients included in the RACE V study, we characterised patients using the 4S-AF scheme. The main findings are 1) the 4S-AF scheme was not associated with progression in patients; 2) the 3S-AF scheme, a modified scheme that excludes the symptoms domain from the 4S-AF scheme, predicted AF progression in patients with self-terminating AF albeit with low predictive value; and 3) the substrate domain explained most of the 4S-AF scheme score, driven mainly by comorbidities.

Characterisation of AF patients. Characterisation of AF patients according to the 4S-AF scheme is based on the stroke risk, symptoms, severity of AF, and substrate. The latter is composed of a number of risk factors, comorbidities, atrial remodelling and older age(12). In this analysis, the median 4S-AF score was lower than reported in previous studies characterising patients with AF. (13,15-17) One of the reasons may be that we only included younger patients with less comorbidities and with paroxysmal self-terminating AF.

According to the 4S-AF scheme definition ,the stroke risk was not low. However, the total CHA_2DS_2-VASc score was lower in the current analysis in comparison to previous studies. For example, Guo *et al* evaluated patients with AF in the Optimal Thromboprophylaxis in Elderly Chinese Patients with Atrial Fibrillation (ChiOTEAF) registry finding patients with slightly higher CHA_2DS_2-VASc score (median 3, IQR 2-5).(16) Similarly, Rivera-Caravaca *et al* and Ding *et al* characterised AF patients using the 4S-AF scheme in the EORP-AF Long-Term General Registry, reporting both slightly higher CHA_2DS_2-VASc score (median 3, IQR 2-4 for both).(13,15) Malavasi *et al* reported a CHA_2DS_2-VASc score with median 2 (IQR 2-5) from the Fibrillazione Atriale in Modena (FAMo) cohort.(17) Higher scores of CHA_2DS_2-VASc may indicate a population with a higher risk of morbidity and mortality.

The severity of symptoms, the second S, was low in the current population. This might be explained by the fact that we excluded symptomatic patients who may want to undergo PVI because of symptoms. A similar proportion of patients had severe symptoms in the EORP-AF Long-Term General Registry (18%) and explained a similar percentage of the total 4S-AF scheme score (15%)(13). On the other hand, the percentage of patients with severe symptoms was higher in FAMo cohort (26%). It was not possible to determine the percentage of the 4S-AF scheme score explained by the symptoms domain.(17)

Since we only included patients with self-terminating AF, the third S, all patients had the same 4S-AF scheme score under this domain. Both the EORP-AF Long-Term General Registry and the FAMo cohort included less than half of the patients with self-terminating AF.(13,15,17) The scores in these two studies were therefore increased by the points awarded to patients with more advanced stages of AF. In the EORP-AF Long-Term General Registry, 18% of the 4S-AF scheme score was explained by the severity of AF burden.

The substrate, the fourth S, depends on the number of comorbidities, atrial remodelling and age. We included relatively young patients with a limited number of comorbidities. However, comorbidities were identified in almost all of our patients. The substrate domain explained more than half of the 4S-AF scheme score in the current study population, driven mainly by comorbidities and risk factors for cardiovascular disease. This is different from the burden of comorbidities reported from the FAMo cohort (67% with zero to two comorbidities); however, the comorbidities included into the definition may have differed.(17) The cardiovascular risk factors

(hypertension, hypercholesterolaemia, diabetes mellitus, coronary artery disease, heart failure and coronary artery disease) reported in the EORP-AF Long-Term General Registry occurred less frequently (12% of patients without cardiovascular risk factors) in comparison to the current study population, with similar definitions.(13) In the latter study, the substrate domain explained less than half of the 4S-AF scheme score whereas in the current analysis it explained most of the score, predominantly in patients with AF progression. The higher reported number of cardiovascular risk factors in the current analysis may be due to more comprehensive deep phenotyping of our patients.(18)

AF Progression. AF progression ranges from 2% to 20% per year(2,4,19) and its progression depends on the AF population investigated, duration of follow-up and type of monitoring of AF progression. We observed lower progression rates as compared to data from 42 meta-analysed studies assessing progression in patients with self-terminating AF.(19) In comparison to previous studies, patients in our study were followed for a relatively short follow-up time and were a relatively healthy population.(19)

In contrast to previous studies, we used continuous monitoring for the detection of AF progression.(5) Most of the data on progression come from registry studies using electrocardiographic or Holter monitoring, or alternatively only symptoms to assess AF progression. In previous studies, follow-up was often longer and AF patients were at higher risk. We included only paroxysmal AF patients. This all may explain differences in AF progression rates between our data and previous studies.(19) In addition, better current handling of comorbidities, for example high percentage of patients with hypertension but with a low average blood pressure in our population, may have reduced the progression rate.(4,11)

We showed that a modified scheme without the symptoms domain, the 3S-AF scheme, was associated with AF progression in the current study. However, the predictive value was low in spite of having patients phenotyped in depth. This association, however, prevailed after adjusting for sex and age. In line with our finding that symptoms seem less relevant for the prediction of AF progression, Ding *et al* also did not find an association between the symptoms domain and all-cause mortality nor for cardiovascular mortality.(13) Needless to say, symptoms are difficult to interpret and depend on the type of patients included as well as the way they are interpreted by the health care professional. To implement symptoms into a score assessing outcome may therefore be

difficult. In our study patients who developed progression had less AF symptoms. The latter may be explained by the institution of a more aggressive rhythm control approach in patients with more severe symptoms. Alternatively, the absence of symptoms could have led to less consultation and therefore less treatment, allowing atrial remodelling to progress.(2,20) A scheme omitting the symptoms severity could probably be a better alternative to predict AF progression. The HATCH score was proposed more than a decade ago to determine the risk of AF progression,(6) however validating results are contrasting.(9) Schnabel *et al* showed in PREFER (PREvention oF thromboembolic events-European Registry) study that the use of the individual components rather than the HATCH score to predict AF progression performed significantly better (C-statistic 0.64 versus 0.52, p = 0.0001) but still with a low predictive value.(2) The CHA_2DS_2VASc score has also shown to predict progression.(4) Since the CHA_2DS_2VASc score includes comorbidities and cardiovascular risk factor components it is expected to be related to AF progression.(4,9)

The 4S-AF is a practical scheme to help characterise patients with AF. However, this scheme remains to be validated. It is a dynamic score that warrants periodic reassessment in all its domains.(12) Incidence of comorbidities included in both the stroke and substrate domain may change punctuation within the domain, may cause progression of atrial remodelling and increasing risk of AF progression.(4) In our current study, unfortunately, the 4S-AF scheme was only assessed at baseline. For assessing the risk on AF progression, a modified 3S-AF scheme may be more informative since symptoms seem not to be relevant for AF progression, in contrast to the severity of the substrate. Further validation of modified 3S-AF scheme to predict AF progression will be needed to prove its utility.

Limitations. The current study involves only patients with self-terminating AF and the results cannot be generalised to the whole AF spectrum. Second, it is an observational study and treatment was at the discretion of the treating physician, having potential impact on AF progression. It was not possible to adjust for treatment strategy. Third, given the relatively healthy population with the majority having a low score in the scheme and the short follow-up time, less progression occurred. Fourth, we validated the score as proposed without making additions, such as P wave intervals; neither did we evaluate dynamic changes in the score, such as comorbidities incidence, which could have modified the score. Among the strengths of the current analysis, AF patients included were in-depth phenotyped and had continuous monitoring providing the opportunity to assess more

accurately AF episodes and burden. Moreover, speckle tracking was used, in place of volume measures, to assess atrial function within the substrate domain. This may have led to the identification of early atrial remodelling as it is less affected by loading conditions in comparison to volumetric methods.

Conclusion

In self-terminating AF patients, the 4S-AF scheme does not predict AF progression. The 3S-AF scheme, however, excluding the symptom domain, may be a more appropriate score to predict AF progression.

References

(1) Padfield GJ, Steinberg C, Swampillai J, Qian H, Connolly SJ, Dorian P, et al. Progression of paroxysmal to persistent atrial fibrillation: 10-year follow-up in the Canadian Registry of Atrial Fibrillation. Heart Rhythm 2017; 14(6):801-807.

(2) Schnabel RB, Pecen L, Engler D, Lucerna M, Sellal JM, Ojeda FM, et al. Atrial fibrillation patterns are associated with arrhythmia progression and clinical outcomes. Heart 2018; 104(19):1608-1614.

(3) Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. Europace 2016; 18(10):1455-1490.

(4) Heijman J, Luermans JGLM, Linz D, Van Gelder IC, Crijns HJGM. Risk Factors for Atrial Fibrillation Progression. Card Electrophysiol Clin 2021; 13(1):201-209.

(5) Nguyen BO, Weberndorfer V, Crijns HJGM, Geelhoed B, Ten Cate H, Spronk H, et al. Prevalence and determinants of atrial fibrillation progression in paroxysmal atrial fibrillation. Heart 2022.

(6) De Vos CB, Pisters R, Nieuwlaat R, Prins MH, Tieleman RG, Coelen RJ, et al. Progression from paroxysmal to persistent atrial fibrillation clinical correlates and prognosis. J Am Coll Cardiol 2010; 55(8):725-731.

(7) Steinberg BA, Hellkamp AS, Lokhnygina Y, Patel MR, Breithardt G, Hankey GJ, et al. Higher risk of death and stroke in patients with persistent vs. paroxysmal atrial fibrillation: results from the ROCKET-AF Trial. Eur Heart J 2015; 36(5):288-296.

(8) Dudink EAMP, Erküner Ö, Berg J, Nieuwlaat R, De Vos CB, Weijs B, et al. The influence of progression of atrial fibrillation on quality of life: a report from the Euro Heart Survey. Europace 2018; 20(6):929-934.

(9) Deng H, Bai Y, Shantsila A, Fauchier L, Potpara TS, Lip GYH. Clinical scores for outcomes of rhythm control or arrhythmia progression in patients with atrial fibrillation: a systematic review. Clin Res Cardiol 2017; 106(10):813-823.

(10) De With RR, Erkuner O, Rienstra M, Nguyen BO, Korver FWJ, Linz D, et al. Temporal patterns and short-term progression of paroxysmal atrial fibrillation: data from RACE V. Europace 2020; 22(8):1162-1172.

(11) Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2021; 42(5):373-498.

(12) Potpara TS, Lip GYH, Blomstrom-Lundqvist C, Boriani G, Van Gelder IC, Heidbuchel H, et al. The 4S-AF Scheme (Stroke Risk; Symptoms; Severity of Burden; Substrate): A Novel

Approach to In-Depth Characterization (Rather than Classification) of Atrial Fibrillation. Thromb Haemost 2021; 121(3):270-278.

(13) Ding WY, Proietti M, Boriani G, Fauchier L, Blomström-Lundqvist C, Marin F, et al. Clinical utility and prognostic implications of the novel 4S-AF scheme to characterize and evaluate patients with atrial fibrillation: a report from ESC-EHRA EORP-AF Long-Term General Registry. Europace 2022; 24(5):721-728.

(14) Pathan F, D'Elia N, Nolan MT, Marwick TH, Negishi K. Normal Ranges of Left Atrial Strain by Speckle-Tracking Echocardiography: A Systematic Review and Meta-Analysis. J Am Soc Echocardiogr 2017; 30(1):59-70.e8.

(15) Rivera-Caravaca JM, Piot O, Roldán-Rabadán I, Denis A, Anguita M, Mansourati J, et al. Characterization of atrial fibrillation in real-world patients: testing the 4S-AF scheme in the Spanish and French cohorts of the EORP-AF Long-Term General Registry. Europace 2022; 24(2):202-210.

(16) Guo Y, Imberti JF, Kotalczyk A, Wang Y, Lip GYH, ChiOTEAF Registry Investigators. 4S-AF scheme and ABC pathway guided management improves outcomes in atrial fibrillation patients. Eur J Clin Invest 2022; 52(6):e13751.

(17) Malavasi VL, Vitolo M, Colella J, Montagnolo F, Mantovani M, Proietti M, et al. Rhythm- or rate-control strategies according to 4S-AF characterization scheme and long-term outcomes in atrial fibrillation patients: the FAMo (Fibrillazione Atriale in Modena) cohort. Intern Emerg Med 2022; 17(4):1001-1012.

(18) Wyse DG, Van Gelder IC, Ellinor PT, Go AS, Kalman JM, Narayan SM, et al. Lone Atrial Fibrillation. J Am Coll Cardiol 2014; 63(17):1715-1723.

(19) Blum S, Meyre P, Aeschbacher S, Berger S, Auberson C, Briel M, et al. Incidence and predictors of atrial fibrillation progression: A systematic review and meta-analysis. Heart Rhythm 2019;16(4):502-510.

(20) Kirchhof P, Camm AJ, Goette A, Brandes A, Eckardt L, Elvan A, et al. Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. N Engl J Med 2020; 383(14):1305-1316.

Chapter 7

Increased plasma levels of NT-proBNP, Troponin T and GDF-15 are driven by persistent AF and associated comorbidities: Data from the AF-RISK study

L.M.G. Meems, V. Artola Arita, M. Velt,a E.A.M.P. Dudink, H.J.G.M. Crijns, I.C. Van Gelder, and M. Rienstra,

Adapted from International Journal of Heart & Vasculature. 2022: 8;39:100987.

Abstract

Atrial fibrillation (AF) is a progressive disease, and early recognition and management may reflect an important strategy to reduce its disease burden. In this study, we evaluated plasma levels of three biomarkers - N-terminal pro-brain natriuretic peptide (NTproBNP), Troponin-T, and growth differentiation factor-15 (GDF-15) - in patients with paroxysmal AF (pAF) (≤7 days of continuous AF, n=323) and persistent AF ((AF duration >7 days and <1 year, n=84) using patients from *AF RISK* study (NCT01510210). In this AF-RISK sub-study, patients with persistent AF experienced more symptoms (higher European Heart Rhythm Association class ($p<0.001$)), had a higher comorbidity burden ($p<0.001$), and had more unfavorable echocardiographic parameters ($p<0.001$). All three biomarker levels were significantly higher in patients with persistent AF as compared to those with pAF ($p<0.001$). Multivariate linear regression analyses showed that age (beta-coefficient for NTproBNP: 0.21; GDF-15: 0.41; Troponin-T: 0.23) and CHA_2DS_2-VASc (beta-coefficient for NTproBNP: 0.20; GDF-15: 0.25; Troponin-T: 0.27) were determinants of all three biomarkers, and that persistent AF determined NTproBNP (beta-coefficient: 0.34), but not Troponin-T and GDF-15. More detailed analysis of CHA_2DS_2-VASc score showed that for all three biomarkers age, coronary artery disease and heart failure were determinants of plasma biomarkers levels, whereas sex determined NTproBNP and Troponin T, and hypertension determined NTproBNP and GDF15. Overall, this study therefore suggests that in AF, Troponin T and GDF15, and especially NTproBNP could be used to detect those patients with more persistent form of AF that may warrant more aggressive treatment of AF and concomitant comorbidities. Future studies, however, are essential to evaluate if more aggressive AF treatment and risk factor management will reduce disease progression and holds a novel therapeutic intervention to reduce the burden of AF.

Atrial fibrillation (AF) is the most common cardiac arrhythmia, with still increasing incidence and prevalence rate. It is expected that 6-12 million people will suffer this condition in 2050 in the US, and 17.9 million people in Europe by 2060 (1). Patients with AF are known to have increased mortality and morbidity rates, including the development of dementia, heart failure (HF) and stroke (2,3), and this causes an important economic and health care burden. AF is a progressive disease, and early recognition and management of increasing AF burden may reflect an important strategy to reduce its disease burden (4).

