GRIGORE T. POPA UNIVERSITY OF MEDICINE AND PHARMACY IASI

Atrial fibrillation in arrhythmology – by left atrial remodeling to thrombogenesis and outcomes

HABILITATION THESIS

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ABBREVIATION LIST

Aa: amino-acid
ACE: angiotensin converting enzyme
AF: atrial fibrillation
Ang II: angiotensin II
ANP: atrial natriuretic peptide
ARB: angiotensin receptor blockers
ARGP: anterior right ganglionated plexi
ASR: asymmetric structural remodeling
ATP: adenosine triphosphate
AUC: area under curve
AVN: atrio-ventricular node
BNP: B-type natriuretic peptide
CA: catheter ablation
CD-NP: Cenderitide
CHADS2: thromboembolic risk scores
CHA2DS2-VASc: thromboembolic risk score
CMR: cardiac magnetic resonance
CMR-LGE: cardiac magnetic resonance – late gadolinium enhancement
CNP:C-type natriuretic peptide
CT: computer tomography
CV: cardiovascular
CVD: cardiovascular disease
CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration
DOACs: direct oral anticoagulants
ECE: endothelin-converting enzyme
ECG: electrocardiogram
ESC: European Society of Cardiology
GFR: glomerular filtration rate
GMP: guanylate mono phosphatase
GP: ganglionated plexi
ET-1: endothelin- 1
HF: heart failure
HFmrEF: heart failure with mid-range ejection fraction
HFpEF: heart failure with preserved ejection fraction
HFrEF: heart failure with reduced ejection fraction
HFS: high-frequency stimulation
IL: interleukin
ILGP: inferior left ganglionated plexi
IRGP: inferior right ganglionated plexi
LA: left atrial
LAA: left atrial appendage
LAV: left atrial volume

LAVI: left atrial indexed volume
LGE-MR: Delayed gadolinium enhancement magnetic resonance imaging
LV: left ventricle
LVEF: left ventricular ejection fraction
mRNA: messenger ribonucleic acid
MACCE: major adverse cardio- and cerebrovascular events
MR-proANP middle range pro atrial natriuretic peptide
MRAs: mineralocorticoid receptor antagonists
MDRD: Modification of Diet in Renal Disease
NAFLD: Non-alcoholic fatty liver disease
NEP: neutral endopeptidase
NHE: natrium-proton exchanger
NPs: natriuretic peptides
NPR-A: natriuretic peptide receptor A
NPR-B: natriuretic peptide receptor B
NPR-C: natriuretic peptide receptor C
NPs: natriuretic peptides
NT-proBNP: N-terminal-Pro-BNP:
pGC: particulate guanylyl cyclase PW: pulsed wave
RAAS: renin-angiotensin-aldosterone system
RVN: right vagus nerve
sGC: soluble guanylyl cyclase
sST2: soluble source of tumorigenicity 2
SAVR: surgical aortic valve replacement
SLGP: superior left ganglionated plexi
SR: sinus rhythm
SSR: symmetric structural remodeling
TDI: tissue Doppler imaging
T2DM: Type 2 diabetic mellitus
TEE: transesophageal echocardiography
TGF ß: transforming growth factor beta

REZUMATUL TEZEI

Teza de abilitare oferă o prezentare generală a activităților mele din domeniul aritmologiei clinice, o ramură relativ nouă a cardiologiei, în cei șapte ani de la finalizarea tezei de doctorat la Universitatea de Medicină și Farmacie "Grigore T. Popa" Iași. Intitulată "Fibrilația atrială în aritmologie – de la remodelarea atrială stângă la trombogeneză şi rezultate terapeutice" teza de abilitare oferă o sinteză a contribuțiilor mele științifice din perioada postdoctorală (din 2012 până în prezent), fiind axată pe patru direcţii de cercetare importante legate de fibrilaţia atrială din perspectiva aritmologiei clinice: evaluarea remodelării atriale stângi determinată de "triggers" şi substrat, în fibrilația atrială paroxistică şi persistentă, aprecierea riscului tromboembolic în fibrilația atrială, analiza rezultatelor în ablația fibrilației atriale şi a relaţiei acestei aritmii cu alte patologii.

Teza de abilitare sintetizează activitatea profesională, academică şi ştinţifică postdoctorală în conformitate cu recomandările Consiliului Național de Atestare a Titlurilor, Diplomelor și Certificatelor Universitare (CNATDCU). De aceea, după rezumat, aceasta are următoarele patru secțiuni: I - evoluţia profesională; II - activitatea de cercetare din perioada postdoctorală; III - planurile de dezvoltare şi proiectele de cercetare viitoare; IV - bibliografia.

Secţiunea întâi a tezei de abilitare descrie activitatea de cercetare realizată după finalizarea tezei de doctorat şi principalele publicații din direcțiile studiate, precum și planul de dezvoltare profesională, academică și de cercetare pentru următorii ani, în care intenția mea a fost să mă concentrez nu doar pe extinderea și diseminarea cunoștințelor de aritmologie clinică ci și asupra aprofundării temelor de cercetare, pe dezvoltarea bazei logistice și pe sporirea diseminării și vizibilității rezultatelor cercetării.

Secţiunea a doua este structurată în 8 subcapitole şi prezintă cele 4 direcţii de cercetare abordate la pacienţii cu fibrilaţie atrială:

1. Prima direcție este evaluarea remodelării atriului stâng prin "triggers" şi substrat în fibrilația atrială paroxistică şi persistentă, din perspectiva aritmologiei clinice, ecocardiografia fiind principala tehnică neinvazivă foarte utilă în evaluarea remodelării atriului stâng.
2. A doua direcție evaluează riscul tromboembolic în fibrilația atrială şi analizează cardiomiopatia atrială, un nou concept în trombogenicitate.
3. A treia direcție studiază fibrilația atrială postoperatorie din perspectiva aritmologiei clinice şi analizează predictorii acesteia.
4. Ultima direcţie abordează factorii de risc (hipertensiunea arterială, diabetul zaharat sau steatoza hepatică) şi comorbidităţile asociate fibrilaţiei atriale (insuficienţa cardiacă şi relaţia ei cu peptidele natriuretice sau anemia), fiind patologii foarte frecvente în practica medicală.

Secțiunea a treia trasează principalele direcții de cercetare și studii clinice pentru viitor pe teme de aritmologie clinică: fibroza atrială din perspectiva trombogenicităţii, aprodundarea studierii "trigger-ilor" asociaţi bolii de reflux gastroesofagian şi a relaţiei osteoporozei cu fibrilaţia atrială. Am pus în discuţie perspectivele din cercetarea clinică cu proiectele viitoare, planurile de implementare precum şi perspectivele din activitatea academică, profesională şi de învăţământ.

Fibrilația atrială, cea mai frecventă tulburare de ritm din practica medicală, pune încă mari probleme de fiziopatologie și implicit de terapie în aritmologia clinică. Având la bază acestea, consider că se pot aborda noi teme de cercetare clinică împreună cu centre internaţionale, pe care să le pot prezenta la manifestările științifice naționale și internaționale şi în care să pot implica doctoranzii cu teme de cercetare de medicină internă, cardiologie sau alte specializări conexe.

Secțiunea a patra cuprinde lista de referințe, care reprezintă o selecţie a celor mai importante titluri bibliografice utilizate în prezentarea tezei de abilitare.

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SUMMARY OF THE THESIS

The habilitation thesis provides an overview of my activities in the field of clinical arrhythmology (a relatively new branch of cardiology) during the seven years since the completion of my doctoral studies at the "Grigore T. Popa" University of Medicine and Pharmacy from Iași. The PhD thesis entitled “Atrial fibrillation in arrhythmology – by left atrial remodeling to thrombogenesis and outcomes” communicates my research in the field of clinical arrhythmology since 2012, focusing on atrial fibrillation.

The habilitation thesis summarizes my postdoctoral professional, academic and scientific activity according to the requirements and recommendations of the National Council for Attesting Titles, Diplomas and Certificates (CNATDCU). As such, the habilitation thesis is structured in four main sections: I - an overview of professional achievements; II - scientific achievements resulting from postdoctoral research; III - future development plans and research projects; IV – references.

After a succinct overview of professional, academic and scientific achievements, the section I of the habilitation thesis provides a synthesis of my scientific contributions from the postdoctoral period. My research into atrial fibrillation from the perspective of clinical arrhythmology has had four main focal points and directions:

1. The assessment of left atrial remodeling due to both *triggers* and *substrate* involved the pathophysiology of atrial fibrillation,
2. The evaluation of thromboembolic risk by analyzing atrial cardiomyopathy,
3. The assessment of predictors in postoperative atrial fibrillation patients, and
4. The relationship of atrial fibrillation with comorbidities (heart failure in relationship with natriuretic peptides and anemia), and risk factors (arterial hypertension, diabetes mellitus, non-alcoholic steato-hepatitis).

Section one describes the research activity carried out after the completion of the PhD thesis and the main resulting publications, as well as the professional, academic and research development plans for the following years. During my entire career, I focused on the expansion, scrutiny, and dissemination of evidence-based knowledge and research results regarding clinical arrhythmology and echocardiography. A closer link between these two is one of my goals as I define my interests and capacity for further research.

Section two is structured in eight sub-chapters summarizing the four main directions of research related to atrial fibrillation from a clinical arrhythmology perspective. The first three chapters are focused on research regarding left atrial remodeling as the main pathophysiological mechanism involved in the occurrence and/or maintenance of atrial fibrillation (triggers and substrate), and echocardiography as the main noninvasive imaging technique used in its evaluation. The fourth chapter focuses on evaluating the thromboembolic risk in atrial fibrillation, and analyzes the link between the latter and atrial cardiomyopathy, a new concept explaining the thrombogenicity in this arrhythmia. Chapters five, six and seven highlight the success rate of atrial fibrillation ablation (both radiofrequency and cryoablation) from the perspective of clinical arrhythmology, as well as the predictors of recurrences and post-ablation outcomes. The last chapter of section two addresses the comorbidities associated with atrial fibrillation, heart failure, arterial hypertension or diabetes mellitus being very common pathologies in medical practice.

The third section highlights the main future research directions related to clinical arrhythmology: atrial fibrosis from the perspective of thrombogenicity, further study of the "triggers" associated with gastroesophageal reflux disease, and the relation of osteoporosis with atrial fibrillation. Here, I place emphasis on the clinical research perspectives in the context of the future research projects implementation plan and my track record of professional activity, including academic and didactic perspectives. Although atrial fibrillation is the most common rhythm disorder in clinical practice, its pathophysiological mechanisms are far from being fully known. This majorly affects the quality of the therapy delivered to patients. Based on these, I firmly believe that new clinical research topics can and should be addressed in collaboration with other international research centers. I believe I have the position and experience to galvanize such research and engage my peers in fruitful exchanges at national and international scientific events. Importantly, this includes continuing my efforts to involve PhD students and residents in internal medicine, cardiology or other specialties.

The fourth section contains a relevant selection of references used in the presentation of the habilitation thesis.

SECTION I - OVERVIEW OF PERSONAL, PROFESSIONAL, ACADEMIC AND SCIENTIFIC ACCOMPLISHMENTS

I am an Associate Professor at the Department of Internal Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, and, throughout my professional development and career, I have integrated clinical practice, teaching, and research. Since October 2019, I am also a senior consultant in internal medicine and cardiology at the Clinical Emergency Military Hospital Iaşi, after having worked at "St Spiridon" Emergency Hospital, the 3rd Clinic of Internal Medicine, between 2008-2019.

The habilitation thesis "Atrial fibrillation in arrhythmology - from left atrial remodeling to thrombogenesis and outcomes" presents the main current and future directions of clinical research related to atrial fibrillation that I have been pursuing following my doctorate. The relevant conceptualization, implementation, results, and perspectives are also visible to the wider scientific community through articles published in peer-reviewed international journals. This work builds on my PhD research and thesis "Left atrial structural remodeling in atrial fibrillation – echocardiographic assessment and clinical implications", supervised by Prof. Dr. Cătălina Arsenescu Georgescu, for which I was awarded the PhD in Medicine in 2012 by the then Ministry of Education, Research, Youth and Sports officially confirmed my PhD in Medicine (Order 6508/19.12.2012, Series H, No. 0015784).

Complementarily, my daily activities with students and residents in internal medicine and cardiology have been instrumental in engaging the younger generations and involving them in the research of atrial fibrillation and, more specifically, clinical arrhythmology.

Professional achievements

After five years of training as a resident, I became a specialist in internal medicine in October 2007. Towards the end of my residency, I benefited from a 2-year fellowship at the Louvain Catholic University (UCL) in Belgium, at Cliniques Universitaires CHU Mont-Godinne. During this time, I acquired substantial knowledge and clinical experience related to rhythm disorders. As a member of the Arrhythmology team led by Professor Luc De Roy, former chair of the Belgian Working Group of Cardiac Electrophysiology, I gained specific skills for electrophysiology laboratory work and I had the opportunity to collaborate with cardiologists, arrhythmologists, neurologists, cardiovascular surgeons, intensive care physicians. The fellowship also leads to continued scientific collaboration and doctoral research, for which I provide more details in the section on scientific activity below.

After completing a second residency in cardiology at the Institute of Cardiovascular Diseases in Iasi, I became a specialist cardiologist in October 2010. Also, since February 2008, I worked as an internist at the 3rd Clinic of Internal Medicine, "St. Spiridon" Hospital Iasi.

From 2010 to 2012, I continued in Belgium with another fellowship in echocardiography (transthoracic, transesophageal, stress) in the same tertiary center at the Catholic University of Louvain, Cliniques Universitaires CHU Mont-Godinne, in the Echocardiography Department. Between November 2011 and October 2012, I coordinated the Echocardiography Unit at this hospital. The experience gained here helped me complete my

training as a cardiologist, as well as obtain national accreditation in general echocardiography (Certificate VIIIB/14684/4.06.2012) and European accreditation in transthoracic echocardiography (EACVI Certificate 558143/2015). In addition, based on my work and collaboration with CHU Mont-Godinne University Hospital, I obtained the right to practice in Belgium as a consultant in internal medicine (INAMI 1-93647-580) as well as in cardiology (INAMI 1-93647-730).

From January 2013, during one year, I worked as cardiologist at Cardiovascular Disease Institute (CCTIFA Unit), and I contributed to the development and implementation of a research project on interventional therapy in AF by means of endocardial ablation with radiofrequency or cryotherapy. During this time, in June 2013, I also successfully passed the required examination for the title of senior specialist/consultant in internal medicine with the overall grade 9.55/10, and received official confirmation soon after (Order no. 1044/2013).

In June 2016, I got the accreditation in general ultrasonography (attestation VIII series B No. 146841/4.06.2016). In June 2017, I became senior specialist/consultant in cardiology, confirmed by WHO 988/2017 with certificate No 13890 of 30.08.2017.

To sum up, I am now a senior specialist/consultant in both internal medicine and cardiology, and I have accreditations in general echocardiography (certificate No 685/3.01.2011, Order No. 418/2005 of the Ministry of Health), general ultrasonography (certificate series VIII B No. 146841/4.06.2012), and an EACVI accreditation in transthoracic echocardiography (Certificate 558143).

Academic activity

I began my academic career as a teaching assistant at the “Grigore T, Popa” University of Medicine and Pharmacy Iași in 2012 and, three years later, I advanced to the position of lecturer. During these years and subsequently, I developed and diversified my teaching and academic skills, starting with courses of internal medicine to3rd year students in General Medicine. In 2016, I also began delivering an optional course to 4th year nursing students entitled "Electrocardiography in intensive care units". My experience as a lecturer grew further and, in the last two academic years, I have also been teaching courses of internal medicine to 4th year medical students and one on arrhythmia ("Les tachyarrhythmies") to 5th year medical students from the French study program, using French as the language of instruction.

To complement and enhance the didactic process, I have also coordinated and coauthored two books on electrocardiography in close collaboration with my students and residents. These books are available in Romanian, French, and English, and they are useful learning resources for 3rd and 4th year medical students, as well as for any young physician still in training.

My commitment to quality undergraduate medical education has been acknowledged and appreciated by the students. In 2017, upon their proposal, I was awarded the title of Professor Bologna during a formal event under the patronage of the Romanian Presidency.

Furthermore, since 2016, the clinical experience gained in the field of rhythm disturbances has allowed me to coordinate and/or teach continuing medical education and training courses on echocardiography and electrocardiography topics. I was lecturer for

general echocardiography accreditation in 2016 and 2017, carried out by our university. Last, but not least I have also expressed an active interest in educational projects, such as "Professional counseling for medical students and an integrated practice program in the field of general and dental medicine" (POSDRU 160/2.1/S/139881, coordinator Prof. Norina Forna), in which I was a member.

Scientific activity

After my graduation, I first became involved with research in 2006, during my aforementioned first fellowship in Belgium. There, I participated for one year in a study on ganglionated plexuses ablation in persistent atrial fibrillation entitled "LA Isolation and Dissection of Fad Pads ". The project was developed by the Catholic University of Louvain and coordinated by Professor Luc de Roy. Work on this grant resulted in three publications in ISI journals (Journal of Cardiovascular Electrophysiology, Annals of Thoracic Surgery and the Anatolian Journal of Cardiology), 5 abstracts published in journals such as Circulation or Acta Cardiologica, and 16 abstracts presented at congresses of cardiology and rhythm disorders (ECAS, Europace, Heart Rythm and AHA). After I completed my fellowship I continued to collaborate with the Cardiology Unit of CHU Mont-Godinne by conducting more clinical research and sharing it by means of other published articles.

Having gained such experience in the field of rhythm disorders, in 2008 I began my doctoral studies at U.M.Ph. Iasi under the supervision of Professor Cătălina Arsenescu Georgescu. My PhD thesis was essentially a clinical study regarding the echocardiographic assessment of left atrial structural remodeling in atrial fibrillation. In the process of conducting the research, I published 6 articles in extenso (Journal of Atrial Fibrillation, Romanian Journal of Cardiology, Medico-Surgical Journal) and delivered 8 presentations at national and international congresses (Europace 2012, Euroecho 2010 and 2011).

During my PhD research, in 2009, I was awarded the 3rd prize Paul-Scholmerich by the Romanian German Academy for the article "Abnormal atrioventricular node conduction and atrioventricular nodal reentrant tachycardia in patients older versus younger than 65 years of age", which I coauthored with Grecu Mihaela and Cătălina Georgescu Arsenescu. I also received an award from the Romanian Society of Cardiology in 2010 and, soon after, the 2nd prize from the Romanian Journal of Cardiology for the article "Asymmetric remodeling of left atrium in atrial fibrillation: correlation with diastolic dysfunction", coauthored by myself and J. Jamart, V. Ambăruș, Cătălina Georgescu Arsenescu Georgescu, published by the journal in 2011(21(4):303-309).

After the successful completion of my doctoral studies, I participated in several research projects. From December 2012 to January 2014, I was a member in the project "Expanding and Upgrading an Atrial Fibrillation Treatment Research Center (CC-TIFA) - as a method of preventing heart failure by developing the research and developing infrastructure", coordinated by Prof. Grigore Tinică at the "Prof. Dr. George IM Georgescu" Institute of Cardiovascular Diseases Iaşi. The work in this project resulted in 10 publications, of which 1 article was published in the ISI-rated journal Europace.

Another study was the post-doctoral research "Atrial Fibrillation - Extraesophageal Manifestation of Gastroesophageal Reflux Disease", which I conducted from June 2014 until

October 2015 as a grant within the project POSDRU/159/1.5/S/133377 coordinated by Prof. Radu Iliescu. This is also when I started the collaboration with Professor Vasile Liviu Drug from the Institute of Gastroenterology and Hepatology. The participation in this research project allowed me to publish 3 articles in ISI journals.

I continued to receive formal recognition for this postdoctoral work. In December 2014, the Romanian Society of Cardiology awarded me with the distinction "Excellence for research in cardiology" (B category) for the article "Predictive value of thromboembolic risk scores before an atrial fibrillation ablation procedure", previously published in the Journal of Cardiovascular Electrophysiology in 2013 (24(2):139-145), for which I had collaborated with coauthors Luc De Roy, Olivier Xhaet, Dominique Blommaert, Jacques Jamart, Marina Gerard, Fabien Dormal, Olivier Deceuninck, Baudouin Marchandise, Erwin Schroeder. In September 2015, during the National Congress of Cardiology, the Romanian Society of Cardiology offered me a prize for the article entitled "Gastroesophageal reflux disease in patients with atrial fibrillation ablation", which I had coauthored with Oana Barboi, Mihaela Grecu, Ciprian Rezuș, Cristina Cijevschi Prelipcean, Gheorghe Bălan, Vasile L. Drug.

Based on the research experience and expertise thus accumulated and acknowledged, since June 2015, I am member of the Research Ethics Committee of the “Grigore T. Popa” University of Medicine and Pharmacy Iasi.

More recently, I was awarded by UEFISCDI with a prize in their program "Awarding the results of research" for the article entitled "Integrity of the Ganglionated Plexi Is Essential to Parasympathetic Innervation of the Atrioventricular Node by the Right Vagus Nerve", authored by Xhaet O, De Roy L, Floria M, Deceuninck O, Blommaert D, Dormal F, Ballant E, LA Meir M, and published in the Journal of Cardiovascular Electrophysiology 2017;28(4):432-437).

Up to now, I am single author and a co-author for 54 articles published in journals indexed by PubMed. Of these, 41 are in ISI-rated journals with impact factors between 0.48 - 5.69. Currently, I have a Hirsh index of 7. I am also single author or a co-author of 12 books on relevant topics. In terms of sharing my research results in scientific events, I have so far delivered 189 oral presentations at national (126 papers, 60 out of these oral presentations) and international congresses (63 papers, 29 out of these oral presentations).

My experience as a scientific author has also enabled me to contribute as a peer-reviewer of 45 manuscripts subsequently published in ISI-rated journals (with impact factor up to 5.69). Similarly, I was a guest editor of the Archive of Clinical Cases with regard to the publication of arrhythmia case reports (Arrhythmology 2017; 4 (2): 52-138), and I also reviewed numerous ESC Congress abstracts for the 2016, 2017 and 2019.

Based on my scientific work and achievements, in 2014 I became a Fellow of the European Society of Cardiology ("scientific excellence"). I am also an active member of the Romanian Society of Cardiology, the Romanian Society of Internal Medicine, the European Society of Cardiology, the European Association of Cardiovascular Imaging and the European Society of Acute Cardiovascular Care.

SECTION II - SCIENTIFIC ACHIEVEMENTS

Chapter 1 LEFT ATRIAL STRUCTURAL REMODELING IN ATRIAL FIBRILLATION

1.1. Introduction

Atrial fibrillation (AF), the most frequent sustained arrhythmia in clinical practice, is associated with a high morbi-mortality and has an epidemic tendency. Its progression and persistence are closely connected to atrial *electrical, mechanical* (contractile) and *structural* (morphological or anatomical) remodeling. Overall left atrial (LA) remodeling reflects the underlying pathophysiological changes associated with arrhythmia progression. These changes include:

- ionic channels alterations,
- cellular energy and neuro-hormonal dysbalance, and
- inflammation.

There is convincing evidence that demonstrates the important pathophysiological association between LA remodeling and AF. It follows that factors preventing, attenuating or capable of stopping LA remodeling may have a major public health impact with respect to the AF *epidemic.*

It has been known for over a decade that in over 90% of cases, AF is initiated by either unique or multiple ectopic foci (*triggers*) arising at the level of the pulmonary veins (1). The evolution of this arrhythmia is progressive by substrate apparition. Accordingly, it is estimated that 14-24% of the paroxysmal AF evolves towards persistent (2). Subsequently, nearly one third of the patients with paroxysmal or persistent AF progress to permanent, most of them during the first 15 years from their diagnose (3). Once appeared, AF firstly determines atrial electrophysiological and mechanical remodeling through action potential duration shortening, effective atrial refractory period mal-adaptation and increase, intra-arterial conduction depression with loss of contractile function and subsequent structural remodeling. The latter is considered an adaptation process similar to the hibernation of the ischemic ventricular myocytes, with the purpose of extending cellular viability. Electrical, mechanic and structural remodeling determines AF maintenance and progression from paroxysmal to the persistent and permanent.

Atrial *electrical remodeling* most frequently appears after 24-48 hours to months from the arrhythmia onset and results in increased and progressive susceptibility, therefore increasing the risk of more frequent AF episodes. The functional and structural atrial changes that appear after AF initiation facilitate its self-perpetuation ("AF begets AF") (4). AF will *generate* AF irrespective of the presence of the initial triggers, leading to arrhythmia perpetuation through substrate alteration (atrial myocardium).

Electrophysiological and cellular changes occur after the first 48 hours after AF onset (5). The changes of the refractory periods, of the intra-arterial conduction as well as the functionality of the sinus node are stages of the atrial electrical remodeling.

Importantly, AF represents in itself a cellular stressor. Physiologically, calcium enters the cells during contraction and contributes to cellular repolarization. The extremely rapid atrial activation at 350-600/min induces a calcium overload inside cardiomyocytes through the L-type calcium channels. This excessive calcium, in the context of high-rate fibrillatory activity leads to the decrease in L-type channels input current, resulting in action potential duration shortening (especially phase 2). The latter, together with the excessive number of atrial "tremors" also contribute to calcium inflow increase. As compensatory mechanisms trying to prevent calcium overload, on the short term the I_{CaL} current is inhibited, while on the long-term the number of I_{CaL} channels themselves diminishes. This L-type calcium channel decreased expression, together with a degree of myolisis determines the atrial contractile dysfunction, first seen through absent "A" waves in the atrial post-cardioversion pressure curve. Furthermore, decreasing the duration of L-type calcium currents will reflect as refractory period shortening and heterogenicity with a decrease in fibrillatory wave's cycle length and the subsequent promotion of micro-reentries and increase in the vulnerability of the substrate (5).

This is the key link of a vicious circle, as the action potential is shortened even more by the shortening of the refractory periods, with their mal-adaptation to high rates. Another essential aspect is the alteration in the activity of several specific phosphatases and kinases, which modulate key proteins involved in the activity of the calcium (as well as the calcineurin). Initially a protective mechanism against calcium overload, the latter becomes yet another vicious circle, which promotes AF.

After AF initiation, other cellular changes occur, such as a decrease in the sodium current, and subsequently of the conduction velocities and increase in the potassium rectifier current. Thus, the cardiomyocyte gains a fetal-type ultrastructural phenotype. In time, the quick sodium channels are submitted to a down-regulation process (the diminishing of their number secondary to the increase in the sodium ions), thus contributing to the slowing of the intra-atrial conduction and to the the wavelength shortening (6).

There are also alterations of the potassium currents: the decrease in the output currents (I_{to}, I_{ksus}, I_{kur}) and in the ATP-dependent currents (I_{KATP}), the increase in the input rectifier current (I_{k1}) and the decrease/increase in the activated acetylcholine currents (I_{KACh}) (7). These changes alter the resting membrane potential and therefore, repolarization. The persistence of the arrhythmia ultimately activates calpain (a cysteine protease), which leads to L-type calcium channels and contractile proteins degradation and subsequent myolysis. The activation of the cysteine proteases initiates and contributes to apoptosis (8). Particularly, cardiomyocytes apoptosis is not complete. Depending on the intensity of the stress to which the atrial myocyte is exposed, the activation of the cysteine proteases may induce atrial remodeling through L-type calcium channels degradation or the disruption of the myofilaments proteins (troponine I or T, actine). This leads to action potential duration shortening, myolysis and contractile dysfunction, with the AF persistence (9). The heat shock proteins – a protein family involved in defending the cell against stress – seem to have the role of preventing atrial remodeling and the progression of the paroxysmal form to the permanent one. Heat shock proteins B1 may relate to the myofibrils and the ionic channels in order to preserve their function and may inhibit the calpain activity (10).

The *mechanic* (contractile) *remodeling* is initiated as fast as the electrical one, namely during the first 48 hours from the atrial fibrillation onset. Both the reduced release of the calcium ion secondary to the "down-regulation" of the responsible channels and the sarcomeres loss (myolysis) also contribute to it. The loss of the mechanic atrial activity will induce atrial dilatation and thrombus formation, as well as atrial fibrillation progression through the creation of a larger space of perpetuation of the fibrillating waves. The recovery of the contractile activity after the conversion to SR is more difficult than the reverse electrical remodeling due to the loss of sarcomeres. Reverse remodeling, namely the recovery of the mechanic function of the atrium after the conversion to SR occurs in a very variable period, of a few hours/days up to 3-4 weeks. It is unknown what conditions this chronologic evolution.

Structural remodeling through the left atrium dilatation increases the surface available to the multiple waves, which together with interstitial fibrosis (that increases the anisotropy and generates conduction blocks) causes atrial fibrillation to become more and more persistent. The model of multiple waves was revealed in the study of the atrial activation pattern in patients undergoing heart surgical interventions (11). Using first mathematical models and them animal models, it was observed that it takes at least 6 microwaves for AF to become persistent (12). The larger the "caught" atrial mass, the higher the tendency to persistence, and the notion of necessary critical mass is a condition for the perpetuation of the AF. The fast atrial activity determines in the intracellular matrix inflammatory phenomena, the activation of the fibroblasts, with fibrosis "in the islands". Interstitial fibrosis is secondary to atrial myocytes apoptosis, glycogen granules accumulation, the loss of myofibrils and gap type cell coupling junctions. This may appear in any heart condition inducing atrial dilatation and is accompanied by the increase in the activity of the conversion enzyme and angiotensin II, multiplied approximately by 3 (13). The interposition of the fibrosis areas with the normal atrial myocytes leads to the lack of homogeneity and conduction anomalies (unidirectional blocks), with arrhythmogenic potential. A histological study that compared the atrial myocardium placed around the pulmonary veins to the one at the level of the left appendage, in patients with AF associated to the mitral valvular disease, revealed that in the perivenous tissue the interstitial fibrosis is 3 times more intense, the density of the myocardial capillaries is significantly reduced and the oxygen diffusion distance is significantly higher. In patients with heart failure (condition that is frequently complicated with atrial fibrillation) at atrial level there were areas with reduced or zero ("scars") electrical activity, as well as delays in impulse conduction, which are processes similar to the ones that appear with age.

Atrial structural remodeling is associated with sustained AF and occurs after weeks or months from the AF initiation, structural alterations were detected both in the preclinical phase and especially in the clinical phase of the AF. Parts of the structural changes that take place during the evolution of this arrhythmia are irreversible. At cellular level, cell hypertrophy, the accumulation of perinuclear glycogen and myolysis were observed most frequently. In patients submitted to a cardiac surgical intervention correcting mitral valvular disease, fibrosis predominates around pulmonary veins, the location of the ectopic sources that initiates frequently AF. The increase of the expression matrix metalloproteinase's and the type I and III collagen, the atrial "stretch" and the insufficient ventricular diastolic filling

contribute to the apparition of structural alterations o the atrial myocardium in atrial fibrillation.

Atrial fibrillation determines also the functional remodeling of the sinus node, which was revealed by the prolonged sinus pause after the electrical cardioversion. Since the term "atrial remodeling" was used for the first time by Wijffels in 1995, this concept has been evolving remarkably and brought an essential contribution to the understanding of the atrial fibrillation mechanisms through animal models and imagines clinical models. Atrial remodeling as a therapeutic target may become effective in the prevention or delay of the atrial fibrillation apparition.

The assessment of the atrial structural remodeling is carried out–by practical judgments–most frequently echocardiographically, by measuring a linear dimension, namely the antero-posterior diameter (2D or M mode). The interest of the more complex assessment of the size of the left atrium grew after the correlation of its antero-posterior diameter with its angiographic dimension was ascertained. The advancement of the ultrasound technology allowed the measuring not only of the linear dimensions but also of the area and volume bi-dimensionally and tri-dimensionally. The area may be measured both, in 4 and 2 apical chamber views, excluding the ostium of the pulmonary veins and the appendage. Its volume is a parameter that uses the area and/or linear dimensions in calculations.

The dilatation of the LA in the AF, secondary to the "stretch" phenomenon, is asymmetric due to its neighboring structures (the aorta, the spine etc.) and the "atrialization" of the antrum of the pulmonary veins. Thus, the atrial dilatation will have different consequences over the anatomic segments of the left atrium, determining the change of its morphology. The mitral annular, the mitral-aortic and the inter-atrial septum are less susceptible to dilatation as they are relatively fixed. Due to the lack of the fibrous components, the rest of the atrial myocardium, namely the junction of the pulmonary veins and their antrum, will modify their morphology. The result will be the change in the atrial geometry, the form of the left dilated atrium becoming trapezoidal. The electrocardiographic evaluation through a single linear dimension (the antero-posterior diameter) is inappropriate in such situations; the changes in the geometry of the LA thus imposed a more complex evaluation. The measurement of the area or volume indexed as a ratio between the atrial volume and the body surface is a parameter closer to its real size, allowing comparisons regardless of gender or body weight.

The American Echocardiography Society and the European Association of Echocardiography recommend the evaluation of the LA as its indexed volume, as a marker of cardiovascular risk, indicator of the existence of a heart condition and predictor of the cardiovascular complications (14,15). The two above mentioned echocardiography societies recommend for the measurement of the atrium volume the use of the ellipsoid method or the Simpson method (the disks method used in calculating the volume of the left ventricle). Moreover, they consider that this determination should become routine in the echocardiography laboratories.

Imaging methods such as the computer-tomography and nuclear magnetic resonance offered new ways of study of the morphology and structure of the atria and marked out the limits of the echocardiography in evaluating them. The echocardiographic measurement of the LA volume correlates to the computer-tomography one, the biplane contrast ventriculography

and the nuclear magnetic resonance. Besides the good correlation, the tendency of the echocardiography to underestimate the left atrium volume also stood out, as it cannot appreciate correctly its maximum sizes (6). Echocardiographically, the size of the left atrium is up to 40% underestimated in comparison to the three-dimensional reconstruction by computer tomography (10).

The echocardiographic functional evaluation of the LA may be obtained by assessing the atrial ejection fraction, the flow in the pulmonary veins and the mitral one. The atrial contractility correlates to the maximum velocity of wave A and the velocity-time integral. The evaluation through the trans-esophageal of the velocity of the flow in the left appendage through pulsed Doppler offers additional information. The maximum velocity during the atrial contraction correlates to the contraction force of the appendage when it is emptie. To this purpose, the determination of the atrial "strain" (deformation/tension of the wall) may also be used.

Other non-invasive parameters that can give information about the atrial remodeling are the following:

- on the surface electrocardiogram:
  - the analysis of the rates of the fibrillatory activity,
  - the P wave on the electrocardiogram mediated by the signal,
  - markers of the autonomous tonus (heart rate variability),
  - extrasystoles with P/T wave;
- on the intra-atrial electrograms:
  - their morphology and amplitude,
  - the analysis of the rates of the fibrillatory activity;
- in the blood:
  - the collagen or its metabolites;
  - mediators of the inflammation (TNF-α, interleukins, protein C reactive, adhesion molecules),
  - platelet markers (factor von Willebrandt, thrombocyte markers, fibrinolysis indices),
  - neurohormonal factors (angiotensin II, aldosterone, the atrial natriuretic peptide, B-type natriuretic peptide);
- molecular and histological markers:
  - the size of the atrial myocytes,
  - interstitial fibrosis,
  - ultrastructural modifications in atrial myocytes,
  - components of the signaling ways.

The echocardiographic identification of mitral wave L (representing the mid-diastolic ventricular filling) in patients with persistent AF is relatively common and indicates an advanced diastolic dysfunction (15). The mitral wave L prevalence is associated with higher E/E' ratio, higher level of B-type natriuretic peptide and with the LA enlargement (15).

The therapy with conversion enzyme inhibitors or antagonists of the angiotensin receivers in patients with LA dilatation may induce reversal structural remodeling (regression of the dilatation), the diminishing of the progression of the AF from paroxysmal to persistent

and the decrease in the number of the relapses of this arrhythmia. Thus, the irbesartan and amiodarone treatment, after the electrical cardioversion, determines a lower relapse rate than the amiodarone treatment exclusively. The angiotensin antagonists combined with the diuretic one decreases the AF incidence after the catheter ablation of the typical atrial flutter. The mechanisms through which the renin angiotensin aldosteron system delays the appearance, prevents the permanent character or reduces the relapses in AF are the following:

- decreasing in the LA pressure and the wall stress,
- preventing of the left atrial and ventricular structural remodeling (fibrosis, dilatation, hypertrophy),
- inhibiting of the neuro-hormonal activation,
- decreasing in the arterial pressure,
- preventing and improving of the heart failure, and
- preventing of the hypokaliemia.

The selective blockers of the atrial potassium channels, such as the dofetilide, increase the atrial refractoriness, resumption of the SR. The blocking of potassium channels both, at atrial and ventricular level is carried out with the risk of the QT interval increasing and torsade de pointes induction. The selective action at the level of potassium channels existent only in the atrial myocardium avoids these proarrhythmic effects.

The voltage-dependent potassium channels are involved in the atrial repolarization through the following currents: I_{to}-type transient outward, output-rectifiers with very fast activation (I_{kur}), fast (I_{kr}) and slow (I_{ks}), input (I_{k1} and activated acetylcholine ($I_{K,A}$ch). Parts of these channels are submitted to electrical remodeling in atrial fibrillation. In chronic atrial fibrillation, the I_{to} currents amplitude reduction is associated to the decrease messenger RNA involved in the synthesis of α subunit of the channel (Kv4.3). The data related to I_{kur} are still unclear and I_{kr} and I_{ks} appear not to be involved. The I_{k1}-type currents are amplified through the increase in the synthesis of the proteins of the subunit α of Kir2.1/Kir2.3 of the channel in question. The activated rectifying currents of acetyl-choline ($I_{k,Ach}$) diminish in atrial fibrillation by the decrease of the synthesis of the subunit Kir3.1/Kir3.4 of the channel. The I_{kur} and $I_{K,Ach}$ channels are to be found at atrial level. Starting from this assertion, there are a series of paraclinical studies with “atrial specific” antiarrhythmics that aim towards these channels. Nevertheless the properties of the atrial channel may change along upon the electrical remodeling and thus the effects of the antiarrhythmics may differ in SR from chronic AF, but not necessarily from the recently installed one. A good example is AVE0118, a derivative of bipyridine, which induces the SR on animal models with AF but not on humans. Electrical remodeling may limit the using of the I_{kur} channel as a possible therapeutic target in AF. Vernkalant is part of the same group but also blocks the sodium channels, which is why it converts and maintains the SR both on animal models and in men.

The studies currently carried out focused on the agents with selective action on these ionic – exclusively atrial – channels, blockers of multiple ionic channels and modifiers of the gap junction. Agents such as vernakalant, AVE0118, AVE1231 and AZD7009 act on some ionic channels specific to the atrium (Ito, INa, Ikur, IKACh, Iks– essentially involved in the repolarization at fast heart rates, I_{kr} – contribute decisively in the repolarization at physiological heart rates). With minimal effects on the ventricular repolarization, the blocking of these currents increase the atrial refractory period, favoring the reinstallation of the SR.

Azimilides, dronedarones and tedisamil – through the blocking of some ionic channels in different stages of the action potential optimize the antiarrhythmic action with minimal proarrhythmic effects. Agents such as rotigaptides (ZP123) and AAP10 act on the connexines (subunits of the gap junctions, which facilitate the electrical communication between cells) and favor the normal intercellular conduction. The 40 and 43 connections are the main components of the gap junctions at the level of the human atrium involved upon the appearance of AF.