In this study, we therefore evaluated plasma levels of three biomarkers in patients with paroxysmal AF (pAF) and persistent AF (persistent AF) using patients from *Identification of a risk profile to guide atrial fibrillation therapy (AF RISK)* study (NCT01510210). Detailed study design and outcomes have been previously described (5). In short, AF RISK was a multicenter, prospective, observational study, including patients aged ≥18 years, with pAF (total AF history <2 years, or total AF history <3 years in case of ≤2 AF episodes of ≤48 hours per month terminating spontaneously) or persistent AF (total AF history <2 years, and total persistent AF duration >7 days and <1 year) in whom a rhythm control strategy was preferred. AF RISK was performed in compliance of the Declaration of Helsinki. The institutional review board approved study protocol and all patients gave written informed consent. After inclusion, all patients underwent baseline assessment including peripheral venous blood sampling for biomarker analyses. Blood samples were processed and EDTA-plasma samples were stored at -80C. For present analysis, we included pAF patients who had sinus rhythm during blood sampling (n=323) and included persistent AF patients who were known with persistent AF, but were in sinus rhythm at baseline visit, either by scheduled cardioversion or spontaneous conversion, and had atrial fibrillation during blood sampling (n=84). Patients with pAF and atrial fibrillation during blood sampling were excluded from this sub-study (n=8).

We analyzed plasma levels of two traditional cardiovascular biomarkers - N-terminal pro-brain natriuretic peptide (NTproBNP) and Troponin-T – and one non-traditional cardiovascular biomarker -growth differentiation factor-15 (GDF-15).Troponin-T, NTproBNP and GDF-15 were measured at baseline using electrochemiluminescence by a Cobase 411 analyser using a standard Roche Diagnostics GmbH method.

Descriptive data of continuous variables are presented as mean± standard deviation in normally distributed data or median (interquartile range) in non-normally distributed data. Categorical variables are presented as numbers with percentages. Differences between groups were evaluated by Student's t-test or Mann–Whitney U test for continuous variables, and Chi-square test was used for categorical variables. A p-value <0.05 was considered statistically significant. Linear regression analyses were performed to determine risk indicators of NTproBNP, GDF15 and Troponin T levels. Biomarkers were logarithmically transformed and standardized to realize constant variance. All patient characteristics and biomarkers were tested using a stepwise approach: univariate variables with $p<0.1$ were investigated in a multivariate model. In the multivariate model, a variable was excluded when $p\geq0.05$. Since CHA_2DS_2-VASc is a surrogate parameter that is a composite score of several components (sex, age, hypertension, diabetes mellitus, coronary artery disease, HF, peripheral artery disease, thromboembolic events) association between three different biomarkers and individual components were tested separately for each biomarker using linear regression analysis. Statistical analyses were performed using R package (Version 3.1.3; R Foundation for Statistical Computing, Vienna, Austria) or STATA (Version 14.2; StataCorp LLC; Tx, USA).

In this AF-RISK sub-study, patients with pAF were younger, more often women and had lower CHA_2DS_2-VASc scores (**Table 1**). Patients with persistent AF experienced more symptoms as reflected by higher European Heart Rhythm Association (EHRA) class ($p<0.001$). They also had mildly impaired kidney function and suffered from more comorbidities, including hypertension, congestive heart failure and a history of coronary artery disease (**Table 1**). Transthoracic echocardiography showed higher left ventricular ejection fraction (LVEF) in patients with pAF (LVEF %, median [IQR]: 58 [58-60]) and smaller left atrial volume indices (LAVI ml/m2, median [IQR]: 32 [25-39]) as compared to those patients with persistent AF (LVEF %, median [IQR]: 55 [50-58], $p<0.001$; LAVI ml/m2, median [IQR]: 39 [31-47], $p<0.001$).

All three biomarker levels were significantly higher in patients with persistent AF as compared to those with pAF: GDF-15, persistent AF: 1152 pg/mL [843-1647] versus pAF: 882 pg/mL [663-1245], $p<0.001$; NTproBNP, persistent AF: 709 pg/mL [321-1441] versus pAF: 100 pg/mL [46-228], $p<0.001$; Troponin T, persistent AF: 8 pg/mL [5-13] versus pAF: 5 pg/mL [3-9], $p<0.001$ (**Table 1**).

Table 1. Baseline characteristics of patients with paroxysmal AF and persistent AF.

	paroxysmal AF n = 323	persistent AF n = 84	p value
Age (years) (SD)	58 ± 12	63 ± 9	P < 0.001
Female sex (%)	130 (40)	19 (23)	P < 0.01
Blood pressure (mmHg)			
systolic	131 ± 18	129 ± 17	P = NS
diastolic	78 ± 9	80 ± 12	P = NS
Smoking (%)	41 (13)	17 (22)	P = NS
Diabetes Mellitus (%)	32 (9)	9 (11)	P = NS
Hypertension (%)	146 (45)	51 (61)	P < 0.05
Hypercholesterolemia (%)	121 (38)	35 (42)	P = NS
Chronic HF (%)	17 (5)	24 (29)	P < 0.001
CAD (%)	18 (6)	17 (20)	P < 0.001
TIA/CVA (%)	20 (6)	6 (7)	P = NS
EHRA class (%)			
I	101 (31)	19 (23)	P < 0.001
II	177 (55)	46 (45)	
III	44 (14)	27 (32)	
CHA_2DS_2VASc	1 [1–2]	2 [1–3]	P < 0.01
eGFR (ml/min/1,73 m2)	86 [76–100]	75 [59–92)	P < 0.001
LVEF (%)	58 [55–60]	55 [50–58]	P < 0.001
LVEDD (mm)	49 [45–53]	50 [45–55]	P = NS
LAVI (ml/m2)	32 [25–39]	39 [31–47]	P < 0.001
Concomitant medication (%)			
Beta-blockers	186 (58)	59 (70)	P < 0.05
ACEi/ARB	131 (40)	46 (56)	P < 0.05
Ca-antagonists	67 (21)	17 (20)	P = NS
Digoxin	7 (2)	11 (13)	P < 0.001
Class IAAD	35 (11)	2 (2)	P < 0.05
Class IIIAAD	19 (6)	7 (8)	P = NS
(N)OAC	194 (60)	78 (93)	P < 0.001
Statin	93 (29)	33 (39)	P = NS
Diuretics	51 (16)	31 (37)	P < 0.001
NTproBNP (pg/mL)	100 [46–228]	709 [321–1441]	P < 0.001
Troponin T (pg/mL)	5 [3–9]	8 [5–13]	P < 0.001
GDF15 (pg/mL)	882 [663–1245]	1152 [843–1647]	P < 0.001

Results are presented as mean ± standard deviation; median [Interquartile ranges] in case of continuous data. For categorical data results are presented as percentages. $P < 0.05$ is considered significant. Differences in continuous data were tested by Student's t-test or Mann-Whitney U test depending on normality of data. Differences in categorical data were tested by $\chi 2$-test. HF = heart failure; CAD = coronary artery disease; TIA = transient ischemic attack; CVA = cerebrovascular accident; EHRA = European heart rhythm association; eGFR = estimated glomerular filtration rate; LVEF = left ventricular ejection fraction; LVEDD = left ventricular end diastolic dimension; LAVI = left atrial volume index; ACEi = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; Ca = calcium; AAD = anti arrhythmic drug; OAC = oral anticoagulant; NOAC = novel oral anticoagulant.

Table 2. Overview of risk indicators per biomarker.

NTproBNP	GDF15	Troponin T
Age	Age	Age
Sex	Smoking	Sex
EHRA category	LVEF (%)	
CHA_2DS_2VASc	CHA_2DS_2VASc	CHA_2DS_2VASc
- Age	- Age	- Age
- Sex	- Hypertension	- Sex
- Hypertension	- Diabetes mellitus	- Coronary artery disease
- Coronary artery disease	- Coronary artery disease	- Chronic heart failure
- Chronic heart failure	- Chronic heart failure	
Persistent AF		
LAVI (mL/m^2)		
LVEF (%)		

EHRA = European heart rhythm association; LAVI = left atrial volume index; LVEF = left ventricular ejection fraction; AF = atrial fibrillation.

Multivariate linear regression analyses showed that age and CHA_2DS_2-VASc are determinants of all three biomarkers, but that other determinants were biomarker-specific. For example, NTproBNP is determined by age, CHA_2DS_2-VASc, increasing EHRA category, LAVI LVEF and presence of persistent AF; GDF15 is determined by age, CHA_2DS_2-VASc, smoking and LVEF; Troponin-T is determined by age, CHA_2DS_2-VASc and sex (**Table 2**). Since CHA_2DS_2-VASc is a composite score of several variables components (sex, age, hypertension, diabetes mellitus,

coronary artery disease, HF, peripheral artery disease, pulmonary embolism or deep venous thrombosis, or cerebrovascular incident) we analyzed the effect of individual components per biomarker in more detail and observed that with regards to CHA_2DS_2-VASc score NTproBNP is determined by age, sex, hypertension, coronary artery disease and HF, that GDF15 is determined by age, hypertension, diabetes mellitus, coronary artery disease and HF, and that Troponin T is determined by age, sex, coronary artery disease and HF. In spite of, the determining effect of CHA_2DS_2-VASc,for all biomarkers, there are some small biomarker-specific effects.

In this multicenter, prospective study we showed that plasma levels of three biomarkers NTproBNP, Troponin-T and GDF-15 are higher in patients with persistent AF than in pAF. In those patients with higher biomarker levels, we observed higher co-morbidity burden and more unfavorable echo-parameters such as enlarged LAVI and slightly reduced LVEF. We furthermore observed that age and CHA_2DS_2-VASc (as surrogate for higher comorbidity burden) are important determinants of all three biomarkers, and that progressive atrial disease, extrapolated from our findings of indicators of progressive atrial disease (higher EHRA category, increased LAVI, decreased LVEF and persistent AF) is a major determinant for plasma NTproBNP levels, but not for GDF15 or Troponin-T levels. Troponin-T and NTproBNP are both traditional cardiovascular biomarkers that are widely used in clinical practice, while GDF15 is a more novel cardiovascular biomarker. Troponin-T reflects myocardial damage and typically increases when ischemia is present or in case of coronary artery disease. Increased levels of troponin- T are associated with increased risk of all-cause mortality and major adverse cardiac events in patients with AF (6). Growth differentiation factor 15 (GDF-15) belongs to the transforming growth factor- β (TGF- β) cytokine superfamily (7) and circulating levels of GDF-15 are influenced by acute and chronic cellular stressors including ageing and disease. In healthy humans, GDF-15 levels are low throughout the entire body (8). Increased plasma levels of GDF-15 are observed in various cardiovascular disease states such as HF and AF (9). In this study we observed that plasma levels of Troponin-T and GDF-15 were not determined by persistent AF, but depended on other factors such as ageing, CHA_2DS_2-VASc, smoking, LVEF and sex. Therefore, these biomarkers may be useful to select those patients with higher co-morbidity burden and more unfavorable echo-measures, but not to assess more progressed AF disease states as persistent AF. NTproBNP, on the other hand, may reflect a biomarker that is helpful to identify those patients who are more likely to suffer from persistent AF than pAF. NTproBNP is a marker of myocardial stretch and is

upregulated when additional stress is assessed on the LV such as in heart failure or AF (10). In patients with AF, higher levels of NTproBNP are associated with worse outcome including death, independent of CHA_2DS_2-VASc score (10). Results of our study demonstrate that progression of AF is associated with increased NTproBNP levels, and it may therefore reflect an additional tool to select those patients with more persistent AF.

This study, however, is a cross-sectional study and results are thus descriptive and do not reflect causality of biomarker levels and AF burden. Because of its cross-sectional design this study cannot differentiate if increased biomarker levels are results of more persistent forms of AF or due to increased comorbidity burden. The fact that there is little biomarker-specific variance in comorbidities suggests that increasing plasma biomarker levels are not a sole effect of comorbidity burden, but future studies are warranted to evaluate this in more detail. Of note, AF-RISK was designed to evaluate AF-progression in patients with AF and did not include participants without AF; we could therefore not compare biomarkers levels in patients with and without AF. Not all AF patients were in an AF episode when blood withdrawal took place. This study was therefore unable to explore the effect of an AF episode on biomarker levels in pAF and persistent AF.

Overall, in this study we show that patients with persistent AF have higher plasma levels of NTproBNP, Troponin-T and GDF15, and that these higher plasma levels are accompanied by higher comorbidity burden and more unfavorable echocardiographic parameters. Additionally, we show that persistent AF determines NTproBNP levels, but not Troponin-T and GDF-15. We therefore suggest that NTproBNP is a better diagnostic biomarker than GDF15 and Troponin-T since NTproBNP is related to more AF, whilst GDF15 and Troponin-T are related to underlying conditions and not a specific AF phenotype. This study also suggest that in AF, Troponin T and GDF15, and especially NTproBNP could be used to detect those patients with high disease burden that may warrant more aggressive treatment of AF and concomitant comorbidities. Future studies, however, are essential to evaluate if more aggressive AF treatment and risk factor management will reduce disease progression and holds a novel therapeutic intervention to reduce the burden of AF.

References

1. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. *Int J Stroke*. 2021;16(2):217-221.

2. Kannel WB, Wolf PA, Benjamin EJ, Levy D. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: Population-based estimates. *Am J Cardiol*. 1998;82(8A):2N-9N.

3. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: The framingham study. *Stroke*. 1991;22(8):983-988.

4. Kirchhof P, Camm AJ, Goette A, et al. Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. *N Engl J Med*. 2020 Oct 1;383(14):1305-1316.

5. De With RR, Marcos EG, Dudink EAMP, et al. Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: Data from the AF-RISK study. *Europace*. 2020;22(3):352-360.

6. Fan Y, Zhao X, Li X, Li N, Hu X. Cardiac troponin and adverse outcomes in atrial fibrillation: A meta-analysis. *Clin Chim Acta*. 2018;477:48-52.

7. Bootcov MR, Bauskin AR, Valenzuela SM, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. *Proc Natl Acad Sci U S A*. 1997;94(21):11514-11519.

8. Assadi A, Zahabi A, Hart RA. GDF15, an update of the physiological and pathological roles it plays: A review. *Pflugers Arch*. 2020;472(11):1535-1546.

9. Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem*. 2017;63(1):140-151.

10. Goetze JP, Bruneau BG, Ramos HR,, et al. Cardiac natriuretic peptides. *Nat Rev Cardiol*. 2020;17(11):698-717.

Chapter 8 **Discussion and Future Perspectives**

The general aim of this thesis was to provide insights on how risk factors, comorbidities and sex differences are associated to AF and its progression. This association was investigated by exploring underlying mechanisms using clinical, echocardiographic and blood biomarkers. In addition, this thesis aimed to explore the role of the atrial cardiomyopathy (ACM) in AF and its progression. To measure ACM, we used non-invasive echocardiographic techniques that could help to assess atrial cardiac remodeling in patients with AF and support earlier detection of ACM. In addition, we explored the role of biomarkers in the determination of progressive states of AF and explore biological pathways that may be involved, including differences between sexes. In **chapter 2**, we assessed atrial ACM using 2D speckle tracking in patients with short-lasting paroxysmal AF. We found that the coexistence of risk factors and comorbidities was associated with severity of ACM measured as an impaired atrial function using 2D speckle tracking. The analysis was performed in The identification of a risk profile to guide atrial fibrillation therapy (AF-RISK) study. In **chapter 3,** we further assessed ACM using 2D speckle tracking to distinguish patients who had both short-lasting paroxysmal AF and heart failure with preserved ejection fraction (HFpEF) from those without HFpEF. This was also an ancillary analysis in the AF-RISK study. We found that atrial function differentiated patients who had both AF and HFpEF, from those without HFpEF, despite both groups having comparable ventricular function. In **chapter 4**, we investigated sex differences in blood biomarkers in patients with short-lasting paroxysmal AF to identify differences between sexes in biological processes involved in ACM. We performed enrichment of biological pathway analyses of 92 cardiovascular blood biomarkers in both women and men from again the AF-RISK study. We then validated our results in women and men with short-lasting paroxysmal AF from the Reappraisal of AF: Interaction Between Hypercoagulability, electrical Remodeling, and Vascular Destabilisation in the Progression of AF (RACE V) study. We found that women had higher levels of inflammatory biomarkers in comparison to men, whereas men had higher levels of vascular remodeling related biomarkers in comparison to women. In **chapter 5**, we explored different biological underlying processes related to ACM in patients with AF and HF, using enrichment of biological pathway analyses. This analysis was performed in the Systems Biology Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) study. We found that the coexistence of AF together with HF was associated with activation of amyloid-beta metabolic processes, amyloid-beta formation, and amyloid

precursor protein catabolic processes. In **chapter 6**, we evaluated the clinical utility of the AF characterization scheme (4S-AF scheme) proposed by the European Society of Cardiology 2020 AF guidelines which include stroke risk, symptom severity, severity of AF burden and substrate. Using data from the RACE V study, we found that a modified scheme, 3S-AF scheme without the symptoms domain, may be more appropriate to assess disease progression in patients with short-lasting paroxysmal AF. In **chapter 7**, we assessed the role of well-established biomarkers (NTproBNP, Troponin-T and GDF-15 with AF progression and risk factors and comorbidities, data from the AF-RISK study. We showed that, independently of Troponin-T and GDF-15, NTproBNP levels were associated with AF progressive states.

AF is a wake-up call because it is not directly fatal, but it is associated with underlying risk factors and comorbidities, and with poor quality of life, stroke, heart failure and worse cardiovascular prognosis.(1,2) AF is a progressive disease and its progression is associated with the number and severity of risk factors and comorbidities.(3-9) AF progression occurs due to more severe atrial remodeling including both electrical and structural changes.(3-9) AF progression can be deleterious for patients given the increase of HF hospitalizations,(4,10) stroke events(11), mortality,(3-9) and impaired quality of life.(12)

ACM assessment in AF patients

Atrial cardiomyopathy (ACM) has been conceptualized in an expert consensus paper as 'any complex of structural, architectural, contractile or electrophysiological changes affecting the atria with the potential to produce clinically-relevant manifestations'.(5) In the same consensus paper, four classes have been determined based on histological findings (I) mainly cardiomyocyte changes; (II) mainly fibrotic changes; (III) combined cardiomyocyte-pathology/fibrosis; (IV) primarily non-collagen infiltration (with or without cardiomyocyte changes). (5) However, clinical and non-invasive parameters are yet to be established for its feasibility in daily clinical practice.(13)

The definition of ACM encompasses structural deterioration of the atria where AF may be a result and/or a contributor to ACM suggesting a reciprocal effect relation.(5,14) Owing to this reciprocal relation, in this thesis we explored different ways to measure ACM within patients with AF. We utilized accessible measures that may correlate to histological atrial remodeling findings, as a proxy to understand underlying ACM and to provide more user-friendly tools for clinicians and less invasive procedures for AF patients.

Assessing ACM in patients with AF can be cumbersome. (5) Atrial fibrosis, as a sign of ACM, is directly related to AF prevalence and burden as shown in human heart tissue (15) and contributes to disease progression, however warrants atrial tissue to diagnose.(16). Electrical remodeling retrieves rapidly when converting AF to sinus rhythm in animal models; however, structural changes persist and may be more relevant in maintaining AF.(17-19) Atrial size increase has been closely associated with structural remodeling (8,20-22) and therefore used as a non-invasive proxy to measure ACM. Studies have shown that structural remodeling is settled before atrial

enlargement. (23-26) New imaging techniques have been suggested to measure ACM more accurately at an early phase and during the course of the disease. (23-26) Early structural remodeling has also been associated with outcomes in AF patients.(23,26,27) Measurement of atrial sizes through echocardiography, however, is a non-invasive approach that is a feasible technique in the regular clinical practice. There are other modalities of imaging to comprehensively quantify left atrial sizes and ACM by means of fibrosis. (**Table 1**)

Speckle tracking is a 2D echocardiographic technique that measures the percentage of deformation of each cardiac chamber.(28,29) Speckle tracking is less affected by loading conditions in comparison to volumetric methods(46), outperforming atrial volume measuring to assess ACM.(47-49) Late gadolinium enhancement magnetic resonance (LGE-MRI) is suggested to assess accurately myocardial fibrosis as well as heart chamber size; however , experience is mostly based on the study of the ventricles.(30-33) LGE-MRI assesses endocardial borders and the presence, pattern and size of myocardial fibrosis in the ventricle(34,35) Reproducibility of the use of LGR-MRI to assess size and fibrosis in the atria is lacking and it can be challenging due to limitations in image resolution, thin atrial walls and variable atrial shape.(31)The Utah classification to measure left atrial fibrosis has been proposed under LGE-MRI modality.(36) The Utah classification consists of four stages as follow, minimal or Utah stage 1; <5%, mild or Utah stage 2; 5–20%, moderate or Utah stage 3; 20–35%, and extensive or Utah stage 4; >35%. (36) The use of the Utah classification and the general use of LGE-MRI in the atrial is yet to be validated and might not be clinically applicable due to its low accessibility, costs and rejection in patients with claustrophobia.

Integrated backscatter (IBS) is a non-invasive ultrasonic tissue technique that characterizes tissue.(37) Through IBS, integrated images are obtained and measured in decibels to generate the temporal profile of cyclic variation and can guide to determine abnormalities.(37-39) However, IBS requires high technical sophistication and special modified ultrasound scanner to obtain the raw ultrasonic signal.

Blood biomarkers of fibrosis, such as Galectin-3 and transforming growth factor beta 1 (TGF-β_1) have been shown to be associated with AF incidence and recurrences after ablation.(40-42). Nevertheless, these biomarkers are not cardiac specific and may be altered by fibrotic processes in

the ventricles and outside the heart. Therefore, studies of more atrial specific biomarkers or combination of biomarkers explaining pathophysiological pathways is warranted.

Cardiac computed tomography has the disadvantage of being affected qualitatively by heart rate (higher than 60 bpm, i.e. AF with a faster and irregular rate), providing dose radiation and being contraindicated in patients with severe renal impairment or contrast sensitivity. In addition, cardiac computed tomography has more limited spatial resolution in comparison to LGE-MRI. In general, imaging techniques that try to capture fibrotic tissue in the atria may face constrains to detect differences in image attenuation given the low thickness of the atria. (24,43-45) Hence, in this thesis we focused on techniques that can detect impaired functionality of the atria and which are more clinically accessible, i.e. by means of echocardiography

The role of left atrial dimensions to assess ACM

Studies have shown that left atrial size is strongly associated with histological fibrosis in patients with AF. (20,46,47) Left atrial size was originally measured in parasternal long axis view which represented the anterior posterior dimension, expressed as left atrium diameter; however, this method was performed under a singular two-plane view making the hard assumption of a symmetrical atria.(48,49) Measuring volume of the left atria depicts a better size assessment than two-dimension (2D) length and area measures(48) and has been shown to be a stronger prognostic parameter in diverse cardiac diseases, including AF.(28,50,51) Debonnaire *et al.* evaluated the incidence of AF in 242 patients with hypertrophic cardiomyopathy without history of AF and followed them for an average of 4.8 years.(52) They found that even though left atrial diameter relate to new-onset AF in hypertrophic cardiomyopathy patients, left atrial volume further refines risk stratification for new-onset AF.(52) *Tsang et al*. retrospectively evaluated new-onset AF in more than 1,600 patients and showed that left atrial volume better stratified risk of AF in comparison to left atrial diameter.(50) Ideally, three-dimension (3D) imaging methods to quantify left atrial size should be performed; however, availability of 3D function might depend on the software and equipment vendor. On the other hand, 2D methods can be acceptably adopted because they have been shown to be correlated to 3D measures (**Figure 1**).(48,53)

Table 1. Imaging studies assessing the prognostic value of atrial remodeling in patients with atrial fibrillation

Study Name	Type of AF patients	Atrial remodeling measurement	n	Age (years)	Female	Follow-up (months)	Intervention	Primary outcome	Results
imaging modalities									
Parwani *et al.*, 2017 (54)	Persistent 100%	2D speckle tracking	102	66±10	33%	15	catheter ablation	AF recurrence	<10% of basal deformation associated with AF recurrence HR 6.4 (95% CI 2.4–16.9)
Yasuda *et al.*, 2015 (55)	paroxysmal 68%, persistent 32%	2D speckle tracking	100	59±11	36%	between 3 and 12 months	catheter ablation	AF recurrence	Basal deformation is predictor of AF recurrence OR: 0.81 (95% CI 0.73 to 0.89)
Yoon *et al.*, 2015 (56) **The Echocardiographic Predictors of Progression of Atrial Fibrillation (E6P) study**	paroxysmal 100%,	2D speckle tracking	313	57±14	38%	26	observational data	AF progression	≤ 30.9% of basal deformation associated with progression HR: 4.224 (95% CI, 1.804–9.892)

Tops *et al.*, 2011 (57)	paroxysmal 76%, persistent 24%	2D speckle tracking	148	54±9	21%	40±4	catheter ablation	LA reverse remodeling (15% or more reduction in maximum left atrial volume	OR:1.813 (95% CI 1.102 to 2.982)
Marrouche *et al.*, 2022 (58) **DECAAF II**	persistent	LGE-MRI	843	Average 63	21 %	12-18	MRI-Guided Fibrosis Ablation	AF recurrence	not significant (fibrosis <20% vs ≥20%)
Chelu *et al.*, 2018 (34) **Atrial Fibrillation Research Registry**	paroxysmal 40%, persistent 60%	LGE-MRI	308	65±12	37%	13.2 (6-36)	catheter ablation	AF recurrence	per every 10% increase in fibrosis HR, 1.4 (95% CI, 1.20–1.76)
King *et al.*, 2017 (59) **AFib research database**	paroxysmal 53%, persistent 31% unknown 16%	LGE-MRI	1,228	64±14	41%	0.23	observational data	major adverse cardiovascular and cerebrovascular events	Utah* stage IV fibrosis versus lower stage I fibrosis HR1.67 (95% CI, 1.01 to 2.76)
Canpolat *et al.*, 2015 (60)	paroxysmal with no identified risk factors/ comorbidities	LGE-MRI, TGF-β_1	41	49 ± 8	41.5 %	18(12-20)	cryoablation	AF recurrence	left atrial fibrosis HR:1.127 (95% CI not provided)

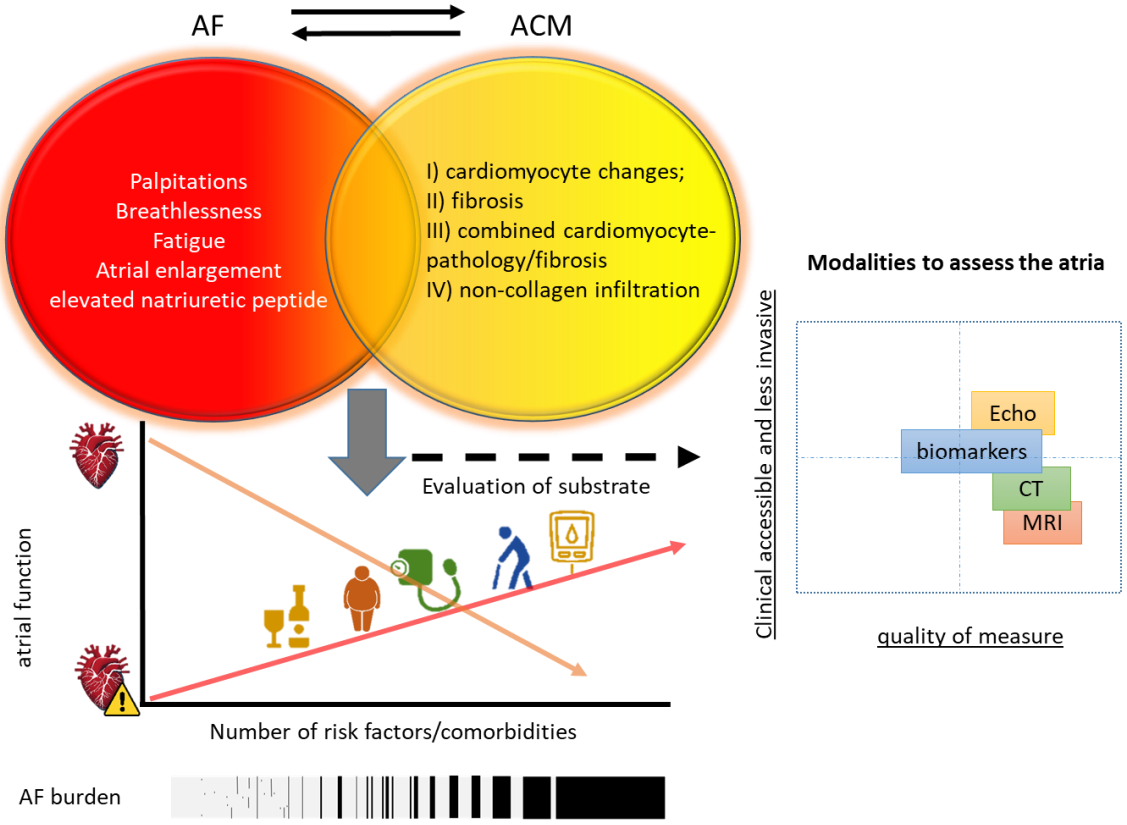
Marrouche *et al.*, 2014 (61) **DECAAF**	paroxysmal 65%	LGE-MRI	272	59 ± 11	32%	16	catheter ablation	AF recurrence	per 1% increase in fibrosis HR 1.06 (95% CI 1.03–1.08)
McGann *et al.*, 2014 (62)	paroxysmal 50% persistent 50%	LGE-MRI	386	mean 65	36%	12	catheter ablation	AF recurrence	in advanced wall structural remodeling HR 4.89
Malcolme-Lawes *et al.*, 2013 (63)	paroxysmal	LGE-MRI	50	60 ± 13	69%	12	cryoballoon or conventional RF ablation	AF recurrence	increased with increasing fibrosis degree
Seitz *et al.*, 2011 (64)	paroxysmal 14% longstanding 36% persistent 50%,	LGE-MRI	22	57 ± 9	18%	3, 6, 8, 12	RF catheter ablation	"Difficulty" AF ablation (RF duration; sinus rhythm achievement; complexity of surface)	strong correlation between fibrosis and difficulty of ablation
Kuppahally *et al.*, 2010 (65)	paroxysmal 38% persistent 62%	LGE-MRI	68	62 ± 14	32%	12	catheter ablation	AF Recurrence	HR 1.04 (95% CI 1.01–1.08)
Oakes *et al.*, 2009 (66)	paroxysmal 51% persistent 49%	LGE-MRI	81	64 ±9 12	36%	9.6 ± 3.7	catheter ablation	AF recurrence	more fibrosis OR 4.88 (95% CI 1.73–13.74)

Ling *et al.*, 2014 (67)	paroxysmal 63% persistent 37%	T1 mapping	132	mean 56	25%	1, 3, 6, 12, 18, 24, 30	catheter ablation	AF recurrence	38% recurrence in patients with T1 time <230 ms vs. 15% in patients with T1 time >230 ms
Sasaki *et al.*, 2014 (68)	paroxysmal 49% persistent 51%	IBS	113	mean 59	16%	13.8 (8.7 – 19.9)	catheter ablation	AF recurrence	per 1dB increase in IBS HR 1.04 (95% CI 1.01–1.07)
Akoum *et al.*, 2011 (36)	paroxysmal (50%) persistent (50%)	LGE-MRI	144	mean 52	-	9	catheter ablation	AF recurrence	increased fibrosis leads to more recurrence, and more ablation is needed for success.
Kubota *et al.*, 2012 (39)	paroxysmal	IBS	27	62 ± 13	11%	37 (34-40)	NA	progression (paroxysmal to persistent)	IBS ≥20 dB vs. <20 dB HR: 8.74 for patients with
den Uijl *et al.*, 2011 (69)	paroxysmal (71%) persistent (29%)	IBS	170	56 ± 9	33%	12±3	RF catheter ablation	AF recurrence	Per 5dB increase in IBS HR 2.8 (95% CI 2.2–3.6)
Wang *et al*-., 2009 (38)	not provided	IBS	70	mean 66	11%	NA	CABG	Post-operative AF	higher IBS in post-operative AF vs. SR

Data are mean ± standard deviation, median (interquartile range) unless otherwise stated.
*Utah fibrosis classification with LGE-MRI= no or minimal Utah stage 1 <5%; mild or Utah stage 2, 5–20%; moderate or Utah stage 3, 20–35%; and extensive or Utah stage 4, >35%.