The vagal activation determines the shortening of the action potential and if the refractory period, it increases the dispersion of the atrial repolarization and it creates an arrhythmogenic sublayer through the $I_{K,Ach}$ type channels. In the chronic atrial fibrillation there was noticed a reduction of the muscarinic receptors for $I_{K,Ach}$. The tertiapin, a selective blocker of $I_{K,Ach}$ prolongs the duration of the action potentials and it converts the AF induces through rapid stimulation at a SR. Ideally, such an agent should block selectively only the constitutive element of the $I_{K,Ach}$ with no effect over the component activated by the agonists because the latter modulates the function of the pacemaker cells and the conduction of the impulses at the level of the sinoatrial node. But the blocking of the $I_{K,Ach}$ currents leaves the sympathetic activity non-counterworked with the increase of the conduction at the level of the atrioventricular node. The long-term use of the quinidine confirms this thing. These ionic channels are not found at a ventricular level; the constitutive component of the $I_{K,Ach}$ appears in case of atrial modeling from the AF and its selective suppression will not create proarrhythmic effects.

Many things remain still uncertain regarding the remodeling of the ionic channels in the AF; one thing is certain: the amiodarone, a blocker of the potassium channels and not only, is the most efficient antiarrhythmic in this arrhythmia with epidemical tendency (16,17).

There is no doubt that structural remodeling has an important role in AF pathophysiology beside of electrical and mechanic remodeling. Over of half of hundred years it is well known that left atrium dilatation and AF are related, but we still don't know who the first is (14). Atrial dilatation could associates with electrical and mechanic remodeling to make arrhyhtmogenic conditions for atrial fibrillation inducibility and perpetuation. The AF onset could induce atrial dilatation by mean of atrial compliance decreasing and contractile dysfunction. It seems that structural remodeling could be used as therapeutical target for preventing and/or delaying of AF progression. Discovery of some therapeutically methods for contractile dysfunction might determine atrial dilatation prophylaxis and implicitly the persistence of this arrhythmia.

LA size is a veritable prognostic marker and predictor of cardiovascular events and its assessment is influenced by its morphology, as well as shape. Determining LA size offers diagnostic and prognostic information in AF patients and those with associated comorbidities, including arterial hypertension, and ischemic heart disease (14,15).

Importantly, LA dilatation leads to a geometrical remodelling and subsequent shape alteration. From this point of view, LA structural remodelling can be either symmetric (SSR) or asymmetric (ASR) (13). In AF patients, LA dilatation tends to be asymmetrical (mostly in the supero-inferior and medio-lateral directions, due to lack of fixed fibrous structures at this level) (18). Taking this into consideration, the two echocardiographic parameters recommended in assessing LA size and subsequent structural remodelling are LA area and

indexed volume (14,15,18). However, LA volume assessment through the ellipsoid formula may be influenced by the presence of ASR (18). Computer tomography (CT), cardiac magnetic resonance imaging (CMR) and ventriculography correlate with each other regarding the accuracy of assessing LA volume and are considered to be the gold standard techniques. More importantly, they all underestimated LA volume when compared to echocardiography (13,15,18).

The relationship between ASR, underlying cardiac rhythm and LA size is still debatable.

Taking into account the atrial remodelling and the relationship with atrial fibrillation I was preoccupied to study this in patients with and without this arrhythmia from echocardiographic point of view. The preoccupations related to atrial remodelling were partially synthesized in the following articles.

1. **Floria M**, Jamart J, Schroeder E, Georgescu CA. *Echocardiographic Parameters Associated with Asymmetrical Structural Remodelling in Patients with or without Atrial Fibrillation.* J Atr Fibrillation 2014;7(4):1159. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5135204/pdf/jafib-07-01159.pdf.
2. **Floria M**, Blommaert D, Lacrosse M, Ambarus V, Dormal F, Dabiri Abkenari L, Jamart J, Rezus C, Cozma D, De Roy L. Assessment of left atrial shape and volume in structural remodeling secondary to atrial fibrillation. J Interv Card Electrophysiol 2009;25(3):167-70. https://link.springer.com/article/10.1007%2Fs10840-008-9349-4

1.2. Aim

The purpose was to assess the distribution of ASR in both AF and non-AF patients with dilated LA and identifying pulsed-wave Doppler (PW) and tissue Doppler Imaging (TDI) echocardiographic parameters of LA function and LV diastolic dysfunction that correlate with ASR.

1.3. Material and methods

Consecutive AF and non-AF patients over 18 years old with a dilated LA according to LA area (>20 cm²) were prospectively included, with AF and LA dilatation being defined according to the recent guidelines (14-17).

Patients who refused participation, those with severe valvular heart disease/valvular prosthesis leading to LA dilatation, congenital heart diseases (irrespective of surgical correction), acute coronary syndromes/ischemic heart disease/wall motion abnormalities, pulmonary hypertension, cardiac implantable devices, amyloidosis, constrictive pericarditis, conduction disorders and neoplasia were excluded. The hospital Ethics Committee approved this and all patients provided written informed consent.

Echocardiographic examinations were standardized and performed with patients lying in left lateral decubitus, with the measurements taken in expiratory apnoea, according to the current guidelines (14,15). All measurements were made by the same operator (MF) on GE Vivid 9 (GE Healthcare) or Philips iE33 machines (Philips Medical Systems), with 3.5 MHz transducers.

The protocols included the following parameters:

- Two-dimensional parameters:
    - LA size (antero-posterior diameter, area, volume - measured with ellipsoid biplane area-length method),
    - LV dimensions (end-diastolic and end-systolic diameters, interventricular septum diameter, posterior wall diameter),
    - LV function (ejection fraction - EF through Simpson's method) and mass (linear method;
- inflow patterns through PW and TDI:
    - peak early diastolic mitral inflow velocity (E),
    - peak late diastolic mitral inflow velocity (A),
    - mid-diastolic mitral annular velocity (L),
    - early diastolic mitral annular velocity (Em),
    - late diastolic mitral annular velocity (Am),
    - systolic mitral annular velocity (Sm),
    - isovolumetric relaxation time (IVRT),
    - E-wave deceleration time (EDT),
    - mitral A-wave duration (Amdur),
    - atrial reverse flow duration (Ardur),
    - peak systolic pulmonary venous flow velocity (S),
    - peak diastolic pulmonary venous flow velocity (D),
- LA function was assessed through the measurement of:
    - peak systolic LA myocardial velocity (Sa),
    - early diastolic LA myocardial velocity (Ea), and
    - late diastolic LA myocardial velocity (Aa), at the level of the LA lateral wall.

The previous 3 parameters evaluate the 3 functions of LA: reservoir (Sa), conduit (Ea) and booster pump (Aa) (19-23). Additionally, PW was used on the right superior pulmonary vein. All of the above-mentioned parameters were averaged after a minimum of 3-5 measurements during a heart rate 60-80 beats/minute at a sweep speed of 100 mm/s.

We defined LA-ASR as a non-ellipsoidal LA shape (basal diameter taken at the intersection between the pulmonary veins and LA > mitral annular dimensions). Patients not falling into this category were considered as having SSR.

Statistical Analysis

SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) and MedCalc (Mariakerke, Belgium) were used in performing the statistical analysis. Continuous variables were expressed as mean and standard deviations, while categorical data as frequency distributions and percentages. Linear regression was used to assess the relation between two variables. Statistically significant variables ($p<0.05$) in univariate analysis were included in stepwise

multivariate analysis (the anterograde and retrograde LR and Wald tests). The area under the curve (AUC) was used to assess predictive variables.

1.4. Results

We included 170 patients, most of them men (61%), with a mean age of 67 ± 11 years, a BMI of 28 ± 5 kg/cm², hypertensive (74%), with coronary artery disease (33%) and diabetes (19%). The majority had AF (59%) and the most frequent form was permanent (61%), as opposed to 24% paroxysmal and 15% persistent, while 70 patients (41%) were in SR. Mean ventricular rate was 72 ± 10 beats per min.

Out of the 112 (66%) patients in which ASR was identified, 62 (55%) had AF and 50 (45%) SR (p=0.002). Patients with ASR had higher ventricular mean mass as compared to those with SSR (150 ± 38% g/m² vs 130 ± 43 g/m², CI: 95%, p=0.002) while the LVEF was similar between groups (49 ± 15% vs 51 ± 12%, CI: 95%, p=0.67).

Interestingly, LA indexed volume was higher in ASR patients, irrespective of underlying heart rhythm, as compared to its value in SSR patients (49 ± 14 ml/m² vs 29 ± 13 ml/m², p<0.001). Moreover, the two showed a statistically significant correlation (r=0.567, p<0.001).

The mean LA-ASR and LA-SSR indexed volumes, assessed by ellipsoid biplane area-length formula in all patients irrespective of the presence of AF or SR, were 49 ± 14 ml/m² (95%CI: 41.8-48) and 29 ± 13 ml/m² (95%CI: 122-138), respectively (p<0.001); they were well correlated (r=0.567, p<0.001). Interestingly, patients with ASR and AF (n=62) had higher LA indexed volumes as compared to those with ASR and SR, n=50 (54 ± 20 ml/m² vs 45 ± 11 ml/m², 95%CI: 49-59.4 and 41.8-48, respectively; p<0.001). Similarly, patients with SSR and AF (n=38) had higher LA indexed volumes as compared to their SR counterparts, n=20 (32 ± 9 ml/m² vs 21 ± 6.5 ml/m², 95% CI: 28.9-34.8 and 17.7-23.8, respectively, p<0.001). **Figure 1** depicts the relationship between LA enlargement as identified by indexed volume and the type of structural remodelling in both AF and SR patients.

Patients with moderate LA dilatation according to LA area (31-40 cm², n=27) had higher ASR/SSR ratio as compared to patients with mild dilatation (20-30 cm², n=140)- 2.8 vs 1.7, CI: 95%, p=0.001. Severe LA dilatation (LA area > 40 cm²) was identified in 3 patients. Associated comorbidities better correlated with the presence of ASR as compared to SSR: r=0.772 vs. r=0.677, p<0.001 for arterial hypertension, r=0.607 vs. r=0.567, p<0.001 for coronary artery disease, r=0.747 vs. r=0.657, p<0.001 for left ventricular hypertrophy, r=0.307 vs. r=0.276, p=0.005 for diabetes mellitus.

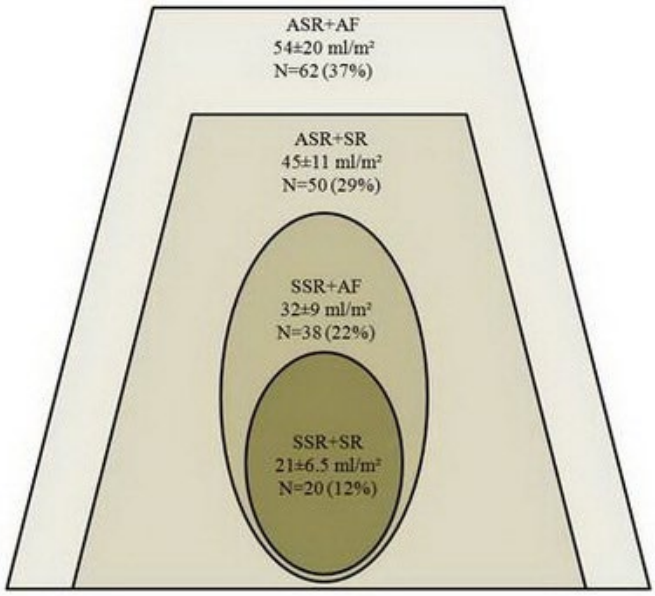


Figure 1. Repartition of patients included in the study according to LA volume, type of LA remodeling and presence of AF or SR. LA: left atrial; SR: sinus rhythm; SSR+SR=patients with symmetrical structural remodelling and sinus rhythm, SSR+AF=patients with asymmetrical structural remodelling and atrial fibrillation, ASR+SR= patients with asymmetrical structural remodelling and sinus rhythm, ASR+AF= patients with asymmetrical structural remodelling and atrial fibrillation.

The TDI and PW echocardiographic parameters associated with ASR by univariate analysis were: L, Amdur, Am-Ar, S, D, Em, Sm and Sa (all $p<0.005$).

Multivariate logistic regression revealed LA systolic myocardial velocity (Sa; $p=0.036$) and peak systolic pulmonary venous flow velocity (S, $p=0.033$) as the best TDI and PW echocardiographic parameters associated with LA-ASR, with a power prediction of 91.8% (95% CI, $p=0.0001$).

When correcting for the number of patients (90% CI), ASR prediction improved to 99.1%, but the statistical significance decreased slightly ($p=0.073$). The sensitivity and specificity of both parameters based on ROC curve analysis were 77 and 70%, respectively (**Figure 2**); the AUC was 0.765 (95% CI: 0.662-0.849, $p=0.0001$).

We were able to asses LA regional function using TDI parameters (Sa, Ea, Aa) in 87% of the patients and pulmonary venous flow using PW (S and D) in 81% of the patients. **Table 1** summarizes the mean values of PW and TDI parameters.

Table 1. Echocardiographic measurement by PW and TDI for all patients included in the study

* Measurements were possible only in patients in SR (n=70) or known with AF but in SR at the time of inclusion (n=24). A=peak late diastolic transmitral flow velocity; Aa=left atrium late diastolic myocardial velocity; AF=atrial fibrillation; Am=late diastolic mitral annular velocity; Amdur=mitral A wave duration; Ardur=atrial reflux duration; ASR=asymmetric structural remodeling; D=peak pulmonary diastolic flow velocity; E=peak early diastolic transmitral flow velocity; Ea=left atrium early diastolic myocardial velocity; Em=early diastolic mitral annular velocity; IVRT=isovolumetric relaxation time; L=mid-diastolic mitral annular velocity; NS=non-significant; PW= Pulse Doppler wave; S=peak pulmonary systolic flow velocity; Sa=left atrium systolic myocardial velocity; Sm= systolic mitral annular velocity; SR=sinus rhythm; SSR=symmetric structural remodeling; TDE=deceleration time of E wave; TDI=Tissue Doppler Imaging.

Echocardiographic parameters	SSR			ASR		
	AF	SR	P value	AF	SR	P value
E (cm/s)	79±23	65±21	0.007	83±28	69±18	0.003
A (cm/s)*	57±21	69±22	NS	74±25	78±25	NS
E/A*	1.2±0.5	1±0.4	NS	1±0.43	0.96±0.44	NS
Sm (cm/s)	5.4±1.1	6.4±1.4	0.01	6.4±1.3	6.3±1.1	NS
Em (cm/s)	8.3±2.4	7.7±2.3	NS	9±2	8±2	<0.001
Am (cm/s)*	8.7±1.6	7.8±2.3	NS	8.8±0.7	8.7±1.7	NS
E/Em	10±3.2	8±2	0.035	9.4±3.6	9.1±2.6	NS
L (cm/s)	35±3	0	NA	26±4	27±9	<0.001
IVRT (ms)	98±17	99±24	NS	102±25	94±29	NS
TDE (ms)	194±74	203±50	NS	186±62	203±56	NS
Amdur (ms)*	132±6	132±8	NS	135±13	131±11	0.05
Ardur (ms)*	106±11	117±8	0.009	110±12	115±12	NS
Am–Ar (ms)*	26±10	15±5	0.006	25±7	17±6	<0.001
S (cm/s)	41±11	39±12	NS	36±14	42±12	0.005
D (cm/s)	41±16	36±14	NS	39±11	33±11	0.003
Sa (cm/s)	6.5±2.6	6.4±2.1	NS	6.6±2.4	7.6±1.9	0.001
Ea (cm/s)	8.6±2.7	7.4±1.9	NS	7.7±2.2	6.9±2.4	NS
Aa (cm/s)*	9.5±2.8	8.3±4.2	NS	9.2±4.4	10.4±3.3	NS

We used S and Sa to create an equation that will estimate the probability of LA-ASR: (ez / (1+ez), where z = - 0.511 - 0.062 x (S) + 0.536 x (Sa). **Figure 3** depicts the scale that may be used in patients with enlarged LA to estimate the latter.

I have also studied the relation between LA structural remodeling and thrombogenesis in patients with AF. LA volume is the preferred parameter in assessing left atrial structural remodeling. Despite this and the fact that LA structural remodeling is associated with increased thromboembolic risk in AF patients, LA volume is still not routinely used in clinical practice and has yet to be included in the thromboembolic risk scores ($CHADS_2$ and CHA_2DS_2-VASc). Mean value of thromboembolic scores ($CHADS_2$ and CHA_2DS_2-VASc) were 1.8 ± 1.2 and 3.2 ± 1.8 respectively.

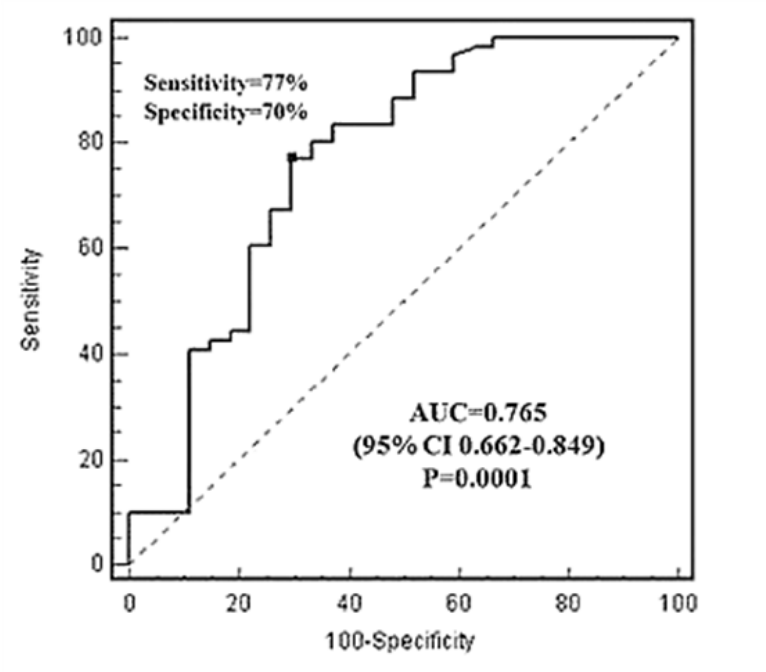


Figure 2. ROC curve for prediction of the left atrium asymmetrical structural remodeling using peak pulmonary vein flow systolic velocity (S) and peak systolic atrial myocardial velocity (Sa). AUC=area under curve; CI=confidential interval.

Sa /S	4	5	6	7	8	9	10	11	12	13	14
25	52	64	76	84	90	94	96	97	98.7	99.2	99.5
30	44	58	70	80	87	92	95	97	98.3	99	99.4
35	36	50	63	74	83	89	94	96	98	98.6	99.2
40	30	42	56	68	78	86	91	95	97	98	98.9
45	24	35	48	61	72	82	88	93	96	98	98.5
50	19	28	40	54	66	77	85	90	94	96	98
55	14	22	33	45	59	71	80	87	92	95	97
60	11	17	27	38	52	64	75	84	90	93	96
65	8	13	20	31	43	57	69	79	86	91	95
70	6	10	16	24	36	49	62	73	82	89	93
75	5	8	13	20	29	41	55	67	78	86	91

Figure 3. Prediction scale of LA-ASR using the best echocardiographic parameters associated with LA-ASR: S (peak pulmonary vein flow systolic velocity) value (rows) and Sa (peak systolic atrial myocardial velocity) value (columns). For example, a patient with S value of 60 cm/s and Sa value of 13 cm/s would have a 93% probability of ASR.

Out of the echocardiographic parameters used in assessing LA enlargement, both $CHADS_2$ and CHA_2DS_2-VASc best correlated with indexed LA volume (R=0.432 and 0.359, respectively; P=0.0001) - **Figures 4 and 5**.

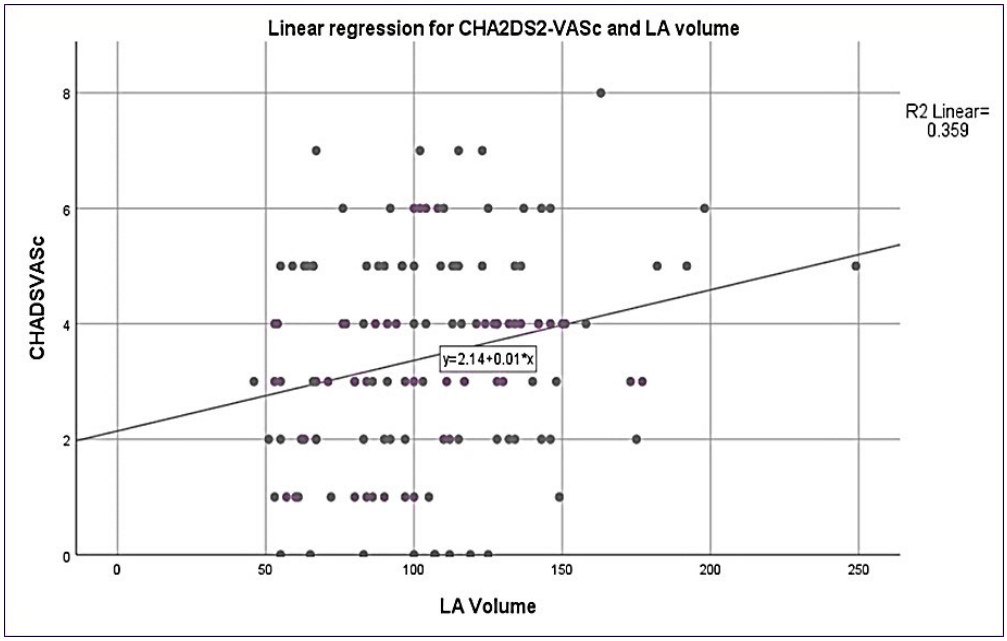


Figure 4. Linear regression for CHA2DS2-VASc and left atrial (LA) volume

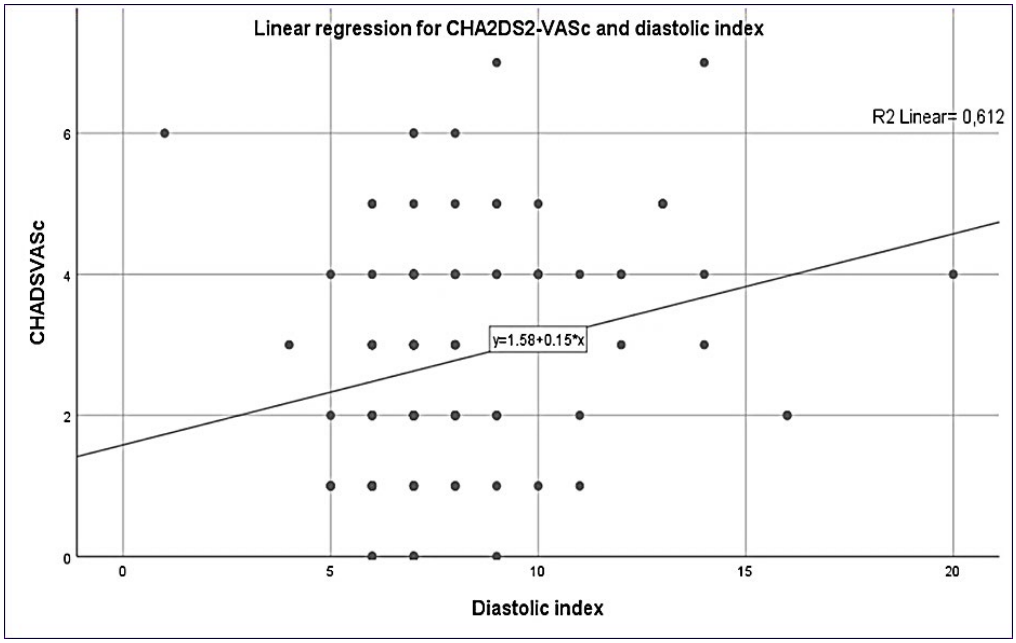


Figure 5. Linear regression for CHA_2DS_2-VASc and diastolic index

Concerning the diastolic LV dysfunction, both scores best correlated with the diastolic index (R=0.356 and 0.612, respectively; P=0.0001) - **Figures 6 and 7**.

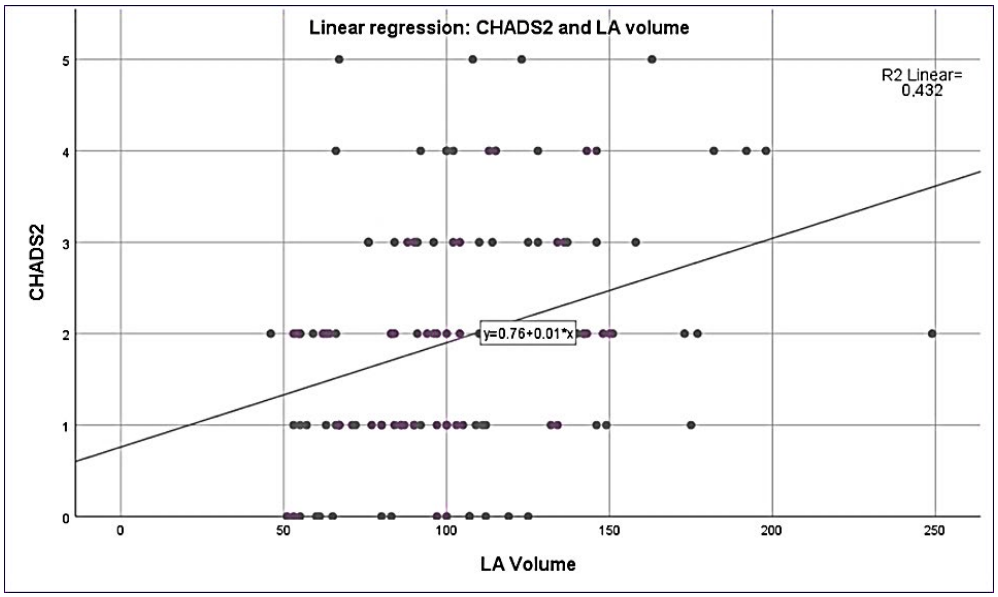


Figure 6. Linear regression for $CHADS_2$ and left atrial (LA) volume

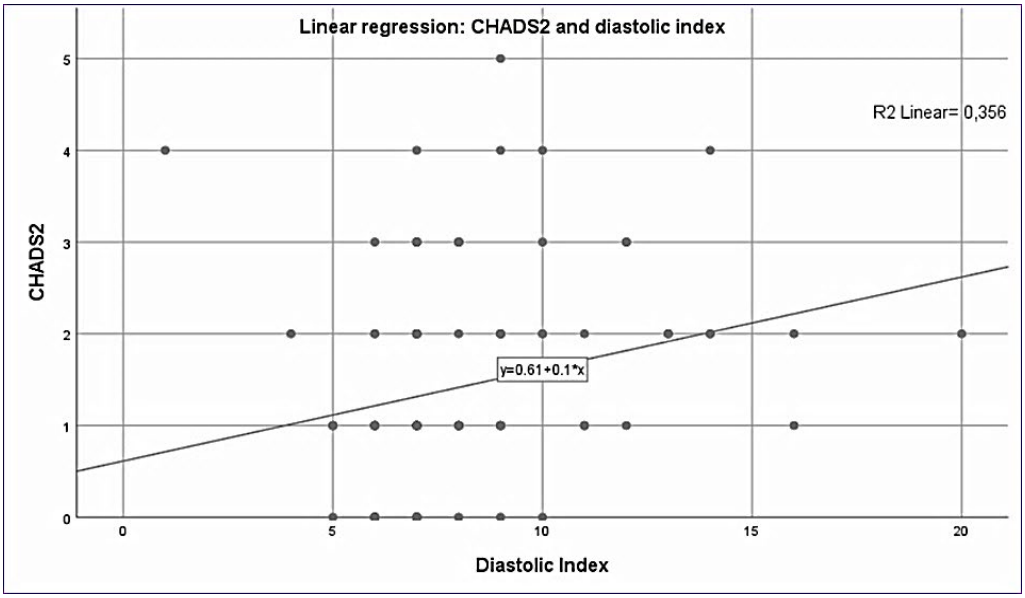


Figure 7. Linear regression for $CHADS_2$ and diastolic index

They failed, however to correlate with L - **Figures 8 and 9**.

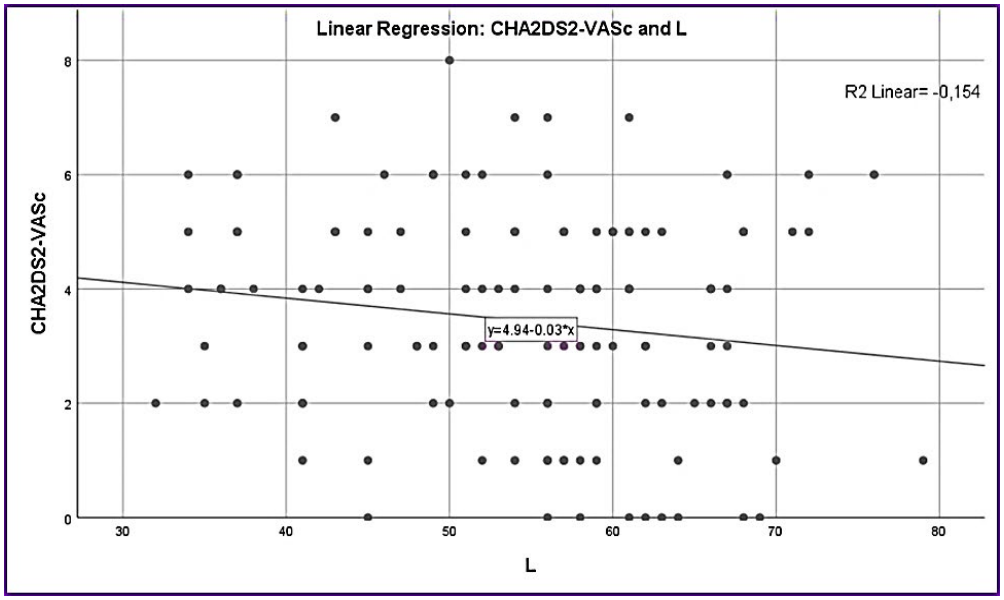


Figure 8. Linear regression for CHA2DS2-VASc and antero-posterior diameter of the left atrium (L)

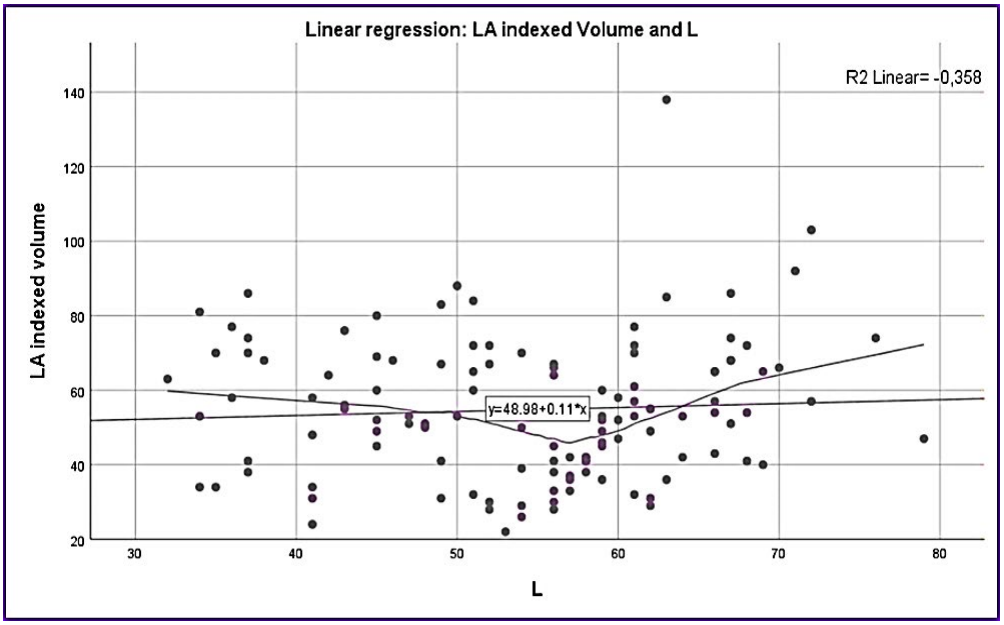


Figure 9. Linear regression for CHADS2 and antero-posterior diameter of the left atrium (L)

Both thromboembolic risk scores increased with LA enlargement, with the exception of L. Interestingly, L showed a negative correlation with both CHA2DS2-VASc (R= - 0.154; P=0.024) and indexed LA volume (R= - 0.358; P=0.001). The AUC for CHADS2, CHA2DS2-VASc and LA volume was 0,76 and 0,791, respectively (**Figures 10 and 11**).

Structural remodeling assessed through LA indexed volume is associated with increased thromboembolic risk, as shown by the correlations with both risk scores. The decrease in antero-posterior diameter concomitant with LA enlargement as assessed through LA volume suggests the asymmetric, possible spherical structural remodeling.

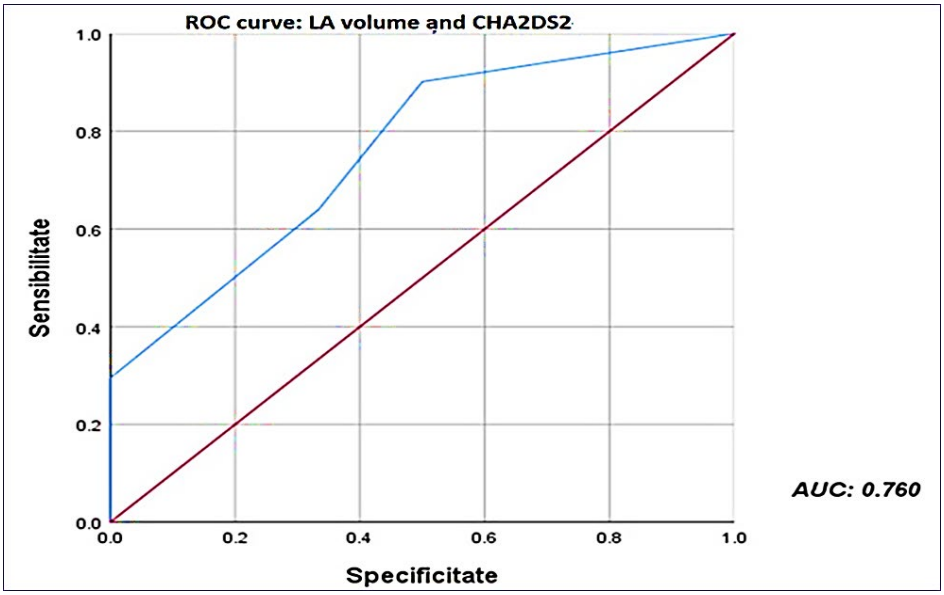


Figure 10. ROC curves: thromboembolic risk scores CHADS2 and left atrial (LA) volume

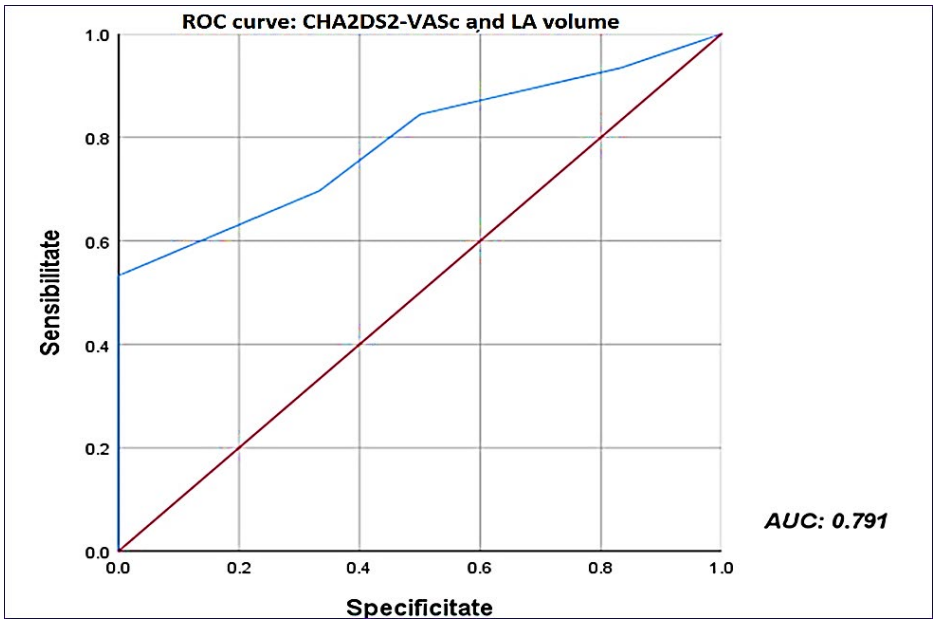


Figure 11. ROC curves: thromboembolic risk scores CHA2DS2-VASc and left atrial (LA) volume

The clinical relevance of this study is linked to the importance of LA as a predictor of cardiovascular and thromboembolic events. Again, LA structural remodeling emerged as being asymmetrical (13). Furthermore, LA enlargement as a parameter of structural remodeling seems to be predictive of AF ablation outcomes and post-procedural recurrence rates and may help in better candidate selection (24).

1.5. Discussions

There is a tight relationship between diastolic dysfunction and LA enlargement, the latter reflecting the severity of the first (14). In addition, pulmonary veins also are an

important site due to their ability to *measure* the LV end-diastolic pressure upon mitral valve opening. Subsequently, increased LV filling pressure leads in time to LA enlargement and change of function (from reservoir during ventricular systole to conduit during early ventricular diastole and booster pump during late ventricular diastole), creating a veritable substrate for arrhythmia initiation and perpetuation. Moreover, the Frank-Starling Law explains that the greater LA volume is, the more enhanced the LA reservoir function and the more increased the LV filing pressure in SR patients (25).

In this study, we analysed the frequency and distribution of ASR in AF and SR patients with enlarged LA, assessed LA function and LV diastolic dysfunction in ASR patients vs SSR patients using PW and TDI parameters and tried to identify independent predictors of ASR in patients with dilated LA (25). The results showed that AF was more prevalent in ASR patients than SSR (37% vs 22%). Interestingly, it was AF patients with ASR that showed the greatest LA indexed volumes (**Figure 1**). However, ASR was equally distributed between AF and SR patients. Despite this, lack of evidence is does not confirm an absence (25). Therefore, although in this study there was no statistically significant difference in the distribution of ASR in AF and SR patients, it doesn't exclude a possible relationship between ASR and AF. More studies, preferably longitudinal are warranted to test this hypothesis.

The fact that ASR/SSR ratio increased with atrial enlargement demonstrates the association between the two. Interestingly, the fact that LA indexed volume correlated in both ASR and SSR patients ($r=0.567$, $p<0.001$) assures us the ellipsoid biplane area-length method in assessing LA volume in all patients, irrespective of the remodelling symmetry was applicable in these patients.

Structural, functional and electrical remodelling are interconnected and contribute to both arrhythmia initiation and progression (4,25). Keeping that in mind, the fact that ASR patients showed increased LA indexed volumes irrespective of underlying rhythm suggests that analysing the symmetry of structural remodelling (presence of ASR) may be superior to LA volume assessment and/or electrical remodelling presence. Moreover, several authors agree that structural remodelling (especially fibrosis) precedes electrical remodelling (23) and is associated with a decreased LA function. In this regard, it seems that a decreased reservoir function increases the risk of AF (26). Several studies have shown that a decreased LA function was predictive of atrial flutter in patients older than 65 years and AF recurrence post catheter ablation (25,26). There may be an association between AF and the presence of ASR; in this case, the pulmonary veins could be arrhythmogenic due to increased LA enlargement leading to increased LA pressure and subsequent stretch-gated channels activation, most of them located at the posterior wall (4,17). However, several studies are required to further test this hypothesis.

The different types of atrial remodelling (structural, electric and functional) are interconnected and clearly linked to both AF initiation and progression and LA enlargement (30).

The results of the study demonstrate that:

- LA enlargement occurs more often asymmetrically;
- This asymmetry frequently involves LA posterior wall and the degree of asymmetry is tightly related to the degree of enlargement (30).