AF=atrial fibrillation; CABG = coronary artery bypass graft; CI = confidence interval; dB=decibels; LGE-MRI = Late gadolinium enhancement magnetic resonance; HR = hazard ratio; IBS = integrated backscatter; n= number; NA=not applicable; OR = odds ratio; RF=radiofrequency; SD=standard deviation; TGF-β_1= Transforming growth factor beta 1

Figure 1. Relation between atrial fibrillation and atrial cardiomyopathy



Atrial cardiomyopathy (ACM) implies cardiovascular deterioration of the atria where atrial fibrillation (AF) may be a result and/or a contributor to ACM suggesting a reciprocal effect relation. This translates into a diminishing of the atrial function (orange line) that can be worsened by the accumulation of risk factors and comorbidities (red line) line represented in the graph under. The worsening of the atrial function may phenotypically be presented as increased AF burden as shown in the bar below the graph symbolizing the frequency and duration of AF episodes. On the right side of the figure, different assessment methods of ACM in patients with AF. They are shown graphically in balance, as for now, based on its clinical applicability in terms of accessibility and less invasive procedures for patients to assess the atria. AF=atrial fibrillation; ACM=atrial cardiomyopathy; CT = computed tomography; MRI= magnetic resonance

C**omorbidities and risk factors for ACM in AF patients**

Even though more risk factors and comorbidities have been associated with the presence of AF, not all studies do deep phenotyping of patients at baseline(**Table 2**).(2) The latter is relevant as available data show that identification and treatment of risk factors and comorbidities is pivotal, also for prevention of recurrent AF.(70) In the Euro Heart Survey on AF, even though not all risk factors and comorbidities associated with AF were registered, more than half of patients already have at least one comorbidity. (4) The incidence of AF increases as well as comorbidities increases with age, however in younger population with AF, risk factors and comorbidities are also present. De With *et al*. showed that 11% of the population did not have identified risk factors and comorbidities in a relatively AF young population from the Phenotyping Young-Onset Atrial Fibrillation Patients study (YOUNG-AF). (71) In addition, comorbidities have been shown to contribute to ACM in AF patients.(72) Therefore, in **chapter 2**, we evaluated ACM using 2D speckle tracking in patients with short-lasting paroxysmal AF. Furthermore, we assessed how ACM was associated with the number of comorbidities. This was a sub-study was of "The identification of a risk profile to guide atrial fibrillation therapy (AF-RISK)" study.(73) The AF-RISK study is an observational study performed in two centers in the Netherland. Patients included in the study had short-lasting paroxysmal AF and were excluded if they had history of heart failure >3 years a history; severe valvular disease; acute coronary syndrome within the previous month; AF classified as post-operative; or a contra-indication for oral anticoagulation. We evaluated seven well-established associated comorbidities or risk factors including, age >65 years, BMI >25 kg/m^2, hypertension, diabetes mellitus, coronary artery disease, moderate to severe mitral valve regurgitation, and kidney dysfunction.(74) We observed that the presence of comorbidities was associated with ACM measured as impaired atrial function. Impaired atrial function was defined as decreased deformation percentage measured through speckle tracking in any of the three functional phases (reservoir, conduit, contractile). In accordance with previous studies, this association was independent of atrial volume indexed, (23-25) implying that in this population strain could be an earlier marker of ACM in patients with short lasting paroxysmal AF.

Moreover, after one year of follow-up, a progression of ACM was observed in patients with no or low number of comorbidities in comparison to those with three or more comorbidities who showed less or no further progression of ACM. These findings suggest that ACM starts early in the

presence of AF along with comorbidities (75) and that in patients with three or more comorbidities ACM is already well established. This supports the idea that early control of comorbidities as well as early rhythm control of AF may be critical for stopping and/or reversing ACM.(76) Needless to say, there are not so well known and newer identified comorbidities/risk factors, such as cancer, obstructive sleep apnoea, and chronic obstructive pulmonary disease that were not incorporated in this analysis.(13) The incorporation of these newer identified comorbidities/risk factors may influence the results and warrants further research.

Heart failure in the context of atrial fibrillation

A common comorbidity associated with AF and its progression is heart failure (HF).(84-86) Despite of being both different conditions, clinical symptoms and risk factors importantly overlap.(84-86) This overlap suggests that there might be similarities in remodeling processes in the heart in both conditions.(84-87) The presence of one of them increases the probability of the other.(85,86,88-90) In addition, the coexistence of both worsens prognosis and is deleterious for quality of life.(91) However, temporality of whether HF or AF comes first has impact in outcomes.(92,93) When HF happens first, the incidence of AF seems to be a sign of worse prognosis and more irreversible pathophysiological mechanisms, which ultimately lead to poor prognosis. (92-95) When AF happens first, the development of HF can be a bystander as it may be caused by a tachycardiomyopathy, which could be reversible, albeit not in all patients having a better prognosis. (92-95) In the context of HF with the reduced ejection fraction (rEF) phenotype, its diagnosis is straightforward by symptoms, results from echocardiography, and B-type natriuretic peptide (BNP) or N-terminal pro-hormone BNP (NTproBNP).(86) Conversely, in the context of HF with the preserved ejection fraction (pEF) phenotype, its diagnosis may be overlooked in the presence of AF, and thus vice versa.(84) Therefore, HFpEF is cumbersome to diagnose due to common symptoms with AF, such as breathlessness, fatigue and elevated NT-proBNP.(84) Even more, scores for its diagnosis have included the presence of AF or ACM as an important criterion (**Table 3**).(88,96) In the H_2FPEF score, diagnosing AF in a patient translates into a 55% probability for the patient having HFpEF.(88) In the HFA-PEFF score, left atrial enlargement translates in two points from the five minimum, 40% of the score, to diagnose HFpEF.(96)

Table 2. Presence of risk factors and comorbidities in AF patients from in-depth phenotyped studies included in chapter 2 (AF-RISK), chapter 6 (RACE V) and other studies including paroxysmal AF patients.

	PAF n(%)	Age, years	HT n(%)	CAD n(%)	HF n(%)		DM n(%)	Overweight n(%)	Kidney dysfunction n(%)	MVR n (%)	no comorbidities n(%)
					pEF n(%)	rEF n(%)					
AF-RISK Registry* n=344	344 (100)	58 ± 12	272 (79)	18 (5)	182 (53)		29 (8)	223 (65)	35 (10)	3 (1)	27 (8)
					174 (51)	8 (2)					
RACE V Registry* n=341	341 (100)	64 ± 10	276 (81)	38 (11)	103 (49)		30 (9)	226 (66)	16 (5)	4 (1)	16 (5)
					97 (46)	6 (2)					
Euro Heart Survey on AF De Vos *et al*. 2010 (4) Registry n=1,219	1,219 (100)	64 ± 13	756 (62)	390 (32)	255 (21)		183 (15)	NA	56 (5)	233 (19)	207 (17)
					NA	NA					
Tayebjee *et al* 2010 (77) Registry n=419	222 (53)	57 ± 10	148 (35)	34 (8)	NA		11(3)	NA	27 (8)	16 (5)	NA
					NA	NA					
Abed *et al* 2013 (70) RCT n=150	86 (57)	60 ± 10	127 (85)	17 (11)	NA		39 (26)	150 (100)	NA	NA	0 (0)
					NA	NA					
ARREST-AF	89 (60)	58 ± 10	126 (85)	20 (13)	NA		26 (17)	NA	NA	NA	NA

Pathak *et al*. 2014 (78) Registry n=149					NA	NA					
CARDIO-FIT Pathak *et al*. 2015 (79) Registry n=308	164 (53)	60 ± 11	232 (75)	39 (12)	NA		84 (27)	(100)	NA	NA	0 (0)
					NA	NA					
PREVEND Vermond *et al*. 2015 (80) Registry n=265	265 (100)	62 ± 9	145 (54)	NA	6 (2)		23 (9)	NA	NA	NA	NA
					NA	NA					
YOUNG-AF* De With *et al* 2018 (71) Registry n=468	329 (70)	49 ± 9	207 (44)	45 (10)	44 (9)		21 (5)	NA	10 (2)	35 (7)	51 (11)
					NA	NA					
REVERSE-AF Middeldorp *et al*. 2018 (81) Registry n=355	196 (55)	63 ± 11	274 (77)	44 (12)	NA		103 (29)	35 (100)	0 (0)	0 (0)	0 (0)
					NA	NA					
RACE 4 Wijtvliet *et al* 2020 (82) RCT	839 (62)	64 ± 11	645 (48)	77 (6)	159 (12)		131 (10)	NA	NA	NA	NA
					NA	NA					

n=1354											
EAST-AFNET Kirchhof *et al*. 2020 (76) RCT	994 (36)	70 ± 8	2450 (88)	NA	798 (29)		NA	NA	13	1251 (45)	NA
					NA	NA					
ACTIVE-AF Elliot *et al.* 2023(83) RCT n=120	78 (62)	70 ± 11	80 (67)	17 (14)	NA		16 (13)	NA	NA	NA	NA
					NA	NA					

CAD: coronary artery disease ; DM: diabetes mellitus; HF: heart failure; HT: hypertension; MVR: mitral valve regurgitation; NA: not available; PAF: short-lasting paroxysmal atrial fibrillation; pEF: preserved ejection fraction; rEF: reduced ejection fraction

***Comorbities included were hypertension, heart failure, age > 65 years, diabetes mellitus; coronary artery disease, Overweight (BMI > 25kg/m^2), moderate or severe mitral valve regurgitation and kidney dysfunction (eGFR < 60 ml/min/1.73m^2)**

Table 3. Proposed clinical scores to diagnose HFpEF in patients with dyspnoea **(88,96)**

H_2FPEF score				**HFA-PEFF score**		
Domain	**Clinical variable**	**Definition**	**Points**	**Domains**	**Criteria definition**	
					Major (2 points)	**Minor (1 point)**
H_2	Heavy	BMI >30kg/m^2	2	**Functional**	-septal e' <7cm/s -lateral e' <10cm/s -average E/e' ratio ≥15 -velocity of tricuspid regurgitation >2.8 m/s (pulmonary artery systolic pressure >35mmHg)	-average E/e' ratio 9-14 -global longitudinal strain <16%
	Hypertension	≥2 antihypertensives	1			
F	atrial Fibrillation	short-lasting paroxysmal or persistent	3	**Morphological**	-LAVI >34ml/m^2 -LVMI ≥199/122g/m^2 (m/w) and relative wall thickness >0.42	-LAVI 29-34 ml/m^2 -LVMI>115/95 g/m^2 (m/w) -relative wall thickness >0.42 -left ventricular wall thickness ≥12mm
P	Pulmonary hypertension	pulmonary artery systolic pressure >35mmHg (echocardiographic Doppler)	1			
E	old agE	Age >60 years	1	**Biomarker (in sinus rhythm**	-NT-proBNP >220 pg/ml -BNP >80pg/ml	-NT-proBNP 125-220 pg/ml -BNP 35-80 pg/ml
F	Filling pressure	E/e' ratio >9 (echocardiographic Doppler)	1	**Biomarker (in atrial fibrillation)**	-NT-proBNP >660 pg/ml -BNP >240pg/ml	-NT-proBNP 365-660 pg/ml -BNP 105-240pg/ml

Interpretation of the probability/likelihood of HFpEF diagnosis		
0-1 points	low probability , unlikely HFpEF	0-1 points
2-5 points	intermediate probability HFpEF	2-4 points
6-9 points	high probability , likely HFpEF	5-6 points

Two recent proposed score to diagnose HFpEF in patients who present dyspnea at consultation H2FPEF and HFA-PEFF.
BNP: B-type natriuretic peptide; LAVI: left atrial volume index; LVMI, left ventricular mass index; m/w: men/women; NT-proBNP: N-terminal pro-B-type natriuretic peptide

Given this interrelation between AF and HF, in **chapter 3** we further explored the value of measuring ACM by means of 2D speckle tracking to distinguish patients who had both AF and HFpEF from those without HFpEF. This was also an ancillary study from the AF-RISK population. We showed that atrial function, from both atria, differentiated patients who had both AF and HFpEF, from those without HFpEF. It is important to acknowledge that the definition of HFpEF used in the current analysis differs from the current suggested algorithms.(88,96) First of all, the definitions of HFpEF have been evolving and two current scores shown in **Table 3** need validation.(86) Second, the current European HF guidelines make a call to use a more clinical and practical criteria to diagnose HFpEF. (86,88,96) Lastly, in order to make our results more comparable and consistent with studies previously done in RACE 3, AF RISK and RACE V studies, we used the same criteria, which included symptoms and signs of heart failure (dyspnea and fatigue, equivalent to NYHA ≥ II) or history of HF hospitalization and N-terminal pro-B-type natriuretic peptide (NT-proBNP) ≥125pg/ml, and one of the following echocardiographic measures: left atrium volume index (LAVI) >34ml/m2, left ventricular mass index ≥115g/m2 for men and ≥95g/m2 for women, average E/e' ≥13cm/s and average e' <9cm/s.(97) Even though HFpEF is characterized and phenotyped as a ventricular disease, we observed no demonstrable differences in left ventricular (LV) function, measured by global longitudinal strain and LV ejection fraction between our patients with AF and HFpEF versus those without HFpEF. These findings suggest that HFpEF is not limited to the ventricle but also affects the atria and that remodeling in patients with HFpEF in the ventricle and in patients with AF in the atria may have common pathways.(87) Patients with AF and patients HF have higher levels of inflammatory biomarkers, such as C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) as compared to controls. (87,98) Neurohumoral activation is another shared mechanism in AF and HF. The sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS) are activated in both conditions, leading to increased vasoconstriction, sodium retention, and myocardial fibrosis. (87,99,100) Oxidative stress is another key factor in the pathogenesis of both AF and HF. Reactive oxygen species (ROS) are generated in response to various stressors, including ischemia, hypoxia, and inflammation, and contribute to cell damage and dysfunction. (87,101-103) The increased production of ROS has been associated with decreased atrial myocardial energetics, as compared to those in the ventricles.(56) Moreover, myocytes from the atria and the ventricle may respond differently during the remodeling processes,

however they may adopt each other's phenotype.(57) In line with our data, in a study including 205 patients with dyspnoea NYHA≥II, short-lasting paroxysmal AF and preserved LV ejection fraction ≥50%, assessing ACM using 2D speckle tracking, defined as left atrial reservoir and left atrial contraction, Katbeh *et al.* found that ACM was associated with the probability of HFpEF, using two scores, H2FPEF and HFA-PEFF.

With the premise that AF and HF usually coexist, in **chapter 5** we further explored different biological underlying processes that could directly or indirectly be related to ACM in patients with AF and HFrEF. We conducted and ancillary study of patients enrolled in the BIOSTAT-CHF study.(104) The BIOSTAT-CHF is a prospective study designed to investigate the effect of personalized treatment for heart failure in patients with chronic heart failure (CHF). The study enrolled over 2500 patients with CHF from 11 different countries in Europe, and the patients were up-titrated according to the guidelines on CHF treatment.(104,105) The presence of AF together with HF was associated with activation of amyloid-beta metabolic processes, amyloid-beta formation, and amyloid precursor protein catabolic processes. These three pathways were validated in an independent validation cohort. Amyloid-beta depositions are commonly described in Alzheimer's disease. Despite Alzheimer's disease is considered a neurological disease, more studies suggest that it can be a systemic disease.(106,107) Recent research has shown that it also accumulates in atrial tissue of patients with AF.(108-110) Donellan *et al*, retrospectively studied 382 patients diagnosed with transthyretin cardiac amyloidosis (ATTR-CA). They showed that AF occurred in 265 (69%) of the patients. Therefore should the role of amyloid-beta within the context of AF and HF and dementia be further investigated, e.g. by performing biopsy studies. Given the cross-sectional approach of the study, we could only hypothesise an association but not causation. There are current initiatives to study the heart-brain connection that could elucidate the association between AF and Alzheimer's disease.(111-113) Our current study was accompanied by an interesting editorial comment discussing the implications of our findings. Keefe *et al* stressed, and we also acknowledge, that to target amyloid-beta in the heart will require direct evidence of cardiac deposition and subsequent experimentation of the therapeutic utility. Our data, thus, may contribute to explore new possibilities of treatments targeting two distant but related diseases as has been considered in terms of preventive interventions discussing that what may benefit the heart, may also benefit the brain. (114-116)

Sex differences in AF

It has been discussed so far that comorbidities and risk factors set the stage for ACM and AF, but risk of AF and clinical presentation of patients with AF differ, in particular between women and men. Men are associated with a 1.5 increased risk to have AF in comparison to women, after adjusting for age and risk factors.(80,117) At the time of AF diagnosing, women are usually older and have more but also different comorbidities than men.(118,119) In The Framingham Heart Study, women with AF had more often valvular heart disease, whereas men had more often coronary artery disease.(120) Other studies showed that women have also more hypertension and HFpEF whereas men had more often coronary artery disease, history of myocardial infarction, as was also the case in the Framingham Heart Study, and impaired left ventricular function. (74,121) In addition, women tend to have more AF symptoms and atypical symptoms. (122-125) and suffer from more adverse effects of antiarrhythmic drugs.(126,127) In the Rate Control Efficacy in Permanent AF: A Comparison between Lenient vs. Strict Rate Control II (RACE II), women had a higher burden of comorbidities in comparison to men (3.7 ± 1.2 in women vs. 2.9 ± 1.4 in men, $P < 0.001$).(74) Therefore, it is imperative to investigate sex related differences in AF.(128)

Blood biomarkers to assess ACM

Differences in biomarker profile by sex

NT-proBNP is a well established blood biomarker of myocardial stretching and it is associated with incident AF. Patton *et al.* studied 5,445 participants in a community-based study and were followed for a median of 10 years. They found that NT proBNP remained the strongest predictors of incident AF after adjusting for potential cofounders with HR 4.0 (95% CI 3.2 – 5.0) irrespective of sex.(129) In another community-based studied, with representations of different ethnical groups, The Multi-Ethnic Study of Atherosclerosis, Patton *et al.* found that NTproBNP was a strong predictor of incident AF in men and women across different ethnical backgrounds.(130) Other biomarkers have also been associated with AF incidence and AF recurrences. Canpolat *et al.* showed that TGF-β_1 was associated with atrial fibrosis measured by LGE-MRI and AF recurrences in AF patients without identified risk factors undergoing ablation.(60) Ho *et al.* found in 3,306 patients from the Framingham Offspring study that Galectin-3 was associated with new-onset AF. (131) However, differences in clinical presentation between women and men may

indicate that underlying biological processes may differ and therefore biomarker profile.(128) For this reason, it is necessary to explore new cardio-specific blood biomarkers and complementary ways to analyse them, i.e assessing predominant biological process through quantifying a large amount of blood circulating proteins. To investigate related biological pathways in a disease, blood biomarkers can be seen as representation of the underlying processes happening in a disease.(132). Therefore, to explore possible underlying biological mechanisms involved in the different clinical presentations between men and women with AF, **in chapter 4**, we investigated blood biomarkers in patients with short-lasting paroxysmal AF. We included patients from the AF- RISK study and we then validated our findings in the RACE V study. For this, we compared an array of 92 cardiovascular blood biomarkers in women and men with AF (Olink cardiovascular panel III). We performed enrichment pathway analyses by adding neighbouring blood biomarkers not measured in our sample but that are closely related in function. The addition of neighbouring blood biomarkers was performed using knowledge from databases of biological processes in which the measured 92 cardiovascular blood biomarkers are involved. The databases used were Gene Ontology (GO) Resource that uses high level groupings established by the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway maps. (132-135) Biomarkers that were significantly different between men and women with AF, from our analysis, were added two neighbouring related, but not measured, biomarkers. This enrichment allowed us to make connection to other potential biomarkers in related biological processes.(132-135) In women, levels of activated leukocyte cell adhesion molecule (ALCAM) and fatty acid binding protein-4 (FABP-4) were significantly higher in comparison to men. ALCAM is a cell adhesion molecule, which is involved in leukocyte recruitment in case of tissue damage. In patients with stroke, ALCAM has been associated with long-term mortality.(136) Ueland *et al* assessed ALCAM levels in 5165 patients admitted with acute coronary syndromes in the PLATelet inhibition and patient Outcomes (PLATO) trial. The results showed that the level of ALCAM was independently associated with adverse outcome including cardiovascular death; this association remained significant after adjusting for inflammatory and cardiac biomarkers.(137) Cell adhesion mechanisms increase the adhesiveness of platelets and leucocytes incrementing the risk of thrombogenesis even when in sinus rhythm.(5) Atherosclerosis has been associated with an increased risk of AF.(138,139) FABP-4 is mainly expressed in adipose tissue and has been associated with systemic pro-inflammatory state, metabolic syndrome and postoperative AF.(140,141) The presence of

inflammation causes and accelerates ACM through pro-inflammatory cytokines and molecules,(8,142)

In men, matrix metalloproteinase-3 (MMP-3) and myoglobin were found to be significantly higher expressed than in women. MMP-3 has been linked to vascular remodeling and has also been suggested as potential therapeutic target in atherosclerosis.(143,144) These differences in biomarker levels between sexes provide further evidence that the predominant pathophysiological mechanisms in women and may differ, with more inflammatory responses in women and with more prominent vascular remodeling processes being present in men. *Chen et al.* found higher infiltration of inflammatory cells in tissue samples from both atria of patients with AF in comparison to those without AF irrespective of sex.(145) Inflammation has been associated with incident AF in women.(146,147) Conen *et al.* in a women cohort, the Women's Health Study, analyzed 25,883 participants with a median of 14 years and found that inflammatory biomarkers were associated incident AF after adjusting for potential risk factors.(146) Previous studies have shown in heart tissue and blood samples that higher levels matrix metalloproteinase biomarkers are associated with the presence and progression of AF. However, the number of participants was not sufficient to stratify the results by sex.(148-150) Clinical parameters, biological system approach using omics, including blood biomarkers, may aid to identify groups that would benefit more from a specific intervention.(151)Given the different clinical presentations of AF between sexes, further research needs to stratify women and men in the study design, rather than in a sensitivity analysis looking for interactions, to clarify differences in mechanisms and possible tailored interventions.

In chapter 7, we utilised well established blood biomarkers in cardiovascular diseases to evaluate the association between AF burden and associated comorbidities, in different AF progressive states. Specifically, we evaluated plasma levels of three biomarkers NTproBNP, Troponin-T and GDF-15. Troponin-T and NTproBNP are known cardiovascular biomarkers that are widely used in clinical practice, while GDF15 is a relatively novel cardiovascular biomarker. This was an ancillary analysis of the AF-RISK study. We showed that NTproBNP was higher in patients with persistent AF irrespective of Troponin-T and GDF-15. GDF-15 belongs to the growth factor- β (TGF- β) cytokine superfamily(152) and it is a marker of oxidative stress and inflammation. Wallentin *et al*. showed that GDF-15 is associated with major bleeding, mortality, and stroke in

patients with AF in the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial after adjusting for other clinical risk factors.(153) GDF-15 has also been associated with increased risk of incident AF.(154) Troponin-T results from myocardial damage and typically increases when ischemia is present or in case of coronary artery disease. High levels of troponin-T are associated with increased risk of all-cause mortality and major adverse cardiac events in AF patients.(155) NTproBNP is a marker of myocardial stretch and its levels increase in the presence of increased dilation on the left ventricle, as what happens in HF or AF.(84,156) Higher levels of NTproBNP are associated with worse outcome in AF patients irrespective of CHA2DS2-VASc score. (84,156) We therefore confirmed that NTproBNP is a better prognostic biomarker for progressed states of AF than GDF15 and Troponin-T.

ACM assessment in progressed forms of AF

AF is a progressive disease that starts with sporadic self-terminating episodes and evolves to more frequent, longer and non-self-terminating episodes.(3)The first score that has been created to predict progression is the HATCH score.(4) The HATCH score results in an ordinal scale with maximum 7 points and is calculated based on the following parameters: Hypertension (1 point), Age>75 years (1 point), Transient ischemic attack or stroke (2 points), Chronic obstructive pulmonary disease (1 point), and Heart failure (2 points).(4) However, the results of the HATCH are contrasting.(10,157). Schnabel *et al* showed in the PREvention oF thromboembolic events-European Registry study that using the individual parameters in contrast to the whole HATCH score to predict progression of performed significantly better (C-statistic 0.64 vs. 0.52, P = 0.0001).(10) Therefore, new measuring techniques such as imaging and blood biomarkers can help improve existing scores or create new ones.(9,158,159) Recently, in the RACE V registry, a score including echocardiographic and biomarkers, in addition to clinical characteristics, has been proposed to predict AF progression. (9) Predictors for AF progression were ACM, male sex, mitral valve regurgitation, waist circumference and blood biomarkers (coagulation, cardiac stretch, cholesterol metabolism, inflammation and the immune system) with a C-statistic of 0.709, pending validation in other cohorts.

Characterization ACM in patients with AF

The 2020 European Society of Cardiology (ESC) AF guidelines proposed a frame to characterize patients with AF, the 4S-AF scheme, which addresses stroke risk, symptom severity, severity of AF burden, and substrate of AF.(85,160) This scheme requires validation to assess its clinical utility. **In chapter 6,** we evaluated the clinical utility of a scheme proposed by the European Society of Cardiology AF guidelines which include risk factors, comorbidities and the markers of ACM discussed in this thesis.(85) Specifically, the 4S-AF scheme addresses Stroke risk, Symptom severity, Severity of AF burden and Substrate of AF to provide a structured phenotyping of AF patients in clinical practice to guide therapy and assess prognosis. We evaluated the clinical utility of this scheme to predict progression in patients with short-lasting paroxysmal AF in the RACE V registry.(9) We found that a slightly modified scheme, the 3S-AF scheme (4S-AF scheme without the domain symptom severity), could be more appropriate to assess AF progression. Symptoms are difficult to interpret because they will depend on individual characteristics of the patient and the interpretation given by health care professionals. Therefore, implementing symptoms into a score assessing outcome may therefore be cumbersome. In previous studies assessing the clinical utility of the 4S-AF scheme in predicting outcomes in patients with AF, Ding *et al* did not find either an association between the symptoms domain and all-cause mortality nor for cardiovascular mortality.(161)

Future perspectives

It is important to assess ACM at early stages of remodeling using accessible tools in clinical practice. ACM represents the atrial remodeling process in AF patients and its measurement is crucial for characterization and prognosis of the individual AF patient.(5,85) Most of the studies on atrial remodeling focus on the left atrium given it anatomical accessibility in imaging studies. Therefore, knowledge on the role of ACM in the right atrium in the characterization and perpetuation of AF is still lacking and could contribute to risk prediction in AF. Further, one of the main topics of the Dutch Heart Foundation EmbRACE network is to recognize early signs of ACM in patients with AF to provide possibilities for early and more personalized treatment. The Dutch EmbRACE is a network of university medical centres, hospitals, and universities in Groningen, Maastricht, Amsterdam, Utrecht, Leiden, Rotterdam, Arnhem, and Eindhoven involving cardiologists, GPs, researchers, and patients. The incorporation of early markers of

ACM into the characterization of patients can provide in a timely manner advice before ACM perpetuates the presence of AF. The utilization of 2D speckle tracking is feasible in the regular clinical setting, non-invasive for patients, and can be utilized in the initiatives to detect early ACM in AF patients. The use of extended ECG can provide a detailed comprehension of the time, duration and burden of AF episodes. Implementation research can provide tools to scale up the use of 2D speckle tracking and provide new evidence from different levels of care.

Blood biomarkers could be useful to help assessment of ACM severity. NTproBNP should be widely used in primary and secondary clinical settings, not only in specialized centers, to improve risk stratification of AF patients and improve the clinical utility of proposed schemes to characterize AF patients. Moreover, the integration of clinical, imaging and biomarkers can better improve prognosis of AF patients. The assessment of new scores or the validation of new ones in AF progression in well phenotyped patients as proposed in RACE V(9) can provide insights of its clinical utility.

Our studies were limited by the use of selected biomarkers, and our current understanding on the role of these biomarkers in specific disease areas. Using unbiased systems biology approach and unsupervised artificial intelligence algorithms may help improve our understanding of unknown unrelated risk factors for AF.