- The presence of ASR was linked to a decreased LA function, especially reservoir.

The clinical implications of this study assessing LA structural remodelling are based on the fact that LA is a marker prognostic of cardiovascular events. Several guidelines recommend the assessment of ASR in enlarged atria, highlighting that patients with increased LA volume have poorer prognosis (14,27). In the above mentioned study, the non-ellipsoidal LA was defined through greater dimensions measured at the intersection between the pulmonary veins and LA as compared to mitral annular dimensions. The importance lies in the fact that this asymmetry may lead to echocardiographic volume underestimation as compared to other imaging techniques such as CT or CMR (13,28). Therefore using the ellipsoidal formula may be inappropriate in patients with ASR. Underestimating LA dimensions may lead to underestimating the risk for cardiovascular events and even misdiagnosis, as in the case of heart failure with preserved ejection fraction, in which a LA indexed volume > 34 mL/m^2 is a diagnostic criteria.

Moreover, properly assessment LA enlargement may call out for specific therapies, such as angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors, with the purpose of inducing a degree of reverse-remodelling and preventing AF initiation and/or progression. Several studies show that these therapies have a favourable effect on LA-LV interaction even in asymptomatic patients (29,30). Studies showing the ongoing LA dissynchrony even in the context of post-pulmonary vein isolation reverse-remodelling emphasize the importance of LA as a veritable biomarker, influencing post-ablation medical therapy (31).

Although CMR is the gold standard in assessing LA structural remodelling through chamber enlargement detection and fibrosis quantification (32-35), 2D and 3D echocardiography remain the most readily available imaging techniques, capable of evaluating both structural and functional LA remodelling (36).

Study Limitations

Despite the fact that LA volumes is the preferred parameter in assessing LA enlargement, LA area was used as inclusion criteria. LA area is included in the ellipsoid biplane area-length formula. Therefore, as expected, LA area and LA volume assessed with the ellipsoid biplane area-length formula, were well correlated ($r=0.837$, $p<0.001$). Taking into consideration both this correlation and the fact that in routine clinical practice, determining LA area is far more easy and rapid, justifies using this parameter.

There were several technical limitations, such as the inability to determine several echocardiographic parameters of pulmonary venous flow and LA function in all patients. It has been shown that the technique is feasible in only 80% of cases (37). Another such limitation was that TDI-based LA function parameters are angle-dependent while LA walls are thin. Moreover, these parameters have not been yet validated for LA function assessment. Myocardial velocity is influenced by translation and tethering, and therefore, it is difficult to distinguish true shortening and lengthening of the atrium from mitral annular and ventricular motion. We carefully adjusted the beam on the lateral wall and gain settings to avoid aliasing and to allow reliable measurement of tissue velocity. Thus, an alternative for LA function measurement that would have been more appropriate is LA strain and strain rate (38,39).

However, it is a less-accessible, emerging technique that is not yet widely used. Inter-vendor variability is still an issue for this new echocardiographic method.

1.6. Conclusions

Asymmetric remodelling is associated with chamber enlargement irrespective of underlying rhythm and with decreased reservoir function; AF seems to be more prevalent in patients with ASR.

Chapter 2 GANGLIONATED PLEXI AND ATRIAL FIBRILLATION

2.1. Scientific context

More than 30% of patients with arrhythmias at hospital admission have AF (16). Major improvements have been made in the treatment of AF in last ten years, offering the potential to cure it. Intrinsic cardiac autonomic nervous system may serve as triggers or perpetuators for AF (40). Both components of autonomic nervous system are present in ganglionated plexi (GP) embedded in the fat pads on the epicardial surface (41). GP function as an integration centre that modulates the autonomic innervation between extrinsic and intrinsic cardiac autonomic nervous system. The parasympathetic activation induces the widening of the atrial vulnerability window while the sympathetic stimulation enhances automatic firing within the pulmonary vein and/or promote atrial ectopic beats at the venoatrial junction (42). The response of GP stimulation is primarily vagal because the parasympathetic fibres represent the majority. GP are located in epicardial fat pads at the border of the pulmonary vein antrum, and can be localized at the time of ablation using endocardial or epicardial high frequency stimulation at specific sites in left atrium to elicit slowing of the ventricular rate during AF. Prior studies showed that four major epicardial GP (anterior right-ARGP, inferior right-IRGP, superior left -SLGP and inferior left -ILGP), as part of intrinsic cardiac autonomic nervous system, were identified on the left atrial posterior wall (43).

Controversies still exist regarding the benefit of atrial denervation in AF. The relationship of major GP response during endocardial approach of ablation and success rate is unknown.

There is a large interactive network among different GP; this network serves as an “integrated center” of the cardiac autonomic innervation (37,38). Vagus nerve exerts its influence on the atrio-ventricular node (AVN) through the epicardial fat pads that are primarily located on the posterior wall of the left atrium. The inferior vena cava-left atrium fat pad (namely also right inferior GP) located around the coronary sinus provides mainly vagal innervations and selectively innervates the AVN in humans (38). It was shown that high frequency stimulation of the right anterior (or superior right atrial vagal GP) and left superior GP (or superior left atrial vagal GP) could also influence the AVN (39). In addition, the influence of the right anterior GP on the AVN appears to be more important than its influence on the left superior GP (39).

A functional neural pathway between the right vagus nerve and the AVN was identified (40), and the integrity of the GP seems to represent a mandatory interconnected network (38). The absence of any alteration in the ventricular rate in response to high frequency stimulation of the right vagus nerve after the ablation of GP suggests that the right vagus nerve is not directly connected to the AVN and that the integrity of the GP is required to produce vagal effects on the AVN (37). Probably there is not a direct pathway between both the right and left vagus nerves and the AVN (38).

At long-term it is not known the influence of GP ablation on the electrophysiology of the AVN. However, the incomplete ablation of the GP can increase the vulnerability of the atria to AF and denervation is likely transient (41,42). In addition, the ablation of the GP that led to parasympathetic denervation of the AVN could play a role in the high ventricular rate response of atrial tachycardia after AF ablation (38). Therefore, GP interaction with AVN and right vagus nerve could provide new insights on that particular mechanism.

There are GP interactions with AVN and right vagus nerve with possible important consequences on vagal denervation in AF ablation (37). However, the role and influence of the GP on the complicated vagal innervation of the heart is far to be clarified.

It is well known that the vagus nerves influences the electrophysiological properties of the atrio-ventricular node (AVN). Despite this, the exact extent of its effects in physiological states are far from being fully known and several authors hypothesized a possible involvement in rhythm disorders. Animal studies suggested that the epicardial fat pads located on the LA posterior wall are the way in which the vagus nerve affects the AVN (41). Interestingly, GP are located inside these fat pads, very close to the pulmonary veins antrum (43). During pulmonary vein isolation, several authors have reported transient bradycardia/conduction disorders, suggesting that radiofrequency ablation may alter the vagus nerve (39,44,45).

Thus, the GP may be targeted during epicardial pulmonary vein isolation in AF patients. However, both the importance and exact mechanisms of the fat pads regarding vagal influence on AVN are not fully known. The interest for this theme of research was materialized in the following publications.

1. Xhaet O, DE Roy L, **Floria M**, Deceuninck O, Blommaert D, Dormal F, Ballant E, LA Meir M. *Integrity of the Ganglionated Plexi Is Essential to Parasympathetic Innervation of the Atrioventricular Node by the Right Vagus Nerve.* J Cardiovasc Electrophysiol 2017;28(4):432-437. doi: 10.1111/jce.13156. https://onlinelibrary.wiley.com/doi/abs/10.1111/jce.13156.
2. **Floria M**, Xhaet O, Antohe I. *Ganglionated plexi interactions with atrio-ventricular node and right vagus nerve.* Anatol J Cardiol 2017;18(5):379. doi: 10.14744/AnatolJCardiol.2017.8087. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5731296/

2.2. Aim. The aim was to assess whether right monolateral thoracoscopic epicardial fat pads ablation reduces the right vagus nerve (RVN) effects on the AVN, tested through selective high-frequency stimulation (HFS) (46) of the RVN and GP in AF patients. We hypothesized that fat pad ablation will render RVN HFS ineffective on AVN conduction.

2.3. Material and methods

We included persistent AF patients over 18 years old, scheduled for epicardial ablation with one of the two characteristics:

(1) history of at least one attempt of electrical cardioversion for persistent AF (47), which was defined as continuous AF for more than 7 days and less than 3 years that was unresponsive to therapeutic doses of several antiarrhythmic drugs;
(2) able to provide written informed consent and participate in all examinations and follow-ups in the present study.

We have excluded patients with:

(1) an LVEF < 35% and/or NYHA class > II
(2) LA thrombus
(3) Advanced forms of chronic obstructive pulmonary disease
(4) Previous cardiothoracic surgeries.

The study was approved and supervised by CHU UCL Namur Ethics Committee and was in accordance with the declaration of Helsinki.

All included patients underwent GP HFS pre and post-ablation (**Figure 12**), FIAB Programmable Cardiac Stimulator 8817, PSA Model 3150–200; St. Jude Medical, St. Paul, MN, USA. We used an electrophysiological catheter (Duo-Dec Super LRG Curl, ref: 401904, St. Jude Medical).

For the ablation procedures, we used two different monopolar energy sources- a 10 microwave Flex ablation device (Guidant Cardiac Surgery, Santa Clara, CA, USA; 65Wand 120 seconds) and a Cobra XL Cooled Surgical Probe (Estech, San Ramon, CA, USA; 65 °C, 60 W, and 150 seconds).

All antiarrhythmics were discontinued (for more than the equivalent of 5 times their half-lives) except for amiodarone in 5 patients. All patients were in AF at the time of the ablation procedure and none converted to SR after the procedure.

The ablation was performed using a multipolar catheter introduces in the transverse sinus, reaching thereafter the 4 pulmonary veins, with the patient under general anesthesia (48) using a double lumen endotracheal tube for unilateral ventilation, while in supine position, rotated to the left at 30^0. Three incisions have been made - two 10 mm incisions in the fifth and sixth intercostal spaces and a 5 mm incision on the anterior axillary line in the fourth intercostal space.

2.4. Results

Influence of the ganglionated plexi on the atrioventricular node conduction properties

We have included 20 patients and performed direct HFS by applying 20Hz for 2 ms and then 20 mA on the right anterior (RAGP), inferior (RIGP) and left superior GP (LSGP). We did

not evaluate left inferior GP (LIGP) due to the difficult access in the context of a thoracoscopic unilateral approach.

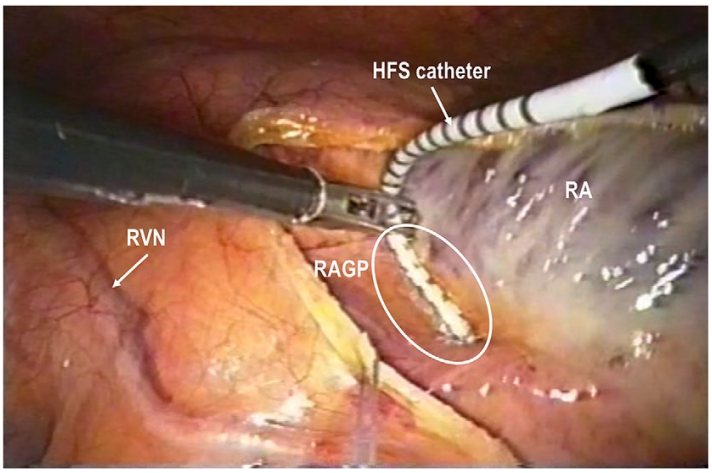


Figure 12. Procedural view of RAPG high frequency stimulation. HFS = high-frequency stimulation; RA = right atrium; RAGP = right anterior ganglionated plexuses; RVN=right vagus nerve.

We took into consideration the normal coefficient of RR variation in AF patients (48,15) and defined HFS as an acute RR lengthening with >25% for at least 5 seconds or an asystole lasting >3s (46). We have repeated HFS in the regions where initially positive, after isolating the LA posterior wall and dissecting the GP. We have stimulated the vagus nerve with the same multipolar catheter after having performed RF ablation.

In 35% of patients (n=7) HFS was positive in all 3 GPs, while in 25% of patients (n=5), HFS was positive in 2 GPs. HFS was positive just in one GP in 25% of patients (n=5). **Figure 13** summarizes the results, while **Figure 14** illustrates pre- and post-procedural positive responses to HFS. Additionally, HFS of the RVN was performed in 10 patients.

After ablation and subsequent dissection, in patients who initially responded to HFS, we were unable to induce HFS responses in both GP and RVN.

2.5. Discussions

In this study, the response to HFS varied across the approached GP, most likely due to:

- The difficulty of accessing all the GP with the pacing catheter from the left unilateral thoracoscopic approach;
- Accessing the RIGP and LSGP required a larger dissection area;
- Dissecting the pericardial reflections could have interacted with the GPs innervation.

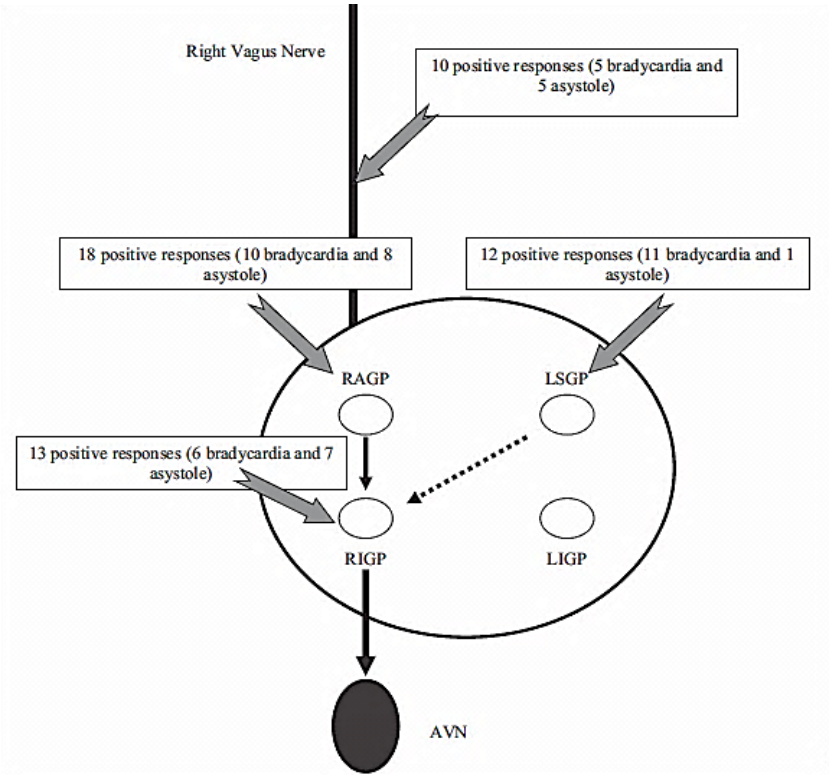
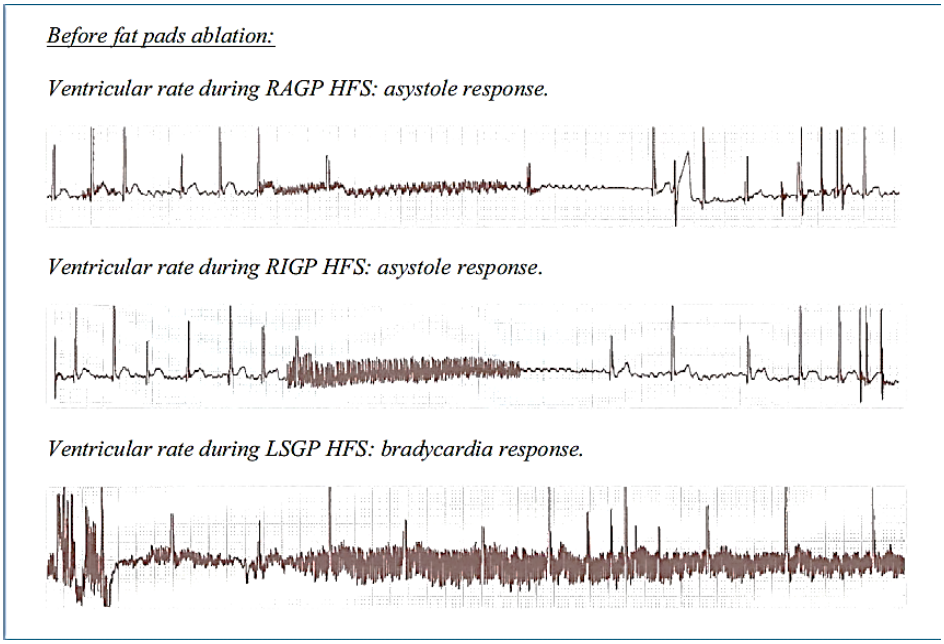


Figure 13. Schematic representation of the posterior wall of the left atrium, the vagus nerve, and the four ganglionated plexi showing the distribution of responses to high-frequency stimulation. The arrows suggest the relative importance of each GP to the other GPs and the AVN (tick lines and dotted lines indicate strong and weak effects, respectively). These arrows must be interpreted with caution because the study protocol does not permit sequential ablation of each GP. AVN = atrioventricular node; RAGP = right anterior ganglionated plexi; LSGP = left superior ganglionated plexi; RIGP = right inferior ganglionated plexi; LIGP = left inferior ganglionated plexi.



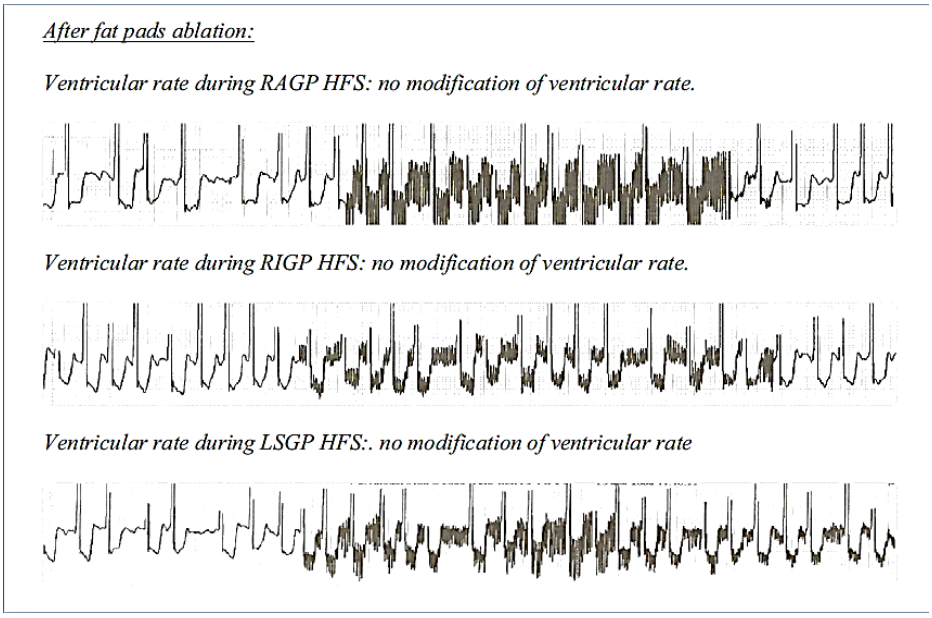


Figure 14. Illustration of the different responses to HFS as a function of the site of stimulation in one patient before and after ablation. RAGP = right anterior ganglionated plexuses; LSGP = left superior ganglionated plexuses; RIGP = right inferior ganglionated plexuses; HFS = high-frequency stimulation.

The most frequent responses to HFS were identified in RAGP, most probably due to anatomic considerations in relation to procedural approach (**Figure 13**). Moreover, the influence of RAPG on AVN tended to be more important than the one of LSPG, findings consistent with the ones of Hue et al. (52).

Post-ablation, all responses of HFS applied to GPs were negative. Several authors have reported similar results, including that AVN was innervated from the RIPG (51,52). Hue et al. highlighted that in dogs, GPs are connected through an interactive network, creating a veritable center responsible of cardiac autonomic innervation (40).

Our results are consistent with other studies in that delivering HFS to the RAGP and LSGP influenced AVN. Their role in cardiac autonomic innervation and their subsequent implication in arrhythmogenesis must, however, be carefully interpreted. The increased incidence of asystole (53%) when delivering HFS to the RIGP compared to other sites suggest that RIGP could be the last GP to have developed.

Interestingly, the lack of ventricular rate changes in response to post-ablation RVN HFS suggest that the vagus nerve modulates cardiac activity via GP and therefore the latter play an important role in conducting the impulses. Therefore, our study demonstrated that the GP network is a vital link between the vagus nerve and the heart, contributing to appropriate autonomic innervation.

The **clinical implications** of this study resides in the impact of GP during AF ablation procedures. Periprocedural, due to the anatomic proximity (the distance between GPs and pulmonary veins ostia is sometimes very small), GP may undergo unintended ablation. This may impact arrhythmogenesis, through a autonomic nervous system disbalance. Importantly,

the cells responsible of the parasympathetic innervation are located in the atrial GP. Several authors reported beneficial outcomes in terms of post-ablation AF recurrence rates when GP ablation was additionally performed. However, the same studies emphasize that incomplete GP ablation could enhance AF risk through autonomic disbalance (25,42,53).

One of the most troublesome reported complication after persistent AF ablation is high ventricular rate atrial tachycardia (54). This could be explained by the unintended and incomplete GP lesions during standard ablation procedures (38,55).

Study limitations

This study had several limitations:

- The thoracoscopic right approach limited the sequential GP ablation and HFS delivery.
- Due to the above mentioned reason, we were unable to assess the veritable network between the GPs and vagus nerve.
- The follow-up duration did not allow us to properly evaluate the long-term effects regarding overall survival, quality of life and arrhythmia-free interval after such a procedure; further studies are warranted.

2.6. Conclusions

Our study demonstrated that there is a connection between GP, autonomic innervation and AF and that ablating GPs in addition to standard pulmonary vein isolation might lead to better outcomes in terms of arrhythmia-free post-procedural outcome; we must emphasize that complete incomplete lesions may in fact promote arrhythmogenesis and even contribute to the development of high-ventricular rate tachycardias and that further studies are required to confirm the beneficial effect on survival.

Chapter 3 ATRIAL FIBRILLATION AND SYMPATOVAGAL BALANCE

3.1. Scientific context

Both gastroesophageal reflux disease (GERD) and atrial fibrillation (AF) are common in general population (68). The available data suggest that AF seems to more frequent that reported in the review. The prevalence of AF increases with age and reaches the highest values (about 20%) in a population older than 60 years. Also, we are assisting in the last two decades to a 13% increase in incidence (48). Over 6 million Europeans suffer from this arrhythmia, and its prevalence is estimated to at least double in the next 50 years as the population ages (35). Atrial fibrillation occurs when structural and/or electrophysiological abnormalities (electrical, mechanical and structural remodeling of left atrium) alter atrial tissue to promote abnormal impulse formation and/or propagation. Beginning with this step AF begets AF, through remodeled atrial myocardium. Left atrial remodeling may be caused by diverse pathophysiologic mechanisms and AF may represent a final common phenotype for multiple disease pathways. In a large majority of patients with AF we can find pathologies

like hypertension, obesity or diabetes mellitus as substrate for left atrial remodeling. Even patients diagnosed with idiopathic AF have more often suffered from insidious coronary artery disease than healthy controls in SR (69).

Most common substrates for both AF and GERD are obesity and aging. In addition, both AF and GERD are associated with other pathologies like sleep apnea or diabetes mellitus. In AF, like in other cardiac arrhythmias, three elementary electrophysiological mechanisms, alone or in combination, may be involved in the initiation and perpetuation of arrhythmia. These are: enhanced or abnormal automaticity, triggered activity and reentry. Enhanced or abnormal automaticity and triggered activity are subcellular mechanisms, whereas reentry is depending on intercellular and tissue properties. The trigger and the substrate are at the origin of AF. The substrate reflects the concept according to which the triggered electrical activation perpetuates in an autonomous way. In addition, the trigger itself can modify the substrate, especially if active for a prolonged period. Sympatho-vagal imbalance is one of the principal mechanisms of AF associated with GERD (70). Although both sympathetic and parasympathetic components play a role in AF, the cholinergic component appears to be important for spontaneous initiation of AF. Electrical stimulation of the left atrial ganglionic plexuses (situated on left atrial posterior wall, close to the esophagus) or the autonomic nerve endings with retrograde activation of the ganglia induces spontaneous firing from pulmonary veins followed by AF (70).

The majority of AF patients with GERD have triggered AF (71). During radiofrequency ablation, these patients may have positive vagal response (71). Gastroesophageal reflux could be only a trigger for AF in paroxysmal AF. Less known, not only GERD may trigger AF, but also AF may determine the occurrence of GERD. It could be speculated that an enlarged and fibrillating left atrium may compress or irritate the neighboring lower esophagus. Indeed, cardiovascular involvement in GERD is less assessed. It is mandatory to extend the research in this field for better understanding the relationship of AF with GERD. Recognizing that morbidity and mortality associated with AF had remained unacceptably high despite all efforts aimed at improving its management, the etiology of AF was placed in the foreground for the first time by the Third Consensus Conference of the Atrial Fibrillation Competence Network/European Heart Rhythm Association (72).

It seems that among traditional cardiovascular risk factors, GERD could be an independent risk factor for AF. Also, we think that AF should be considered as possible extraesophageal syndrome in the GERD classification (73).

Atrial fibrillation is the most frequent cardiac arrhythmia and its incidence is rising, especially in regions with aging inhabitants (74). Gastroesophageal reflux disease (GERD) is a frequently encountered benign disease affecting the upper esophageal tract (73). Taking into consideration both the anatomic proximity of the esophagus and atria and their common autonomic innervation, it has been hypothesized that there might be a link between the development of GERD and AF. The sympatho-vagal disbalance is regarded as one of the main possible mechanisms explaining this association (68).Both sympathetic and para-sympathetic nervous system play an important role in AF pathophysiology. Several factors may affect this association and contribute to AF either through inflammatory process or sheer mechanical compression, such as hiatal hernia, esophagitis or an enlarged LA.

The relationship between AF and GERD is still controversial (48), as most studies are retrospective and included national registries data or various self-reporting questionnaires without objectively evaluating autonomic disbalance (48,75). A very useful non-invasive tool in this respect is heart rate variability (HRV), which decreases in highly sympathetic states and increases under the effects of parasympathetic innervation (48,76).

HRV is an important tool in assessing autonomic disbalance and out of the several methods available for its evaluation, the most frequently used are related to time and frequency domains. The prospective studies assessing sympathovagal disbalance in relation to arrhythmogenesis in GERD patients are scarce.

I studied the relationship between AF and GERD through the perspective of electrocardiography and echocardiography. This preoccupation was synthetized in a few articles mentioned in the following box.

1. **Floria M**, Bărboi O, Grecu M, Cijevschi Prelipcean C, Balan G, Drug VL. *Atrial fibrillation and sympathovagal balance in patients with gastroesophageal reflux disease*.Turk J Gastroenterol 2017;28(2):88-93. IF=1.107. http://www.turkjgastroenterol.org/sayilar/298/buyuk/88-931.pdf
2. **Floria M**, Bărboi O, Rezuș C, Ambărus V, Cijevschi-Prelipcean C, Balan Ghe, Drug LV. *Atrial Fibrillation and Gastro-Oesophageal Reflux Disease – Controversies and Challenges.* Current Pharmaceutical Design 2015;21(26):3829-34. IF=3.052 http://www.eurekaselect.com/129660/article
3. **Floria M**, Drug VL. *Atrial fibrillation and gastroesophageal reflux disease: From the cardiologist perspective.* World J Gastroenterol 2015; 21(10); 3154-3156. doi: 10.3748/wjg.v21.i10.3154. IF=2.78. http://www.ncbi.nlm.nih.gov/pubmed/25780320

3.2. Aim

The aim of this study was to prospectively compare HRV parameters (both in time and frequency domains) in patients with and without GERD, with and without AF using 24 hours electrocardiographic (ECG) HOLTER monitoring.

The **secondary aims** were to assess the relative risk (RR) of developing AF in GERD patients and to evaluate the degree of LA structural remodeling through chamber enlargement assessed through transthoracic echocardiography.

3.3. Material and methods

Our team consisted of a gastroenterologist and a cardiologist who successively included the following patients:

(1) Adults (older than 18 years);

(2) Absent history of confirmed gastrointestinal diseases/ evaluation (naïve patients);

(3) Having symptoms suggestive of GERD (mild heartburn and/or regurgitations twice a week and/or moderate/severe symptoms more than 1 day/week) reported as troublesome according to Montreal definition (73);

and excluded the following:

(1) Patients (younger than18 years) and those who refused inclusion;
(2) Documented valvular AF/under anticoagulation therapy;
(3) More than mild valvular hear disease;
(4) Implantable cardiac devices, including pacemakers and defibrillators;
(5) Previous myocardial infarction/ stroke/ transient ischemic attack;
(6) Chronic gastrointestinal diseases (inflammatory bowel diseases, celiac disease);
(7) Known thyroid disorders;
(8) Other inflammatory diseases and patients under immunosuppressive therapies; patients under non-steroidal anti-inflammatory drugs or aspirin (> 100 mg/day) during the 30 days prior to initial admission;
(9) Active neoplasia, dementia, other neurological and/or debilitating psychiatric disorders;

GERD and AF were diagnosed according to current guidelines (73,74). Subsequently, valvular AF was defined as AF in patients with either valvular prosthesis or moderate/severe native mitral stenosis (74). Due to ethical reasons, antiarrhythmic therapies were not discontinued in AF patients. Proton pump inhibitor therapy was interrupted 8 weeks prior to inclusion. The diagnosis of GERD was clinically made by the gastroenterologist, based on patients' symptoms and in accordance with the Montral Consensus (73). All patients underwent both a gastroenterological and cardiological exam, irrespective of symptoms, including upper gastric endoscopy, transthoracic echocardiography and 24 hours HOTLER monitorization. We included the following clinical parameters after having taken a detailed medical history: age, gender, obesity (BMI > 30 kg/m^2), and noted the presence of arterial hypertension, heart failure, coronary artery disease, peripheral artery disease, dyslipidemia, diabetes. The upper gastrointestinal endoscopies was performed after 24-72h after the HOLTER monitorization by the same experienced gastroenterologist on a Olympus Exera CV-160 endoscope. We noted the presence of hiatal hernia (contributing to GERD), esophagitis and Barrett's esophagus.

Two-dimensional transthoracic echocardiography was performed on a Sonoscape SSI 8000 ultrasound machine (Providian Medical Equipment LLC, OH, USA by a single experienced operator. Left atrial structural remodeling was assessed through chamber enlargement and diastolic left ventricular function (E/A and E/Em ratio). The 2 channels 24 hours ECG HOLTER monitoring (EC-2H 2-Channel, Cardiospy, Labtech Holter ECG System, Hungary) was used to assess HRV parameters (both time and frequency domains) and the patients continued their daily activities. We analyzed the following HRV parameters: as a time-domain parameter we used the standard deviation of normal-to-normal interval (SDNN; ms), while as a frequency domain we assessed the low-frequency (LF)/high-frequency (HF) ratio (determined through a fast Fourier transformation), indicating a sympathovagal disbalance. Intervals < 200 ms and > 2000 ms were considered artifacts and therefore, were rejected. The HRV data was analyzed by an experienced electrophysiologist.

This study was in accordance with the University Ethics Committee's principles and with the Declaration of Helsinki.

Statistical Analysis

SPSS version 15.0 was used for the statistical analysis (SPSS, Inc., Chicago, IL, USA). We expressed categorical values as frequencies and percentages, while continuous variables were expressed as mean ± standard deviation. Taking into consideration the variable types, we used the $\chi 2$, Cochran, and Wilcoxon rank sum tests, Kruskal Wallis, and Anova test to compare variables between groups. A p value < 0.05 was considered statistically significant.

3.4. Results

We prospectively included 135 patients during July 2014 and February 2015 and divided them into 2 groups: 61 GERD patients (group I) and 74 non-GERD patients (control group II). Male gender, age and BMI were similarly distributed across groups (41% versus 46% male gender, 61.5±9 versus 58±9 years mean age, 28.8±4 kg/m^2 versus 29±4 kg/m^2 mean BMI; all p values > 0.05). **Table 2** summarizes the included clinical data for both groups.

We identified no statistically significant differences in the clinical data between the GERD group and control. AF was similarly distributed in the two groups (33 % in GERD group vs 39% in control, p=0.52). We identified a RR of 1.17 (95% confidence interval [CI] 0.78–1.75; p=0.34) for the development of AF in GERD patients.

Table 2. Clinical parameters and those relative risks in patients with gastroesophageal reflux disease (group I, n=61) versus without gastroesophageal reflux disease (group II, n=74)

PARAMETERS	Group I n (%)	Group II n (%)	Odd Ratio (95% CI)	Relative risk (95%CI)
Men	25 (41,0)	28 (37,8)	1,14 (0,57-2,28)	1,07 (0,74-1,56)
Age≥60 years	38 (62,3)	34 (45,9)	1,94 (0,97-3,88)	1,45 (0,98-2,14)
Obesity	24 (39,3)	33 (44,6)	0,81 (0,38-1,70)	1,13 (0,77-1,65)
Dyslipidemia	43 (70,5)	58 (78,4)	0,66 (0,28-1,54)	1,24 (0,84-1,84)
Hypertension	43 (70,5)	58 (78,4)	0,66 (0,28-1,54)	1,24 (0,84-1,84)
Diabetes mellitus	11 (18,0)	11 (14,9)	1,26 (0,46-3,44)	0,89 (0,56-1,41)
Heart failure	17 (27,9)	18 (24,3)	1,20 (0,53-2,79)	0,91 (0,60-1,36)
Ischemic heart disease	19 (31,1)	15 (20,3)	1,78 (0,76-4,20)	0,74 (0,51-1,09)
Peripheral arterial disease	2 (3,3)	1 (1,4)	2,44 (0,17-69,8)	0,68 (0,30-1,54)
Atrial fibrilation	20 (32,8)	29 (39,2)	0,76 (0,35-1,63)	1,17 (0,78-1,75)

All p values were >0.05 (Student's t test).

Table 3 comparatively summarizes ECG HOLTER, echocardiographic and upper gastrointestinal endoscopy parameters of the two groups. In GERD patients, we identified a statistically significant lower SDNN mean (97.6±13.7 ms versus 139.9±44.6 ms, p=0.001). Similarly, HRV frequency domain parameter – LF/HF ratio mean was also lower in these patients, however without attaining statistical significance (0.75±0.17 versus 0.76±0.24, p=0.930).

We identified a significantly lower E/A ratio in GERD patients as compared to control (0.97±0.40 versus 1.31±0.67; p=0.007) while LA area, as a marker of structural remodeling was similar between groups (25±5.4 cm2 versus 25±5 cm2; p=0.781).

Esophagitis was more frequent in GERD patients (n=32) as compared to control (n=8)- odds ratio (OR)=9.61, 95% CI 3.74–22.15; RR=2.62, 95% CI 1.86–3.68; p=0.0019, while hiatal hernia showed a similar distribution between groups (11 versus 13 patients; OR=1.03, 95% CI 0.43–2.50; RR=1.02, 95% CI 0.63–1.65; p=0.999). All patients with esophagitis were classified as Los Angeles Class A. We identified Barret's esophagus in one patient (GERD group).

Table 3. Comparative data of electrocardiogram, echocardiography and upper gastrointestinal endoscopy parameters in patients with gastroesophageal reflux disease (group I) versus without gastroesophageal reflux disease (group II).

PARAMETER	GROUP I (n=61)	GROUP II (n=74)	P value (Student' t test)
ECG HOLTER			
SDNN[+] (ms)	97.6±13.7	139.9±44.6	0.001
LF/HF ratio[++]	0.75±0.17	0.76±0.24	0.930
ECHOCARDIOGRAPHY			
E/A ratio[§]	0.97±0.40	1.31±0.67	0.007
E/Em ratio[§§]	8.1±2.3	7.8±2.9	0.592
Left atrium area (cm^2)	25±5.4	25±5	0.781
UPPER GASTROINTESTINAL ENDOSCOPY			
Esophagitis (%)	52.5	10.8	0.001
Hiatus hernia (%)	18.0	17.6	0.999

[§]E/A ratio=E wave velocity/A wave velocity ratio; [§§]E/Em ratio=E wave velocity/Em velocity ratio; [++]LF/HF ratio=low frequency/high frequency ratio; [+]SDNN=the standard deviation of normal to normal N-N intervals.

Table 4 summarizes the comparative data between HOLTER ECG, echocardiographic and upper gastrointestinal endoscopy parameters in patients with AF and GERD (n=36) and AF without GERD (n=39) versus SR and GERD (n=25) and SR without GERD (n=35).

We identified that the mean SDNN values were lower in AF patients with GERD as compared to those with AF but without GERD (114±58 ms and 273±100, p=0.001). There were no differences between the mean frequency domain HRV parameter (LF/HF ratio) between the 4 groups (p=0.749). However, AF patients with GERD had higher LF/HF mean ratio as compared to those without GERD (0.71±0.16 versus 0.69±0.17, p=0.862), but the difference was not statistically significant. There were statistically significant differences in the means of LV diastolic dysfunction parameters (E/A, E/Em and LA area). LA area, as a

marker of atrial structural remodelling differed between AF patients with and without GERD (25.8±5.1 cm^2 versus 27.3±5.1 cm^2, p=0.04).

Table 4. Comparative data of electrocardiogram, echocardiography and upper gastrointestinal endoscopy parameters in patients with atrial fibrillation (AF) versus sinus rhythm (SR) depending on the gastroesophageal reflux disease (GERD) diagnosis.

PARAMETER	AF+GERD n=36	AF–GERD n=39	SR+GERD n=25	SR–GERD n=35	p value Anova test
ECG HOLTER					
$SDNN^{+}$ (ms)	114±58	273±100	88±53	146±33	0.001
LF/HF ratio^{++}	0.71±0.16	0.69±0.17	0.76±0.24	0.72±0.17	0.749
ECHOCARDIOGRAPHY					
E/A ratio§	1.02±0.49	1.65±0.87	0.94±0.34	1.10±0.42	0.001
E/Em ratio§§	8.7±2.3	8.5±3.9	7.3±2.1	7.2±1.3	0.046
Left atrium area (cm^2)	25.8±5.1	27.3±5.1	23.9±5.8	22.9±3.4	0.002
UPPER GASTROINTESTINAL ENDOSCOPY					
Esophagitis (%)	50.0	7.7	56.0	14.3	0.001*
Hiatus hernia (%)	19.4	15.4	16.0	20.0	0.942*

**Kruskal-Wallis Test*
AF+GERD: patients with AF and GERD; AF-GERD: patients with AF without GERD; SR+GERD: patients with SR with GERD; SR-GERD: patients with SR without GERD
§E/A ratio=E wave velocity/A wave velocity ratio; §§E/Em ratio=E wave velocity/Em velocity ratio; $^{++}$LF/HF ratio=low frequency/high frequency ratio; $^{+}$SDNN=the standard deviation of normal to normal N-N intervals.

We identified esophagitis in 16 patients with AF and GERD and in 2 AF patients without GERD (RR=8.53, 95% CI 2.14–34.0; p=0.001). GERD patients had more frequently esophagitis, irrespective of heart rhythm. Similarly, hiatal hernia was more frequent in GERD patients, irrespective of AF or SR. Hiatal hernia was identified in 23.2 % patients with GERD and AF and in 9.4% with AF, but without GERD (RR=2.49, 95% CI 0.71–8.75; p=0.251).