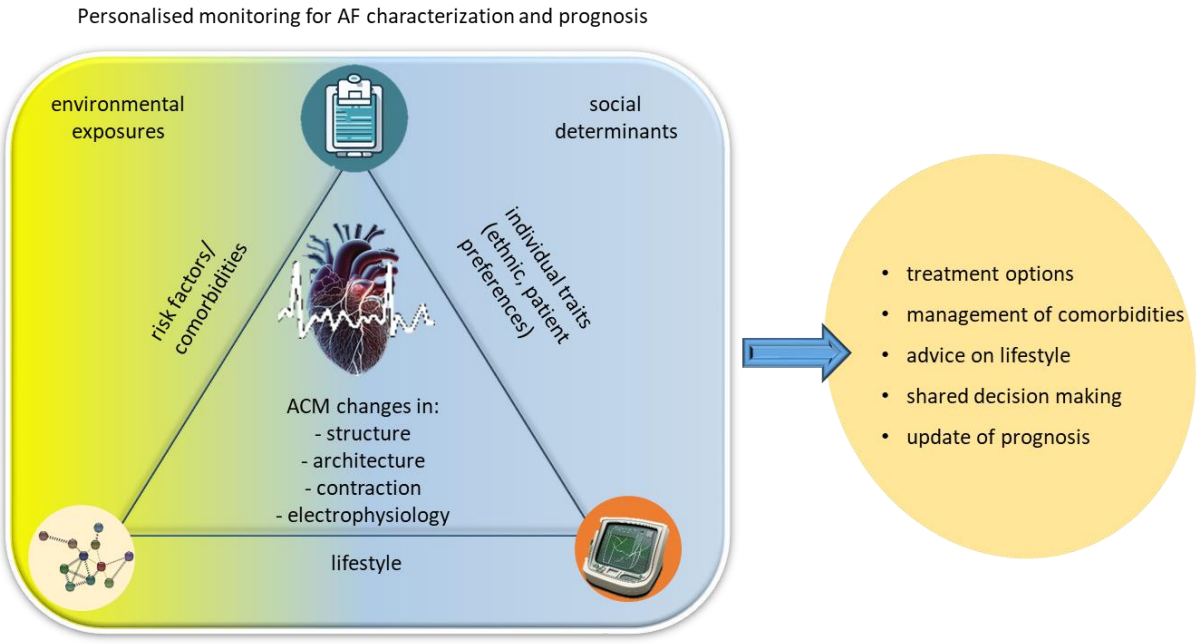
Comorbidities may play different roles in the settling of ACM in the left and right atria, which requires further investigation. Cardiovascular health scores addressing modifiable risk factors have been associated to the incidence and progression of AF.(162,163) Risk factor management through lifestyle modification reduces AF burden and severity.(77,85,86,89) Therefore, search for comorbidities is relevant to provide an integrated to AF patients and software tools can help into the identification of comorbidities (**Figure 2**). The EHRA-PATHS project aims to transform and improve the clinical practice in the field of AF to holistic, inclusive and personalised treatment strategies. The EHRA-PATHS project gathers multidisciplinary expertise from the academy, clinical practice and the industry to address multimorbidity in elderly AF patients through interdisciplinary through patient-centred, systematic care pathways.(164)

Attempting to characterize patients based on clinical parameters and biological parameters without contextualizing the patients' environment, could miss a holistic view of a person. Most of the

studies characterise patients in a point in time and avoid the dynamics of patients' characteristics, comorbidities and lifestyle decisions. In addition, available data on AF patients is derived mainly from older Western European populations being results not generalizable to the whole world population.(165-167) Attempts should be done whether markers of ACM in AF patients apply to multiethnic populations(163) with the different modalities utilized in this thesis. eHealth tools can be used to characterize patients based on changes of clinical parameters, exams, biomarkers and lifestyle decisions (**Figure 2)**. Further research is warranted into estimating the mediation effect of lifestyle on the incidence and progression of AF taking into account dynamic changes of health status, cardiovascular aging and, sex and ethnic differences. Currently, the LifestYle pReventIon of Cardiovascular Ageing (LYRICA) project aims to identify markers of cardiovascular aging, besides traditional risk factors, to diagnose and predict cardiovascular diseases and assess the effect of lifestyle interventions from an ethnical and dynamic health status perspective.

Ultimately, understanding the environment in which people live and develop their daily activities may influence the risk of AF and its progression as well as the underlying risk factors and comorbidities. These environmental exposures, define within the context of exposome,(168) and the social determinants such as sports facilities, place where people live, income and healthy eating options may provide a better contextualization of clinical parameters for each individual **(Figure 2)**.(169,170)

Figure 2. Ideal monitoring of AF for personalized assessment and management



In conclusion, identification of ACM by feasible and easy to use tools in clinical practice can help to better characterize patients with AF. In addition to assessment of the severity of the ACM, the identification of comorbidities is crucial. Together they may contribute to provide a personalised management strategy to AF patients. Dynamic changes in patients should be taken into account when analysing and adjusting management of patients with AF.

References

(1) Heijman J, Luermans, J. G. L. M., Linz D, van Gelder IC, Crijns, H. J. G. M. Risk Factors for Atrial Fibrillation Progression. Card Electrophysiol Clin 2021 Mar;13(1):201-209.

(2) George WD, Van Gelder Isabelle C, Ellinor Patrick T, Go Alan S, Kalman Jonathan M, Narayan Sanjiv M, et al. Lone Atrial Fibrillation. J Am Coll Cardiol 2014;63(17):1715-1723.

(3) Padfield GJ, Steinberg C, Swampillai J, Qian H, Connolly SJ, Dorian P, et al. Progression of paroxysmal to persistent atrial fibrillation: 10-year follow-up in the Canadian Registry of Atrial Fibrillation. Heart Rhythm 2017 Jun;14(6):801-807.

(4) de Vos CB, Pisters R, Nieuwlaat R, Prins MH, Tieleman RG, Coelen RJ, et al. Progression from paroxysmal to persistent atrial fibrillation clinical correlates and prognosis. J Am Coll Cardiol 2010 Feb 23;55(8):725-731.

(5) Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. Europace 2016 Oct;18(10):1455-1490.

(6) Wong JA, Conen D, Van Gelder IC, McIntyre WF, Crijns HJ, Wang J, et al. Progression of Device-Detected Subclinical Atrial Fibrillation and the Risk of Heart Failure. J Am Coll Cardiol 2018 Jun 12;71(23):2603-2611.

(7) Blum S, Meyre P, Aeschbacher S, Berger S, Auberson C, Briel M, et al. Incidence and predictors of atrial fibrillation progression: A systematic review and meta-analysis. Heart Rhythm 2019 Apr;16(4):502-510.

(8) Nattel S, Harada M. Atrial remodeling and atrial fibrillation: recent advances and translational perspectives. J Am Coll Cardiol 2014 Jun 10;63(22):2335-2345.

(9) Nguyen BO, Weberndorfer V, Crijns HJ, Geelhoed B, Ten Cate H, Spronk H, et al. Prevalence and determinants of atrial fibrillation progression in paroxysmal atrial fibrillation. Heart 2022 Jul 20.

(10) B Schnabel R, Pecen L, Engler D, Lucerna M, Sellal JM, Ojeda FM, et al. Atrial fibrillation patterns are associated with arrhythmia progression and clinical outcomes. Heart 2018 Oct;104(19):1608-1614.

(11) Steinberg BA, Hellkamp AS, Lokhnygina Y, Patel MR, Breithardt G, Hankey GJ, et al. Higher risk of death and stroke in patients with persistent vs. paroxysmal atrial fibrillation: results from the ROCKET-AF Trial. Eur Heart J 2015 Feb 1;36(5):288-296.

(12) Dudink, E. A. M. P., Erküner Ö, Berg J, Nieuwlaat R, de Vos CB, Weijs B, et al. The influence of progression of atrial fibrillation on quality of life: a report from the Euro Heart Survey. Europace 2018 Jun 1;20(6):929-934.

(13) Kornej J, Börschel CS, Benjamin EJ, Schnabel RB. Epidemiology of Atrial Fibrillation in the 21st Century: Novel Methods and New Insights. Circ Res 2020 Jun 19;127(1):4-20.

(14) Ning Y, Tse G, Luo G, Li G. Atrial Cardiomyopathy: An Emerging Cause of the Embolic Stroke of Undetermined Source. Front Cardiovasc Med 2021 Aug 9;8:674612.

(15) Goette A, Juenemann G, Peters B, Klein HU, Roessner A, Huth C, et al. Determinants and consequences of atrial fibrosis in patients undergoing open heart surgery. Cardiovasc Res 2002 May;54(2):390-396.

(16) Burstein B, Qi X, Yeh Y, Calderone A, Nattel S. Atrial cardiomyocyte tachycardia alters cardiac fibroblast function: a novel consideration in atrial remodeling. Cardiovasc Res 2007 Dec 1;76(3):442-452.

(17) Everett TH4, Li H, Mangrum JM, McRury ID, Mitchell MA, Redick JA, et al. Electrical, morphological, and ultrastructural remodeling and reverse remodeling in a canine model of chronic atrial fibrillation. Circulation 2000 Sep 19;102(12):1454-1460.

(18) Ausma J, Wijffels M, Thoné F, Wouters L, Allessie M, Borgers M. Structural changes of atrial myocardium due to sustained atrial fibrillation in the goat. Circulation 1997 Nov 4;96(9):3157-3163.

(19) Schotten U, Ausma J, Stellbrink C, Sabatschus I, Vogel M, Frechen D, et al. Cellular mechanisms of depressed atrial contractility in patients with chronic atrial fibrillation. Circulation 2001 Feb 6;103(5):691-698.

(20) Hoit BD, Shao Y, Gabel M, Walsh RA. Left atrial mechanical and biochemical adaptation to pacing induced heart failure. Cardiovasc Res 1995 Apr;29(4):469-474.

(21) Shi Y, Ducharme A, Li D, Gaspo R, Nattel S, Tardif JC. Remodeling of atrial dimensions and emptying function in canine models of atrial fibrillation. Cardiovasc Res 2001 Nov;52(2):217-225.

(22) van de Vegte, Yordi J, Siland JE, Rienstra M, van der Harst P. Atrial fibrillation and left atrial size and function: a Mendelian randomization study. Sci Rep 2021 Apr 19;11(1):8431-8.

(23) Gupta DK, Shah AM, Giugliano RP, Ruff CT, Antman EM, Grip LT, et al. Left atrial structure and function in atrial fibrillation: ENGAGE AF-TIMI 48. Eur Heart J 2014 Jun 7;35(22):1457-1465.

(24) To ACY, Flamm SD, Marwick TH, Klein AL. Clinical utility of multimodality LA imaging: assessment of size, function, and structure. JACC Cardiovasc Imaging 2011 Jul;4(7):788-798.

(25) Kojima T, Kawasaki M, Tanaka R, Ono K, Hirose T, Iwama M, et al. Left atrial global and regional function in patients with paroxysmal atrial fibrillation has already been impaired before enlargement of left atrium: velocity vector imaging echocardiography study. Eur Heart J Cardiovasc Imaging 2012 Mar;13(3):227-234.

(26) Delgado V, Di Biase L, Leung M, Romero J, Tops LF, Casadei B, et al. Structure and Function of the Left Atrium and Left Atrial Appendage: AF and Stroke Implications. J Am Coll Cardiol 2017 Dec 26;70(25):3157-3172.

(27) Sachdeva S, Desai R, Andi K, Vyas A, Deliwala S, Sachdeva R, et al. Reduced left atrial strain can predict stroke in atrial fibrillation - A meta-analysis. Int J Cardiol Heart Vasc 2021 Aug 24;36:100859.

(28) Badano LP, Kolias TJ, Muraru D, Abraham TP, Aurigemma G, Edvardsen T, et al. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. Eur Heart J Cardiovasc Imaging 2018 Jun 1;19(6):591-600.

(29) Voigt JU, Pedrizzetti G, Lysyansky P, Marwick TH, Houle H, Baumann R, et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. J Am Soc Echocardiogr 2015 Feb;28(2):183-193.

(30) Wu KC, Weiss RG, Thiemann DR, Kitagawa K, Schmidt A, Dalal D, et al. Late gadolinium enhancement by cardiovascular magnetic resonance heralds an adverse prognosis in nonischemic cardiomyopathy. J Am Coll Cardiol 2008 Jun 24;51(25):2414-2421.

(31) Mandoli GE, D'Ascenzi F, Vinco G, Benfari G, Ricci F, Focardi M, et al. Novel Approaches in Cardiac Imaging for Non-invasive Assessment of Left Heart Myocardial Fibrosis. Front Cardiovasc Med 2021 Apr 15;8:614235.

(32) Garg P, Assadi H, Jones R, Chan WB, Metherall P, Thomas R, et al. Left ventricular fibrosis and hypertrophy are associated with mortality in heart failure with preserved ejection fraction. Sci Rep 2021 Jan 12;11(1):617-6.

(33) Di Marco A, Anguera I, Schmitt M, Klem I, Neilan TG, White JA, et al. Late Gadolinium Enhancement and the Risk for Ventricular Arrhythmias or Sudden Death in Dilated Cardiomyopathy: Systematic Review and Meta-Analysis. JACC Heart Fail 2017 Jan;5(1):28-38.

(34) Chelu MG, King JB, Kholmovski EG, Ma J, Gal P, Marashly Q, et al. Atrial Fibrosis by Late Gadolinium Enhancement Magnetic Resonance Imaging and Catheter Ablation of Atrial Fibrillation: 5-Year Follow-Up Data. J Am Heart Assoc 2018 Dec 4;7(23):e006313.

(35) Siebermair J, Kholmovski EG, Marrouche N. Assessment of Left Atrial Fibrosis by Late Gadolinium Enhancement Magnetic Resonance Imaging: Methodology and Clinical Implications. JACC Clin Electrophysiol 2017 Aug;3(8):791-802.

(36) Akoum N, Daccarett M, McGann C, Segerson N, Vergara G, Kuppahally S, et al. Atrial fibrosis helps select the appropriate patient and strategy in catheter ablation of atrial fibrillation: a DE-MRI guided approach. J Cardiovasc Electrophysiol 2011 Jan;22(1):16-22.

(37) Yamada S, Komuro K. Integrated backscatter for the assessment of myocardial viability. Curr Opin Cardiol 2006 Sep;21(5):433-437.

(38) Wang G, Shen L, Wang L, Li H, Zhang Y, Chen H. Relationship between integrated backscatter and atrial fibrosis in patients with and without atrial fibrillation who are undergoing coronary bypass surgery. Clin Cardiol 2009 Sep;32(9):56.

(39) Kubota T, Kawasaki M, Takasugi N, Imai H, Ishihara Y, Okubo M, et al. Left atrial pathological degeneration assessed by integrated backscatter transesophageal echocardiography as a predictor of progression to persistent atrial fibrillation: results from a prospective study of three-years follow-up. Cardiovasc Ultrasound 2012 Jun 29;10:28-28.

(40) Wu C, Hu Y, Chou C, Lin Y, Chang S, Lo L, et al. Transforming growth factor-β1 level and outcome after catheter ablation for nonparoxysmal atrial fibrillation. Heart Rhythm 2013 Jan;10(1):10-15.

(41) Park S, On YK, Kim JS, Choi J, Ju E, Jeong DS, et al. Transforming growth factor β1-mediated atrial fibrotic activity and the recovery of atrial mechanical contraction after surgical maze procedure. Int J Cardiol 2013 Apr 5;164(2):232-237.

(42) Rosenberg MA, Maziarz M, Tan AY, Glazer NL, Zieman SJ, Kizer JR, et al. Circulating fibrosis biomarkers and risk of atrial fibrillation: The Cardiovascular Health Study (CHS). Am Heart J 2014 May;167(5):723-8.e2.

(43) Chen C, Liu Y, Chen C, Wen M, Hung C, Wan Y. Radiation dose exposure of patients undergoing 320-row cardiac CT for assessing coronary angiography and global left ventricular function. Int J Cardiovasc Imaging 2012 Jun;28 Suppl 1:1-5.

(44) Chinnaiyan KM, Bilolikar AN, Walsh E, Wood D, DePetris A, Gentry R, et al. CT dose reduction using prospectively triggered or fast-pitch spiral technique employed in cardiothoracic imaging (the CT dose study). J Cardiovasc Comput Tomogr 2014;8(3):205-214.

(45) Walters TE, Ellims AH, Kalman JM. The role of left atrial imaging in the management of atrial fibrillation. Prog Cardiovasc Dis 2015;58(2):136-151.

(46) Henry WL, Morganroth J, Pearlman AS, Clark CE, Redwood DR, Itscoitz SB, et al. Relation between echocardiographically determined left atrial size and atrial fibrillation. Circulation 1976 Feb;53(2):273-279.

(47) Ma J, Chen Q, Ma S. Left atrial fibrosis in atrial fibrillation: Mechanisms, clinical evaluation and management. J Cell Mol Med 2021 Mar;25(6):2764-2775.

(48) Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015 Mar;16(3):233-270.

(49) Vyas H, Jackson K, Chenzbraun A. Switching to volumetric left atrial measurements: impact on routine echocardiographic practice. Eur J Echocardiogr 2011 Feb;12(2):107-111.

(50) Tsang TS, Barnes ME, Bailey KR, Leibson CL, Montgomery SC, Takemoto Y, et al. Left atrial volume: important risk marker of incident atrial fibrillation in 1655 older men and women. Mayo Clin Proc 2001 May;76(5):467-475.