3.5. Discussions

The effects of GERD on the cardiovascular system have rarely been studied. The presence of a trigger is one of the pathophysiological mechanisms in paroxysmal and persistent AF (77). AF development requires the presence of both trigger and substrate (either anatomic or functional) able to initiate and perpetuate AF. As noticed by Haissaguerre et al., an ectopic beat originating in the pulmonary veins acts like a trigger for AF (3). This led to the acknowledgement of the importance of the pulmonary veins and LE posterior wall on the autonomic innervation. In this regard, GERD may trigger AF through sympathovagal disbalance (70). LA structural, functional and electrical remodeling may alter atrial myocardium and create the necessary substrate for AF, as could the appearance of a trigger. However, this LA remodeling may in return be due to several other comorbidities with

different mechanisms. Taking this into consideration, it is preferable to differentiate AF from the different mechanisms point of view rather than considering it a homogenous disease (78).

In this study, we noticed that hypertension, coronary artery disease and diabetes were equally distributed between GERD and non-GERD patients (79). Moreover, the same observation holds true for AF distribution. Thus, AF was not more frequently diagnosed in GERD patients. Only one study including over 5000 patients, conducted by Bunch et al. and published in 2008, reported that after excluding several other risk factors, GERD was not associated with a higher risk of AF (80). Our study is the second to report that the frequency of AF is not significantly higher in GERD patients. When comparing our study with the one conducted by Bunch et al, we have assessed LA structural remodeling and autonomic disbalance (HRV parameters) and found that in GERD patients, LA diastolic dysfunction was more frequent, without, however showing significantly higher values of LA area as chronic echocardiographic marker of the latter.

With respect to the LA structural remodeling in the 4 subgroups, AF patients had significantly larger LA areas irrespective of GERD. It follows that LA remodeling may be due to the presence of AF and not GERD. Considering LA remodeling as an AF substrate in GERD patients is therefore difficult, although it can be speculated that an enlarged palpating LA may irritate/compress the lower esophageal region due to anatomic proximity.

Throughout studies assessing short-term recordings, time-domain HRV parameters such as SDNN are more frequently used then frequency parameters. Out of the first, SDNN is the preferred parameter with a value of < 50 ms being indicative of a high risk, 50-100 ms moderate and > 100 ms normal (80). A value of < 50 ms translates into a decreased HRV, found in both cardiovascular and non-cardiac pathological states (76). It has been suggested that lower values may be associated with an overall poorer prognosis, including increased mortality and several chronic affections and age (76). The lower SDNN mean found in GERD patients in our study translates into an increased arrhythmic risk. Regarding SDNN value, in GERD patients, a lower value is associated with a moderate increase in arrhythmic risk, while in non-GERD patients there is no association with arrhythmic risk.

As for frequency domain parameters, LF/HF ratio evaluates the disbalance between sympathetic and parasympathetic nervous systems. A higher ratio reflects a sympathetic predominance, while lower values are associated with a highly active parasympathetic system. In our study (79), GERD patients had lower LF/HF ratio values than non-GERD patients, suggesting the dominance of the parasympathetic nervous system. However, this difference was statistically non-significant. When performing subgroup analysis, AF and GERD patients had higher LF/HF mean values (statistically non-significant), suggesting a decreased parasympathetic activity, most probably due to concomitant heart failure. As expected, esophagitis was more frequently diagnosed in GERD patients. Apparently, the pattern of autonomic disbalance depends on the concomitant erosive esophagitis (81). From this perspective, patients with non-erosive GERD had higher LF/HF ratios (82). However, in endoscopically confirmed esophagitis patients (including the asymptomatic), the autonomic tone is lower, probably due to the importance of the esophagus in the modulating autonomic balance, rather than symptoms (83).

Another important associated risk factor with both GERD and AF is obesity. Increased body fat correlates with HRV (84). In our study, obesity was equally distributed across the

two groups. Despite this, the RR of developing GERD in the obese was 1.13 (95% CI 0.77–1.65). It is known that GERD patients may present with a mixed autonomic neuropathy (85). Authors have shown that impaired parasympathetic activity is linked to GERD (86), most probably due to the vagus nerve effects on the lower esophageal sphincter (87).

Our study was the second to demonstrate that AF is not statistically significant more frequent in GERD patients. It seems that autonomic balance seems to be affected in GERD patients, with the possible predominance of the parasympathetic activity. A similar study reported similar results (88), without however having assessed the diagnosis of AF or LA structural remodeling.

Study limitations

The interpretation of HRV parameters is difficult due to the numerous concomitant cardiovascular diseases and other factors that may influence them. In our study, however, the distribution of cardiovascular diseases was similar in both GERD and non-GERD patients.

Another limitation is the effect of antiarrhythmic therapy (which was no interrupted in our patients due to ethical reasons) on HRV.

Taking into consideration the possible pro-arrhythmic or antiarrhythmic effects of proton pump inhibitors, assessing HRV before and after treatment could reveal new findings (89,90). A larger sample of patients is necessary.

However, we did not identify statistically more frequent AF in GERD patients (79).

2.6. Conclusions

GERD patients have an autonomic nervous disbalance, with the predominance of the parasympathetic activity and that the latter may contribute to arrhythmia development.

This research study was conducted from June 2014 until October 2015 during the post-doctoral program at the "Gr. T. Popa" University of Medicine and Pharmacy within the project entitled "Program of Doctoral and Postdoctoral Diversity Research in Multidisciplinary Diseases in Chronic Diseases, POSDRU/159/1.5/S/133377. The theme of the study was "Atrial fibrillation - extraesophageal manifestation of gastroesophageal reflux disease", the project director being Professor Dr. Iliescu Radu.

During this study, I collaborated with colleagues from Institute of Gastroenterology and Hepatology, especially with Professor Vasile Liviu Drug and Oana Barboi.

Chapter 4 THROMBOEMBOLIC RISK SCORES IN ATRIAL FIBRILLATION

4.1. Scientific context

Thromboembolic events, ranging from stroke to systemic embolisms, are one of the most serious and frequent complications of AF, an arrhythmia with a current epidemic tendency. Leading to the development of heart failure with a subsequent increase in mortality and decrease in quality of life, AF is associated with LA thrombi formation (especially in the

LA appendage). This arrhythmia is linked to increased atrial fibrosis degree (as a marker of LA structural fibrosis) and a hypercoagulability state; the previous two can both cause and promote AF through the induction of a veritable atrial cardiomyopathy (93).This thrombogenic atrial cardiomyopathy may create a vicious pathophysiological circle with resulting LA thrombi formation (94).

Both recommended thromboembolic risk scores ($CHADS_2$- *Congestive heart failure [CHF] or left ventricular ejection fraction [LVEF] ≤ 40%*, *Hypertension*, *Age* ≥ 75 years, *Diabetes mellitus*, previous *Stroke* and/or transient ischemic attack [TIA] and CHA_2DS_2-VASc-with the addition of the following parameters to the previous ones used in $CHADS_2$: history of vascular disease, age between 65 and 74 years, and gender; compared to the CHADS2, age≥75 years and previous stroke carry a double risk weight) used in AF patients to assess anticoagulation therapy indication are far from being accurate in determining the thromboembolic risk (93). Following the idea that both $CHADS_2$ and CHA_2DS_2-VASc are suboptimal thromboembolic risk scores, transesophageal echocardiography could be used in non-paroxysmal AF patients with higher risk scores values despite a proper anticoagulation therapy. Until now, there is no ideal assessment tool that could be used in routine clinical practice to better stratify patients according to their thromboembolic risk and allow the implementation of the appropriate therapy.

CHA_2DS_2-VASc is recommended since 2010 guidelines to assess thromboembolic risk in non-valvular AF patients irrespective of type (both paroxysmal and non-paroxysmal). A recently published study proposed a new version, CHA_2DS_2-VASc-RAF, by adding two new parameters to the previous risk score: AF type and renal dysfunction (GFR < 57 mL/min/1.73m^2), with a seemingly improvement in prediction power and prognostic and thromboembolic risk assessment accuracy.

So far, AF type has not been included in thromboembolic risk scores based on the results of several studies that reported no difference in the aforementioned risk between paroxysmal and non-paroxysmal AF type (96). However, when carefully analyzing these studies, it becomes obvious that the anticoagulation therapy was heterogenous and there was an erroneous randomization between AF types. Interestingly, ROCKET-AF reported a higher mortality and thromboembolic risk for persistent AF patients as compared to paroxysmal, in the context of similarly distributed anticoagulation therapies and similar rates of major bleeding (96). A recent metanalysis which included 18 studies and more than 200 000 patients, revealed that the thromboembolic risk was higher in non-paroxysmal AF patients with similar major bleeding rates between the latter and paroxysmal AF (97). The introduction of AF type as an additional parameter to CHA_2DS_2-VASc by Kapłon-Cieślicka A. might improve the overall risk score predictive power, assigning 4 points to persistent AF and 10 points to long-lasting persistent AF (95). The authors suggested that AF patients with an initial CHA_2DS_2-VASc of 0 or 1 *and* non-paroxysmal AF should not be “should not automatically be attributed low thromboembolic risk and disqualified from anticoagulant treatment" (95).

Renal dysfunction has also failed to enter current thromboembolic risk scores in AF patients, although there is a strong association with other CHA_2DS_2-VASc parameters (ie. heart failure, arterial hypertension, diabetes). Moreover, authors have shown that for every 10 mL/min/1.73 m^2 decrease in GFR, the thromboembolic risk increases with 7%, while for

every 25 mg/mmol increase in the albumin-creatinine ratio, the risk of stroke increases with 10% (98). It seems that renal dysfunction enhances thromboembolic risk through the enhancement of pro-inflammatory (seen through increased C-reactive protein, interleukin-6 levels) and pro-coagulant pathways (increased fibrinogen, factors VII and VIII, D-dimers and plasmin-antiplasmin complex levels), as well as endothelial alterations with a subsequent increase in arterial stiffness. These changes seem to be present early in the development of renal dysfunction and promote both atherosclerosis and thrombosis (99). Moreover, the structural and functional alteration present in the cardio-renal metabolic syndrome promote arterial stiffness (99). **Figure 15** depicts the possible links between renal and heart dysfunction and atrial cardiomyopathy, highlighting the subsequent pro-inflammatory and procoagulant pathways.

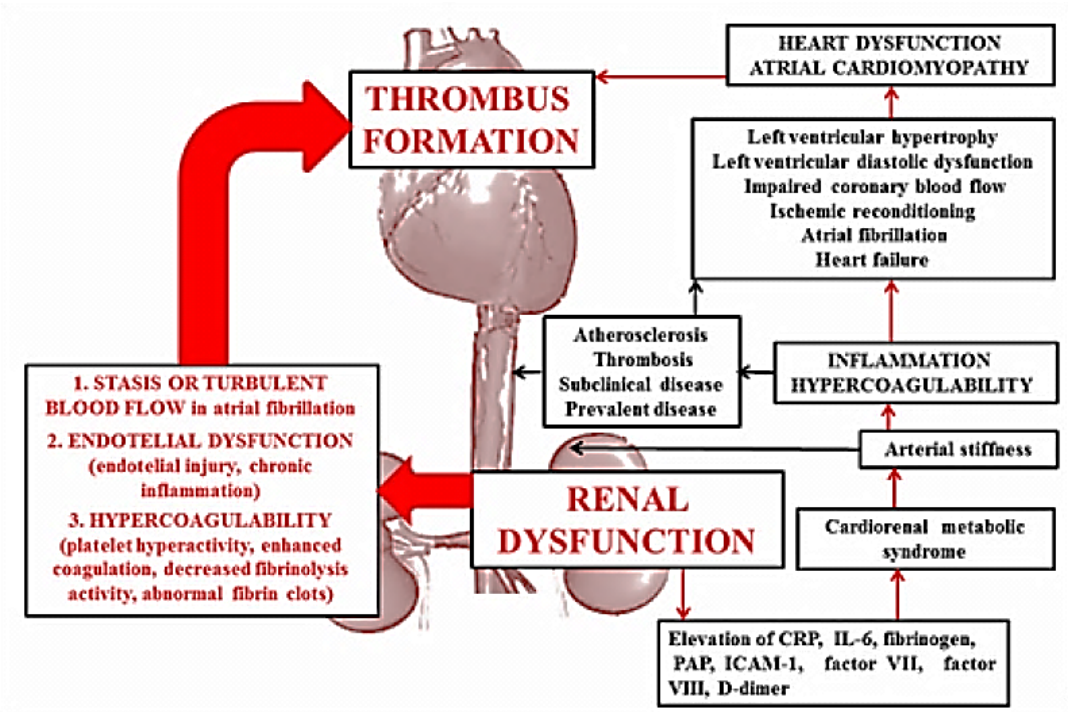


Figure 15. The interactions between renal dysfunction and thrombus formation pathways through heart dysfunction and atrial cardiomyopathy. *CRP: C-reactive protein; ICAM-1: intercellular adhesion molecule 1; IL-6:interleukin-6; PAP: plasmin-antiplasmin.*

Although not included in CHA_2DS_2-VASc, a different score was proposed - R_2CHADS_2, which assigned 2 points for a creatinine clearance < 60 mL/min/1.73 m^2 determined with Cockcroft-Gault formula (96).

ATRIA (Anticoagulation and Risk Factors in Atrial Fibrillation) thromboembolic risk score also included renal dysfunction as a parameter, assigning 1 point for proteinuria and eGFR<45 mL/min/ end-stage renal diseases) (100). Despite this, neither R_2CHADS_2 nor ATRIA have been routinely used in clinical practice, needing further validation through large clinical trials.

Renal dysfunction is also associated with increased bleeding risk, as proven by the inclusion of its various parameters in several bleeding risk scores, such as HASBLED (which assigns 1 point for a creatinine >2.26 mg/dL or >200 µmol/L/dialysis/ renal transplant),

ATRIA (1 point for an eGFR <30 mL/min or dialysis), ORBIT (1 point for an eGFR < 60 mL/min), HEMORR$_2$HAGES and SAMe-TT$_2$R2 (both assign 1 point for renal dysfunction) (93). Out of these, HASBLED is the only one to have been validated for the assessment of bleeding risk in AF patients. In patients with end-stage renal disease, it is recommended to carefully assess the ratio between risk and benefit in what concerns thromboembolic risk versus hemorrhagic risk (93). These patients maintain their thromboembolic risk, and therefore, anticoagulation therapy should not be withheld, but carefully chosen and closely monitored, while addressing other risk factors that may predispose to bleeding events (93).

CHA$_2$DS$_2$-VASc-RAF assigned 2 points for renal dysfunction, defined as an eGRF <56 mL/min/1.73m^2. The clinical relevance of the implementation of such a risk score goes beyond a better overall thromboembolic risk assessment. Renal dysfunction also influences the choice of the anticoagulant, with patients with severe renal dysfunction/dialysis being able to receive only vitamin K antagonists, as direct oral anticoagulants (DOACs) are contraindicated in the setting of a GFR < 15 mL/min/1.73 m^2. Another debate focuses on the appropriate formula to determine GFR in non-valvular AF patients with respect to their thromboembolic risk. There are several formulas, including Cockcroft-Gault formula, MDRD (Modification of Diet in Renal Disease), and CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation, that defined renal dysfunction as a GFR < 60 mL/min/1.73m^2. While MDRD underestimates GFR, the Cockcroft-Gault formula overestimates it in healthy individuals with a GFR > 60 mL/min/1.73m^2, and both overestimate it at GFR > 60 mL/min/1.73m^2 in patients with reduced muscle mass (proven to have an increased thromboembolic risk) (101). According to the risk of stroke, it seems that using Cockcroft-Gault is more appropriate.

Adding renal dysfunction to the new risk score CHA$_2$DS$_2$-VASc-RAF may improve its accuracy in determining the thromboembolic risk, but also its complexity. The latter however is addressed by the numerous widely available electronic devices.

Renal dysfunction and AF type are both associated with atrial fibrosis as a marker of LA structural remodeling (94). This induces in time, a veritable fibrotic bi-atrial cardiomyopathy, a substrate for both atrial arrhythmias promotion and progression and thromboembolic events (94). The latter should be better explored in each AF patient.

Catheter ablation is the recommended therapy in highly symptomatic AF patients which fail to respond to antiarrhythmic drugs in which rhythm control is desirable. However, one of the procedure's contraindications is the presence of LA and/or LA appendage (LAA) thrombi. In this context, there is no agreement among various scientific societies on the indication and/or appropriate timing of performing a pre-procedural transesophageal echocardiography (TEE) (59,77). On the other hand, guidelines focusing on anticoagulation recommend the use of AF cardioversion guidelines peri-procedural (22,35). The latter recommend peri-procedural TEE in all AF patients for > 48h/ unknown duration in the absence of prior (proper) anticoagulation for 3 weeks before the procedure.

The most widely used thromboembolic risk scores in AF patients are CHADS$_2$ congestive heart failure [CHF] or left ventricular ejection fraction [LVEF] ≤ 40%, hypertension, age ≥ 75 years, diabetes mellitus, previous stroke and/or transient ischemic attack [TIA]-2 points) and CHA$_2$DS$_2$-VASc (it includes the history of vascular disease, age between 65 and 74 years, and female gender as additional risk factors as compared to

$CHADS_2$; it assigns 2 points for age >75 years and previous stroke). However, neither has been validated to assess thromboembolic risk in AF patients during pre-pulmonary vein isolation (PVI) TEE (102,103).

Thrombogenicity in AF and the relationship with atrial remodeling, atrial cardiomyopathy and atrial failure is an important field for research in arrhythmology. The main researches about this theme were synthetized in the following articles.

1. **Floria M**, De Roy L, Xhaet O et al. Predictive Value of Thromboembolic Risk Scores before an Atrial Fibrillation Ablation Procedure. J Cardiovasc Electrophysiol 2013;24(2):139-145. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1540-8167.2012.02442.x
2. **Floria M**, De Roy L, Blommaert D, Deceuninck O, Schroeder E. Excluding the Presence of Left Atrial Thrombus Before Pulmonary Vein Isolation. J Cardiovasc Electrophysiol 2013;24(3):E6-7. https://onlinelibrary.wiley.com/doi/abs/10.1111/jce.12103
3. **Floria M**, De Roy L, Xhaet O, Blommaert D, Schroeder E. Transesophageal Echocardiography before Pulmonary Vein Isolation in Nonvalvular Atrial Fibrillation: A Tentative for Better Evaluation. J Cardiovasc Electrophysiol 2013; 24(5):E9-10. https://onlinelibrary.wiley.com/doi/abs/10.1111/jce.12135.
4. **Floria M**, Blommaert D, Deceuninck O, Xhaet O, De Roy L. Incidence of left atrial thrombus prior to catheter ablation of atrial fibrillation: Is it time for atrial cardiomyopathy evaluation? J Cardiovasc Electrophysiol 2018;29(1):E4. https://onlinelibrary.wiley.com/doi/abs/10.1111/jce.13393.
5. **Floria M**, Tanase DM. Atrial fibrillation type and renal dysfunction: new challenges in thromboembolic risk assessment. Heart 2019;105(17):1295-1297. https://heart.bmj.com/content/105/17/1295.long.

4.2. Aim

During this study I aimed to assess the relationship between the thromboembolic risk scores, the presence of LA/LAA thrombi/ spontaneous echo contrast (SEC) (together with a decreased LAA peak emptying velocity), and LA volume and to identify the predictive power of the risk scores that could justify a pre-procedural TEE in low and intermediate risk patients (103).

4.3. Material and methods

It was included 681 consecutive non-valvular AF patients scheduled for PVI that underwent a 24h pre-procedural TEE (103). Each patient provided informed consent and the study was approved by the Hospital Ethics Committee and was in accordance with the declaration of Helsinki.

Two investigators, blinded to previous echocardiographic data, reviewed past medical records and included clinical data from the 3 months prior to scheduled ablation. In patients having undergone several TEEs, we only included the first investigation. We determined LVEF and LA volume (ellipsoid formula) using two-dimensional transthoracic echocardiography and defined LA enlargement as a indexed LA volume >28 mL/m^2.

At the time of the TEE, we determined both $CHADS_2$ (0-6 points) and CHA_2DS_2-VASc (0-9 points) risk scores (59,77). Patients having either $CHADS_2$ or CHA_2DS_2-VASc 0 or 1 were considered to be at low and intermediate thromboembolic risk, respectively. We defined the type of AF in accordance to the European Society of Cardiology guidelines (77) and considered patients that described AF episodes characteristic of more than 1 category to have the most frequent pattern described. All patients were anticoagulated for at least 4 weeks prior to the procedure, irrespective of both AF type and $CHADS_2$ with an international normalized ratio (INR) between 2-3. The latter was weekly verified by the general practitioner. INR value was monitored in patients with LA or LAA thrombi. Both patients and general practitioners were routinely contacted to ensure both proper anticoagulation (therapeutic INR) and assess subsequent thromboembolic risk. Patients were switched to low-molecular weight heparin (LMWH) three days prior to admission and endocardial PVI followed guidelines recommendation and standard protocols (77,104).

TEE was performed 24 hours prior to PVI using a multiplane phase array transducer. Both TEE image acquisition and esophageal intubation were in accordance with the guidelines (105,106). We defined LA/LAA thrombus as an homogenously echodense intracavitary circumscribed mass, distinguishable from both endocardium border and pectinate muscles, seen in more than one incidence (107). We defined SEC as an echo-dense characteristically swirling appearance while using appropriate gain settings and visually assessed it as either mild, moderate and severe (102). Moderate/severe SEC (sludge) was described as a more gelatinous and precipitant echodense appearance, without having a proper distinguishable mass during the entire cardiac cycle. We considered a LAA peak emptying velocity of ≤ 20 cm/s as being decreased. The electrophysiologist decided for each patient whether to cancel the AF ablation procedure based on the detection of LA/LAA sludge on the TEE (102). The procedure was aborted in all patients with a LA/LAA thrombus.

Statistical Analysis

All statistical analysis was performed with either SPSS version 15.0 (SPSS Inc., Chicago, IL, USA) or Med Calc (Mariakerke, Belgium). We reported categorical variables as frequencies and percentages and continuous data as means ± standard deviations. $\chi 2$, Cochran and Wilcoxon rank sum tests were used to compare subgroups. Correlations were assessed using Spearman's rank coefficient. ROC curves F-distribution were used to determine the predictive power, sensitivity and specificity of the thromboembolic risk scores. The confidence interval (CI) was 95% and all tests were 2-tailed (statistical significance at a p value of < 0.005).

4.4. Results

We included 681 non-valvular AF patients, mean age 57±11 years, the majority men (78%), mean BMI of 28 ± 3 kg/m2, the majority with paroxysmal AF (69%). **Table 5** summarizes clinical and echocardiographic data in patients with and without LA/LAA thrombus.

Table 5. Clinical and echocardiographic parameters of patients with or without thrombus in the LA/LAA

Clinical and Echocardiographic Parameters	**Patients With Thrombus (n =7)**	**Patients Without Thrombus (n = 674)**	**P-Value**
Clinical parameters Mean age (years) Men	 63 ± 7 5 (71%)	 56 ± 11 529 (78%)	 NS NS
Mean BMI (kg/m^2)	31 ± 5	28 ± 5	NS
Persistent AF	6(86)	203(30)	0.002
Coronary heart disease	0(0%)	70(10%)	NS
Hypertension	4(57%)	261(39%)	NS
Heart failure	6(86%)	56(8%)	<0.001
Diabetes mellitus	2(29%)	42 (6%)	0.017
Previous TIA/stroke	0 (0%)	5 (1%)	NS
Mean $CHADS_2$ Mean CHA_2DS_2-VASc	3.1 ± 1.2 3.9 ± 1.1	0.8 ± 0.9 1.2 ± 1.2	<0.001 <0.001
Echocardiographic parameters			
SEC (%) absent mild moderate or severe (sludge)	 1 (14%) 3 (43%) 3 (43%)	 642 (95%) 27 (4%) 5 (1%)	 <0.001
Low LAA emptying velocity (cm/s)	6 (86%)	19 (3%)	<0.001
Mean LA volume index (mL/m^2)	31 ± 5	37 ± 8	NS
Mean LVEF (%)	39±13	45 ± 9	NS
LVEF ≤ 40%	2(28%)	27 (4%)	NS

AF = atrial fibrillation; BMI = body mass index; LAA = left atrial appendage; LA = left atrium; LVEF = left ventricular ejection fraction; SEC = spontaneous echo contrast; TIA = transient ischemic attack; NS = non-significant.

Prior to PVI, we identified thrombi in 7 patients (1%), all with negative medical history for stroke/TIA. All of the thrombi were located in the LAA and were laminated and organized in two patiens. Except for 1 patient (paroxysmal AF), the rest had received appropriate anticoagulation therapy 1 month prior to the procedure and had persistent AF. The patient had a INR of 1.6 before having been switched to LMWH, a $CHADS_2$ of 3, and a CHA_2DS_2-VASc of 4. LAA thrombi were therefore more prevalent in persistent AF patients (3% vs 0.2%) - **Table 6**. Congestive heart failure (CHF) was present in 87% (n=6) of the patients.

Table 6. Clinical and Echocardiographic Parameters of Patients Depending on the Type of Atrial Fibrillation

Clinical and Echocardiographic Parameters	Paroxysmal AF (n = 472 or 69%)	Persistent AF (n = 209 or 31%)	P-Value
Clinical parameters			
Mean age (years)	56 ± 11	58 ± 10	0.033
Men	357 (76%)	176 (85%)	0.004
Mean BMI (kg/m2)	28 ±5	29 ± 5	<0.001
Coronary heart disease	47 (10%)	23 (11%)	NS
Hypertension	169 (36%)	93 (45%)	0.022
Heart failure	21 (4%)	39 (19%)	<0.001
Diabetes mellitus	25 (5%)	19 (9%)	0.044
Previous TIA/stroke	1 (0.2%)	4 (2%)	NS
Mean CHADS2	0.7 ± 0.9	1.0 ± 1.1	<0.001
Mean CHA2DS2-VASc	1.2 ± 1.2	1.4 ± 1.3	0.009
Echocardiographic parameters			
SEC (%)			
Absent			
Mild	462 (98%)	179 (87%)	<0.001
Moderate or severe (sludge)	10 (2%)	20 (10%)	
Low LAA emptying velocity (cm/s)	0 (0%)	8 (3%)	
Presence of thrombus	9 (2%)	16 (8%)	<0.001
Mean LA volume index (mL/m2)	1 (0.2%)	6 (3%)	0.001
Mean LVEF (%)	34 ±6	40 ± 9	<0.001
LVEF ≤ 40%	46 ±8	43 ± 10	NS
	8 (1.7%)	21 (10%)	NS

AF = atrial fibrillation; BMI = body mass index; LAA = left atrial appendage; LA = left atrium; LVEF = left ventricular ejection fraction; SEC = spontaneous echo contrast; TIA = transient ischemic attack; NS = non significant.

Patients in which a thrombus was identified had higher means of both thromboembolic risk scores (**Table 7**), with a minimum $CHADS_2$ of 1 and a minimum CHA_2DS_2-VASc of 2. Consequently, LAA thrombi were more frequently found in high thromboembolic risk patients (both $CHADS_2$ and CHA_2DS_2-VASc >2) as compared to intermediate and low-risk: 4.9% and 3.1% vs 0.2% and 0%, p<0.001.

The presence of LAA thrombi correlated with persistent AF (p=0.002), CHF (p<0.001), diabetes mellitus (p= 0.017), the presence of SEC (p<0.001), as well as decreased LAA peak emptying velocity (p<0.001). LA indexed volume was similar between subgroups (with/without thrombi). In patients with thrombi, both risk scores showed a weak, however statistically significant correlation with BMI (r=0.187, p<0.001 and r=0.108, p=0.007) and LVEF (r=–0.494, p< 0.001 and r= –0.384, p<0.001).

Two patients had sustained > 48 hours post-procedural TIAs, confirmed by an MRI scan only in the first patient (52 years old woman with persistent AF, $CHADS_2$=2, CHA_2DS_2-VASc=3) and not in the other (47 years old male with paroxysmal AF, $CHADS_2$=1, CHA_2DS_2- VASc=1). None of the two had preprocedural LAA thrombi or SEC, therefore the most probable cause was inappropriate anticoagulation during the procedure. No complications arose during TEE in any of the patients.

Table 7. Distribution of Patients With and Without Thrombus in the LAA Depending on the Type of Atrial Fibrillation and Thromboembolic Risk (CHADS2 and CHA2DS2-VASc Scores ≥ 2 Correspond to Patients at High Thromboembolic Risk; CHADS2 and CHA2DS2-VASc Scores < 2 Correspond to Patients at Low or Intermediate Thromboembolic Risk)**.**

	Paroxysmal AF Without /With Thrombus		**Persistent AF Without /With Thrombus**		**Total Without /With Thrombus**	
CHADS2 score						
0–1	400 (84.9%)	0	152 (74.9%)	1	552 (81.9%)	1 (14.3%)
≥2	71 (15.1%)	1 (100%)	(16.7%)		122 (18.1%)	6 (85.7%)
Overall	471 (100%)	1 (100%)	51 (25.1%)	5	674 (100%)	7 (100%)
			(83.3%)			
			203 (100%)	6 (100%)		
CHA_2DS_2-VASc score						
0–1	329 (69.9%)	0	122 (60.1%)	0	451 (66.9%)	0
≥2	142 (30.1%)	1 (100%)	81 (39.9%)	6 (100%)	223 (33.1%)	7 (100%)
Overall	471 (100%)	1 (100%)	203 (100%)	6 (100%)	674 (100%)	7 (100%)

AF = atrial fibrillation

Thrombogenic milieu

We reported the presence of SEC in 5.6% of the patients (n=38), with mild SEC seen in 4.4% (n=30) and moderate/severe in 1.2% (n=8) patients. SEC was observed in 2% paroxysmal AF patients. With respect to the presence of moderate/severe SEC, 2.4% patients had a $CHADS_2$ of 0, 4.2% a $CHADS_2$ of 1, 3.8% had a $CHADS_2$ of 2 while a $CHADS_2$ ≥3 was present in the majority of patients (76.9%), p<0.001. Consequently, 35.6% of patients with moderate/severe SEC had a CHA_2DS_2-VASc ≥ 3, with 0.9% having had a CHA_2DS_2-VASc of 0, 3.7% of 1 and 7.1% of 2, p<0.001.

Sludge was more prevalent in patients with increased thromboembolic risk ($CHADS_2$ and CHA_2DS_2-VASc ≥ 2) as compared to low/intermediate risk patients: 8.6% vs 3.1%, p=0.005 and 7.8% vs 2.2%, p<0.001. All patients had persistent AF (3.4% vs 0%, p<0.001).

Both LAA thrombi and sludge were found in 2.2% patients. The procedure was cancelled in 15 patients, 7 with a LAA thrombus and 8 with moderate/severe SEC. All persistent AF patients with a thrombus had a decreased LAA emptying velocity.

Risk Scores

The majority of patients had a low/intermediate thromboembolic risk according to both risk scores: 81.9% (n=552) had a $CHADS_2 < 2$ and 66.9% (n=451) had a CHA_2DS_2-VASc < 2 (**Table 7**).

We reported a maximal negative predictive power for LA/LAA thrombi of 99.8% for a $CHADS_2<2$ and 100% for CHA_2DS_2-VASc<2 (95% CI: 99-100), while the positive predictive power of both scores<2 was 5% (95% CI: 2-10) and 3% (95%CI: 1-6), respectively.

When both scores were lower than 2, a maximal negative predictive value was almost reached for the presence of LA/LAA thrombi: 99.8% for CHADS2 and 100% for CHA2DS2-VASc (95% CI: 99–100). Similarly, the positive predictive values for CHADS2 and CHA2DS2-VASc scores <2 were 5% (95% CI: 2–10) and 3% (95% CI: 1–6), respectively. The sensitivity and specificity of a $CHADS_2 \geq 2$ in predicting the presence of a LA/LAA thrombus were 86% (95% CI: 42–100) and 82% (95% CI: 79–85), with an area under the curve of 0.928 (95% CI: 0.906–0.946), while a CHA_2DS_2-VASc ≥ 2 showed a 100% sensitivity (95% CI: 59–100) and 67% specificity (95% CI: 63–70) with an area under the curve of 0.933 (95% CI: 0.912–0.951), respectively (**Figure 16**).

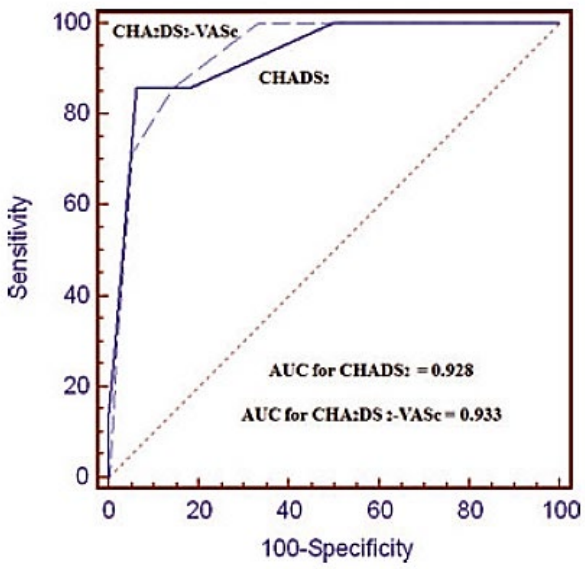


Figure 16. ROC curves for CHADS2 (thick line) and CHA2DS2-VASc (thin line) scores ≥2 representing the sensibility and specificity of the two scores for the detection of a thrombus in the left atrium by transesophageal echocardiography.

Patients with a LAA thrombus had higher thromboembolic risk scores means (mean $CHADS_2$ of 3.1, mean CHA_2DS_2-VASc of 3.9) as compared to the others (0.8 and 1.2, respectively), p<0.001. Similarly, patients with decreased LAA emptying velocities (< 20 cm/s) had higher $CHADS_2$ (1.6 vs 0.8, p=0.001) and CHA_2DS_2-VASc (2.3 vs 1.2; p<0.001). In these patients, LA indexed volume failed to correlate with either risk score: r = 0.013, p=0.85 for $CHADS_2$ and r = 0.034, p=0.624 for CHA_2DS_2-VASc.

Table 6 summarizes clinical and echocardiographic parameters in AF patients according to AF type (paroxysmal vs persistent). Persistent AF patients had more frequently decreased LAA peak emptying velocity, LA enlargement, SEC, LAA thrombi (3% vs 0.2%, $p<0.05$) and sludge ($p<0.001$). Both risk scores had higher means in persistent AF patients.

4.5. Discussions

Our study showed a low overall prevalence of both LA/LAA thrombi and combined thrombi/sludge in properly anticoagulated patients during the 1 month prior to AF ablation procedure. However, there was a high prevalence of thrombi in persistent AF patients. These findings are similar to those reported by several authors (108,109). This could be due to the fact that almost 50% of the patients had low thromboembolic risk according to $CHADS_2$. When using CHA_2DS_2-VASc, patients were similarly distributed as low, intermediate and high risk, demonstrating the better discriminative abilities of CHA_2DS_2-VASc (102).

It is known that the more risk factors there are (110) and the higher the $CHADS_2$ score is (111), the more probable it becomes to identify a LA/LAA thrombus, facts also confirmed by our study (**Table 7**). Except for one patient who had paroxysmal AF (and also a subtherapeutic INR of 1.6 with a high thromboembolic risk), all patients with thrombi had persistent AF and higher thromboembolic risk scores values. This emphasizes that AF patients need to be appropriately anticoagulated according to thromboembolic risk scores, irrespective of AF type. No patient with either $CHADS_2$ or CHA_2DS_2-VASc of 0 had LA/LAA thrombi. In contrast, a median of 3 and 4 of $CHADS_2$ and CHA_2DS_2-VASc, respectively, correlated with LAA thrombus, finding consistent with other studies (109).

LA indexed volume had similar values when comparing patients with thrombi to those without and varied significantly in paroxysmal versus persistent AF patients. This could be due to the very small number of patients in which a thrombus was identified (all in the LAA). Reversely, all of these patients had an enlarged LA, but the latter did not correlate with either $CHADS_2$ or CHA_2DS_2-VASc. LA echocardiographic parameters are not included in the thromboembolic risk scores while LAA parameters, such as peak emptying velocity show a weak correlation with overall LA parameters (112). However, it seems that LA and LAA function is more tightly linked to thromboembolic events prediction than chamber size in these patients.

We identified no thrombus in appropriately anticoagulated low thromboembolic risk AF patients and in only 1 intermediate risk persistent AF patient. According to both risk scores, there were 81.9% intermediate risk patients and 66.9%, respectively. From this point of view, our findings are similar to another study (111). In a different study, Wallace et al. reported an increased number of LA thrombi out of which three had paroxysmal AF; however the included number of patients was small and the paroxysmal AF patients had concomitant CHF (114).

Our results suggest that a value < 2 of either thromboembolic risk scores practically excludes the possibility of LA/LAA thrombi in properly anticoagulated patients (an INR in therapeutic range for at least 1 month prior to AF ablation).

Clinical Implications

The **clinical implications of this study** are that in appropriately anticoagulated AF patients with either $CHADS_2$ or CHA_2DS_2-VASc <2, performing an TEE is not necessary to exclude a LA/LAA thrombus prior to an ablation procedure. However, in appropriately anticoagulated persistent AF patients with increased thromboembolic risk according to both risk scores, performing a pre-procedural TEE seems mandatory. We have proposed a flowchart to identify the requirements of performing a TEE in AF patients (**Figure 17**).

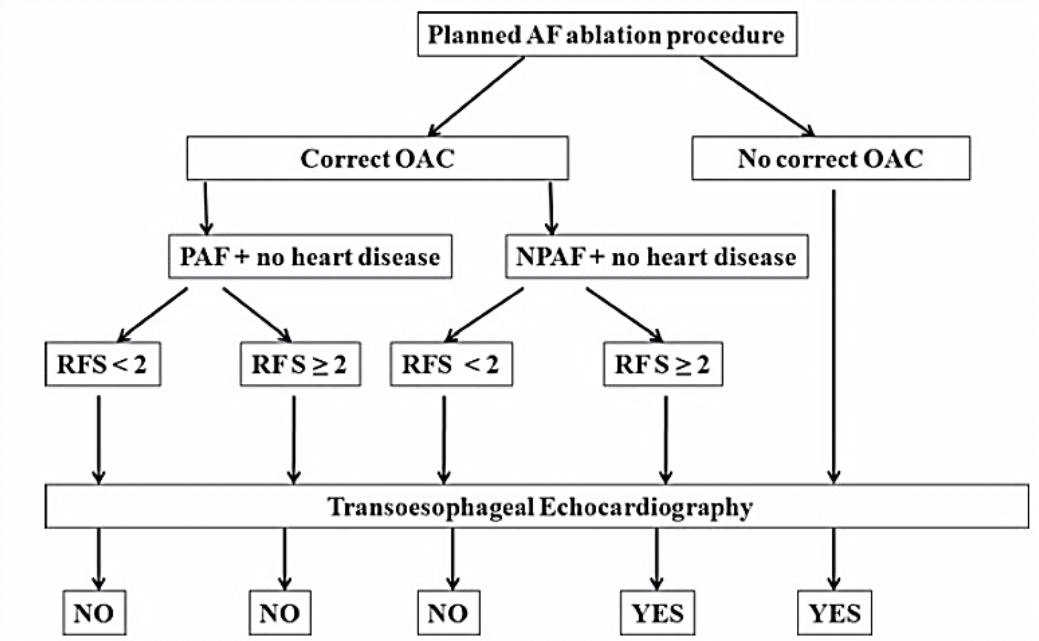


Figure 17. Proposed algorithm for determining the need for transesophageal echocardiography before an atrial fibrillation ablation procedure. *AF = atrial fibrillation; NPAF = nonparoxysmal atrial fibrillation; OAC = oral anticoagulation therapy for 4 weeks; PAF = paroxysmal atrial fibrillation; RFS = risk factor score; TEE = transesophageal echocardiography (102).*

According to our proposed algorithm, appropriately anticoagulated paroxysmal AF patients do not require a pre-procedural TEE, irrespective of the thromboembolic risk scores. Similarly, persistent AF patients with a thromboembolic risk score < 2 do not require pre-ablation TEE. In contrast, non-paroxysmal AF patients with increased thromboembolic risk (>2) require pre-procedural TEE, despite proper anticoagulation therapy. Lastly, we would suggest that TEE might be taken into consideration in patients with known heart disease and that TEE is required in inappropriately anticoagulated patients or in those with an unknown anticoagulation status.