(51) Pritchett AM, Jacobsen SJ, Mahoney DW, Rodeheffer RJ, Bailey KR, Redfield MM. Left atrial volume as an index of left atrial size: a population-based study. J Am Coll Cardiol 2003 Mar 19;41(6):1036-1043.

(52) Debonnaire P, Joyce E, Hiemstra Y, Mertens BJ, Atsma DE, Schalij MJ, et al. Left Atrial Size and Function in Hypertrophic Cardiomyopathy Patients and Risk of New-Onset Atrial Fibrillation. Circ Arrhythm Electrophysiol 2017 Feb;10(2):e004052. doi: 10.1161/CIRCEP.116.004052.

(53) Badano LP, Miglioranza MH, Mihăilă S, Peluso D, Xhaxho J, Marra MP, et al. Left Atrial Volumes and Function by Three-Dimensional Echocardiography: Reference Values, Accuracy, Reproducibility, and Comparison With Two-Dimensional Echocardiographic Measurements. Circ Cardiovasc Imaging 2016 Jul;9(7):e004229. doi: 10.1161/CIRCIMAGING.115.004229.

(54) Parwani AS, Morris D, Blaschke F, Huemer M, Pieske B, Haverkamp W, et al. Left atrial strain predicts recurrence of atrial arrhythmias after catheter ablation of persistent atrial fibrillation. Open Heart 2017 Apr 28;4(1):e000572-000572. eCollection 2017.

(55) Yasuda R, Murata M, Roberts R, Tokuda H, Minakata Y, Suzuki K, et al. Left atrial strain is a powerful predictor of atrial fibrillation recurrence after catheter ablation: study of a heterogeneous population with sinus rhythm or atrial fibrillation. Eur Heart J Cardiovasc Imaging 2015 Sep;16(9):1008-1014.

(56) Yoon YE, Oh I, Kim S, Park K, Kim SH, Park J, et al. Echocardiographic Predictors of Progression to Persistent or Permanent Atrial Fibrillation in Patients with Paroxysmal Atrial Fibrillation (E6P Study). J Am Soc Echocardiogr 2015 Jun;28(6):709-717.

(57) Tops LF, Delgado V, Bertini M, Marsan NA, Den Uijl DW, Trines, Serge A I P, et al. Left atrial strain predicts reverse remodeling after catheter ablation for atrial fibrillation. J Am Coll Cardiol 2011 Jan 18;57(3):324-331.

(58) Marrouche NF, Wazni O, McGann C, Greene T, Dean JM, Dagher L, et al. Effect of MRI-Guided Fibrosis Ablation vs Conventional Catheter Ablation on Atrial Arrhythmia Recurrence in Patients With Persistent Atrial Fibrillation: The DECAAF II Randomized Clinical Trial. JAMA 2022 Jun 21;327(23):2296-2305.

(59) King JB, Azadani PN, Suksaranjit P, Bress AP, Witt DM, Han FT, et al. Left Atrial Fibrosis and Risk of Cerebrovascular and Cardiovascular Events in Patients With Atrial Fibrillation. J Am Coll Cardiol 2017 Sep 12;70(11):1311-1321.

(60) Canpolat U, Oto A, Hazirolan T, Sunman H, Yorgun H, Şahiner L, et al. A prospective DE-MRI study evaluating the role of TGF-β1 in left atrial fibrosis and implications for outcomes of cryoballoon-based catheter ablation: new insights into primary fibrotic atriocardiomyopathy. J Cardiovasc Electrophysiol 2015 Mar;26(3):251-259.

(61) Marrouche NF, Wilber D, Hindricks G, Jais P, Akoum N, Marchlinski F, et al. Association of atrial tissue fibrosis identified by delayed enhancement MRI and atrial fibrillation catheter ablation: the DECAAF study. JAMA 2014 Feb 5;311(5):498-506.

(62) McGann C, Akoum N, Patel A, Kholmovski E, Revelo P, Damal K, et al. Atrial fibrillation ablation outcome is predicted by left atrial remodeling on MRI. Circ Arrhythm Electrophysiol 2014 Feb;7(1):23-30.

(63) Malcolme-Lawes LC, Juli C, Karim R, Bai W, Quest R, Lim PB, et al. Automated analysis of atrial late gadolinium enhancement imaging that correlates with endocardial voltage and clinical outcomes: a 2-center study. Heart Rhythm 2013 Aug;10(8):1184-1191.

(64) Seitz J, Horvilleur J, Lacotte J, O H-Ici D, Mouhoub Y, Maltret A, et al. Correlation between AF substrate ablation difficulty and left atrial fibrosis quantified by delayed-enhancement cardiac magnetic resonance. Pacing Clin Electrophysiol 2011 Oct;34(10):1267-1277.

(65) Kuppahally SS, Akoum N, Badger TJ, Burgon NS, Haslam T, Kholmovski E, et al. Echocardiographic left atrial reverse remodeling after catheter ablation of atrial fibrillation is predicted by preablation delayed enhancement of left atrium by magnetic resonance imaging. Am Heart J 2010 Nov;160(5):877-884.

(66) Oakes RS, Badger TJ, Kholmovski EG, Akoum N, Burgon NS, Fish EN, et al. Detection and quantification of left atrial structural remodeling with delayed-enhancement magnetic resonance imaging in patients with atrial fibrillation. Circulation 2009 Apr 7;119(13):1758-1767.

(67) Ling L, McLellan AJA, Taylor AJ, Iles LM, Ellims AH, Kumar S, et al. Magnetic resonance post-contrast T1 mapping in the human atrium: validation and impact on clinical outcome after catheter ablation for atrial fibrillation. Heart Rhythm 2014 Sep;11(9):1551-1559.

(68) Sasaki N, Okumura Y, Watanabe I, Nagashima K, Sonoda K, Kogawa R, et al. Transthoracic echocardiographic backscatter-based assessment of left atrial remodeling involving left atrial and ventricular fibrosis in patients with atrial fibrillation. Int J Cardiol 2014 Oct 20;176(3):1064-1066.

(69) den Uijl DW, Delgado V, Bertini M, Tops LF, Trines SA, van de Veire, Nico R, et al. Impact of left atrial fibrosis and left atrial size on the outcome of catheter ablation for atrial fibrillation. Heart 2011 Nov;97(22):1847-1851.

(70) Abed HS, Wittert GA, Leong DP, Shirazi MG, Bahrami B, Middeldorp ME, et al. Effect of weight reduction and cardiometabolic risk factor management on symptom burden and severity in patients with atrial fibrillation: a randomized clinical trial. JAMA 2013 Nov 20;310(19):2050-2060.

(71) De With RR, Marcos EG, Van Gelder IC, Rienstra M. Atrial fibrillation progression and outcome in patients with young-onset atrial fibrillation. Europace 2018 Nov 1;20(11):1750-1757.

(72) Cosio FG, Aliot E, Botto GL, Heidbüchel H, Geller CJ, Kirchhof P, et al. Delayed rhythm control of atrial fibrillation may be a cause of failure to prevent recurrences: reasons for change to

active antiarrhythmic treatment at the time of the first detected episode. Europace 2008 Jan;10(1):21-27.

(73) De With RR, Marcos EG, Dudink, E. A. M. P., Spronk HM, Crijns, H. J. G. M., Rienstra M, et al. Atrial fibrillation progression risk factors and associated cardiovascular outcome in well-phenotyped patients: data from the AF-RISK study. Europace 2020 Mar 1;22(3):352-360.

(74) Kloosterman M, Crijns, H. J. G. M., Mulder BA, Groenveld HF, Van Veldhuisen DJ, Rienstra M, et al. Sex-related differences in risk factors, outcome, and quality of life in patients with permanent atrial fibrillation: results from the RACE II study. Europace 2020 Nov 1;22(11):1619-1627.

(75) Wijffels MC, Kirchhof CJ, Dorland R, Allessie MA. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. Circulation 1995 Oct 1;92(7):1954-1968.

(76) Kirchhof P, Camm AJ, Goette A, Brandes A, Eckardt L, Elvan A, et al. Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. N Engl J Med 2020 Oct 1;383(14):1305-1316.

(77) Tayebjee MH, Creta A, Moder S, Hunter RJ, Earley MJ, Dhinoja MB, et al. Impact of angiotensin-converting enzyme-inhibitors and angiotensin receptor blockers on long-term outcome of catheter ablation for atrial fibrillation. Europace 2010 Nov;12(11):1537-1542.

(78) Pathak RK, Middeldorp ME, Lau DH, Mehta AB, Mahajan R, Twomey D, et al. Aggressive risk factor reduction study for atrial fibrillation and implications for the outcome of ablation: the ARREST-AF cohort study. J Am Coll Cardiol 2014 Dec 2;64(21):2222-2231.

(79) Pathak RK, Elliott A, Middeldorp ME, Meredith M, Mehta AB, Mahajan R, et al. Impact of CARDIOrespiratory FITness on Arrhythmia Recurrence in Obese Individuals With Atrial Fibrillation: The CARDIO-FIT Study. J Am Coll Cardiol 2015 Sep 1;66(9):985-996.

(80) Vermond RA, Geelhoed B, Verweij N, Tieleman RG, Van der Harst P, Hillege HL, et al. Incidence of Atrial Fibrillation and Relationship With Cardiovascular Events, Heart Failure, and Mortality: A Community-Based Study From the Netherlands. J Am Coll Cardiol 2015 Sep 1;66(9):1000-1007.

(81) Middeldorp ME, Pathak RK, Meredith M, Mehta AB, Elliott AD, Mahajan R, et al. PREVEntion and regReSsive Effect of weight-loss and risk factor modification on Atrial Fibrillation: the REVERSE-AF study. Europace 2018 Dec 1;20(12):1929-1935.

(82) Wijtvliet, E P J Petra, Tieleman RG, van Gelder IC, Pluymaekers, Nikki A H A, Rienstra M, Folkeringa RJ, et al. Nurse-led vs. usual-care for atrial fibrillation. Eur Heart J 2020 Feb 1;41(5):634-641.

(83) Elliott AD, Verdicchio CV, Mahajan R, Middeldorp ME, Gallagher C, Mishima RS, et al. An Exercise and Physical Activity Program in Patients With Atrial Fibrillation: The ACTIVE-AF Randomized Controlled Trial. JACC Clin Electrophysiol 2023 Apr;9(4):455-465.

(84) Kotecha D, Lam CS, Van Veldhuisen DJ, Van Gelder IC, Voors AA, Rienstra M. Heart Failure With Preserved Ejection Fraction and Atrial Fibrillation: Vicious Twins. J Am Coll Cardiol 2016 November 15;68(20):2217-2228.

(85) Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J 2021 Feb 1;42(5):373-498.

(86) McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur Heart J 2021 Sep 21;42(36):3599-3726.

(87) Coats AJS, Heymans S, Farmakis D, Anker SD, Backs J, Bauersachs J, et al. Atrial disease and heart failure: the common soil hypothesis proposed by the Heart Failure Association of the European Society of Cardiology. Eur Heart J 2021 Dec 7.

(88) Reddy YNV, Carter RE, Obokata M, Redfield MM, Borlaug BA. A Simple, Evidence-Based Approach to Help Guide Diagnosis of Heart Failure With Preserved Ejection Fraction. Circulation 2018 Aug 28;138(9):861-870.

(89) Lubitz SA, Benjamin EJ, Ellinor PT. Atrial fibrillation in congestive heart failure. Heart Fail Clin 2010 Apr;6(2):187-200.

(90) Santhanakrishnan R, Wang N, Larson MG, Magnani JW, McManus DD, Lubitz SA, et al. Atrial Fibrillation Begets Heart Failure and Vice Versa: Temporal Associations and Differences in Preserved Versus Reduced Ejection Fraction. Circulation 2016 Feb 2;133(5):484-492.

(91) Lam CSP, Santema BT, Voors AA. Atrial Fibrillation in Heart Failure: A Common and Deadly Combination. JACC Heart Fail 2017 August 01;5(8):575-577.

(92) Smit MD, Moes ML, Maass AH, Achekar ID, Van Geel PP, Hillege HL, et al. The importance of whether atrial fibrillation or heart failure develops first. Eur J Heart Fail 2012 Sep;14(9):1030-1040.

(93) Pallisgaard J, Greve AM, Lock-Hansen M, Thune JJ, Fosboel EL, Devereux RB, et al. Atrial fibrillation onset before heart failure or vice versa: what is worst? A nationwide register study. Europace 2023 Feb 16;25(2):283-290.

(94) Van Deutekom C, Van Gelder IC, Rienstra M. Atrial fibrillation and heart failure temporality: does it matter? Europace 2023 Feb 16;25(2):247-248.

(95) Piccini JP, Abraham WT, Dufton C, Carroll IA, Healey JS, van Veldhuisen DJ, et al. Bucindolol for the Maintenance of Sinus Rhythm in a Genotype-Defined HF Population: The GENETIC-AF Trial. JACC Heart Fail 2019 Jul;7(7):586-598.

(96) Pieske B, Tschöpe C, de Boer RA, Fraser AG, Anker SD, Donal E, et al. How to diagnose heart failure with preserved ejection fraction: the HFA-PEFF diagnostic algorithm: a consensus

recommendation from the Heart Failure Association (HFA) of the European Society of Cardiology (ESC). Eur Heart J 2019 Oct 21;40(40):3297-3317.

(97) Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure. Rev Esp Cardiol (Engl Ed) 2016 December 01;69(12):1167.

(98) Dzeshka MS, Lip GY, Snezhitskiy V, Shantsila E. Cardiac Fibrosis in Patients With Atrial Fibrillation: Mechanisms and Clinical Implications. J Am Coll Cardiol 2015 Aug 25;66(8):943-959.

(99) von Lueder TG, Kotecha D, Atar D, Hopper I. Neurohormonal Blockade in Heart Failure. Card Fail Rev 2017 Apr;3(1):19-24.

(100) Hartupee J, Mann DL. Neurohormonal activation in heart failure with reduced ejection fraction. Nat Rev Cardiol 2017 Jan;14(1):30-38.

(101) Lam CSP, Voors AA, de Boer RA, Solomon SD, van Veldhuisen DJ. Heart failure with preserved ejection fraction: from mechanisms to therapies. Eur Heart J 2018 Aug 7;39(30):2780-2792.

(102) van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. Eur J Heart Fail 2019 Apr;21(4):425-435.

(103) Samman Tahhan A, Sandesara PB, Hayek SS, Alkhoder A, Chivukula K, Hammadah M, et al. Association between oxidative stress and atrial fibrillation. Heart Rhythm 2017 Dec;14(12):1849-1855.

(104) Voors AA, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, et al. A systems BIOlogy Study to TAilored Treatment in Chronic Heart Failure: rationale, design, and baseline characteristics of BIOSTAT-CHF. Eur J Heart Fail 2016 Jun;18(6):716-726.

(105) Voors AA, Ouwerkerk W, Zannad F, van Veldhuisen DJ, Samani NJ, Ponikowski P, et al. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. Eur J Heart Fail 2017 May;19(5):627-634.

(106) Wang J, Gu BJ, Masters CL, Wang Y. A systemic view of Alzheimer disease - insights from amyloid-β metabolism beyond the brain. Nat Rev Neurol 2017 Sep 29;13(10):612-623.

(107) Troncone L, Luciani M, Coggins M, Wilker EH, Ho C, Codispoti KE, et al. Aβ Amyloid Pathology Affects the Hearts of Patients With Alzheimer's Disease: Mind the Heart. J Am Coll Cardiol 2016 Dec 6;68(22):2395-2407.