Although the risks are minimum, TEE remains a less desirable procedure and patients can refuse it, especially if repeated. Therefore, to ensure its efficiency, it is necessary to properly identify those in need of such an exploration.

Study Limitations

There are several limitations to this study:

(1) this is a retrospective analysis, although 2 independent physicians were in charge of verifying the echocardiographic data accuracy;
(2) Switching to LMWH at least 72 hours pre-ablation might have affected the thrombogenic milieu. Some authors have reported performing ablations with patients under anticoagulation (113), but our study was conducted between 2000 to 2010, before this trend was started (102);
(3) Grading the severity of SEC into mild, moderate or severe is subjective; however there is no other reported quantification method;
(4) The accuracy of the thromboembolic risk scores and the monitoring of appropriate INR values was made by contacting either the cardiologist in charge of the appointed general practitioner. We have reported a low number of thrombi, most probably due to the 1 month pre-procedural appropriate anticoagulation;
(5) We must highlight that predicting thromboembolic events in AF patients is not perfect and that rather than solely relying on the $CHADS_2$ risk score, the European Society of Cardiology has switched from classifying patients in low/moderate/high risk to a more factor-based approach (77). As such, the newer CHA_2DS_2-VASc risk score is recommended to complement this assessment.

The CHA_2DS_2-VASc risk score takes into consideration more thrombogenic risk factors than its predecessor does. In the SPORTIF trial (Stroke Prevention Using Oral Thrombin Inhibitor in Atrial Fibrillation), CHA_2DS_2-VASc had a negative predictive value for thromboembolic events of 99.5% (116). Another study comparing the c-statistics of the two thromboembolic risk scores reported a value of 0.653 (95% CI, 0.50–0.81) for $CHADS_2$ and 0.898 (95% CI, 0.84–0.96) for CHA_2DS_2-VASc (117). The latter better identifies low-risk patients than its predecessor and is at least equally efficient in identifying high-risk patients. Although some authors have reported thrombi in AF patients with a $CHADS_2$ of 1, similar study designs and patients are needed in order to compare findings across studies (108,118).

Our study included only non-valvular AF patients (103). CHA_2DS_2-VASc score better identified the low/intermediate from high thromboembolic risk patients and had a high predictive ability of a thrombus in properly anticoagulated patients without, however, cardiac diseases (103).

Puwanant showed that the presence of heart disease is associated with thrombus formation in $CHADS_2$=1 patients with CHF (108). Regarding patients treated before 2006, it is unclear whether they received LMWH as a bridging therapy 72 hours pre-procedural. Not only that determining the thromboembolic risk is far from perfect, but also the same holds true with the prevention, as newer strategies are under development (119). Nevertheless, it must be emphasized that these studies require similar designs and included population in order to be compared later on.

This study included pre-ablation AF patients with the purpose of determining the thromboembolic risk scores' predictive value of LA/LAA thrombi (102). In patients with both

risk scores < 2, there was a maximal negative predictive value of the presence of LA/LAA thrombi. An algorithm was developed in which the decision of whether a TEE was performed was based firstly on the paroxysmal versus non-paroxysmal AF type, and secondly, on the values of the thromboembolic risk scores. The only remark lies with the presence of associated heart disease, patients in which it may be reasonable to perform TEE irrespective of risk scores' values due to the associated LA structural remodeling which is the substrate of AF and enhances thromboembolic risk. In our study, LAA thrombi were more prevalent in persistent AF patients (3% vs 0.2% in paroxysmal AF patients) (103).

Interestingly, patients with high thromboembolic risk (both risk scores ≥2) had more frequently LAA thrombi than intermediate/low thromboembolic risk patients (4.9% and 3.1% vs. 0.2% and 0%, respectively, p<0.001) (100). More frequently, the presence of LAA thrombi was associated with persistent AF (p=0.002), SEC (p<0.001), low LAA peak emptying velocity (p< 0.001), heart failure (p< 0.001) and diabetes mellitus (p=0.017). We canceled our ablation procedures in 2.2% of patients (7 with LAA thrombi and 8 with moderate/severe SEC) (102). The rare occurrence of both LA/LAA thrombi and of concomitant LA/LAA thrombus and sludge was reported in several other studies (108,109,111). There is still no consensus regarding the proper timing and indication of pre-procedural TEE and the 2011 update of the Venice chart suggests that non-anticoagulated AF patients should be the ones to undergo TEE (122).The same document admits that TEE is however performed in high-risk patients, with a special focus on persistent AF patients (122). This routine clinical practice heterogenicity only contributes to the lack of agreement. In this setting, our study aimed to better stratify this risk and to create a practical algorithm. The risk of developing LA/LAA thrombi will never be absent in AF patients, however, a better stratification according to CHA_2DS_2-VASc score will allow us to avoid unnecessary TEEs, thus decreasing patients' associated discomfort and procedural costs and rare, but possible risks. At the same time, our results emphasize the need of such an exploration in high thromboembolic risk persistent AF patients and paroxysmal AF patients with concomitant heart disease.

We must at the same time take into consideration that nearly 25% of atrial thrombi fail to dissolve with anticoagulation therapy; this brings a supplementary difficulty in interpreting echocardiographic masses/ SEC and judging anticoagulation preventive efficiency (102).

The alternative of performing a CT to a TEE has not been validated (120). Several authors even report a worrisome low sensitivity and positive predictive abilities of the CT (29% and 20%) (113). At the same time, we must emphasize that a CT is more costly, has increased risks, including radiation exposure, and the use of contrast may be impossible in some patients due to either allergies or renal dysfunction. TEE can be used pre-procedural to assess the presence of SEC and/or a permeable foramen ovale (121).

Contrastingly, several studies suggest that the presence of LA/LAA thrombus doesn't seem to be connected to either appropriate anticoagulation (123) or type of pharmacological agent used (either vitamin-K antagonists or non-vitamin K antagonists) (120), nor does it seem do depend strictly on other concomitant risk factors (both clinical- such as a CHA2DS2 -VASc ≥2, non-paroxysmal AF, CHF and imagistic (123)- LA enlargement, the presence of hypertrphic cardiomyopathy, LAA morphology with more than 3 lobes), and not even associated with SR during the TEE (124). Taking this into consideration, the attention should

be focused on determining the amount of atrial fibrosis, tightly linked to thrombogenicity. Hypercoagulability may determine and enhance atrial fibrosis and therefore promote AF (125) through a veritable fibrotic atrial cardiomyopathy (94). Moreover, authors have proven that atrial fibrosis as a hallmark of structural remodelling is related to increased thromboembolic risk (126). This association should be analysed in each patient (124), which would lead to a more patient-tailored approach and therefore, an appropriate patient care. Rather than other factors, this fibrotic atrial cardiomyopathy will help us understand pre-procedural LA/LAA thrombus formation (124).

4.6. Conclusions

The results of our study suggest that properly anticoagulated AF patients with either $CHADS_2$ or CHA_2DS_2-VASc scores <2 do not need a pre-AF ablation TEE, irrespective of AF type (paroxysmal/persistent). Performing a pre-procedural TEE might be necessary in persistent AF patients with $CHADS_2$ or CHA_2DS_2-VASc scores > 2 and paroxysmal AF patients with concomitant heart disease. More studies are warranted to confirm these findings.

Chapter 5 POSTOPERATIVE ATRIAL FIBRILLATION PREDICTION

5.1. Scientific context

The most frequent complication post cardiac surgery is post-operative atrial fibrillation (POAF), with an estimated incidence of 40-50% after valvular surgeries (183). POAF increases mortality, thromboembolic events risk and patient care costs (184). Several authors have reported age as an independent risk (185,186). The increasing overall life-expectancy has led to an increased number of aortic valve replacement procedures, and a subsequent increase in POAF. Aortic valve patients already have an increased mortality and arrhythmic risk as such prophylactic measures are required, such as either antiarrhythmic or anti-inflammatory drugs (5) or intraoperative atrial pacing (187,188). Despite this data, routine pharmacological prophylaxis is rarely used in cardiac surgery departments, most probably due to medication side effects (189-191).

As the POAF's etiology is not completely understood, there is a general reluctance in adopting available prophylactic therapies. Recent recommendations suggest that prophylaxis should be considered only in high-risk patients (192).

The interest for this theme of research namely pre-and intraoperative risks factors for POAF was synthetized in an ISI article.

1. Iliescu AC, Salaru DL, Achitei I, Grecu M, **Floria M**, Tinica G. Postoperative atrial fibrillation prediction following isolated surgical aortic valve replacement. Anatol J Cardiol 2018;19(6):394-400. https://www.journalagent.com /anatoljcardiol/pdfs/AJC-70745-ORIGINAL_INVESTIGATION-FLORIA.pdf

5.2. Aim

The aims of this study was to identify the pre-and intraoperative risks for POAF in surgical aortic valve replacement (SAVR) patients and to create a model that could help predict POAF and therefore justify the implementation of prophylactic measures.

5.3. Methods

We retrospectively included 8740 patients during January 2000 and June 2014 scheduled for cardiac surgeries, out of which 1191 patients underwent isolated SAVR and performed a cross-sectional analysis (**Figure 22**). We included patients > 18 years old, who have submitted informed consent. We excluded patients who refused to sign the informed consent, AF patients and other heart rhythm disorders, patients with implanted pacemakers/defibrillators, and those in need of aorto-coronary bypass/mitral valve surgery. The study was approved by the hospital Ethics committee.

We included the following pre-operative parameters: 24 hours HOLTER monitoring, echocardiography (and classified patients according to their LVEF in normal LV systolic function- LVEF> 50%, reduced – 30-50% or severely reduces- <30%), biological tests and both intra-operative and early-postoperative data.

We defined POAF according to the 2014 Guidelines on Atrial Fibrillation of the American College of Cardiology/American Heart Association (193) as AF lasting for more than 15 minutes. We continuously monitored patients during the first 72 hours and twice daily when symptomatic. We maintained a close monitorization during their early post-operative period.

Statistical Analysis

We used SPSS 17.0 (SPSS inc., Chicago, IL, USA) and Microsoft Office Excel 2013 to perform the statistical analysis. We considered a $p<0.05$ as significant. Continuous data were expressed as mean ± standard deviation and categorical data as numbers and percentages. We used Kolmogorov-Smirnov test to test for the normality of the distribution and Fisher's exact test and chi-square test to compare the nominal variables. We used the t-test and Mann-Whitney test (when appropriate, depending on the variable type) to compare subgroups. Spearman's coefficient was used to assess heterogeneously distributed data.

We used logistic regression (forward/backward selection; entry p-value=0.02; removed when $p>0.05$) to create a model able to identify high-risk patients and predict POAF. We evaluated the model significance using the Wald test and the power of variables association by determining 95%CI. We then used the AUC of the ROC to better discriminate the models.

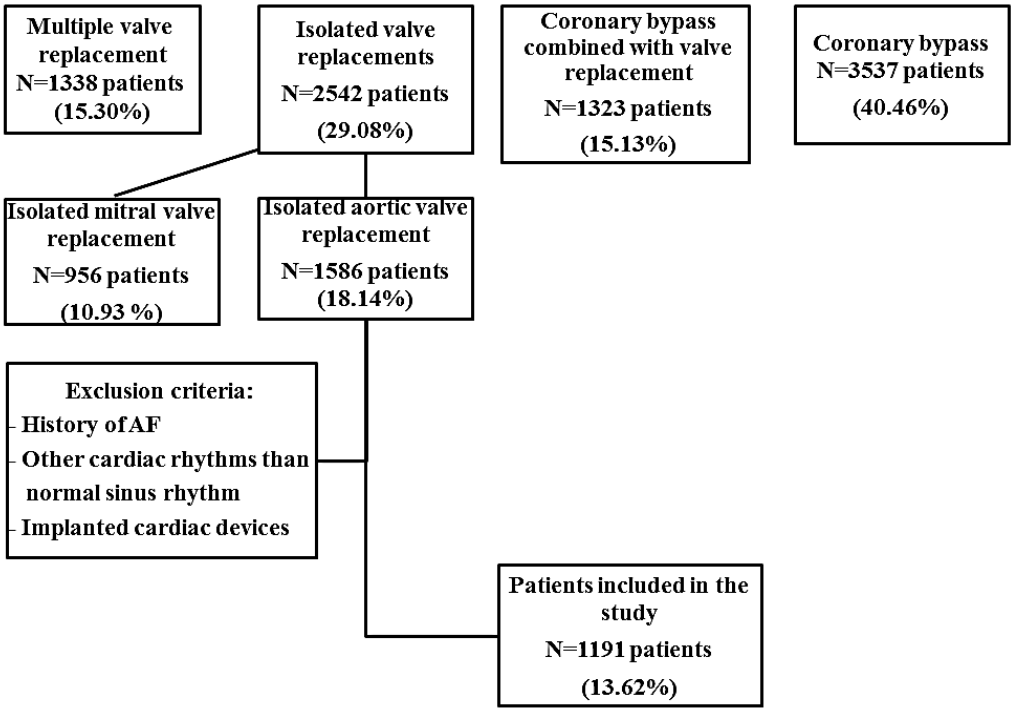


Figure 22. Flow chart with patients included in the study (with isolated aortic valve replacement).

CHAID (Chi-square automatic interaction detection) classification tree represents a decisional flowchart used for prediction. It starts with including all the case (root node) and thereafter divides into several child nodes (subgroup cases). After analyzing the predictive power of all variables included, partitioning is made. In the terminal nodes, the subgroups are mostly homogenous with respect to the dependent variable (193,194). The advantage of this method is that it is non-parametric and that is capable of analyzing how both continuous and/or categorical independent variables may best associate, with resulting homogenous groups. In our study, we considered post-operative AF as the dependent variable.

5.3. Results

In our hospital, the most frequently performed procedure was isolated coronary artery by-pass (40.46%), the second being isolated valve surgery (29.08%). There were 1191 (13.62%) procedures performed (isolated SAVR) (**Figure 22**). We divided the included patients into 2 groups- 28.7% (n=342) POAF patients and 71.3% (n=849) SR patients. Mean age was 64±13 years, 67.8% male. **Table 12** summarizes demographic data.

Table 13 summarizes the pre-operative echocardiographic findings, with the mean thickness of interventricular septum being higher in POAF patients being the only difference between the groups.

Table 12. Preoperative characteristics of patients with postoperative atrial fibrillation (POAF) compared to sinus rhythm patients

PARAMETER	POAF group N=342 patients (28.7%)	Sinus rhythm group N=849 patients (71.3%)	P value
Age (years; median± SD)	69.03±10.57	64.47±14.11	**0.005**
Men (%)	63.2	69.8	0.52
Smoker (%)	49.1	39.1	0.059
Arterial hypertension (%)	25.6	28.3	0.76
Hyperlipidemia (mg/dl)	129.98±95.47	148.84±87.50	**0.02**
Diabetes mellitus (%)	12	12	0.725
EuroSCORE II	9.00±2.87	5.78±1.97	**0.001**
Serum creatinine >200 μmol/l (%)	45.3	26.7	**0.002**
Preoperative medical treatment			
Betablockers (%)	23.4	21.7	0.12
Calcium blockers (%)	7.8	5.8	0.091
ACEI (%)	17.7	18.9	0.523
ARBs (%)	5.2	7.2	0.34
Diuretics (%)	6.1	5.4	0.71

ACEI: angiotensin converting enzyme inhibitors; ARBs: angiotensin II receptor blocker; IVS: interventricular septum; LA: left atrium; LVEF: left ventricular ejection fraction, LVED: left ventricular end diastolic; NS: non-statistically significant; PAP: systolic pulmonary artery pressure; SD: standard deviation.

Table 13. The preoperative echocardiographic parameters

PARAMETER	POAF group N=342 patients (28.7%)	Sinus rhythm group N=849 patients (71.3%)	P value
LVEF <30% (%)	37	38	**0.039**
LVEF=30-50% (%)	52	110	**0.041**
LVEF>50% (%)	359	746	0.16
LVED volume (mm)	54.70±9.71	54.61±10.47	0.072
LA volume (ml)	49.42±11.70	47.30±10.37	0.091
Indexed LA volume (ml/m2)	26.71±12.30	26.27±11.52	**0.007**
Indexed LA volume≥35 ml/m2 (%)	36.25	9.62	**<0.001**
IVS (mm)	14.55 ±2.87	14.38±2.55	**0.032**
Aortic annulus (mm)	23.42±5.55	23.55±5.31	0.43
Aortic regurgitation severity			
Mild	88 (30.24%)	203 (69.75%)	0.72
Moderate	113 (34.45%)	215 (65.54%)	0.54
Severe	97 (26.50%)	259 (70.76%)	0.91
PAP (mm Hg)	47±14	43±12	0.52

IVS: interventricular septum; LA: left atrium; LVED: left ventricular end diastolic; LVEF: left ventricular ejection fraction; PAP: systolic pulmonary artery pressure

Regarding the etiology of the aortic disease, the most common was degenerative (aortic calcification) - 87.74% of the cases (n=1045), followed by bicuspid aortic valve in 12.51% (n=149) patients and unicuspid in only one patient (0.08%). Endocarditis was reported in 10.24% cases (n=122), being associated with age> 65 years (p<0.01).

Separate stiches were used for the insertion of all prosthesis. Mechanical valves were implanted in 78.16% (n=931) patients, out of which 75.61% (n=704) CarboMedics, 23.63% (n=220) Edwards Mira, 0.75% (n=7) single-disc Medronic Hall and 0.10% (n=1) St.Jude Medical.

In the 21.83% (n=260) patients implanted with bioprosthesis, Medtronic Hancock II was the most frequently used (95%, n=247), followed by Carpentier Edwards's Lifescience (5%, n=13). Out of these patients, 41.53% (n=108) developed POAF, compared to the 27.46% (n=255) POAF in mechanical valve patients, p<0.001.

In POAF patients, the aortic clamping time was longer than in SR group (95.00±42.30 min vs 92.49±24.50 min, p>0.05). The extracorporeal circulation time was also longer in POAF patients: 136.01±57.76 min vs 128.05±59.70 min, p=0.085.

We reported statistically significant (p=0.001) post-operative complications in the two groups (POAF vs SR): endocarditis in 77.86% (n=95) patients vs 22.13% (n=27), prolonged ventilation in 2.47% (n=21) vs 10.52% (n=36) patients, stroke (>72h): 3.18% (n=27) vs 13.47% (n=47), other neurological complications: 4.82% (n=41) vs 16.37% (n=56) patients and acute renal failure: 5.3% (n=45) vs 19% (n=65) patients. We reported no statistical significance in the other complications, including sepsis, mediastinitis, MSOF, transient ischemic attacks and coma >24 hours.

Table 14 summarizes the performed multivariate analysis that highlighted six parameters linked to increased risk of POAF: age, BMI, tricuspid regurgitation (moderate), prolonged ventilation time and prolonged hospitalization in the intensive care unit, as well as an LAV of > 35 ml/m^2.

Table 14. Predictors of new-onset postoperative atrial fibrillation.

PREDICTOR	B	SE	Wald	df	Sig.	Exp (B)	95% CI for Exp(B)	
							Lower	Upper
BMI (kg/m2)	0.029	0.016	3.179	1	0.075	1.030	0.970	1.032
Tricuspid regurgitation more than mild	0.333	0.157	4.484	1	0.034	1.396	1.194	2.135
Prolonged ventilation	0.168	0.267	0.396	1	0.529	1.183	0.768	1.599
Long intensive care staying (more than 3 days)	2.070	0.800	6.698	1	0.010	7.925	1.770	38.959
LA volume (ml/m2)	0.020	0.007	7.221	1	0.007	1.020	1.009	1.037
Age ≥65 years	0.280	0.07	17.452	1	0.0001	1.028	1.022	1.047

BMI: body mass index; LA: left atrium

We entered these variables into a model that was able to predict POAF in 64.7% cases (Chi-square =62.291, p=0.000) with a 10.5% variation for POAF. According to the ROC curve analysis (**Figure 23**), this model presented a moderate discriminative ability with an AUC of 0.65, p=0.001, CI 0.571-0.771.

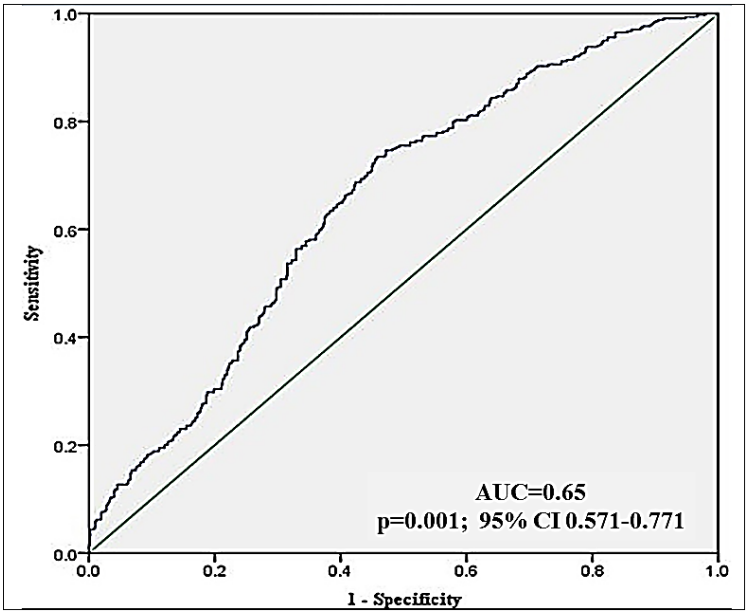


Figure 23. Receiver-operator characteristic (ROC) curve of point score as a predictor of new-onset postoperative atrial fibrillation (POAF) in patients undergoing aortic surgery.

CHAID classification tree

Figure 24 depicts the use of the CHAID decision tree in the analysis, with a maximum tree depth of four. The dependent variable was POAF, while the independent variables entered in the model were: age, arrhythmia history, BMI, LAV, LVEF, tricuspid regurgitation (either moderate/severe), prolonged ventilation time, prolonged intensive care stay, diabetes and Euro SCORE value.

The analysis revealed age as the most important predictor, with four subsequent risk levels: <46.8 years= very low-risk, 46.8-57 years= low risk, 57-68 years= intermediate risk and > 68 years= high-risk. In low risk patients, a LAV> 40 mL was the second best variable according to its predictive power. In intermediate risk patients, the history of AF was the next best predictive variable, followed by a BMI > 27 kg/m^2 in patients without known AF. In high-risk patients, moderate/severe tricuspid regurgitation was the next variable according to its predictive value. The highest risk was identified in patients older than 81 years.

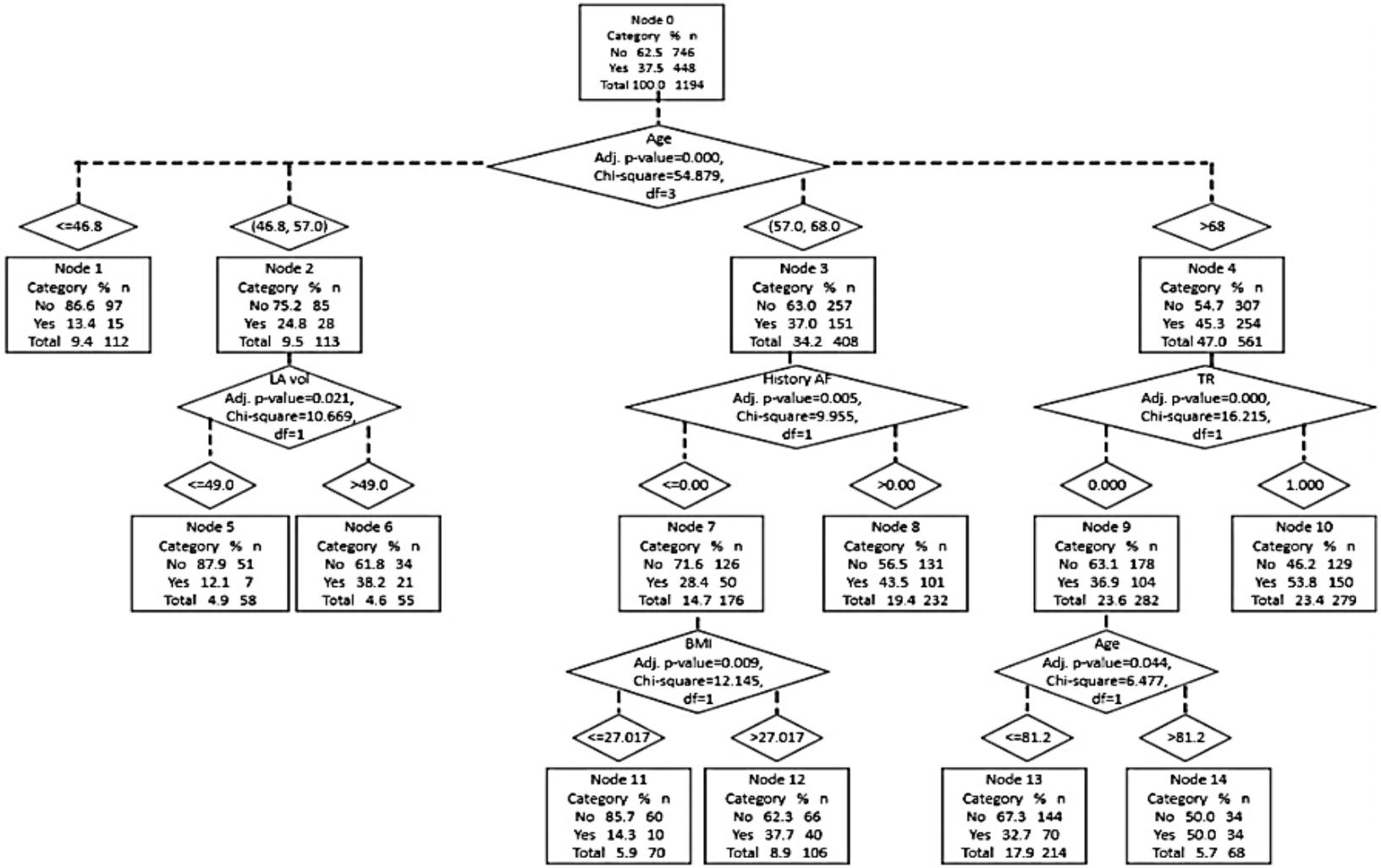


Figure 24. Tree model based on CHAID model (Chi-squared Automatic Interaction Detection) for patients with postoperative atrial fibrillation (POAF).
LA vol.: left atrial volume; AF: atrial fibrillation; TR: tricuspid regurgitation; BMI: body mass index

5.4. Discussion

In our study, we reported a general incidence of POAF of 28.71%, results similar to other studies (183-185). Logistic regression was used to create a model predictive of POAF; the model was influenced both by the introduced variables and by the number of included patients. Although there are authors that investigated risk factors for POAF, there are very few prediction models created (the available ones mostly for either coronary artery by-pass or other combined procedures) (191,192,195-197).

Previous predictive models were based on either smaller patients samples or excluded LV systolic dysfunction or severe renal disease, thus limiting their predictive power in real-life settings (198-200). In 2002, Mahoney et al. created a predictive model (predictive power of 0.665) for valve surgery patients that included age and the presence of chronic obstructive pulmonary disease (184). Similarly, Tran et al. included in their prediction score developed in 2015 age > 65 years, the presence of mitral valve disease and a dilated LA, reporting a 82% sensitivity and 39.2% specificity (195). In 2014, Mariscalco et al. developed on 12938 cardiac surgery patients a POAF risk score that included age, chronic pulmonary obstructive disease, glomerular filtration rate, the need for an emergency surgery, the use of an preoperative intra-aortic balloon pump, LV systolic dysfunction and valve surgery with moderate predictive abilities (202).

Age was a predictive variable in all of the studies (184, 191-202). Out of the six variables entered in our model, we also included age. It is known that increased age is linked to increased atrial fibrosis, which in turn translates into increased arrhythmogenesis. Moreover, increased age is at the same time linked to numerous risk factors and comorbidities, explaining the increased rates of POAF in patients implanted with a biological prosthesis.

Indexed LAV also emerged as a powerful POAF predictor ($p=0.007$). Importantly, 36.25% of POAF patients had an indexed LAV > 35 mL/m^2, as compared to the 9.62% SR patients.

Tricuspid regurgitation and prolonged ventilation time could be linked to chronic pulmonary disease having been included in other models (184,202); however these variables did not attain statistical significance in our study.

Aortic clamping time and extracorporeal circulation time did not emerged as having statistical significance; however it is known that the latter involves a pro-inflammatory response that should be further studied through CRP and interleukine-6 levels.

The aim of this study was to evaluate POAF, including its prediction factors in SAVR patients. We excluded patients with known AF from the multivariate analysis.

The clinical relevance of this study lies in the importance of properly identifying high-risk patients for POAF and implementing in this population appropriate prophylactic measure, thus avoiding antiarrhythmic drugs side-effects and unnecessary costs in low-risk individuals. In our model, age was the variable with the highest discriminative abilities, determining that patients over the age of 68 years are at high-risk. AF history in intermediate-risk patients could justify antiarrhythmic drug prophylaxis. This model could also be applied in high-risk patients, when judging the risk-benefits ratio.

Study limitations

We identified several limitations to our study:

1. This is a retrospective study by design, although data were prospectively included;
2. We recorded AF strictly during hospital stay, without having taken into consideration the possibility of out-of-hospital AF episodes;
3. The overall model accuracy remains moderate, despite having included variables with increased predictive value. This could be due to the multiple concomitant risk factors and associated comorbidities with a possible arrhythmogenic effect, requiring at the same time different therapies with subsequent effects and interactions (203-205).

According to the 2016 ESC Atrial Fibrillation guidelines, post-operative beta-blockers are recommended for POAF prophylaxis (class IB) (193). In high-risk patients, peri-procedural amiodarone is recommended with a class IIaB strength. Despite this, side-effects alter clinical decision making. The use of prophylactic intravenous amiodarone has been recently studied (206) and revealed that serious side effects such as conduction disorders and severe bradycardia could limit its use in degenerative aortic valve disease (207).

Despite numerous studies including cardiac surgery patients, the underlying POAD mechanisms are far from being identified. Further research is warranted, especially focusing on inflammation and newer derived parameters based on the latter.

5.5. Conclusions

The conclusion of our study is that properly stratifying patients undergoing aortic valve replacement according to their POAF risk is necessary in order to implement antiarrhythmic prophylaxis in high-risk individuals, thus limiting both costs and unnecessary side effects. The CHAID derived model is an easily applied tool useful in determining POAF risk and guiding prophylaxis protocols.

Chapter 6 ATRIAL FIBRILLATION AND COMORBIDITIES

6.1. Heart failure and natriuretic peptides

6.1.1. Introduction

Heart failure is an important complication of AF, but also an important substrate. The incidence of heart failure (HF) is increasing. Better understanding of HF is important in the relationship with this arrhythmia. Therefore studying HF could be a step in the optimization of AF management.

If for HF with reduced ejection fraction (HFrEF) there are well-established methods of diagnosis and treatment, this is far from true in HF with preserved ejection fraction (HFpEF) patients. This increasing incidence justifies the need for proper diagnostic, therapeutic and prognostic tools. In the era of cardiac biomarkers, the natriuretic peptides (NPs) have a well-

established role in HF pathophysiology and patient management. However, both the lack of a consensus regarding NPs in HFpEF and the heterogeneous population makes diagnosis and management difficult and unstandardized, leading to a decrease in quality of life and increase in mortality and hospitalization. This subject is a potential theme for researches. Therefore, I made a review that was recently published in a journal with IF=4.183.

1. Tanase DM, Radu S, Al Shurbaji S, Baroi GL, Florida Costea C, Turliuc MD, Ouatu A, **Floria M**. Natriuretic Peptides in Heart Failure with Preserved Left Ventricular Ejection Fraction: From Molecular Evidences to Clinical Implications. Int J Mol Sci 2019;20(11). pii: E2629. doi: 10.3390/ijms20112629. https://www.mdpi.com/1422-0067/20/11/2629

2. Sirbu O, Sorodoc V, Jaba IM, **Floria M**, Stoica A, Profire L, Tuchilus C, Rusu G, Sorodoc L. The Influence of Cardiovascular Medications on Iron Metabolism in Patients with Heart Failure. Medicina-Lithuania 2019;55(7). pii: E329. doi: 10.3390/medicina55070329. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6681074/pdf/medicina-55-00329.pdf.

3. Sîrbu O, **Floria M**, Dascalita P, Stoica A, Adascalitei P, Sorodoc V, Sorodoc L.Anemia in heart failure - from guidelines to controversies and challenges. Anatol J Cardiol 2018;20(1):52-59. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6237795/pdf/AJC-20-52.pdf

6.1.2. Heart failure with preserved left ventricular ejection fraction

Heart failure represents a clinical syndrome affecting 2-3% of the general population (133). Its prevalence is increasing and it leads to increased risk of death, hospitalization rates, a decrease in quality of life and higher costs through complex therapeutic strategies (134,135).

One of the classifications of HF is made by determining the left ventricular ejection fraction (LVEF). As such, HF can be either with preserved ejection fraction – HfpEF (LVEF > 50%) or reduced ejection fraction – HfrEF (LVEF<40%), with patients with a LVEF between 40 and 50% being labeled as HF with mid-range EF- HFmrEF. European Society of Cardiology (ESC) highlights the fact that HFpEF diagnosis is challenging, emphasizing the current lack of consensus. According to ESC guidelines, there are four diagnostic criteria for HFpEF: the presence of HF symptoms and signs (dyspnea, orthopnea, cough), a LVEF of >50% (with the remark that patients with a LVEF 40-49% may be classified as HFmrEF, being included in clinical trials as HFpEF), increased levels of NPs (B-type natriuretic peptide: BNP> 35pg/mL and/or N-terminal-Pro-BNP: NT-proBNP > 125 pg/mL) and imagistic evidence of structural heart disease, including LV hypertrophy, diastolic dysfunction and/or left atrial (LA) enlargement.

Biomarkers reflect myocardial damage and stress, systemic inflammation and fibrosis and their routine measurements mirror myocardial structural disease (134). Natriuretic peptides are the most frequently used biomarkers in HF patients, as their elevated levels constitute a diagnostic criterion irrespective of LVEF (133-136). However, the underlying mechanisms behind their increase differ in the two types of HF, with HFpEF patients showing lower levels of NPs (134-137). In these patients, higher levels of circulating NP are

given by the increased LV diastolic filling pressure and end-diastolic wall stress (134,136). As compared with HFrEF, the circulating levels of NPs are lower, especially in the context of lower myocardial stretch, LV end-diastolic wall stress and volume overload (136). ESC guidelines recommend taking into consideration lower diagnostic thresholds for BNP and NT-proBNP when assessing a potential HFpEF patients (135).

Essential hypertension and myocardial ischemia are frequent causes of HFpEF and one third of these patients have concomitant atrial fibrillation (AF) (136). The presence of AF impacts NPs circulating levels, different studies showing that AF patients with HFpEF had higher mean NPs levels as compared with sinus rhythm patients (135-138). As such, NPs values must be interpreted differently in AF patients both when diagnosing HFpEF and when assessing prognosis (134, 136, 139,140). Several other factors affect NP levels (137-141). Obese patients tend to have lower baseline NPs, while renal dysfunction, feminine gender and age are associated with increased levels (139).

6.1.3. Natriuretic peptides: from molecular evidences to clinical implications

There are three endogenous NPs secreted as pre-prohormones: atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). Subsequently, there are three natriuretic peptide receptors (NPR): natriuretic peptide receptor A (NPR-A), natriuretic peptide receptor B (NPR-B) and natriuretic peptide receptor C (NPR-C or clearance receptor) (139-148). These peptides act as hormones with pleiotropic effects by binding to the first 2 receptors, contributing to cardiovascular homeostasis and pressure and volume overload counter-regulatory mechanisms (143). Moreover, all NPs have various molecular forms, different in healthy subjects from HF patients, such as different ANP forms and glycosylated proBNP (153).

Biologically active ANP is a 28 amino acid (aa) peptide. Initially secreted as pre-proANP (151 amino acids), this molecule is cleaved into proANP (126 amino-acids), which is deposited in granules inside atrial myocardium. A transmembrane protease cleaves the secreting proANP into its biologically active short-lived form (ANP- 28 amino acids) and its inactive form, NT-proANP (98 amino-acids) (139-143). The latter has a much longer half-life (60-120 minutes); however, its numerous subsequent fragments make it a still unpractical biomarker in routine clinical practice.

The majority of ANP is secreted in the atria in response to myocardial stretch, atrial concentrations being 1000 higher as compared to ventricular ANP levels (133). Ventricular myocardium produces small amounts of ANP; however, in HF patients, hypertrophied ventricular myocardium becomes able to secrete ANP (142). Extracardiac sources include hypothalamus, lung and thyroid gland (137).

There are 3 forms of circulating ANP: αANP, βANP and pro-ANP (153). Found only in the human atria, the biosynthesis pathways of the βANP are still unclear. Its structure is of a anti-parallel dimer of αANP. When compared to αANP, βANP has lower bioavailability (40%), slower onset of action and lower receptor affinity.

ANP has numerous biological effects, including blood pressure lowering effects and renin-angiotensin-aldosterone system (RAAS) inhibition (148-152). It may affect apical Na channel and Na/K ATPasis basal activity, determining a decreased sodium reabsorption and

increased Na excretion, leading to increased natriuresis (151). Moreover, it inhibits RAAS through renin (at the juxtaglomerular cyclic guanosine monophosphate- cGMP dependent cells level) and aldosterone inhibition (158), leading to blood pressure reduction. Moreover, studies have shown that ANP acts at the level of adrenal glands, directly inhibiting aldosterone production (148). Not only that it inhibits RAAS, but it also possesses antifibrotic and antihypertrophic effects through cGMP dependent angiotensin II and endothelin inhibition (144). A study conducted by revealed that ANP might counteract myocardial hypertrophy by inhibiting calcium mediated epinephrine response through cGMP (147). A different mechanism through which ANP affects blood pressure may be through baroreflex modulation, stimulating vagal afferent fibers (148).

Recent studies focusing on AF patients found that NT-proANP levels correlate with AF type and LA dimensions (150). Seewoster et al. revealed that NT-proANP correlated with LA dimension as determined by cardiac magnetic resonance (149).

The gene coding ANP contains 3 exons: the first exon codes the 5' region (not translated), a signal peptide formed of 25aa (16aa of proANP). The second exon is responsible for coding proANP while the third exon has a role in coding terminal 3' tyrosine . Recent studies have shown that a chorionic transmembrane enzyme cleaves proANP into pre-preoANP and NT-proANP.

The biologically active form of human BNP is BNP_{32} (140), widely varying across different species. The gene coding BNP is also formed of 3 exons: the first exon codes a 26 aa signal peptide and the first 15aa of proBNP; the second exon codes the majority of proBNP and the third exon codes terminal tyrosine and 3' region (144). mRNA BNP is translated into pre-proBNP with 134 aa after which the signal peptide is removed, resulting BNP-108 (133). Different forms of BNP exist in the atria and the ventricles- BNP 32 and 108, respectively (144). BNP 32 is mostly found in the atria, while BNP 108 is found in the ventricular myocardial (152). At the level of the Golgi apparatus, proBNP is cleaved into BNP and NT-proBNP, to be later released in plasma (152). As opposed to ANP, which is mostly stored in vesicles, BNP is secreted in response to myocardial stretch.

Several studies identified higher BNP concentrations at the level of anterior interventricular vein and coronary sinus, which supports the fact that BNP is mainly secreted by ventricular myocardium (133). The difference between BNP and ANP mRNA resides in a repetitive unit which determines mRNA BNP degradation in a fashion similar to oncogenes (136). BNP gene expression differs from that of ANP, being more dynamic (141). BNP possess vasodilator effects, promoting natriuresis and diuresis. At the myocardial level, BNP inhibits fibrosis and necrosis (148-151). Also, it possesses anti-inflammatory effects through monocytes, B lymphocytes and natural killer cells regulation. Importantly, BNP interferes with post-skeletal muscles ischemia angiogenesis.

Importantly, it seems that HF patients have decreased amounts of BNP32; the latter is cleaved to either BNP3-32 or BNP8-32 by dipeptidyl peptidase IV (DPP IV). The two forms are less biologically active than BNP32, probably due to faster degradation and may account for the presumed resistance to NPs in HF patients (133, 148).

Both ANP and BNP can undergo post-translational changes, such as phosphorylation and glycosylation. The physiological impact of a phosphorylated proANP form is still uncertain (153). As one of the most frequent change, glycosylation stabilizes proteins, thus preventing

further processing. The glycosylation pattern is strikingly different between ANP and BNP (153). *O*-glycosylation of proBNP inside the Golgi apparatus is multi-sited (approximately 7 sites) and its degree may vary among patients. Heavily glycosylated pro-BNP molecules are recognized as a cause of decreased conversion to its biologically active form, with a subsequent increase in pro-BNP/total BNP ratio. Interestingly, in acute HF the glycosylation level of proBNP decreases as there is a tendency towards forming more mature BNP. In acute HF, the increased proBNP production is accompanied by decreased glycosylation and increased furin activity, leading to elevated BNP and NT-proBNP levels.

C-type natriuretic peptide is secreted in the myocardium, endothelium, chondrocytes, brain and blood cells (144). C-type natriuretic peptide gene also contains 3 exons, with the first exon containing 23 aa signal peptide and 7 aa proCNP. The second exon contains the proCNP sequence while the third the exon 3' terminal. After removal from the 126 aa pre-proCNP of the 23 aa signal sequence results 103 aa proCNP. An intracellular endopeptidase cleaves proCNP into 53 aa CNP. CNP_{53} is cleaved in return to CNP_{22}. Both forms have the same functions; however, CNP_{53} is found in the myocardium while CNP_{22} is found in plasma and brain (152). CNP also possess vasodilator effects, being secreted by endothelial cells in response to vascular lesions. Also, it inhibits fibrosis, platelet aggregation and tissue plasminogen activation. CNP levels tend to increase in advanced HF as compared to incipient HF.

NPR-A and NPR-B determine NP functions. ANP and BNP activate NPR-A while CNP activates NPR-B (142, 148). NPR-A is found in the lungs, kidneys, adrenal glands, while NPR-B is predominantly found inside fibroblasts (150-152).

NPR-C is found in the brain, atrium, lungs and aorta. These receptors have an extracellular region for the ligand attachment and a intracellular region with a cGMP dependent proteinkinase (138, 155).

As a second messenger, cGMP is formed from 2 possible precursors, either soluble guanylyl cyclase- *sGC* (found in cytosol; it requires nitric oxide binding) and particulate guanylyl cyclase (pGC), found in the cellular membrane and activated via NPR. As such, this activation leads to an increase in cGMP, which in turn increases protein-kinase G (PKG) levels (156-158). The latter phosphorylates several proteins, including myocardial cytoskeletal titin (157). Moreover, decreased levels of cGMP and subsequently of PKG have been associated with myocardial remodeling through increased cardiomyocyte hypertrophy and resting tension (157) (**Figure 25)**.

HTN, AF, chronic kidney disease- frequently found comorbidities in HFPEF patients, determine a decrease in cGMP through a pro-inflammatory state and subsequent decrease of nitric oxide. Importantly, augmenting cGMP concentrations may constitute therapeutic targets in HFpEF.

NPs have a wide range of biological effects, including endocrine and paracrine. They promote diuresis and natriuresis, vasodilation and inhibit sympathetic nervous system and renin-angiotensin-angiotensinogen. In advanced HF, there is a resistance to NPs effects, together with either, an increased turnover, increased biologically inactive NP secretion or decreased NPR-A activation due to secondary receptors dephosphorylation (133-137).

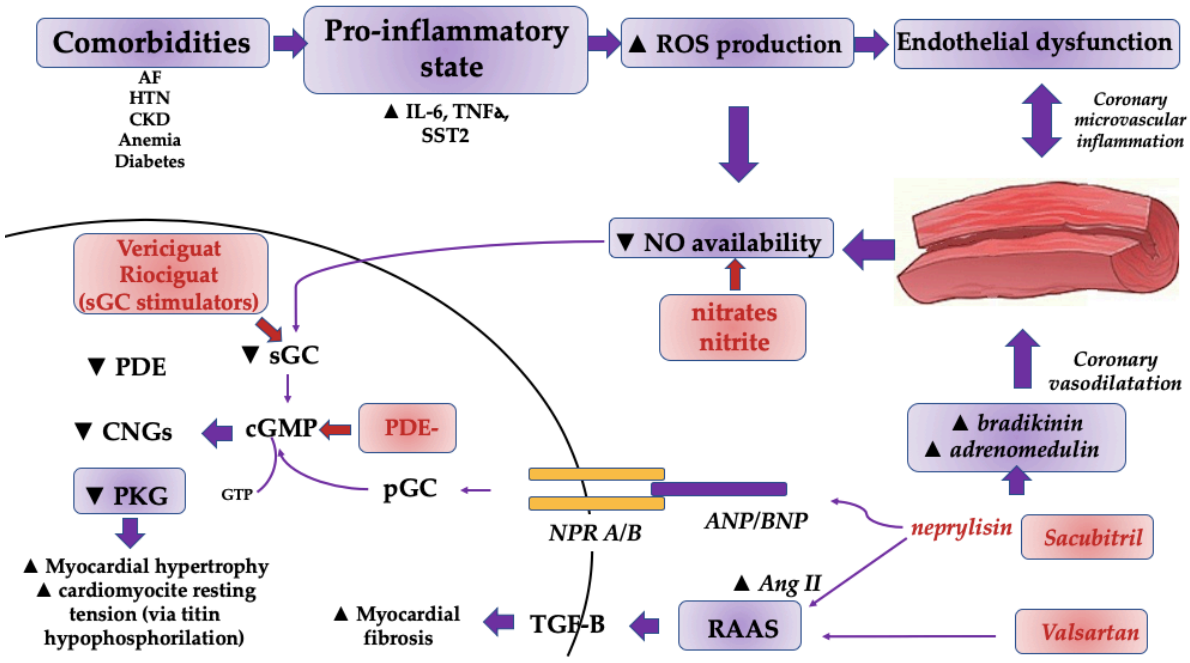


Figure 25. NPs, cGMP and RAAS in HFpEF patients and possible therapeutic targets. *AF: atrial fibrillation; Ang II: angiotensin II; cGMP: cyclic guanosine monophosphate; CKD: chronic kidney disease; CNGs: cyclic nucleotide gated-ion channels; HTN: hypertension; IL: interleukin; NO: nitric oxide; NP: natriuretic peptide; NPR: natriuretic peptide receptor; pGC: particulate guanylyl cyclase; PKG: protein kinase G; PDE: phosphodiesterase; PDE-: phosphodiesterase inhibitors; RAAS: renin-angiotensin-aldosterone system; sGC: soluble guanylyl cyclase, TGF-B: transforming growth factor beta.*

If ANP levels are influenced by atrial pressures, BNP concentrations are determined by ventricular stretch in response to underlying pressure and/or volume overload. ANP short half-life (derived from its higher affinity for NPR-C) precludes its utilization in routine practice while BNP's stability makes it a desirable biomarker in HF diagnosis and prognosis.

A novel NP, middle-range proANP (MR-proANP) has emerged as having a greater stability and both diagnostic and prognostic values, especially in HFpEF patients. It derives from an intermediate region of NT-proANP and exhibits increased stability. Moreover, it correlates with increased LA dimensions and it seems that its diagnostic (154) and prognostic (144) utility might be superior to that of NT-proBNP in HFpEF. Moreover, it correlates with NYHA class in several studies (154).

NPs degradation can be either receptor- mediated (NPR-C) or enzyme-mediated (137-142) and is recognized as a therapeutic target in both hypertension and HF (144, 148). While receptor mediated NP degradation is based on internalization (Claritin-mediated) and hydrolysis (148), enzyme-mediated NP degradation occurs mainly through the neutral endopeptidase (NEP) zinc-dependent neprylisin (136,142). Although it is expressed by varying tissues, it can mostly be found in the proximal renal tubules, myocardium, fibroblasts and endothelial cells (136-139). This enzymatic degradation makes both ANP and BNP unstable in serum, their plasmatic levels being used in routine clinical practice. Contrary, NT-proBNP shows increased serum stability. Inhibiting NEP leads to an increase in NP levels,

which benefits HF patients (152). Insulin degrading enzyme has also proven to degrade NPs, specifically ANP in addition to insulin (137).

6.1.3.1. Implications of natriuretic peptides in heart failure with preserved left ventricular ejection fraction diagnosis

Diagnosing HFpEF remains difficult due to a lack of consensus, patients' heterogeneity and multiple concurrent pathologies that may mimic not only HF symptoms, but also lead to either increased (AF) or decreased (obesity) NP levels (158). HFpEF diagnosis requires clinical and imagistic criteria, as well as elevated NPs levels. Given that one third of HFpEF have normal NPs levels (158), relying solely on their values for diagnosis is not recommended and their value must be interpreted in the clinical context. As such, the diagnostic gold standard is cardiac catheterization showing increased LV filling pressures.

The longer plasma half-life of BNP and NT-proBNP (22 minutes and 70 minutes, respectively) as compared to ANP (2 minutes) makes the two the preferred NPs for guiding HF diagnosis and, probably, therapy. Moreover, despite having a longer half-life, NT-proANP failed to emerge as a diagnostic marker due to the increased number of cleaved fragments that limit its detection. European Society of Cardiology considers a BNP level of > 35 pg/mL and/or NT-proBNP > 125 pg/mL suggestive of chronic heart failure, with higher values being recommended in acute settings- over 100 pg/mL and > 300 pg/mL, respectively (133, 135). Moreover, it is agreed upon that acute HF patients exhibit increased NPs levels regardless of LVEF (135, 159-167). In a study, acute HFpEF patients showed NT-proBNP levels between 600-1000 pg/mL (168). Although HFpEF patients/ patients previously treated with diuretics may exhibit lower levels, there is no consensus in what regards NPs diagnostic threshold in these patients. Given that NPs levels are affected by several factors, including the presence of AF and body mass index, their use rather resides in excluding the diagnosis of HF with a subsequent high-negative predictive value (0.94-0.98) (135). As such, especially in what concerns the diagnosis of HFpEF, NPs levels must be corroborated with both clinical context and other imagistic parameters. In the light of numerous affections that may mimic HFpEF symptoms, the ESC guidelines propose these NPs diagnostic thresholds as to limit overdiagnosing HFpEF.

Several studies agree that both BNP and NT-proBNP retain their diagnostic performance (although with a decrease in sensitivity and specificity) in acute heart failure, irrespective of LVEF (164). However, factors that affect their levels should be taken into consideration when attempting HFpEF diagnosis. Both cardiac and non-cardiac causes may lead to a subsequent increase in NPs, including AF, recent cardioversion, myocarditis, acute coronary syndrome, age, severe renal impairment, pulmonary embolism, sepsis and critical illness (164). It has been shown that NPs predictive values drop from 0.95 to 0.82 in patients > 75 years old (136). As such, the expected NP values will be higher in the elderly, even in the absence of HF. Age-adjusted NT-proBNP have been proposed for acute HF diagnosis, considering cut-off values of >450 pg/mL, 900 pg/mL and >1800 pg/mL for patients <50 years, > 50 years and >75 years old, respectively (137). In contrast, NPs levels tend to be lower in obese patients, irrespective of volume status (136). The fact that NPs concentrations

vary among HFpEF patients is demonstrated by different medians across studies. The I-PRESERVE trial revealed a median NT-proBNP concentration of 341 pg/mL in HFpEF patients (133), while a different study highlighted that nearly a third of HFpEF patients had BNP levels below 100 pg/mL while displaying increased LV filling pressures as measured by cardiac catheterization (165).

Recent studies pointed out the need of different thresholds in HFpEF in regard to sinus rhythm patients. HFpEF and AF coexist in 30% of patients (170) while AF in itself is one of the most frequent causes of HFpEF. AF patients tend to have higher NT-proBNP levels and exercise intolerance in the absence of HF (170). Moreover, they have increased LA dimensions and LV filling pressures, making echocardiographic findings of HFpEF difficult to interpret (133, 170). Although the use of higher levels is recommended, ESC has failed to provide a clear cut-off value for HFpEF diagnosis in AF patients. Several studies reported the use of different NPs cut-off values as inclusion criteria with respect to underlying cardiac rhythm (sinus versus atrial fibrillation): 600 pg/mL in the SOCRATES trial (171) and >900 pg/mL in PARAGON trial (172).

The use of different diagnostic methods in adjunction with increased NPs level is recommended by ESC guidelines. Structural and functional alterations as determined by transthoracic echocardiography include a left atrial indexed volume (LAVI) of >34 mL, increased LV mass index (115g/m2 for men and 95 g/m2 for females), E/e' >13 and mean septal and lateral wall e' of <9cm/s (135). This is supported by the correlation between BNP and structural and functional alterations in HFpEF. In a study conducted by Iwanaga et al, BNP levels correlated with both left ventricular end-diastolic pressure and end-diastolic wall stress, more significantly with the latter (173). Moreover, it seems that a BNP of > 100 pg/mL or a NT-proBNP of > 600 pg/mL indicates a LV restrictive filling pattern (164). NP levels also correlate with LA dimensions, this correlation being stronger in HFpEF patients (167). Although LVEF is preserved, it seems that global systolic function is altered in HFpEF patients. In a study conducted by Kraigher-Krainer et al, the decreased LV systolic strain (both longitudinal and circumferential) noticed in HFpEF patients correlated with NT-proBNP levels (169). The disposition of LV hypertrophy also influences BNP levels, being significantly elevated in patients with concentric as compared to eccentric LV hypertrophy (137).

In the setting of an acute coronary syndrome, ANP levels show an early elevation with a rapid decline, as opposed to BNP levels, who tend to exhibit a bimodal elevation (137). The first peak has been reported in the first two days post-myocardial infarction, with the second occurring nearly one week after the event, reflecting the extent of LV remodeling.

Not only that the diagnostic performance remains constant, but the treatment is similar in decompensated HF with regard to LVEF (135, 172-179). In chronic HF, however, the treatment differs significantly between HFpEF and HFrEF, being well-established for the latter.

Renal function is tightly related to NPs levels. NPs tend to be elevated in CKD and end-stage renal disease, up to a value of 200 pg/mL even in the absence of overt HF (137). Haemodialysis but not peritoneal dialysis has been shown to lower BNP levels with nearly 40% (175). Proposed cut-off values of NT-proBNP for diagnosing HF in CKD patients

include a level of > 1200 pg/mL, with higher levels being suggested in older patients (137,176).

Recently, a new NP has emerged as a potential HFpEF diagnostic tool. Cui et al. have revealed increased MR-proANP levels in HFpEF patients, with a significantly higher AUC when compared to NT-proBNP (0.844 versus 0.518, p<.001) (154). Moreover, when comparing their levels based on NYHA class, MR-proANP concentrations differ in regard with NYHA class, as compared to NT-proBNP, which showed no variation. Taking into consideration the link with echocardiographic parameters, MR-proANP correlated with LAVI, as opposed to NT-proBNP. In another study, MR-proANP showed non-inferiority to NT-proBNP in acute HF diagnosis, being elevated even in patients who showed non-diagnostic NT-proBNP levels (159). The authors of the BACH study found that a MR-proANP of >120pmol/l was suggestive of HF; adding this parameter to BNP increased its diagnostic performance to 73.6% (159).

Not only that NPs have proven their diagnostic utility, but several studies questioned their ability to identify patients at risk for HF development. STOP-HF trial referred patients with BNP levels of > 50pg/mL to further echocardiographic investigations, leading to a decrease in LV dysfunction (174).

Given its difficulties, the diagnosis of HFpEF in its characteristically heterogeneous population usually requires more than one biomarker. As the separation of HF in reduced and preserved EF occurred rather recently, more studies focused on HFrEF. Although both European and American guidelines have yet to consider different thresholds for the two classes of HF, increasing evidence links their distinct pathophysiologies to unique therapeutic strategies. Moreover, taking into consideration the increasing HFpEF incidence and altered prognosis, proper identification of these patients become vital.

6.1.3.2. Therapeutic Implications of Natriuretic Peptides in Heart Failure with Preserved Left Ventricular Ejection Fraction

NPs have been shown to inhibit RAAS, suppressing angiotensin II mediated vasoconstriction, sodium reabsorption (proximal tubule) and aldosterone, endothelin and renin secretion (137, 141). Their use in HF therapy is bimodal, both as a therapeutic target per se and as an indicator evaluating therapy response. However, their use in HFpEF remains controversial.

The rationale behind using NPs as therapeutic target in HF therapy (177, 194) resides in the seemingly abnormal BNP processing with a subsequent deficiency in active forms and resistance to their biological effects in these patients (137). It seems that HF patients are deficient in biologically active BNP_{32} with a subsequent increase in BNP_{1-108} (177). Augmenting their effects can be due by either administering NPs or reducing their breakdown.

6.1.3.3. Prognostic Implications of Natriuretic Peptides in Heart Failure with Preserved Left Ventricular Ejection Fraction

It was agreed upon that although NPs values tend to be lower in HFpEF patients with no consensus regarding diagnostic thresholds, they retain their prognostic utility irrespective of LVEF (135).

In a different study, increased BNP levels were associated with increased mortality, irrespective of LVEF. Also, it seems that higher BNP levels translate into increased risk for developing both AF and transient ischemic attacks.

Prognostic value of BNP and NT-proBNP

The NPs concentrations retain their prognostic utility albeit their lower baseline levels in HFpEF patients. Several studies reported similar death risks for a given NPs concentration irrespective of HF phenotype. Levy and Anand compared patients from I-PRESERVE and VALHEFT trials, showing that a 1-log increase in NT-proBNP levels carries a mortality HR of 1.7 regardless of LVEF. However, the same authors demonstrated that the mortality of HFrEF patients was two thirds higher than of those with HFpEF. Similarly, Salah et al. reported that hospitalized HFpEF patients have lower mortality rates when compared to HFrEF; however the difference is minor and the risk of death tends to equalize after discharge. More importantly, although patients with HFpEF display lower baseline NPs concentrations, for the same NT-proBNP level, prognosis is similar regardless of LVEF. This paradox can be firstly be explained by the different mechanisms involved in NPs secretion between the two phenotypes. It is known that at the same end-diastolic LV pressure, HFpEF patients display lower NT-proBNP levels. This may be due to NPs correlation with diastolic wall stress which, according to the law of La Place, correlates with wall pressure and cavity diameter and is inversely related to wall thickness. As patients with HFpEF typically exhibit a concentric LV remodelling with increased wall thickness, this may explain in part their lower NPs levels for a given increased wedge pressure as compared to the HFrEF patients (who typically have an eccentrical LV remodelling). Secondly, this paradox can be explained by the distribution of comorbidities in regard with HF phenotype. As such, HFpEF patients tend to be older, with increased incidence of arterial hypertension, chronic kidney disease, AF and anemia, comorbidities that may account for similar prognosis between HF phenotypes despite lower NTpro-BNP of HFpEF. This drives attention to the need of also addressing non-cardiovascular diseases in order to improve outcomes in HFpEF patients. The fact that while the same NP concentration may point out to the same relative risk of death and overall prognosis still differs between HF phenotypes underlines exactly the contribution of other risk factors and comorbidities to the mortality. That's why it is not advisable to regard NP levels as surrogate markers of mortality.

It appears that different NPs concentrations threshold might be necessary for prognosis in HFpEF patients as compared to HFrEF. Accordingly, NPs prognostic values vary across different studies, several authors highlighting that NPs increase in addition to their baseline levels holds prognostic importance. Moreover, relying on an NP prognosis threshold may not be necessary, as prognosis can and should be continuously assessed.

Anand et al. associated a baseline NT-proBNP of 339 pg/mL with a 4 years mortality of 21.1%, which translates into a 5% annual mortality for an NT-proBNP level between 300-500 pg/mL.

As NT-proBNP is also influenced by AF, increasing its concentration irrespective of HF presence, these levels should be judged accordingly when determining prognosis. Kristensen et al. showed that different NT-proBNP levels should be used in AF versus non-AF patients to determine prognosis. While an NT-proBNP level of < 400 pg/mL was associated with a better prognosis irrespective of rhythm; the presence of AF accounted for the different mortality rates in HFpEF patients with a NT-proBNP >400 pg/mL. However, higher NT-proBNP AF patients had increased risks of hospitalization as compared to patients with HFrEF and the same NPs level. In addition, it seems that patients with lower baseline NPs levels would benefit on long-term from supplementary explorations.

A recent study launched the possibility of increased NT-proBNP playing a causative role in AF. This idea is controversial as several comorbidities that promote AF lead in turn to an increase in NT-proBNP, which remains a biologically inactive product. Moreover, an increase in both ANP and BNP in sacubitril/valsartan patients led to atrial reverse-remodeling, emphasizing their antifibrotic and antihypertrophic effects.

Prognostic value of natriuretic peptides and heart failure with preserved ejection fraction therapy

There are controversies regarding the prognostic value of NPs in sacubitril/valsartan treated patients. As the formerly known LCZ696 interacts with NPs levels, there still are questions regarding their accuracy in determining HF patients' prognosis. Some studies agree that both BNP and NT-proBNP retain their prognostic utility in these patients, emphasizing the fact that during therapy initiation, NT-proBNP is the preferred biomarker (several months are required for BNP levels stabilization). However, the currently available trials did not include HFpEF patients treated with sacubitril/valsartan.

Another issue regarding NPs prognostic value in HFpEF is whether these patients would benefit from NPs guided therapy. So far, studies are controversial, especially taking into consideration that these patients respond differently to HF therapy that those with reduced LVEF. A study conducted by Khan et al. including patients with both HFrEF and HFpEF came to the conclusion that NP guided therapy was not beneficial. A different study emphasized that regardless of LVEF, a constantly elevated NPs should not determine changes in patient management. Maeder et al. revealed in TIME-CHF trial that NT-proBNP guided therapy was not beneficial in HFpEF patients, as compared to HFrEF. Although it included HFrEF patients, GUIDE-IT trial concluded that biomarker directed therapy may not benefit these patients. The opposite was shown in a meta-analysis conducted by Troughton et al. The authors stated that BNP-guided therapy improved outcomes in patients < 75 years old and reduced HF hospitalization rates regardless of age and LVEF. Brunner la Roca et al. agreed that NP guided therapy either through BNP or NT-proBNP is safe and more importantly, cost-effective. A British team stated that BNP-guided therapy might be cost-effective in HFpEF patients < 75 years old.

Recent studies have focused on MR-proANP as a possible biomarker to guide HF therapy. So far, it seems that MR-proANP levels might be able to predict incident HF, as it shown in a study conducted by Sabatine et al. Interestingly, it seems that MR-proANP might predict cardiac resynchronization therapy responders which showed lower levels of the biomarker as compared to the non-responders.

Regardless of the biomarker used, NP guided medical therapy remains controversial, especially in HFpEF patients. In these patients, more studies are required to assess the benefits of optimizing medical therapy after serial NP measurements. The most studied NPs are BNP and its inactive form, NT-proBNP, especially due to their proven diagnostic capacities. The novel MR-proANP, with its increased half time as compared to ANP shows promise both in prognosis and guiding medical therapy, but more studies are required to confirm its safety profile and utility.

6.1.4. Future perspectives

Several on-going studies on NPs in HFpEF have been announced. The results of PARAGON-HF trial will clear the impact of sacubitril/valsartan therapy in HFpEF, while another on-going study conducted by Mayo clinic (ClinicalTrials.gov Identifier: NCT03506412) will determine how this therapy affects NPs levels and cGMP in HFpEF patients. A different study finishing in December 2019 will assess the effects of sacubitril/valsartan as compared to either enalapril or valsartan in HFpEF patients (ClinicalTrials.gov Identifier: NCT03066804).

Moreover, in the context of the pro-inflammatory state of HfpEF, there is increasing body of evidence linking non-NPs biomarkers to HFpEF as a better diagnostic tool. Emerging markers reflecting myocardial fibrosis such as soluble source of tumorigenicity 2 (sST2), growth differentiation factor-15, galectin 3 and interleukins (1 and 6) are showing promise, correlating in different degrees with transthoracic echocardiographic parameters of diastolic dysfunction. Their utility in comparison to NPs remains to be evaluate.

6.1.5. Conclusions

The number of HFpEF patients is increasing; moreover, these patients show similar prognosis with HFrEF patients.

The role of NPs in HF is both diagnostic and prognostic. Guidelines regard a BNP > 35 pg/mL or an NT-proBNP > 125 pg/mL as being diagnostic in non-acute setting, while emphasizing the fact that although several factors alter NP concentrations, their diagnostic utility rather lies in their negative predictive ability. For decompensated HF, either a BNP > 100 pg/mL, an NT-proBNP > 300 pg/mL or a MR-proANP of > 120pmol/L is diagnostic. Authors agree that lower levels may be considered for HFpEF, without any thresholds being agreed upon. The absence of a consensus regarding diagnostic thresholds for NPs in HFpEF led to inhomogeneous inclusion criteria in large clinical trials, thus affecting the results.

NPs' role go beyond diagnosis, as they can be regarded as a therapeutic target per se through nesiritide and the novel ARNI, sacubitril/valsartan. Augmenting NPs concentrations leads to lower blood pressure levels, symptoms, and quality of life improvement. A degree of

reverse remodelling with a subsequent decreasing in myocardial fibrosis and LA dimensions could determine.

Higher NPs levels are associated with poorer prognosis, especially in the acute settings, irrespective of LVEF. Moreover, BNP and MR-proANP predict not only incident HF, but also AF, and as AF is one of the most frequent causes of HFpEF, this becomes of outmost importance. For AF patients it must be reminded that NP levels tend to be higher, irrespective of the presence of HF. Also, for the same BNP concentration, they show a better prognostic as compared with their sinus-rhythm counterparts.

Finally, guiding medical therapy by serial NP measurements is controversial, especially in HFpEF. Authors recommend in the absence of a consensus that no change in patient management should be take if the NPs levels remain constantly elevated throughout treatment.

The need for further studies assessing firstly diagnostic NPs thresholds and prognostic values in HFpEF patients is enormous. Furthermore, given the novel therapies that interact with NPs, such as sacubitril/valsartan, these levels might be altered.

6.2. Heart failure and anemia

Anemia associated with heart failure (HF) is a frequent condition, which may lead to heart function deterioration by the activation of neuro-hormonal mechanisms. Therefore, a vicious circle is present in the relationship of heart failure and anemia. The consequence is reflected upon the patients' survival, quality of life, and hospital readmissions. Anemia and iron deficiency should be correctly diagnosed and treated in patients with heart failure. The etiology is multifactorial but certainly not fully understood. There is data suggesting that the following factors can cause anemia alone or in combination: iron deficiency, inflammation, erythropoietin levels, prescribed medication, hemodilution, and medullar dysfunction. There is data suggesting the association among iron deficiency, inflammation, erythropoietin levels, prescribed medication, hemodilution, and medullar dysfunction. The main pathophysiologic mechanisms, with the strongest evidence-based medicine data, are iron deficiency and inflammation. In clinical practice, the etiology of anemia needs thorough evaluation for determining the best possible therapeutic course. In this context, we must correctly treat the patients' diseases; according with the current guidelines we have now only one intravenous iron drug. This paper is focused on data about anemia in heart failure, from prevalence to optimal treatment, controversies, and challenges.

Heart failure represents a highly prevalent worldwide disease. It is estimated that its incidence will significantly rise within the near future. The proportion of patients with HF in the United States may increase by 46% and to >8 million by 2030 (1). Despite significant progress in therapeutic methods and tools, a large number of patients remain symptomatic, with limited capacity at exertion and a high mortality risk. These patients often associate other conditions that may complicate disease management, the most frequent being chronic kidney disease, anemia, and diabetes mellitus.

Despite anemia being a common occurrence in patients with HF, a direct causal link has not yet been established with either HF or the presence of other comorbidities. Accordingly, in practice, the etiology of anemia needs thorough evaluation to determine the

best possible therapeutic course. The mechanisms responsible for anemia in patients with HF are still unclear. The following six factors have been suggested to cause anemia alone or in combination: iron deficiency, inflammation, erythropoietin levels, prescribed medication, hemodilution, and medullar dysfunction. The main pathophysiologic mechanisms, with the strongest evidence-based medicine data, are iron deficiency and inflammation.

In the presence of chronic anemia, low tissue oxygenation results, which leads to the development of compensatory mechanisms: hemodynamic and non-hemodynamic.
The two main non-hemodynamic mechanisms are erythropoiesis stimulation, which leads to an increased capacity of oxygen transport, and a lowering of hemoglobin affinity for oxygen, leading to a rise in oxygen levels being transported to the tissues. These are rapid and reversible mechanisms, allowing for immediate changes in binding and releasing oxygen to peripheral tissue (22).

The hemodynamic compensatory mechanisms are significantly more complex and slower and associate numerous unfavorable effects. Initially, there is a reduction in the peripheral vascular resistance as consequence of both low hematocrit values and the vasodilation mediated by high levels of nitric oxide. These events lead to low blood pressure, which causes a reflex rise in cardiac debit, to maintain balanced blood pressure and tissue perfusion. A rise in sympathetic and RAAS activity determines vasoconstriction and low renal perfusion. Water and salt retention results, with plasmatic and extracellular expansion. Over the long term, these mechanisms are responsible for the development of HF in patients with severe anemia (<7 g/dL).

Anemia is frequently associated with HF. The etiology of this association is multifactorial and certainly not fully understood. The main pathophysiologic mechanisms, with the strongest evidence-based medicine data, are iron deficiency and inflammation. ACEIs and ARBs are essential drugs recommended in HF, but they might represent one of the causes of anemia related to this chronic disease. The adrenergic beta 1, beta 2, and alpha blockers such as carvedilol or digoxin, useful drugs recommended in HF, were also incriminated.

It seems that there are not enough evidences regarding oral iron in this aspect. The current guidelines of HF recommend only one intravenous iron (ferric carboxymaltose) for the treatment of anemia. However, in the literature, there is no clear way to proceed after the correction of iron deficiency. The clinical judgment is important here, but we also need to know the risks of iron overload: bacterial infections, increased oxidative stress, which could lead to endothelial dysfunction, and increased risk of coronary events. In this context, further randomized trial should be performed to evaluate the long-term impact of iron treatment on patients and the right period of treatment.

Several recent studies have raised questions regarding the influence of different drugs on iron metabolism, and even more questions with respect to the mechanism involved. There are additional data regarding a probable drug-induced etiology of iron deficiency. It seems that betablockers and amlodipine could influence the iron status in patients with heart failure. Practitioners should therefore be aware of the potential impact of therapeutic recommendations, as this may imply more intensive monitoring of the biochemical parameters of iron deficiency in such patients.

Chapter 7 ATRIAL FIBRILLATION AND CARDIOVASCULAR RISK FACTORS

Arterial hypertension, diabetes mellitus and non-alcoholic fatty liver disease are some of risk factors for AF. The optimization and better, the correction of risk factors of AF, is mandatory for an improvement of the prognosis in this arrhythmia associated with a high comorbidities and even death. In the years, I tried to understand better these pathologies known as risk factors for AF. These subjects about the risk factors associated with AF and other arrhythmias were synthetized in some reviews published in the last years. The concern for this branch of research was materialized in the articles below.

1.Tanase DM, Gosav EM, Radu S, Ouatu A, Rezus C, Ciocoiu M, Costea CF, **Floria M.** Arterial Hypertension and Interleukins: Potential Therapeutic Target or Future Diagnostic Marker? Int J Hypertens 2019;2019:3159283. doi: 10.1155/2019/3159283 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6521461/pdf/IJHY2019-3159283.pdf

2.Grigorescu ED, Lacatusu CM, **Floria M**, Mihai BM, Cretu I, Sorodoc L. Left Ventricular Diastolic Dysfunction in Type 2 Diabetes-Progress and Perspectives. Diagnostics (Basel) 2019;9(3). pii: E121. doi: 10.3390/diagnostics9030121. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6787758/pdf/diagnostics-09-00121.pdf

3.Sîrbu O, **Floria M**, Dăscălița P, Şorodoc V, Şorodoc L. Non-alcoholic fatty liver disease-From the cardiologist perspective. Anatol J Cardiol 2016;16(7):534-41. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5331403/pdf/AJC-16-534.pdf

4. Lăcătușu CM, Grigorescu ED, **Floria M**, Onofriescu A, Mihai BM. The Mediterranean Diet: From an Environment-Driven Food Culture to an Emerging Medical Prescription. Int J Environ Res Public Health 2019;16(6). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6466433/pdf/ijerph-16-00942.pdf

7.1. Arterial hypertension and interleukins through fibrosis

Hypertension (HTN) as a multifactorial pathology is one of the most important cardiovascular risk factors, affecting up to 30-40% of the general population. Complex immune responses are involved in the inflammatory mechanism of hypertension, with evidence pointing to increased inflammatory mediators even in prehypertensive patients (264). Increased vascular permeability, thrombogenesis, and fibrosis, effects that are associated with sustained hypertension, could be attributed to chronic inflammation (**Figure 26**).

Cardiovascular risk factors such as dyslipidemia, obesity, and diabetes, through their inflammatory state, promote endothelial dysfunction. Mast cells, T lymphocytes, dendritic

cells, activated neutrophils, and platelets interact to produce an inflammatory response, with increased production of proinflammatory cytokines, ROS, and adhesion molecules (265-267).

Given the increasing prevalence of HTN and its effects on (cardiovascular) mortality and comorbidity, there a constant focus on better understanding its pathogenesis. Inflammation plays an essential role in HTN. Chronic inflammation enhances endothelial and tissue cells functions by promoting pro-inflammatory cytokine synthesis- IL-1β, IL-6, IL-8, IL17, IL23, TGFβ and TNFα.

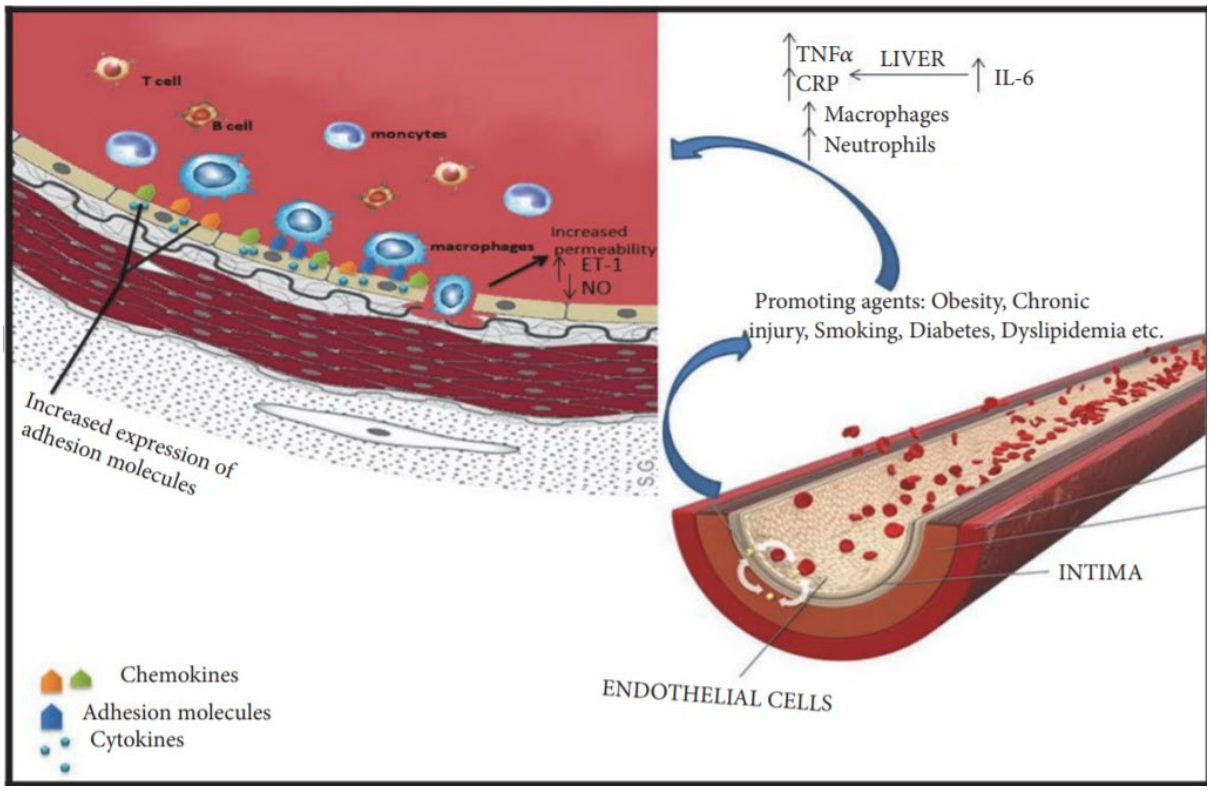


Figure 26. Etiology of the infammatory process. Low level chronic infammation increases the concentrations of markers and of infammatory cells, leading to increased production of C-reactive protein (CRP) by the liver, in response to interleukin-6 (IL-6), which provokes a reduction in vasodilation and an increase in vascular damage. *TNF-a: tumor necrosis factor alpha; IL-6: interleukin-6; CRP: C-reactive protein; NO: nitric oxide; ET-1: endothelin-1.*

These cytokines contribute to elevated blood pressure with structural and myogenic modifications via elevated levels of CRP, VCAM-1, NO, EDRF, PGI2,H2S, Ang II and ET-1 release. IL-1β in essential hypertension, promoting VCAM-1, ICAM-1 and E-selectin expression with atherosclerotic effects. Different ethnic studies revealed that specific plasma levels of IL-1β polymorphisms are associated with higher blood pressure.

IL-6 could be an important cardiovascular risk biomarker. Likewise, various IL-6 polymorphisms remains correlated with higher HTN risk in different populations. IL-17 and IL-23 family were linked to atherogenic effects, endothelial dysfunction, hypertension, CHF and Ang II induced HTN with subsequent renal injury. Over the years, cytokines such IL-8, IL-12, IL-15 ,IL-18, IL-22 were associated with inflammation and HTN. Additionally, IL-4

and IL10 exert their anti-inflammatory effect by suppressing IL-1, IL-6, IL-12 and TNF, HLA II and adhesion molecules.

Taking into consideration the increase in HTN prevalence and the complex pathogenesis mechanism, targeting inflammation as a possible anti-hypertensive therapy is a new option. Evidence showed that several cardiovascular drugs like statins, ACEI, ARBs and calcium channel blockers, lowered blood pressure and serum inflammatory cytokines concentrations. Even if anti-hypertensive effects of monoclonal antibodies remain controversial. It seems that IL-6 inhibition in hypertensive specimens decreased myocardial fibrosis and reduced albuminuria, emphasizing the role of the pro-inflammatory cytokine in developing end-organ damage.