(108) Tanabe J, Ouchi T, Watanabe N, Tanabe K. Wild-type transthyretin cardiac amyloidosis diagnosed in a patient in his 50s. BMJ Case Rep 2020 Jun 30;13(6):e236656. doi: 10.1136/bcr-236656.

(109) Kumar S, Bhaskaran A. Atrial Fibrillation in Transthyretin Cardiac Amyloidosis: The Growing Need to Look Forward. JACC Clin Electrophysiol 2020 Sep;6(9):1128-1130.

(110) Donnellan E, Wazni OM, Hanna M, Elshazly MB, Puri R, Saliba W, et al. Atrial Fibrillation in Transthyretin Cardiac Amyloidosis: Predictors, Prevalence, and Efficacy of Rhythm Control Strategies. JACC Clin Electrophysiol 2020 Sep;6(9):1118-1127.

(111) Ritz K, van Buchem MA, Daemen MJ. The heart-brain connection: mechanistic insights and models. Neth Heart J 2013 Feb;21(2):55-57.

(112) Hooghiemstra AM, Bertens AS, Leeuwis AE, Bron EE, Bots ML, Brunner-La Rocca H, et al. The Missing Link in the Pathophysiology of Vascular Cognitive Impairment: Design of the Heart-Brain Study. Cerebrovasc Dis Extra 2017;7(3):140-152.

(113) Miller M. Emotional Rescue: The Heart-Brain Connection. Cerebrum 2019 May 1;2019:cer-Jun.

(114) Barnes DE. The mediterranean diet: good for the heart = good for the brain? Ann Neurol 2011 Feb;69(2):226-228.

(115) Richardson LA, Izuora K, Basu A. Mediterranean Diet and Its Association with Cardiovascular Disease Risk Factors: A Scoping Review. Int J Environ Res Public Health 2022 Oct 6;19(19):12762. doi: 10.3390/ijerph191912762.

(116) Román GC, Jackson RE, Gadhia R, Román AN, Reis J. Mediterranean diet: The role of long-chain ω-3 fatty acids in fish; polyphenols in fruits, vegetables, cereals, coffee, tea, cacao and wine; probiotics and vitamins in prevention of stroke, age-related cognitive decline, and Alzheimer disease. Rev Neurol (Paris) 2019 Dec;175(10):724-741.

(117) Schnabel RB, Yin X, Gona P, Larson MG, Beiser AS, McManus DD, et al. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. Lancet 2015 Jul 11;386(9989):154-162.

(118) Nielsen PB, Skjøth F, Overvad TF, Larsen TB, Lip GYH. Female Sex Is a Risk Modifier Rather Than a Risk Factor for Stroke in Atrial Fibrillation: Should We Use a CHA(2)DS(2)-VA Score Rather Than CHA(2)DS(2)-VASc? Circulation 2018 Feb 20;137(8):832-840.

(119) Kassim NA, Althouse AD, Qin D, Leef G, Saba S. Gender differences in management and clinical outcomes of atrial fibrillation patients. J Cardiol 2017 Jan;69(1):195-200.

(120) Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA 1994 Mar 16;271(11):840-844.

(121) Andrade JG, Deyell MW, Lee AYK, Macle L. Sex Differences in Atrial Fibrillation. Can J Cardiol 2018 Apr;34(4):429-436.

(122) Scheuermeyer FX, Mackay M, Christenson J, Grafstein E, Pourvali R, Heslop C, et al. There Are Sex Differences in the Demographics and Risk Profiles of Emergency Department (ED) Patients With Atrial Fibrillation and Flutter, but no Apparent Differences in ED Management or Outcomes. Acad Emerg Med 2015 Sep;22(9):1067-1075.

(123) Ball J, Carrington MJ, Wood KA, Stewart S, SAFETY Investigators. Women versus men with chronic atrial fibrillation: insights from the Standard versus Atrial Fibrillation spEcific managemenT studY (SAFETY). PLoS One 2013 May 29;8(5):e65795.

(124) Xiong Q, Proietti M, Senoo K, Lip GYH. Asymptomatic versus symptomatic atrial fibrillation: A systematic review of age/gender differences and cardiovascular outcomes. Int J Cardiol 2015 Jul 15;191:172-177.

(125) Blum S, Muff C, Aeschbacher S, Ammann P, Erne P, Moschovitis G, et al. Prospective Assessment of Sex-Related Differences in Symptom Status and Health Perception Among Patients With Atrial Fibrillation. J Am Heart Assoc 2017 Jun 30;6(7):e005401. doi: 10.1161/JAHA.116.005401.

(126) Rienstra M, Van Veldhuisen DJ, Hagens VE, Ranchor AV, Veeger, Nic J G M, Crijns, Harry J G M, et al. Gender-related differences in rhythm control treatment in persistent atrial fibrillation: data of the Rate Control Versus Electrical Cardioversion (RACE) study. J Am Coll Cardiol 2005 Oct 4;46(7):1298-1306.

(127) Piccini JP, Simon DN, Steinberg BA, Thomas L, Allen LA, Fonarow GC, et al. Differences in Clinical and Functional Outcomes of Atrial Fibrillation in Women and Men: Two-Year Results From the ORBIT-AF Registry. JAMA Cardiol 2016 Jun 1;1(3):282-291.

(128) Linde C, Bongiorni MG, Birgersdotter-Green U, Curtis AB, Deisenhofer I, Furokawa T, et al. Sex differences in cardiac arrhythmia: a consensus document of the European Heart Rhythm Association, endorsed by the Heart Rhythm Society and Asia Pacific Heart Rhythm Society. Europace 2018 October 01;20(10):1565-1565ao.

(129) Patton KK, Ellinor PT, Heckbert SR, Christenson RH, DeFilippi C, Gottdiener JS, et al. N-terminal pro-B-type natriuretic peptide is a major predictor of the development of atrial fibrillation: the Cardiovascular Health Study. Circulation 2009 Nov 3;120(18):1768-1774.

(130) Patton KK, Heckbert SR, Alonso A, Bahrami H, Lima JAC, Burke G, et al. N-terminal pro-B-type natriuretic peptide as a predictor of incident atrial fibrillation in the Multi-Ethnic Study of Atherosclerosis: the effects of age, sex and ethnicity. Heart 2013 Dec;99(24):1832-1836.

(131) Ho JE, Yin X, Levy D, Vasan RS, Magnani JW, Ellinor PT, et al. Galectin 3 and incident atrial fibrillation in the community. Am Heart J 2014 May;167(5):729-34.e1.

(132) The Gene Ontology Consortium. The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Res 2019 Jan 8;47(D1):D330-D338.

(133) Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000 Jan 1;28(1):27-30.

(134) Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res 2017 Jan 4;45(D1):D353-D361.

(135) Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res 2015 Jan;43(Database issue):447.

(136) Smedbakken L, Jensen JK, Hallén J, Atar D, Januzzi JL, Halvorsen B, et al. Activated leukocyte cell adhesion molecule and prognosis in acute ischemic stroke. Stroke 2011 Sep;42(9):2453-2458.

(137) Ueland T, Åkerblom A, Ghukasyan T, Michelsen AE, Becker RC, Bertilsson M, et al. ALCAM predicts future cardiovascular death in acute coronary syndromes: Insights from the PLATO trial. Atherosclerosis 2020 Jan;293:35-41.

(138) Rimmele DL, Borof K, Jensen M, Behrendt CA, Cheng B, Debus ES, et al. Association Between Carotid Atherosclerosis and Atrial Fibrillation, Cardiac, and Renal Function. Eur J Vasc Endovasc Surg 2022 Apr;63(4):641-647.

(139) Heeringa J, van der Kuip, Deirdre A M, Hofman A, Kors JA, van Rooij, Frank J A, Lip GYH, et al. Subclinical atherosclerosis and risk of atrial fibrillation: the rotterdam study. Arch Intern Med 2007 Feb 26;167(4):382-387.

(140) Terra X, Quintero Y, Auguet T, Porras JA, Hernández M, Sabench F, et al. FABP 4 is associated with inflammatory markers and metabolic syndrome in morbidly obese women. Eur J Endocrinol 2011 Apr;164(4):539-547.

(141) Rader F, Pujara AC, Pattakos G, Rajeswaran J, Li L, Castel L, et al. Perioperative heart-type fatty acid binding protein levels in atrial fibrillation after cardiac surgery. Heart Rhythm 2013 Feb;10(2):153-157.

(142) Ihara K, Sasano T. Role of Inflammation in the Pathogenesis of Atrial Fibrillation. Front Physiol 2022 Apr 14;13:862164.

(143) Beyzade S, Zhang S, Wong YK, Day IN, Eriksson P, Ye S. Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. J Am Coll Cardiol 2003 Jun 18;41(12):2130-2137.

(144) Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002 Feb 22;90(3):251-262.

(145) Chen M, Chang J, Liu W, Yang C, Chen Y, Tsai T, et al. Increased inflammatory cell infiltration in the atrial myocardium of patients with atrial fibrillation. Am J Cardiol 2008 Oct 1;102(7):861-865.

(146) Conen D, Ridker PM, Everett BM, Tedrow UB, Rose L, Cook NR, et al. A multimarker approach to assess the influence of inflammation on the incidence of atrial fibrillation in women. Eur Heart J 2010 Jul;31(14):1730-1736.

(147) Gomez SE, Parizo J, Ermakov S, Larson J, Wallace R, Assimes T, et al. Evaluation of the association between circulating IL-1β and other inflammatory cytokines and incident atrial fibrillation in a cohort of postmenopausal women. Am Heart J 2023 Apr;258:157-167.

(148) Li M, Yang G, Xie B, Babu K, Huang C. Changes in matrix metalloproteinase-9 levels during progression of atrial fibrillation. J Int Med Res 2014 Feb;42(1):224-230.

(149) Nakano Y, Niida S, Dote K, Takenaka S, Hirao H, Miura F, et al. Matrix metalloproteinase-9 contributes to human atrial remodeling during atrial fibrillation. J Am Coll Cardiol 2004 Mar 3;43(5):818-825.

(150) Anné W, Willems R, Roskams T, Sergeant P, Herijgers P, Holemans P, et al. Matrix metalloproteinases and atrial remodeling in patients with mitral valve disease and atrial fibrillation. Cardiovasc Res 2005 Sep 1;67(4):655-666.

(151) Tromp J, Ouwerkerk W, Demissei BG, Anker SD, Cleland JG, Dickstein K, et al. Novel endotypes in heart failure: effects on guideline-directed medical therapy. Eur Heart J 2018 Dec 21;39(48):4269-4276.

(152) Bootcov MR, Bauskin AR, Valenzuela SM, Moore AG, Bansal M, He XY, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc Natl Acad Sci U S A 1997 Oct 14;94(21):11514-11519.

(153) Wallentin L, Hijazi Z, Andersson U, Alexander JH, De Caterina R, Hanna M, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. Circulation 2014 Nov 18;130(21):1847-1858.

(154) Chen M, Ding N, Mok Y, Mathews L, Hoogeveen RC, Ballantyne CM, et al. Growth Differentiation Factor 15 and the Subsequent Risk of Atrial Fibrillation: The Atherosclerosis Risk in Communities Study. Clin Chem 2022 Jul 27;68(8):1084-1093.

(155) Fan Y, Zhao X, Li X, Li N, Hu X. Cardiac troponin and adverse outcomes in atrial fibrillation: A meta-analysis. Clin Chim Acta 2018 Feb;477:48-52.

(156) Goetze JP, Bruneau BG, Ramos HR, Ogawa T, de Bold MK, de Bold AJ. Cardiac natriuretic peptides. Nat Rev Cardiol 2020 Nov;17(11):698-717.

(157) Deng H, Bai Y, Shantsila A, Fauchier L, Potpara TS, Lip GYH. Clinical scores for outcomes of rhythm control or arrhythmia progression in patients with atrial fibrillation: a systematic review. Clin Res Cardiol 2017 Oct;106(10):813-823.

(158) Magnussen C, Niiranen TJ, Ojeda FM, Gianfagna F, Blankenberg S, Njolstad I, et al. Sex Differences and Similarities in Atrial Fibrillation Epidemiology, Risk Factors, and Mortality in Community Cohorts: Results From the BiomarCaRE Consortium (Biomarker for Cardiovascular Risk Assessment in Europe). Circulation 2017 October 24;136(17):1588-1597.

(159) Vieira MJ, Teixeira R, Gonçalves L, Gersh BJ. Left atrial mechanics: echocardiographic assessment and clinical implications. J Am Soc Echocardiogr 2014 May;27(5):463-478.

(160) Potpara TS, Stankovic GR, Beleslin BD, Polovina MM, Marinkovic JM, Ostojic MC, et al. A 12-year follow-up study of patients with newly diagnosed lone atrial fibrillation: implications of arrhythmia progression on prognosis: the Belgrade Atrial Fibrillation study. Chest 2012 Feb;141(2):339-347.

(161) Ding WY, Proietti M, Boriani G, Fauchier L, Blomström-Lundqvist C, Marin F, et al. Clinical utility and prognostic implications of the novel 4S-AF scheme to characterize and evaluate patients with atrial fibrillation: a report from ESC-EHRA EORP-AF Long-Term General Registry. Europace 2021 Nov 22.

(162) Díaz-Gutiérrez J, Martínez-González MÁ, Alonso A, Toledo E, Salas-Salvadó J, Sorlí JV, et al. American Heart Association's life simple 7 and the risk of atrial fibrillation in the PREDIMED study cohort. Nutr Metab Cardiovasc Dis 2023 Jun;33(6):1144-1148.

(163) Ogunmoroti O, Michos ED, Aronis KN, Salami JA, Blankstein R, Virani SS, et al. Life's Simple 7 and the risk of atrial fibrillation: The Multi-Ethnic Study of Atherosclerosis. Atherosclerosis 2018 Aug;275:174-181.

(164) Heidbuchel H, Van Gelder IC, Desteghe L, EHRA-PATHS Investigators. ESC and EHRA lead a path towards integrated care for multimorbid atrial fibrillation patients: the Horizon 2020 EHRA-PATHS project. Eur Heart J 2022 Apr 14;43(15):1450-1452.

(165) Volgman AS, Bairey Merz CN, Benjamin EJ, Curtis AB, Fang MC, Lindley KJ, et al. Sex and Race/Ethnicity Differences in Atrial Fibrillation. J Am Coll Cardiol 2019 Dec 3;74(22):2812-2815.

(166) Shen AY, Contreras R, Sobnosky S, Shah AI, Ichiuji AM, Jorgensen MB, et al. Racial/ethnic differences in the prevalence of atrial fibrillation among older adults--a cross-sectional study. J Natl Med Assoc 2010 Oct;102(10):906-913.

(167) Norby FL, Benjamin EJ, Alonso A, Chugh SS. Racial and Ethnic Considerations in Patients With Atrial Fibrillation: JACC Focus Seminar 5/9. J Am Coll Cardiol 2021 Dec 21;78(25):2563-2572.

(168) Lee EY, Akhtari F, House JS, Simpson RJJ, Schmitt CP, Fargo DC, et al. Questionnaire-based exposome-wide association studies (ExWAS) reveal expected and novel risk factors associated with cardiovascular outcomes in the Personalized Environment and Genes Study. Environ Res 2022 Sep;212(Pt D):113463.

(169) Essien UR, Kornej J, Johnson AE, Schulson LB, Benjamin EJ, Magnani JW. Social determinants of atrial fibrillation. Nat Rev Cardiol 2021 Nov;18(11):763-773.

(170) Abdel-Qadir H, Akioyamen LE, Fang J, Pang A, Ha ACT, Jackevicius CA, et al. Association of Neighborhood-Level Material Deprivation With Atrial Fibrillation Care in a Single-Payer Health Care System: A Population-Based Cohort Study. Circulation 2022 Jul 19;146(3):159-171.