Cardiovascular drugs and immunosuppressant molecules reduced plasma levels of different interleukins in hypertensive subjects. Even if, few monoclonal antibodies were proven to exert some effects, so far no monoclonal antibody has been approved for HTN treatment. These findings emphasize the role of cytokines in the pathogenesis of hypertension and end-organ damage, pointing out towards a possible therapeutic target.

7.2. Diabetes mellitus and left ventricular diastolic dysfunction

Type 2 diabetic (T2DM) patients may develop underlying CVD without experiencing or recognizing the telltale signs and symptoms until too late. Subclinical manifestations are therefore difficult to study and report, which makes the real scale of the problem an important question in an otherwise well-understood associated pathology. Systolic dysfunction associated with left ventricular ejection fraction reduction was proven as an insufficiently indicative of heart failure diagnoses, as symptoms may also occur with mid-range as well as preserved ejection left ventricular fractions (recently redefined as ≥ 50%). In this context, diastolic dysfunction is a more useful indicator of early heart failure if the patient is not experiencing any symptoms, and standard ejection fraction assessment does not raise any red flags (242).

The latest, 2016 guideline appears to serve best in identifying the more severe cases. Moreover, the echocardiographic parameters, such as the diastolic index E/e', were found to be predictors of adverse cardiovascular events. At the same time, while it does not fully cater for the subclinical domain, it does create an opportunity to select the cases classified as "indeterminate" for the purpose of screening for silent cardiovascular disease and subsequent monitoring. Concurrently, upon reviewing available studies and in relation to other recent reviews, editorials, and commentaries, we have become aware of the challenges of full investigations and accurate interpretations: equipment model and availability, methodological heterogeneity, patient profiling and matching, inter-observer variability, and clinician experience and expertise. Moreover, the underlying demographic and anthropological profile of the population to which the patient belongs may further interfere with interpretation and results, in conjunction with known associated comorbidities. Such difficulties have imprinted a significant degree of heterogeneity onto existing scientific endeavors and outcomes. As guideline improvements are constantly sought, retrospective and comparative studies are

useful but limited to those databases affording analyses with updated parameters and formulae. With or without the 'usual suspects' in the picture (aging, obesity, metabolic syndrome, hypertension), diabetes can distort diastolic function by itself and contribute to the snowball effect of serious cardiac complications such as heart failure. In the daily clinical practice of managing the wide spectrum of complications related to diabetes, not all clinicians may fully appreciate the significance of detecting diastolic dysfunction early, or lack methodological capacity to do it. Therefore, the widespread screening of diabetic patients, aiming to detect subclinical manifestations as early as possible, makes clinical sense. Current concerns regarding the poor cost efficiency of such programs could be outweighed by more scientific evidence into the long term benefits of early detection and prevention.

While researchers have been including patients with T2DM in their cohorts, practitioners could learn more from further studies focusing exclusively on the aforementioned diabetic profile via robust methodologies of matching subjects with control groups. Moreover, more longitudinal research is needed to trace the slow progression, regression, or stagnation of subclinical cardiac manifestations over longer periods of time. Such studies would require rigorous monitoring and planning in order to account for inherently confounding factors (personal, clinical, and institutional).

Given the scale and complexity of the issues, technological advances in the field of big data, artificial intelligence, and online collaboration should be taken advantage of. A multi-centric, inter-disciplinary, semi-automated approach to collecting, processing, interpreting, and studying such information would, on one hand, help reduce variability and heterogeneity while, at the same time, advancing our understanding of asymptomatic cardiovascular disease in type 2 diabetes to enable preventative screening.

The prevalence of diabetes is steadily rising, and once it occurs, it can cause multiple complications with a negative impact on the whole organism. Complications of diabetes may be macrovascular: such as stroke and ischemic heart disease as well as peripheral vascular and microvascular diseases—retinopathy, nephropathy, and neuropathy. Key factors that cause cardiovascular disease in people with diabetes include hyperglycemia, dyslipidemia, obesity, insulin resistance, inflammation, hypertension, autonomic dysfunction, and decreased vascular response capacity. Microbes can be considered a complex endocrine system capable of ensuring the proper functioning of the body but are also responsible for the development of numerous pathologies (diabetes, coronary syndromes, peripheral arterial disease, neoplasia, Alzheimer's disease, and hepatic steatosis). Changes in the intestinal microbiota may influence the host's sensitivity to insulin, body weight, and lipid and carbohydrate metabolism. Dysbiosis causes activation of proinflammatory mechanisms, metabolic toxicity, and insulin resistance.

Atherosclerosis of the large arteries and coronary arteries leads to macrovascular complications such as stroke, ischemic heart disease, and peripheral vascular disease. Atherosclerosis of small arteries causes diabetic nephropathy and is related to cardiovascular morbidity. Diabetes, regardless of its effect on atherosclerosis, is associated with changes in cardiac structure and function leading to myocardial dysfunction, called "metabolic cardiomyopathy" (268). The presence of a metabolic syndrome exposes patients to an increased risk of cardiovascular complications. Moreover, in addition to classical risk factors, there are other unconventional factors that cause vasoconstriction and thrombosis, endothelial

dysfunction, inflammation, oxidative stress, and vascular wall abnormalities that also contribute to an increased cardiovascular risk (292).

7.3. Non-alcoholic fatty liver disease and cardiovascular diseases

Non-alcoholic fatty liver disease (NAFLD) is associated with cardiovascular disease is widely recognized, as well as the fact that NAFLD patient mortality rises when such an association is present. In particular, NAFLD is associated with coronary and carotid atherosclerosis, endothelial dysfunction and arterial rigidity, ventricles function, valves morphology, congestive heart failure, and arrhythmias (especially atrial fibrillation). Additionally, the hypercoagulability status in NAFLD patient may be suggested by the presence of inflammatory and coagulation markers. The presence of a hypercoagulability status linking NAFLD with a high risk of developing cardiovascular disease has been suggested based on the inflammatory status associated with this pathology and on epidemiological studies. It seems that NAFLD could affect both left ventricular systolic and diastolic function. In addition, NAFLD could affect cardiac valves and is strongly associated with an increased risk of prevalent aortic valve sclerosis (270). Therefore, we could talk about an increased rate of congestive heart failure in this patient population (271). Obviously, due to these changes in cardiac and valvular function, there is also a high risk of arrhythmias, especially atrial fibrillation (271).

NAFDL is not the only gastroenterological disease associated with cardiovascular disease. P-wave dispersion measured on the electrocardiogram reflects the conduction of the sinus electrical stimulus at the atrial level. Increased values are found in inflammatory bowel disease patients, and it indicates the heterogeneity of intra-atrial and interatrial conduction as well as discontinuous propagation of electrical impulses, which predisposes to atrial fibrillation (272). Chronic inflammation also affects the success rate of cardioversion and maintenance of the sinus rhythm in patients with inflammatory bowel disease and atrial fibrillation (273).

III. FUTURE DIRECTIONS

Chapter 8 FUTURE EVOLUTION AND DEVELOPMENT PLANS

8.1. Perspectives in research activity

The main research directions will be focused on rhythm disorders (especially AF) and substrate assessment by either echocardiography or other imaging modalities (like cardiac magnetic resonance - CMR). In the following pages I synthetized the main projects which I want to put in practice. Therefore, I realized an extensive review of literature, presented in the following box, about the left atrial structural remodelling in non-valvular AF and the role of CMR in atrial fibrosis detection.

Floria M, Radu S, Gosav EM, Cozma D, Mitu O, Ouatu A, Tanase DM, Scripcariu V, Serban LI. Left Atrial Structural Remodelling in Non-Valvular Atrial Fibrillation: What Have We Learnt from CMR? Diagnostics (Basel) 2020;10(3). pii: E137. doi: 10.3390/diagnostics10030137. https://www.mdpi.com/2075-4418/10/3/137/htm

I will continue to be focused on AF from pathogenesis and risk factors to thrombogenesis and outcomes. An interdisciplinary team will be the solution for many issues in this arrhythmia. Therefore, I will proposed three theme of research based on the collaboration with the radiologist, gastroenterologist and endocrinologist. The main theme will be:

1. Atrial fibrosis and non-valvular AF
2. Atrial fibrosis and risk scores in non-valvular atrial fibrillation
3. Heart rate variability and gastroesophageal reflux disease assessed by impedance-pH-metry
4. Osteoporosis and specific treatment – the relationship with AF

1. Atrial fibrosis and non-valvular atrial fibrillation

Clinical arrhythmology is an essential field in cardiology for both electrophysiologists and cardiologists: in the case of the former to prevent the specialist from becoming a technician and in the case of the latter to fully understand the specialty. However, clinical arrhythmology needs to be more promoted among students and residents. The clinical guidelines reaffirm this idea while also recommending an increasingly widespread interventional approach throughout the range of arrhythmias.

Atrial fibrillation is the most frequent cardiac arrhythmia and is associated with increased risk of stroke, mortality and decreased quality of life (273). Left atrial structural, functional and electrical remodelling are linked to AF pathophysiology and mirror the phrase "atrial fibrillation begets atrial fibrillation" (274). The different types of atrial remodelling are interconnected, as structural remodelling leads to LA dysfunction and subsequent electrical changes in the cardiomyocytes (274-278).

Fibrosis is the hallmark of left atrial structural remodelling and is associated with increased risk of stroke, heart failure development and/or progression and poorer catheter ablation outcomes with increased recurrence rates. Therefore, given the therapeutic and prognostic implications of left atrial remodelling and the subsequent atrial cardiomyopathy in atrial fibrillation patients, properly identifying it becomes necessary. Fibrosis is best imaged through late-gadolinium enhancement cardiac magnetic resonance. This offers diagnosis and prognostic information and influences therapeutic choices.

Subsequently, imaging structural remodelling is necessary given its impact on catheter ablation candidate selection, technique and post-procedural outcomes and prognosis. Cardiac magnetic resonance with late-gadolinium enhancement (CMR-LGE) is the gold standard in imaging fibrosis (279), however it is not widely available and different centres failed to reach an agreement regarding scanning protocols. We will further analyse the utility of various CMR-derived imaging parameters of LA structural remodelling, including size, shape and fibrosis (both extension and architecture) in relation to the index ablation procedure timing in non-valvular AF patients in terms of candidate selection, ablation strategy and post-procedural outcomes.

Atrial cardiomyopathy and left atrial remodeling

LA is a thin-walled structure of varying thickness (1 to 15 mm), postero-superior to the right atrium with its four PVs located postero-superiorly in a dome-like shape [279]. The left atrial appendage (LAA) is narrower than that of the right atrium with over 90% of the thrombi of AF patients forming at this level. [273,279]. Its morphology varies with non-chicken wing morphology being associated with increased thromboembolic risk [274].

The importance of LA function resides in its contribution with nearly 30% to the ventricular stroke volume [279]. LA behaves like a reservoir during ventricular systole, a conduit in early ventricular diastole and as a booster pump in late systole. Subsequently, its dysfunction has been associated with increased risk of stroke [280], poorer ablation outcomes and overall prognosis [280-283].

LA remodelling can be defined as the time-dependent structural, functional and/or electrical alterations in response to mechanical (pressure and/or volume overload), metabolic or electrical stressors, being the substrate for a veritable atrial cardiomyopathy [274,279]. Initially reversible (< 1 week of exposure) and adaptive, in time the cellular, electrical and autonomic nervous alterations will become permanent and maladaptive [274].

Several conditions including heart failure, arterial hypertension, and valvular heart disease promote atrial remodelling through either pressure and/or volume overload. Atrial arrhythmias, especially AF alter atrial structure, leading to irreversible changes in shape and function [279,280]. Moreover, the aforementioned diseases promote AF through LA remodelling (‘AF begets AF’).

The different types of LA remodelling (structural, functional, electrical) are interconnected [274], influencing both therapeutic options and prognosis. Fibrosis associated with structural remodelling leads to conduction heterogenicity, promoting re-entry and abnormal foci [280]. Furthermore, low-voltage areas correlate with fibrotic regions in AF patients [281] and LGE-CMR fibrotic burden is linked to LA dysfunction [281]. However,

fibrosis and associated dysfunction may appear early during remodelling, preceding chamber enlargement [281]. They are linked to increased risk of stroke even in non-AF patients [276,281].

Given the therapeutic and prognostic implications of LA remodelling and the subsequent atrial cardiomyopathy in AF patients, properly identifying it becomes necessary. As such, we will further review LA structural remodelling with the available imaging techniques and its utility in routine clinical practice.

Left atrial structural remodeling

The hallmark of LA structural remodelling is myocardial fibrosis [274], atrial enlargement being the final expression of the latter. It has been shown that 6 weeks of AF increase fibrosis amounts [280]. From this perspective, some authors have highlighted a fibrotic atrial cardiomyopathy as being the substrate for AF maintenance and/or progression and its increased risk of thromboembolic events [281].

Until recently, atrial fibrosis was regarded as a consequence of AF, but studies have shown that increased amounts precedes and contributes to AF development in sinus rhythm patients [280,283]. This can be explained by the fibrotic effect of several comorbidities regarded as AF risk factors (i.e. arterial hypertension, heart failure, diabetes) [274]. At a molecular level, this is supported by the pro-fibrotic effects of angiotensin II, aldosterone, TGF-β1 and pro-inflammatory cytokines and the reported revers-remodelling following therapy with either aldosterone receptor blockers or angiotensin converting enzyme inhibitors [274].

In time, a fibrotic LA progresses to overt enlargement. The latter is the most easily imaged facet of structural remodelling (however the chronologically last), as echocardiography is readily available and there are much firmer agreements on the recommended parameters [284]. Recently, there has been a shift from focusing on LA size to LA shape, as it is known that its dilatation is asymmetrical in the beginning, progressing from a discoid shape towards a sphere [285]. Echocardiographic or CMR-derived sphericity index seems to predict post-ablation recurrence rates and overall prognosis [285-288].

Different imaging techniques including echocardiography, computer tomography (CT) and CMR have been used to assess structural remodelling. This is useful in developing a patient-tailored approach for AF ablation as it may improve candidate selection, influence ablation strategies and determine prognosis. However, incorporating the results into clinical decision-making tools remains a challenge mainly because there is yet a universal definition of both LA structural remodelling and reverse-remodelling.

Left atrial size

It is agreed upon that AF patients have increased LA dimensions and these are associated with a poor overall prognosis, including poorer ablation outcomes and increased recurrence rates [283]. From this perspective, LA is currently being regarded as a veritable biomarker predictive of cardiovascular and thromboembolic events even in non-AF patients [274].

LA diameter underestimates its dimensions, as such it is no longer recommended in assessing enlargement [273,283]. Although increased antero-posterior diameter is associated with post-CA recurrences, LA dilatation is asymmetrical and preferentially occurs in two directions: medial-lateral and supero-inferior [288]. It follows that assessing LA dimensions by diameter is inaccurate and LA volume (LAV) is preferred [283]. The ESC considers an indexed LAV of >34 mL/m2 indicative of an enlarged LA [283]. Although routinely evaluated through echocardiography, CMR is the gold-standard in chamber dimensions quantification.

A recent metanalysis including 21 studies and a total number of 3822 patients assessed the relation between LAV and AF recurrence (288). The authors concluded that patients with recurrences had higher LAV and indexed LAV (LAVI). Moreover, both emerged as independent predictors (288). For each 1 mL increase in LAV/LAVI, there was a 3% increase in AF recurrence risk while a 1.84 mm increase in diameter was equivalent to a 0.8 mL increase in LAV. In addition, increased LA dimensions predicted AF development. Habibi et al. showed that increased LAV was an independent predictor of AF in a asymptomatic population (282) and that a 5 mm increase in LA diameter nearly doubled the risk of AF. Not only this, but increased dimensions are associated with LA dysfunction and increased risk of stroke independent of AF and CHA2DS2-VASc risk score (283, 288, 292).

Left atrial shape

Given the asymmetrical pattern of LA dilatation, several authors have focused on the LA shape. It seems that while dilating, LA's shape evolves towards a sphere (285-288). This geometrical structural remodelling is also associated with poorer ablation outcomes and recurrences (288).

The sphericity index compares the LA shape as determined by 3D CMR to a sphere and expresses this similarity through a percentage. It seems that patients with higher sphericity indexes have 11 higher risk of developing 1 year post-CA recurrences as compared to those with discoid LA (289). In the LAGO-AF study, LA sphericity index emerged as the sole independent predictor of recurrences (289). Subsequently, geometrical structural remodelling may be superior to size in predicting CA outcomes and arrhythmia recurrences. Moreover, persistent AF patients tend to have a more spherical LA (285) and increased baseline sphericity index is associated with poorer ablation outcomes.

In a study conducted by Moon et al, a sphericity index of > 0.87 strongly correlated with increased recurrences and weakly with LAV (284).

Left atrial fibrosis

Late-gadolinium enhancement CMR (LGE-CMR) is the gold standard in assessing myocardial fibrosis (283,292). A gadolinium-based contrast agent enhances fibrosis detection due to its accumulation in the extracellular space (increased in fibrotic tissues) and its delayed clearance. This strengthens the T1 weighted signal due to gadolinium's paramagnetic properties (280). Images are acquired 15 to 20 minutes after contrast agent administration; however, adjustments are made based on injected amounts and patient's renal function.

Left atrial fibrosis as a predictor of post-ablation recurrences

After the CA scar in AF patients was identified on LGE-CMR scans (274, 292), this technique was intensely studied for its ability to evaluate AF substrate and predict therapeutic response and post-ablation recurrence rates (274, 275,279, 293). Oakes et al. showed that fibrosis extension correlated with increased arrhythmia recurrence rates at 6 months follow-up post CA (291). In a different study, patients with persistent AF and > 35% LA fibrosis had increased recurrence rates (294). Specifically, the recurrence risk was 1.5 higher for each 10% increase in the LA-LGE. Similarly, Marrouche et al. characterized AF patients fibrosis severity using Utah classification in stage I (<10%), stage II (10-20%), stage III (20-30%) and stage IV (>30%) and reported a 6% recurrence risk for each 1% LA fibrosis increase (275).

In a recent multivariate analysis comparing the impact of different CMR-derived remodelling parameters (including LA volume, sphericity index, LA ejection fraction and fibrosis degree) on CA outcomes, only LA fibrosis emerged as a predictor of late AF recurrences (a median follow up of nearly 7 years) (294). On the other hand, LA volume and fibrosis don't always correlate, emphasizing the idea that a normally-sized LA could be extensively fibrotic (295).

It seems that the size of the largest fibrotic region is also important (293). A different analysis of the DECAAF study revealed that the dimensions of the largest fibrosis patch predicts recurrences in Utah stages II and III patients (297).

Interestingly, fibrosis disposition is inhomogeneous with its different locations being linked to arrhythmia recurrences. The posterior wall and left inferior pulmonary vein are preferentially affected, especially in persistent AF patients (298,299). This is in accordance with histological studies (279). Moreover, it seems that the posterior wall is the most affected irrespective of AF history. Consequently, a theory emerged that the increased wall stress at the level of the pulmonary veins and posterior wall (due to its proximity to the descending aorta) could in time lead to fibrosis and contribute to AF maintenance and/or post-ablation recurrence (299).

Left atrial fibrosis and thromboembolic risk

The extension of baseline LA fibrosis also means higher thromboembolic risk (276, 280,300), and subsequently, higher risk for developing major adverse cardio- and cerebrovascular events (MACCE) (301). In a study conducted by King et al., baseline LA fibrosis reported as Utah stages correlated with both thromboembolic risk scores CHADS2 and CHA2DS2-VASc (301). Patients in Utah IV (>35% LA fibrosis) had a higher incidence of MACCE (defined as either transient ischemic attacks, myocardial infarction, acute decompensated HF or CV death) and were four times more likely to develop a transient ischemic attack. Several studies also report that patients with increased LA fibrosis are most likely to have had sustained a stroke/transient ischemic attack (302) and that in the majority of them, transoesophageal echocardiography identifies a LA thrombus (303). Moreover, the

overall fibrosis extension had better c-statistics then both thromboembolic risk scores in predicting LAA thrombi (0.87 versus roughly 0.7) (302).

The presence of a fibrotic atrial cardiomyopathy might explain the persistence of the increased thromboembolic risk even in patients maintaining sinus rhythm post-CA (301). Notably, it seems that this risk remains constant even with long-term sinus rhythm maintenance.

The same fibrotic atrial cardiomyopathy correlated with embolic strokes of undetermined origin even in non-AF patients (276). Patients with more than 12% fibrosis burden presented with strokes even in the absence of confirmed AF (276).

As such, anticoagulation might be an option even in sinus-rhythm patients with increased LA fibrosis and might justify continuing anticoagulation in patients with fibrotic LA who maintained post-ablation sinus rhythm. At a molecular level, this is supported by the pro-inflammatory environment and subsequent thrombogenic endothelial dysfunction found in fibrotic atria irrespective of the underlying rhythm (280). In other words, blood stasis is not the only thrombogenic mechanisms and fibrosis in itself may lead to higher thromboembolic risk.

Spronk et al. launched a different perspective, - that hypercoagulability in itself may stimulate fibroblasts and increase fibrosis (300). The fact that anticoagulating goats (nadroparin) resulted in decreased fibrosis could shift the current point of view regarding anticoagulation therapy from strictly preventing thromboembolic events to influencing the substrate by reducing fibrosis degree (300).

Left atrial fibrosis and LA dysfunction

There is a connection between LA dysfunction, LA fibrosis, increased thromboembolic risk and post-ablation recurrence rates. An altered reservoir function has been associated with both increased thromboembolic risk (304) and recurrence rates (305). Another study emphasized the link between LA reservoir dysfunction (standard deviation time to peak strain) and increased thromboembolic risk [34]. The authors reported that adding parameters of mechanical LA dyssynchrony to the thromboembolic risk scores might CHA2DS2-VASc c-statistics from 0.75 to 0.82 (306).

Left atrial fibrosis and heart failure

Sustained AF leads to HF development and/or progression (273,274) and HF in turn is worsened in terms of prognosis and quality of life by the superposition of AF. It has been shown that AF patients with concomitant HF have increased LA fibrosis and subsequently, individuals with higher degrees of LA structural remodelling have lower baseline left ventricular ejection fraction (LVEF) (307). Out of these, patients with less LGE extension benefit most from CA procedures in terms of LVEF improvement (308). The mechanism proposed was that an intensely fibrotic LA is unable to contribute with the usual 10-15% to the ventricular filling, because it is stiffer and will not contract efficiently even if sinus rhythm is restored. Moreover, it seems that AF patients have increased LV fibrosis as

determined by T1 weighed CMR scans, also contributing to the systolic dysfunction of these patients and worse prognosis (309).

The fact that AF is tightly linked from a pathophysiological point of view with HF in terms of development, impact on patients' quality of life through symptoms worsening and overall prognosis support the use of CA to restore sinus rhythm in patients with reduced LVEF. The CASTLE-AF trial emphasized that HF patients with reduced LVEF and AF benefit in terms of survival and hospitalization rates from sinus rhythm restoration using CA procedures (309).

However, there is a delicate balance between risk and benefits in ablating AF HF patients. A very extensive ablation scar could in turn, determine HF development and/or worsening (307). Taking into consideration that a higher fibrotic LA would require additional substrate ablation (and therefore, additional ablation lines) and that exactly these patients are more likely to have concomitant HF, ablating these patients becomes even more difficult. It is exactly for this reason that further studies are needed to determine a LA fibrosis threshold that would justify, on one hand, performing substrate ablation and on the other, exclusion from ablation procedures due to lack of symptomatic and prognostic benefit.

Left atrial appendage structural remodelling

Although there is an agreement on the importance of LA remodelling in AF pathophysiology and patients' management, there is a lack of consensus regarding the remodelling of LA appendage (LAA). The lack of this agreement is even more striking since almost 90% of the AF thrombi occur at this level (311,312).

The most studied parameter of LAA was its morphology, with non-chicken wing type being associated with increased thromboembolic risk (313). Khurram et al. revealed that while the morphology per se showed no correlation with thromboembolic risk, out of the LAA morphological parameters analysed, a more trabeculated LAA with a narrower orifice was associated with an increased risk of stroke, most likely due to increased blood stasis (313). The authors highlighted that categorizing LAA through a pre-determined morphology is user-dependent and unreliable due to inter-observer variability. This in turn could account for the lack of correlations between LAA morphologies and thromboembolic risk across various studies.

Recently, authors focusing on LAA remodelling revealed higher post-ablation arrhythmia recurrence risk with each 1% increase in LAA fibrosis as assessed by LGE-CMR (314). Moreover, the authors did not find a correlation between the degree of LA and LAA structural remodelling, most probably due to their different embryologic origin. Ma Nan et al. recently revealed that LAA fibrosis determined on histological specimens correlated with AF duration and post-ablation recurrence risk (315). However, there are no studies reporting the direct correlation between LAA LGE extension and histological specimens.

The importance of assessing LAA remodelling lies beyond understanding AF pathophysiology; it might justify using additional ablation lines at this level. A different study showed that the LAA was the source of ectopic foci in 9% of the patients requiring a re-do procedure (316). However, authors highlight that this procedure may be associated with increased rates of cerebrovascular thromboembolic events and subsequent LAA thrombi formation (315).

2. Atrial fibrosis and risk scores in non-valvular atrial fibrillation

I want to extend and develop the research on left atrial structural remodeling. Therefore I have written a design for a study entitled: "Association of Atrial FIBrosis assessed by late enhancement gadolinium MRI scans with CHA_2DS_2-VASc and HATCH SCOREs in patients with nonvalvular atrial fibrillation- a prospective study (A-FIB-SCORE)".

Atrial fibrillation is the most frequent arrhythmia, being linked to left atrial (LA) structural, functional and electrical remodeling. Quantifying the degree of structural remodeling through LA enlargement and especially fibrosis may influence therapeutic choices (ie. ablation) and prognosis. Delayed gadolinium enhancement magnetic resonance imaging (LGE-MRI) is the gold standard in characterizing myocardial tissue, being able to highlight the presence of atrial cardiomyopathy (**Figure** 27), strongly related to increased arrhythmogenic and thrombogenic risk.

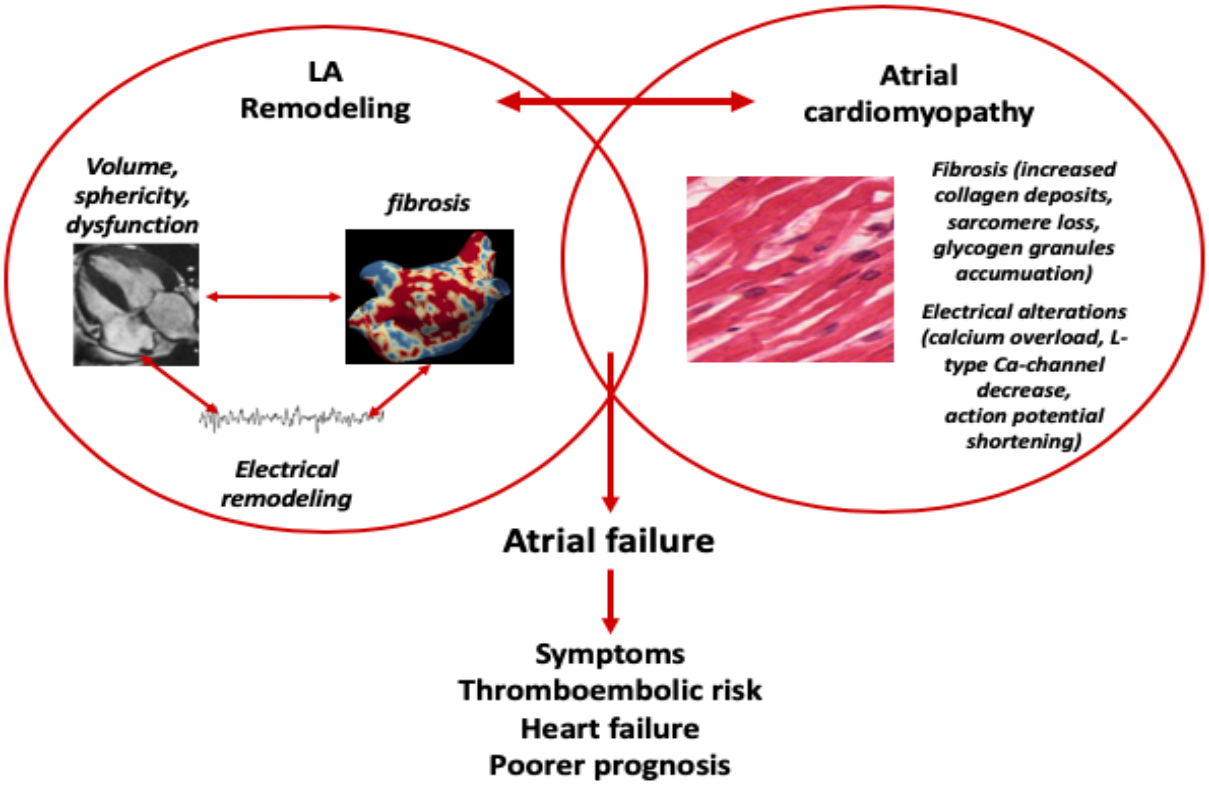


Figure 27. The relationship between LA remodeling, atrial cardiomyopathy and atrial failure.

Higher degrees of LA fibrosis, irrespective of LA size, have been linked to increased rates of strokes even in non-AF patients, higher risk of developing AF, increased rates of post-ablation recurrences and poorer outcomes. As such, early quantification of atrial fibrosis by noninvasive methods may influence interventional therapeutic choices, preventing AF occurrence, maintenance and related complications.

HATCH (AF progression), CHA_2DS_2-VASc (thromboembolic risk) and HAS-BLED (bleeding risk) scores may be good clinical tools to assess atrial fibrosis in AF patients,

provided that they correlate with the latter as determined by LGE-CMR (using DECAAF protocol) and graded according to Utah stages. If they correlate, they may influence catheter ablation technique and candidates selection, with increased risk score values (and subsequent higher atrial fibrosis degree) translating into reduced success rate and/or higher post-ablation recurrence rates. Increased atrial fibrosis accelerates AF progression, which may be linked to higher HATCH scores value.

A clinical prediction algorithm of AF progression in relation with atrial fibrosis and thrombosis due to left atrial remodeling might be created.

AF is the most common arrhythmia and is associated with a degree of structural, functional and electrical LA remodelling (317). Functional remodelling may precede structural remodelling (assessed by increased fibrosis and/or chamber enlargement). Fibrosis promotes re-entry through conduction heterogenicity and subsequent unidirectional block and increased amounts correlate with stroke even in non-AF patients (317). Moreover, the relationship between atrial fibrotic changes and AF occurrence and persistence has been demonstrated in histological analyses obtained from surgical specimens (318). As such, the extent of atrial fibrosis translates into poorer ablation outcomes, higher post-ablation recurrence rates and increased risk of stroke. Consequently, chamber enlargement, best quantified through indexed left atrial volume index (LAVI) as a mean of structural remodelling have also shown positive correlations (319, 320).

While cardiac magnetic resonance offers the most reliable chamber dimension assessment, LGE-CMR is the gold-standard method in assessing and quantifying myocardial fibrosis (321-323), preferably using the DECAAF protocol (323).

The size of the largest fibrosis patch and the LAV also correlate with poorer outcomes. Moreover, recent studies showed that increased atrial fibrosis correlates with stroke irrespective of patients' underlying cardiac rhythm (319,324) and with AF progression towards permanent (325).

In parallel, research focusing on functional LA remodeling has shown that altered LA function (reservoir, conduit, booster pump) is also associated with increased stroke risk, poorer ablation outcomes and increased arrhythmia recurrence (326, 327). However, assessing LA strain and strain rate is difficult as there are no cut-off values agreed upon, as they tend to vary according to different vendors. Although the more newer CMR fast-tracking techniques could overcome some of the limitations of echocardiographic LA functional assessment, the lack of consensus is still valid (327).

CHA_2DS_2-VASc score is recommended by current guidelines to assess thromboembolic risk in AF patients (328). It seems that its values increase with LA dimensions, suggesting that LA structural remodeling may be associated with higher thromboembolic risk (329). Moreover, CHA_2DS_2-VASc is predictive of postoperative AF after cardiac surgery and may be helpful in identifying high-risk patients (330).

HATCH score evaluates AF progression risk, with nearly 50% of the patients with paroxysmal AF and HATCH score >5 progressing to persistent AF (331). Taking into consideration that both increased atrial fibrosis and higher HATCH scores lead to arrhythmia progression, a correlation between the two would contribute to the development of a patient-tailored approach.

Left atrium is characterized by an asymmetric structural remodeling (increased LAVI, sphericity index) (326, 332-333), with increased fibrosis being an arrhythmogenic and thrombogenic substrate. LA fibrosis also contributes to arrhythmia development in sinus rhythm patients (334-337). Once developed, the arrhythmia in turn leads to increased fibrosis, supporting the dogma that AF begets AF (337-340). More importantly, functional remodeling through reduced strain and strain rates also been associated with increased recurrence rates (339, 340).

Despite the importance of LA structural and functional remodeling and its proven correlations with poorer outcomes and stroke, there is yet a clinical tool that, through its correlations with fibrosis (fibrotic atrial cardiomyopathy) and/or chamber enlargement, may help stratify patients according to AF progression and thromboembolic risk. The still high post-ablation recurrence rate may be due to inappropriate candidates' selection with subsequent increased fibrosis and urges the creation of a wide applicable clinical tool.

By conducting a clinical prospective observational study designed to assess the relationship between clinical risk scores in AF patients and LA structural and functional remodeling parameters, we aim to analyze:

a) LA fibrosis extension (Utah scale) and the size of the largest fibrosis area by LGE-CMR (DECAAF protocol)
b) The relationship of fibrosis with clinical risk scores
c) The predictors of post-ablation AF recurrence risk
d) The relationship between LA morphology and fibrosis degree
e) Analyze the relationship between LA structural and functional remodeling and stroke in AF patients for a better understanding of atrial cardiomyopathy.

Arrhythmia initiation and progression is tightly linked to LA structural and functional remodelling. LA fibrosis is an arrhythmogenic and thrombogenic substrate, correlating with stroke irrespective of cardiac rhythm (341, 342).

We, therefore, expect that the risk scores will correlate LA fibrosis, enlargement and strain, allowing the creation of a clinical tool that may influence patient management. CHA_2DS_2-VASc, HATCH and HAS-BLED clinical risk scores might correlate with fibrosis extension (Utah scale on LGE-CMR) and/or size of the largest fibrotic area. CHA_2DS_2-VASc, HATCH and HAS-BLED clinical risk scores might correlate with spheroidal LA structural remodelling (sphericity index) and AF post-ablation recurrences. Fibrosis extension, LA size and function might be strongly associated with stroke
Decreased LA functions (reduced strain, strain rate) could be strongly linked to higher thrombogenicity and, implicitly, clinical risk scores.

Atrial fibrosis will be determined by LGE-MRI scans (**Figure 28**), using routine protocol (343).

Patients will undergo 3D LGE-MRI scans along with contrast enhanced magnetic resonance angiography and cine imaging. High-resolution respiration navigated, ECG-triggered gradient-echo pulse sequence images will be acquired. Inversion recovery preparation will be applied every heart beat The scans will be performed 20 minutes after the administration of gadolinium, with adjustments made according to the dose of the injected contrast agent. Fibrosis will be defined as a 2- standard deviations pixel intensity difference

and will be graded according to Utah classification as following: stage I- ≤ 10%, stage II- 10-20%, stage III- 20-30% and stage IV ≥ 30% (343).

Nowadays we are talking more and more about atrial fibrosis as marker of structural remodeling than atrial size. A good correlation between atrial fibrosis and above mentioned risk scores and/or left atrial size and a cut-off vale for one of these risk scores correlated with severe atrial fibrosis will be very important for clinical practice.

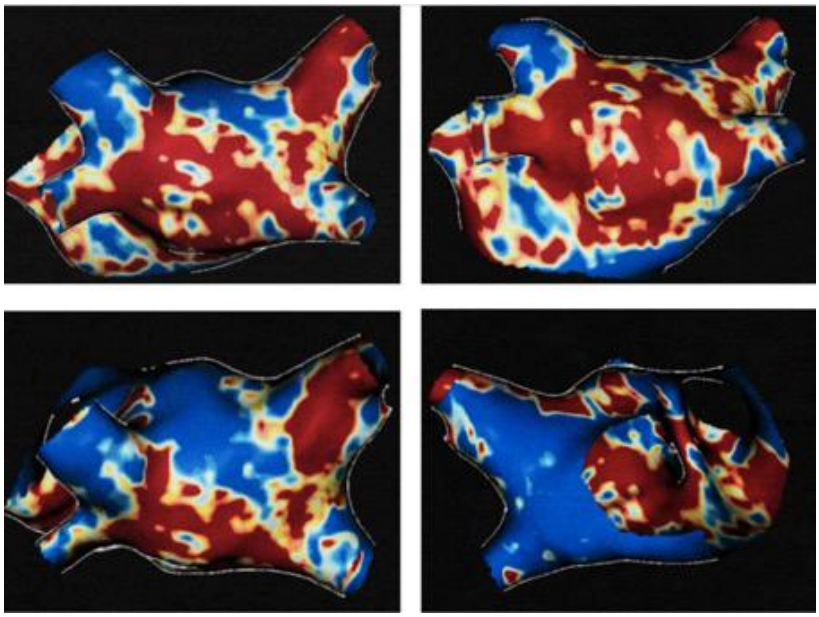


Figure 28. LGE-LA of 35.4% (Utah IV) in a persistent AF patient with repetitive ablations. Increased fibrosis at the posterior wall and pulmonary veins. Red demonstrates the presence of fibrosis. *AF: atrial fibrillation; LGE-LA- late gadolinium enhancement in the left atrium.*

It will be needed to make any efforts to control structural remodeling progression in patients with minimum and mild atrial fibrosis. Electrical or pharmacological cardioversion is not appropriate in patients with severe atrial fibrosis. We will be able to avoid unnecessary medical invasive or less invasive intervention in these patients, using a simple assessment of these scores. The problem of rate or rhythm control in this arrhythmia (associated with high mortality and many comorbidities) continue to be a debate. The benefits or the adverse consequences will be much more accurate assessed in these patients.

Less hospitalization related to pharmacological and electrical cardioversion by avoiding these procedures may be another positive result of this study because of cost efficiency. On the other hand, early evaluation of patients with AF could contribute to the medical measures for prevention of AF progression. This mean less comorbidities related to AF with a large social and economic impact.

3. Heart rate variability in patients with gastroesophageal reflux disease

Another proposed study will extend the post-doctoral theme related to the relation between AF and gastroesophageal reflux disease (GERD). This study will be entitled: "The relationship between heart rate variability and esophageal impedance-pH monitoring parameters in patients with gastroesophageal reflux disease: atrial fibrillation risk prediction".

Understanding the association between GERD and non-valvular AF is extremely important in the global multimodality treatment strategies to improve outcomes in patients with AF. Common autonomic innervation of the esophagus and left atrium, sympatho-vagal imbalance and inflammation from esophageal acid exposure seem to explain the association between non-valvular AF with GERD. It remains unclear what degree of esophageal acid exposure may induce sympatho-vagal imbalance and trigger AF. We aimed to prospectively assess sympatho-vagal imbalance through heart rate variability (HRV) parameters (in time and frequency domains) using 24 hours electrocardiogram (ECG) HOLTER monitoring in patients with/without GERD, off acid-suppression therapy with proton pomp inhibitors. The patients will be included based on both clinical symptoms and upper gastrointestinal endoscopy by a joint team consisting of gastroenterologist and cardiologist. Simultaneous with 24 hour ECG HOLTER, esophageal impedance-pH monitoring (for % and time acid and bolus exposure) will be performed. 24 hour ECG HOLTER monitoring will be repetead after 8 weeks on acid-suppression therapy with proton pomp inhibitors. In addition, LA structural remodeling parameters by transthoracic echocardiography and the presence of hiatal hernia and esophagitis by upper gastrointestinal endoscopy will be noted. The correlation between HRV and impedance-pH monitoring parameters during esophageal acid exposure depending on degree of severity will be assessed. The variation of HRV parameters off and on acid-suppression therapy with proton pomp inhibitors in relation with left atrium structural remodeling parameters will be analyzed; these parameters this will be compared also in patients with/without GERD. A prediction model of AF risk in patients with GERD using HRV, impedance-pH monitoring and echocardiography parameters will be created.

Scientific context and motivation

AF is the most common arrhythmia in clinical practice with a high morbi-mortality; its incidence has risen in countries with rapidly aging populations, with 120 000–215 000 newly diagnosed patients per year (23). One of four middle-aged adults in Europe and the US will develop AF. By 2030, 14–17 million AF patients are anticipated in the European Union (73). GERD is one of the most frequent benign disorders of the upper gastrointestinal tract (73). The range of GERD prevalence estimates in Europe has broadened slightly, ranging from 8.8% to 25.9% (75). Due to the close positioning of the esophagus and the atria and their similar autonomic innervations, it has been proposed that the development of GERD could be associated with the occurrence of AF (75, 344). Sympatho-vagal imbalance seems to be one of the principal mechanisms of both, AF and GERD (75, 322). Hiatus hernia, esophagitis or dilated left atrium seems to be also implicated in this association due to a potential mechanical effect or inflammatory process. It seems that GERD significantly increased the risk of AF only in the presence of esophagitis (80); in addition it remains unclear what degree of esophageal acid exposure may trigger AF. Despite of the fact that association between GERD and AF is supported by clinical and experimental studies, this relationship is still not completely undetstood (75). However, most of these studies about the association between AF and GERD are based on retrospective data of national registries or self-reported questionnaires and did not evaluate the autonomic imbalance in these patients (75, 345).

Heart rate variability is a noninvasive tool that has been successfully used to estimate modulation of autonomic tone (345). HRV is known to decrease when sympathetic activity predominates, whereas it increases when parasympathetic activity predominates (345). Different methods are available for the analysis of HRV (76). The most widely-used methods are those in time and frequency domains. There are few prospective data about autonomic (sympatho-vagal) imbalance and arrhythmias risk in patients with GERD (76, 85, 86, 345).

Esophageal impedance-pH monitoring is considered the most sensitive tool for assessing all types of GERD (acidic, weakly acidic and weakly alkaline), their composition, proximal extent, duration and clearing. Normal values for impedance-pH monitoring off acid-suppression therapy have been determined from US and European studies (346). The key clinical measurement for impedance-pH testing is the number and duration of acid and non-acid reflux episodes as well as their relationship with the symptoms. In patients on proton pomp inhibitors, qualitative analysis of the reflux-symptom association using symptom index or symptom association probability is essential (347). Baseline clinical and reflux parameters off and on therapy with proton pomp inhibitors may provide important clinical clues to association of GERD with AF.

Understanding potential atrial arrhythmogenic mechanisms in patients with GERD, such as impaired autonomic stimulation and mechanical irritation due to anatomical proximity of the left atrium and the oesophagus is extremely important in the global multimodality treatment strategies to improve outcomes in patients with AF (348). Treatment of GERD to avoid AF or to reduce AF burden might represent a future treatment perspective. Tools for the prediction of AF may identify high-risk individuals more likely to benefit from preventive measures. Therefore, I will assess sympatho-vagal imbalance off and on therapy with proton pomp inhibitors simultaneous with impedance-pH monitoring in patients with GERD. Developing a prediction model of AF risk in patients with GERD using HRV, impedance-pH and echocardiography parameters will be of great importance in clinical practice.

I will prospectively assess sympatho-vagal imbalance through HRV parameters (in time and frequency domain) by 24 hour electrocardiogram (ECG) HOLTER monitoring in patients with/without GERD, off acid-suppression therapy with proton pomp inhibitors. I will prospectively include patients with GERD symptoms (study group) and patients without GERD (control group) referred to Clinic Emergency Military Hospital of Iaşi during at least 15 months. The patients will be successively included based on both clinical symptoms and upper gastrointestinal endoscopy by a joint team consisting of gastroenterologist and cardiologist.

Gastroesophageal reflux disease will be diagnosed by the gastroenterologist based on clinical symptoms (heartburn and/or regurgitation of at least 2-3 time/week perceived as "troublesome" of the patient) and by upper gastrointestinal endoscopy (esophagitis, Barrett esophagus). Therapy with proton pump inhibitors (if any before inclusion in the study) will be interrupted 8 weeks before the inclusion in the study.

Understanding potential atrial arrhythmogenic mechanisms in patients with GERD, such as impaired autonomic stimulation and mechanical irritation due to anatomical

proximity of the structural remodeled left atrium and the esophagus is extremely important in the global multimodality treatment strategies to improve outcomes in patients with AF.

I expect that in patients with GERD esophageal acid exposure will determines changes in impedance-pH parameters and also simultaneous HRV parameters variation. To assess the degree of these changes could bring information related to AF risk in patients with GERD. Baseline clinical and reflux parameters off and on therapy with proton pomp inhibitors (known with pro and antiarrhythmic capacities) may provide important clinical clues to association of GERD with AF and especially AF risk decreasing in these patients. Treatment of GERD to avoid AF or to reduce AF burden might represent a future treatment perspective.

Developing a risk prediction model of AF in patients with GERD may identify high-risk individuals more likely to benefit from preventive measures in order to decrease the morbi-mortality of the most frequent arrhythmia in clinical practice. Obviously this objective will determine important social and economic consequences. A multicentric study as the first step to a national registry with patients with GERD with/without AF would be a great importance for future cohort studies and a better understanding of this association.

4. Non-valvular atrial fibrillation risk in patients with vitamin D deficiency

Nonvalvular AF and vitamin D insufficiency are common public health problems, very often associated with severe and life threatening comorbidities. Recent research has linked inadequate vitamin D status to cardiovascular diseases and overall mortality (349-352). Vitamin D deficiency could have direct electromechanical effects on the left atrium, which could increase the incidence of AF about two fold (349).

Heart rate variability techniques are used to explore the role of autonomic nervous system alterations in disease mechanisms, especially those conditions, like atrial fibrillation, in which sympathovagal factors are thought to play an important role (350, 351). We aimed to compare prospectively atrial arrhytmogenicity risk of patients with/without vitamin D deficiency, without atrial fibrillation history. All patients will undergoing 24 hour holter monitoring for heart rate variability measurement as non-invasive method of atrial vulnerability and assessment of 25 hydroxyvitamin D level with a chemiluminescence assay. Besides clinical and osteodensitometry parameters, echocardiographic data like left atrium and left ventricle size and functions will be assessed. Other imunologic or biochemical parameters will be measured to serve as exclusion criteria or to analyse better vitamin D deficiency occurence.

Univariate and multivariate analysis associated with ROC curves for cut-off values will be used for assessing this relationship between vitamin D level and heart rate variability in terms of time and frequency domain parameters. A risk score for atrial fibrillation in patients with vitamin D deficiency based on clinical, biological and echocardiografic parameters could be created. Initiation of the strategies for enhancement of vitamin D status in the population could lower AF risk if a causal link between low vitamin status and atrial vulnerability would be demonstrated, enabling prevention or termination of atrial fibrillation.
. Patients with/without vitamin D deficiency and without of AF will undergo detailed anamnesis, biological exams, osteodensitometry, 24 hour holter monitoring and

echocardiography will be done in all included patients. Medical history and physical exam will be performed in all patients. All parameters included in risk scores and biologic data of the patients will be obtained by medical history and preoperative routine exams.
The biological parameters, echocardiographic data, tTime and frecquency domain parameters of heart rate variability will be performed. The presence and degree of severity of osteoporosis will be established by osteodensitometry.

8.2. Perspectives in professional activity

Medical education must address and ensure the adequate integrated development of all aspects of a future physician's emerging professional identity. Apart from building a substantial foundation of knowledge and understanding, the teaching act must enhance communication skills and emotional intelligence by occasioning demonstrations, simulations and guided practice including soft skills, underlying attitudes, and ethical behaviors that are relevant and applicable in clinical settings, but also more broadly in society, e.g. clinical examination and oral presentation skills, emergency/crisis management skills etc. To be successful in such a complex undertaking, one must embrace the challenges and opportunities of local/national/international collaboration and partnerships across the spectrum of stakeholders

As a seasoned professional and faculty member, I enlist myself as an active promoter of the following clinical, scientific and academic perspectives and specific enterprises:

- facilitating residents (the futures PhD students) access to ultrasonography as a necessary tool for a thorough clinical examination;
- encouraging and supervising junior research initiatives by graduating students, manifested by their taking part in clinical studies and reporting on such work in their graduation theses;
- collaborating with other research centers in the country and abroad, ensuring the kind of information and experience exchange which underpins high quality research;
- facilitating interdisciplinary research by carrying out joint projects both within the Faculty of Medicine and within the University (also with students from the Faculty of Pharmacy and the Faculty of Medical Bioengineering);
- integrating residents into complex research projects and teams such as the different studies carried out in the clinic, doctoral projects, studies of drug companies etc.

In doing so, I will be pursuing one of my main educational goals, which is to make cardiology more accessible and attractive to both residents and students. Specifically, my intention is to familiarize medical students with ultrasonography, in particular with Focused Cardiac Ultrasound Imaging - FOCUS and Point-of-Care Ultrasound - POCUS. Recent guidelines emphasize that these imaging techniques should be routinely performed in order to complete the standard physical exam (354). Moreover, there are explicit recommendations to include FOCUS and POCUS in the medical curriculum. Importantly, several authors highlight that introducing ultrasound in undergraduate medical education automatically calls for the implementation of a peer-mentoring system, by which senior students guide their junior peers and alleviate some of the existing shortage of readily available senior teachers

(354). Taking into consideration the development of the newer hand-held pocket ultrasonography devices (353), access to ultrasonography will grow, directly mirroring the increasing needs in both medical education and patient care.

The Electrocardiography and Echocardiography Student and Resident Research Group might become an important source for futures PhD students. Currently, to adapt to the overwhelmingly positive response and demand, we are holding separate sessions for beginners and for advanced, twice a week, with two of the founding members now actively teaching sessions for beginners. In the advanced sessions, we alternate, holding an ECG meeting one week and an echocardiography session the following week. We are also organizing both ECG and practical hands-on FOCUS ultrasound workshops.

To summarize, my goals regarding futures PhD students are:

- teaching ECG and cardiac ultrasound to residents in a friendly environment; encouraging residents' initiatives and projects, such as writing research projects; showcasing the role of cardiac ultrasound in completing routine clinical examination, as recommended by current guidelines; bringing the idea of FOCUS and POCUS ultrasound examination techniques closer to the residents; showcasing the importance of teaching cardiac ultrasound to residents and its usefulness in understanding cardiac anatomy, physiology and semiology;
- encouraging participants to teach each other; facilitating the access of residents to summer practice scholarships abroad in various Arrhythmology Departments.

For the near future, I have two projects for the Electrocardiography and Echocardiography Student and Resident Research Group:

1. Creation of a research group by selecting among the residents involved in the Electrocardiography and Echocardiography Student and Resident Research Group. The main concern will be ultrasonography. In this regard, during the Congressis 2020 Edition, on 4th April, we will be holding Chest Ultrasound Masterclass with two special guests: doctor Natalia Buda of the Medical University of Gdańsk, Poland and doctor Tudor Toma, Consultant respiratory physician, Senior Clinical Lecturer, University Hospital Lewisham and Greenwich, King's College Medical School, London, United Kingdom.

   Dr. Natalia Buda is an Internal Medicine specialist who has started a similar activity to our Circle, teaching ultrasonography to medical students at her university and encouraging a similar peer-teaching scheme involving the more experienced students as teachers in her stead after having mastered the basics.

   Dr. Tudor Toma is a Respiratory Medicine Specialist who also focuses on the use of ultrasound in managing and treating patients; he is interested in popularizing the use of ultrasound among young residents and students. We have worked together and published a book on Ultrasound, including a chapter on cardiac ultrasound, entitled "Ecografia clinică a toracelui".

2. Creation of an echocardiography laboratory dedicated to clinical research in which residents with aptitudes for clinical studies will work. I will continue the activities of the

Electrocardiography and Echocardiography Student and Resident Research Group that will engage the residents interested in scientific research.

This framework will facilitate the mainstreaming of research as an activity of ongoing interest to the community by providing the necessary perspective, standards, and means. In this setting, resources and information will be shared through practical demonstrations about how to research and appraise information (critical reading, using specialized search engines and databases), how to write a research project or present a scientific paper (oral communication skills, public speaking, argumentation), how to work in a team (maximizing meeting time, brainstorming).

Collaboration between students, residents, young physicians and teachers in an organized environment, by combining enthusiasm and capacity with professional experience, establishes the prerequisites for achieving high results.

The registries are very useful for further clinical, epidemiological and cost controle studies. A multicentric national study may be developed using a pilot study. A local and than a national registry of patients with AF I want to develop.

IV. REFERENCES

1. Haissaguerre M, Jais P, Shah DC, et al. Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins. N Engl J Med. 1998;339:659-666.
2. Jahangir A, Lee V, Friedman PA, et al. Long-term progression and outcomes with aging in patients with lone atrial fibrillation: A 30-year follow-up study. Circulation 2007;115:3050-3056.
3. Schoonderwoerd BA, Van Gelder IC, Van Veldhuisen DJ, et al. Electrical and structural remodeling: Role in the genesis and maintenance of atrial fibrillation. Prog Cardiovasc Dis. 2005;48:153-168.
4. Wijffels MC, Kirchhof CJ, Dorland R, et al. Atrial fibrillation begets atrial fibrillation. A study in awake chronically instrumented goats. Circulation. 1995; 92:1954–68.
5. Allessie M, Ausma J, Schotten U. Electrical, contractile and structural remodeling during atrial fibrillation. Cardiovasc Res. 2002;54:230-246.
6. Shiroshita-Takeshita A, Brundel BJ, Nattel S. Atrial fibrillation: Basic mechanisms, remodeling and triggers. J Interv Card Electrophysiol 2005; 13:181-193.
7. Brundel BJ, Henning RH, Kampinga HH, et al. Molecular mechanisms of remodeling in human atrial fibrillation. Cardiovasc Res 2002;54:315-324.
8. Communal C, Sumandea M, De Tombe P, et al. Functional consequences of caspase activation in cardiac myocytes. Proc Natl Acad Sci USA 2002;99:6252–6256.
9. Zhang Z, Biesiadecki BJ, Jin JP. Selective deletion of the NH2-terminal variable region of cardiac troponin T in ischemia reperfusion by myofibril-associated mu-calpain cleavage. Biochemistry 2006;45:11681–11694.
10. Brundel BJJM, Ke L, Dijkhuis AJ, et al. Heat shock proteins as molecular targets for intervention in atrial fibrillation. Cardiovascular Research 2008; 78: 422–428.
11. Allessie MA, Boyden A, Camm J, et al. Pathophysiology of Atrial Fibrillation. Circulation 2001;103 :769.
12. Dai Y, Wang X, Cao L, et al. Expression of extracellular signal regulated kinase and angiotensin-converting enzyme in human atria during atrial fibrillation. J Huazhong Univ Sci Technolog Med Sci 2004; 24:32.
13. Corradi D, Callegari S, Benussi S, et al. Regional left atrial interstitial remodeling in patients with chronic atrial fibrillation undergoing mitral-valve surgery. Virchows Archiv 2004; 445(5):498-505.
14. Lang RM, Biering M, Devereux RB et al. Recommendations for chamber quatification. American Society of Echocardiography and European Association of Echocardiography. Eur J Echocardiography 2006; 7:79-108.
15. Lang RM, Badano LP, Mor-Avi V, 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;16(3):233-70.
16. Fuster V, Rydén LE, Cannom DS, et al. ACC/AHA/ESC 2006 guidelines for the management of patients with atrial fibrillation-executive summary: A report of the American College of Cardiology/American Heart Association Task Force on practice guidelines and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Revise the 2001 Guidelines for the Management of Patients with Atrial Fibrillation). Eur Heart J 2006; 27:1979-2030.
17. Kirchhof P, Benussi S, Kotecha D, et al. ESC Scientific Document Group. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J 2016;37(38):2893-2962.

18. Knackstedt C, Visser L, Plisiene J, et al. Dilatation of the pulmonary veins in atrial fibrillation: a transesophageal echocardiographic evaluation. Pacing. Clin Electrophysiol 2003;26:1371-8.
19. Paulus WJ, Tschöpe C, Sanderson JE, et al. How to diagnose diastolic heart failure: a consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the Heart Failure and Echocardiography Associations of the European Society of Cardiology. Eur Heart J 2007; 28: 2539–2550.
20. Thomas L, Levett K, Boyd A, Leung DYC, Schiller NB, Ross DL. Changes in regional Lleft atrial function with aging/ evaluation by Doppler tissue imaging. Eur J Echocardiogr 2003;4:92-100.
21. Di Salvo G, Caso P, Lo Piccolo R, et al. Atrialmyocardial deformation properties predict maintenance of sinus rhythm after external cardioversion of recent-onset lone atrial fibrillation: a color Doppler myocardial imaging and transthoracic and transoesophagean echocardiographic study. Circulation 2005; 112:387-95.
22. Kojima T, Kawasaki M, Tanaka R, 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 J Echocardiogr 2012. 13:227-34
23. Muranaka A,Yuda S, Tsuchihashi K, et al. Quantitative Assessment of Left Ventricular and Left Atrial Functions by Strain Rate Imaging in Diabetic Patients with and without Hypertension. Echocardiography 2009;26:262-71.
24. Altman DG, Bland JM. Absence of evidence is not evidence of absence. BMJ 1995; 311-485.
25. Hirose T, Kawasaki M, Tanaka R, et al. Left atrial function assessed by speckle tracking echocardiography as a predictor of new-onset non-valvular atrial fibrillation: results from a prospective study in 580 adults. Eur J Echocardiogr 2012; 13:243-50.
26. Abhayaratna WP, Fatema K, Barnes ME, 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 2008;101:1626-9.
27. Evangelista A, Flachskampf F, Lancellotti P, et al. European Association of Echocardiography recommendations for standardization for performance, digital storage and reporting of echocardiographic studies. Eur J Echocardiography 2008;9:438.
28. Camm AJ, Kirchhof P, Lip GY, et al. Guidelines for the management of atrial fibrillation: the Task Force for the Management of Atrial Fibrillation of the European Society of Cardiology (ESC). Eur Heart J 2010; 31: 2369-2429.
29. Miyoshi H, Oishi Y, Mizuguchi Y et al. Association of left atrial reservoir function with left atrial structural remodeling related to left ventricular dysfunction in asymptomatic patients with hypertension: evaluation by two-dimensional speckle-tracking echocardiography. Clin Exp Hypertens 2015;37(2):155-65.
30. Miyoshi H, Oishi Y, Mizuguchi Y et al. Influence of comorbid cardiovascular risk factors on left atrial-left ventricular interaction in asymptomatic patients: clinical application of two-dimensional speckle-tracking echocardiography. Int Heart J 2014;55(2):138-45.
31. Kobayashi Y, Okura H, Kobayashi Y et al. Assessment of Atrial Synchrony in Paroxysmal Atrial Fibrillation and Impact of Pulmonary Vein Isolation for Atrial Dyssynchrony and Global Strain by Three-Dimensional Strain Echocardiography. J Am Soc Echocardiogr 2014 ; 27(11):1193-9.
32. McGann C, Akoum N, Patel A, et al. Atrial fibrillation ablation outcome is predicted by left atrial remodeling on MRI. Circ Arrhythm Electrophysiol 2014;7(1):23-30.

33. Schaaf M, Andre P, Altman M, et al. Left atrial remodelling assessed by 2D and 3D echocardiography identifies paroxysmal atrial fibrillation. Eur Heart J Cardiovasc Imaging 2017;18(1):46-53.
34. Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr 2009; 22:107-33.
35. Mochizuki A, Yuda S, Oi Y, et al. Assessment of left atrial deformation and synchrony by three-dimensional speckle-tracking echocardiography: comparative studies in healthy subjects and patients with atrial fibrillation. J Am Soc Echocardiogr 2013;26:165–74.
36. Wu VC, Takeuchi M, Otani K, et al. Effect of through-plane and twisting motion on left ventricular strain calculation: direct comparison between two-dimensional and three-dimensional speckle-tracking echocardiography. J Am Soc Echocardiogr 2013;26:1274–81.
37. Bisbal F, Guiu E, Calvo N, et al. Left atrial sphericity: a new method to assess atrial remodeling. Impact on the outcome of atrial fibrillation ablation. J Cardiovasc Electrophysiol 2013;24:752–9.
38. Aksu T, Güler TE, Mutluer FO, Oto MA. Vagal denervation in atrial fibrillation ablation: A comprehensive review. Anatol J Cardiol 2017;18(2):142-148.
39. Pappone C, Oreto G, Rosanio S, et al. Atrial electroanatomic remodeling after circumferential radiofrequency pulmonary vein ablation: Efficacy of an anatomic approach in a large cohort of patients with atrial fibrillation. Circulation 2001;104:2539-2544.
40. Hou Y, Scherlag BJ, Lin J, et al. Ganglionated plexi modulate extrinsic cardiac autonomic nerve input: effects on sinus rate, atrioventricular conduction, refractoriness, and inducibility of atrial fibrillation. J Am Coll Cardiol 2007; 50:61-68.
41. Hirose M, Leatmanoratn Z, Laurita KR, Carlson MD. Partial vagal denervation increases vulnerability to vagally induced atrial fibrillation. J Cardiovasc Electrophysiol 2002; 13:1272-1279.
42. Oh S, Zhang Y, Bibevski S, Marrouche NF, Natale A, Mazgalev TN. Vagal denervation and atrial fibrillation inducibility: Epicardial fat pad ablation does not have long-term effects. Heart Rhythm 2006; 3:701-708.
43. Lemery R, Birnie D, Tang AS, Green M, Gollob M. Feasibility study of endocardial mapping of ganglionated plexuses during catheter ablation of atrial fibrillation. Heart Rhythm 2006;3:387-396.
44. Nakagawa H, Jackman WM, Scherlag BJ, Lazzara R. Pulmonary vein isolation during atrial fibrillation: Insight into the mechanism of pulmonary vein firing. J Cardiovasc Electrophysiol 2003;14:261-262.
45. Hsieh MH, Chiou CW, Wen ZC, et al. Alterations of heart rate variability after radiofrequency catheter ablation of focal atrial fibrillation originating from pulmonary veins. Circulation 1999;100:2237-2243.
46. Scherlag BJ, Nakagawa H, Jackman WM, et al. Electrical stimulation to identify neural elements on the heart: Their role in atrial fibrillation. J Interv Card Electrophysiol 2005;13:37-42.
47. Calkins H, Brugada J, Packer DL, et al. Heart Rhythm Society, European Heart Rhythm Association, European Cardiac Arrhythmia Society, American College of Cardiology, American Heart Association; Society of Thoracic Surgeons: HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of atrial fibrillation: Recommendations for personnel, policy, procedures and follow-up. A report of the Heart Rhythm Society (HRS) task force on catheter and surgical ablation of atrial fibrillation. Heart Rhythm 2007;9:335-379.

48. La Meir M, De Roy L, Blommaert D, Buche M. Treatment of lone atrial fibrillation with a right thoracoscopic approach. Ann Thorac Surg 2007;83:2244-2245.
49. van den Berg MP, Haaksma J, Brouwer J, Tieleman RG, Mulder G, Crijns HJ. Heart rate variability in patients with atrial fibrillation is related to vagal tone. Circulation 1997;96:1209-1216.
50. Kirsh JA, Sahakian AV, Baerman JM, Swiryn S. Ventricular response to atrial fibrillation: Role of atrioventricular conduction pathways. J Am Coll Cardiol 1988;12:1265-1272.
51. Quan KJ, Lee JH, Van Hare GF, Biblo LA, Mackall JA, Carlson MD. Identification and characterization of atrioventricular parasympathetic innervation in humans. J Cardiovasc Electrophysiol 2002;13:735-739.
52. Shah P, Ellenbogen KA,Wood MA,Gilligan DM, Dan D. Identification of preferential sites of parasympathetic input to the atrioventricular node in man. Heart Rhythm 2004;1:27-32.
53. Sakamoto S, Schuessler RB, Lee AM, Aziz A, Lall SC, Damiano RJ Jr. Vagal denervation and reinnervation after ablation of ganglionated plexi. J Thorac Cardiovasc Surg 2010;139:444-452.
54. Patel AM, d'Avila A, Neuzil P et al. Atrial tachycardia after ablation of persistent atrial fibrillation: Identification of the critical isthmus with a combination of multielectrode activation mapping and targeted entrainment mapping. Circ Arrhythm Electrophysiol 2008;1:14-22.
55. Nayebpour M, Talajic M, Villemaire C, Nattel S. Vagal modulation of the rate-dependent properties of the atrioventricular node. Circ Res 1990;67:1152-1166.
56. Calò L, Martino A. Insidious coronary artery disease: cause, consequence, or innocent bystander for idiopathic atrial fibrillation? Heart Rhythm 2012; 9: 1930-1931
57. Reddy YM, Singh D, Nagarajan D et al. Atrial fibrillation ablation in patients with gastroesophageal reflux disease or irritable bowel syndrome-the heart to gut connection! J Interv Card Electrophysiol 2013; 37: 259-265.
58. Velagapudi P, Turagam MK, Leal MA, Kocheril AG. Atrial fibrillation and acid reflux disease. Clin Cardiol 2012; 35: 180-186.
59. Kirchhof P, Lip GY, Van Gelder IC et al. Comprehensive risk reduction in patients with atrial fibrillation: emerging diagnostic and therapeutic options--a report from the 3rd Atrial Fibrillation Competence NETwork/European Heart Rhythm Association consensus conference. Europace 2012;14: 8-27.
60. Vakil N, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. Am J Gastroenterol 2006; 101:1900-1920.
61. January TC, Wann LS, Alpert JS, et al. American College of Cardiology/American Heart Association Task Force on Practice Guidelines: 2014 AHA/ACC/HRS Guideline for the Management of Patients With Atrial Fibrillation: Executive Summary. JACC 2014; 64: 2246-80.
62. Roman C, Bruley des Varannes S, Muresan L, Picos A, Dumitrascu DL. Atrial fibrillation in patients with gastroesophageal reflux disease: A comprehensive review. World J Gastroenterol 2014; 20:9592-9.
63. Pumprla J, Howorka K, Groves D, Chester M, Nolan J. Functional assessment of heart rate variability: Physiological basis and practical applications. Int J Cardiol 2002; 84: 1-14.
64. Calkins H, Kuck KH, Cappato R, et al. 2012 HRS/EHRA/ECAS Expert Consensus Statement on Catheter and Surgical Ablation of Atrial Fibrillation: Recommendations for

Patient Selection, Procedural Techniques, Patient Management and Follow-up, Definitions, Endpoints, and Research Trial Design. Europace 2012; 14: 528-606.
65. Dewland TA, Vittinghoff E, Harris TB, et al. Inflammation as a mediator of the association between race and atrial fibrillation. Results from the Health ABC Study (Health, Aging, and Body Composition). JACC Clin Electrophysiol 2015; 1: 248-55.
66. Bunch TJ, Packer DL Jahangir A, et al. Long-term risk of atrial fibrillation with symptomatic gastroesophageal reflux disease and esophagitis. Am J Cardiol 2008; 102: 1207-11.
67. Montenero AS, Mollichelli N, Zumbo F, et al. Helicobacter pylori and atrial fibrillation: a possible pathogenic link. Heart 2005; 91:960-1.
68. Chen CL, Orr WC, Yang CC, Kuo TB. Cardiac autonomic regulation differentiates reflux disease with and without erosive esophagitis. Scand J Gastroenterol 2006; 41: 1001-6.
69. Lee YC, Wang HP, Lin LY, et al. Heart rate variability in patients with different manifestations of gastroesophageal reflux disease. Auton Neurosci 2004; 116: 39-45.
70. Hillebrand S, Swenne CA, Gast KB et al. The role of insulin resistance in the association between body fat and autonomic function. Nutr Metab Cardiovasc Dis 2015; 25: 93-9.
71. Dobrek L, Nowakowski M, Mazur M, Herman RM, Thor PJ. Disturbances of the parasympathetic branch of the autonomic nervous system in patients with gastroesophageal reflux disease (GERD) estimated by short-term heart rate variability recordings. J Physiol Pharmacol 2004; 55(Suppl 2): 77-90.
72. Dobrek L, Nowakowski M, Syguła A et al. 24-hour heart rate variability in patients with gastroesophageal reflux disease. Folia Med Cracov 2005; 46: 53-64.
73. Błaut U, Dobrek Ł, Laskiewicz J, Thor PJ. Disturbances of the autonomic nervous system in gastroesophageal reflux disease. Folia Med Cracov 2001; 42: 63-73.
74. Stollberger C, Finsterer J. Treatment of esophagitis/vagitis-induced paroxysmal atrial fibrillation by proton-pump inhibitors. J Gastroenterol 2003; 38: 1109.
75. Milovanovic B, Filipovic B, Mutavdzin S, et al. Cardiac autonomic dysfunction in patients with gastroesophageal reflux disease. World J Gastroenterol 2015; 21: 6982-9.
76. Linz D, Hohl M, Vollmar J, Ukena C, Mahfoud F, Böhm M. Atrial fibrillation and gastroesophageal reflux disease: the cardiogastric interaction. Europace 2016; 19(1):16-20.
77. Hirsh BJ, Copeland-Halperin RS, Halperin JL. Fibrotic atrial cardiomyopathy, atrial fibrillation, and thromboembolism: Mechanistic links and clinical inferences. J Am Coll Cardiol 2015;65:2239–2251.
78. Kapłon-Cieślicka A, Budnik M, Gawałko M, et al. Atrial fibrillation type and renal dysfunction as important predictors of left atrial thrombus. Heart 2019;105(17):1310-1315.
79. Steinberg BA, Hellkamp AS, Lokhnygina Y, et al. ROCKET-AF Steering Committee and Investigators. Higher risk of death and stroke in patients with persistent vs. paroxysmal atrial fibrillation: results from the ROCKET-AF Trial. Eur Heart J 2015;36:288-96.
80. Lilli A, Di Cori A, Zacà V. Thromboembolic risk and effect of oral anticoagulation according to atrial fibrillation patterns: A systematic review and meta-analysis. Clin Cardiol 2017;40:641-647.
81. Masson P, Webster AC, Hong M, et al. Chronic kidney disease and the risk of stroke: a systematic review and meta-analysis. Nephrol Dial Transplant 2015;30:1162-9.
82. Jia G, Aroor AR, Sowers JR. Arterial Stiffness: A Nexus between Cardiac and Renal Disease. Cardiorenal Med 2014;4:60-71.
83. Piccini JP, Stevens SR, Chang YC, et al. for the ROCKET AF Steering Committee and Investigators. Renal dysfunction as a predictor of stroke and systemic embolism in patients with nonvalvular atrial fibrillation: validation of the R2CHADS2 index in the

ROCKET AF (Rivaroxaban Once-daily, oral, direct factor Xa inhibition Compared with vitamin K antagonism for prevention of stroke and Embolism Trial in Atrial Fibrillation) and ATRIA (AnTicoagulation and Risk factors In Atrial fibrillation) Study Cohorts. Circulation 2013;127:224–232.
84. Barra S, Providência R, Faustino C, et al. Performance of the Cockcroft-Gault, MDRD and CKD-EPI Formulae in Non-Valvular Atrial Fibrillation: Which one Should be Used for Risk Stratification? J Atr Fibrillation 2013;6:896.
85. Natale A, Raviele A, Arentz T, et al. Venice Chart international consensus document on atrial fibrillation ablation. J Cardiovasc Electrophysiol 2007;18:560-580.
86. Flachskampf FA, Decoodt P, Fraser AG, Daniel WG, Roelandt JRTC; for the Subgroup on Transesophageal Echocardiography, Valvular Heart Disease, on behalf of theWorking Group on Echocardiography of the European Society of Cardiology: Recommendations for performing transesophageal echocardiography. Eur J Echocardiogr 2001;2:8-21.
87. Flachskampf FA, Badano L, Daniel WG, et al. European Association of Echocardiography; Echo Committee of the European Association of Cardiothoracic Anaesthesiologists. Recommendations for transoesophageal echocardiography: Update 2010. Eur J Echocardiogr 2010;11:557-576.
88. Aschenberg W, Schluter M, Kremer P, Schroder E, Siglow V, Bleifeld W. Transesophageal two-dimensional echocardiography for the detection of left atrial appendage thrombus. J Am Coll Cardiol 1986;7:163-166.
89. Puwanant S, Varr BC, Shrestha K et al. Role of the CHADS2 score in the evaluation of thromboembolic risk in patients with atrial fibrillation undergoing transesophageal echocardiography before pulmonary vein isolation. J Am Coll Cardiol 2009;54:2032-2039.
90. Dorekamp M, Sohns C, Vollmann D, et al. Detection of left atrial thrombus during routine diagnostic work-up prior to pulmonary vein isolation for atrial fibrillation: Role of transoesophageal echocardiography and multidetector computed tomography. Int J Cardiol 2013; 163(1):26-33.
91. Wazni OM, Tsao HM, Chen SA, et al. Cardiovascular imaging in the management of atrial fibrillation. J Am Coll Cardiol 2006;48:2077-2084.
92. Rader VJ, Khumri TM, Idupulapati M, Stoner CN, Magalski A, Main ML. Clinical predictors of left atrial thrombus and spontaneous echocardiographic contrast in patients with atrial fibrillation. J Am Soc Echocardiogr 2007;20:1181-1185.
93. Agmon Y, Khandheria BK, Meissner I, et al. Are left atrial appendage flow velocities adequate surrogates of global left atrial function? A population-based transthoracic and transesophageal echocardiographic study. J Am Soc Echocardiogr 2002;15:433-440.
94. McCready JW, Nunn L, Lambiase PD et al. Incidence of left atrial thrombus prior to atrial fibrillation: Is pre-procedural transoesophageal echocardiography mandatory? Europace 2010;12:927-932.
95. Wallace TW, Atwater BD, Daubert JP, et al. Prevalence and clinical characteristics associated with left atrial appendage thrombus in fully anticoagulated patients undergoing catheter-directed atrial fibrillation ablation. J Cardiovasc Electrophysiol 2010;21:849-852.
96. Gopinath D, LewisWR, Di Biase L, Natale A. Pulmonary vein antrum isolation for atrial fibrillation on therapeutic coumadin: Special considerations. J Cardiovasc Electrophysiol 2011;22:236-239.
97. Lip GY, Frison L, Halperin JL, Lane DA. Identifying patients at high risk for stroke despite anticoagulation. A comparison of contemporary stroke risk stratification schemes in an anticoagulated atrial fibrillation cohort. Stroke 2010;41:2731–2738.

98. Staa TP, Setakis E, Di Tanna GL, Lane DA, Lip GY. A comparison of risk stratification schema for stroke in 79884 atrial fibrillation patients in general practice. J Thromb Haemost 2011;9:39–48.
99. Scherr D, Dalal D, Chilukuri K, et al. Incidence and predictors of left atrial thrombus prior to catheter ion of atrial fibrillation. J Cardiovascular Electrophysiol. 2009;20(4):379–384.
100. Raviele A, Natale A, Calkins H, et al. Venice chart international consensus document on atrial fibrillation ablation: 2011 update. J Cardiovasc Electrophysiol 2012 ;23(8):890-923.
101. Zhu WG, Xiong QM, Hong K. Meta-analysis of CHADS2 versus CHA2DS2-VASc for predicting stroke and thromboembolism in atrial fibrillation patients independent of anticoagulation. Tex Heart Inst J 2015;42(1):6-15.
102. Gunawardene MA, Dickow J, Schaeffer BN, et al. Risk stratification of patients with left atrial appendage thrombus prior to catheter ablation of atrial fibrillation: An approach towards an individualized use of transesophageal echocardiography. J Cardiovasc Electrophysiol 2017;28(10):1127-1136.
103. Laish-Farkash A, Suleiman M. Evaluation of left atrial thrombus prior to catheter ablation of atrial fibrillation: Is it time for an individualized approach? J Cardiovasc Electrophysiol 2017;28(10):1137-1139.
104. Spronk HM, De Jong AM, Verheule S, et al. Hypercoagulability causes atrial fibrosis and promotes atrial fibrillation. Eur Heart J 2017;38(1):38-50.
105. Daccarett M, Badger TJ, Akoum N, et al. Association of left atrial fibrosis detected by delayed-enhancement magnetic resonance imaging and the risk of stroke in patients with atrial fibrillation. J Am Coll Cardiol 2011;57(7):831-8.
106. Nedios S, Koutalas E, Sommer P, et al. Asymmetrical left atrial remodelling in atrial fibrillation: relation with diastolic dysfunction and long-term ablation outcomes. Europace 2017;19(9):1463-1469.
107. Lo LW, Chen SA. Cardiac Remodeling After Atrial Fibrillation Ablation. J Atr Fibrillation 2013;6(1):877.
108. Shrivastava R, Smith B, Caskey D, Reddy P. Atrial fibrillation after cardiac surgery: does prophylactic therapy decrease adverse outcomes associated with atrial fibrilation. J Intensive Care Med 2009;24:18.
109. Mahoney EM, Thomson TD, Veledar E, Williams J, Weintraub WS. Cost-effectiveness of targeting patients undergoing cardiac surgery for therapy with intravenous amiodarone to prevent atrial fibrillation. J Am Coll Cardiol 2002;40:737-45.
110. Aranki SF, Shaw DP, Adams DH, Rizzo RJ, Couper GS, VanderVliet M, et al. Predictors of atrial fibrillation after coronary artery surgery. Current trends and impact on hospital resources. Circulation 1996;94:390-7.
111. Zaman AG, Archbold RA, Helft G, Paul EA, Curzen NP, Mills PG. Atrial fibrillation after coronary artery bypass surgery: a model for preoperative risk stratification. Circulation 2000;101:1403-8.
112. Lomivorotov VV, Efremov SM, Pokushalov EA, Karaskov AM. New onset atrial fibrillation after cardiac surgery: pathophysiology, prophylaxis and treatment. J Cardiothorac Vasc Anesth 2016;1:200-16.
113. Archbold RA, Schilling RJ. Atrial pacing for the prevention of atrial fibrillation after coronary artery bypass graft surgery: a review of the literature. Heart 2004; 90:129-133.
114. Benjo A, Garcia D, Kumar S, Garcia W, Macedo FY, Benjo C, et al. Post-operative Biatrial Pacing Consistently Decreases Atrial Fibrillation in Cardiac Surgery: A Randomized-controlled Trial Meta-analysis. Circulation 2013;128: A17324.

115. Steinberg BA, Zhao Y, He X, et al. Management of postoperative atrial fibrillation and subsequent outcomes in contemporary patients undergoing cardiac surgery: Insights from the Society of Thoracic Surgeons CAPS-Care Atrial Fibrillation Registry. Clin Cardiol 2014;37:7-13.
116. Raiten JM, Ghadimi K, Augoustides JGT, et al. Atrial fibrillation after cardiac surgery: clinical update on mechanisms and prophylactic strategies. J Cardiothorac Vasc Anesth 2015;29(3):806-16.
117. Price J, Tee R, Lam BK, Hendry P, Green MS, Rubens FD. Current use of prophylactic strategies for postoperative atrial fibrillation: a survey of Canadian cardiac surgeons. Ann Thorac Surg 2009;88:106–10.
118. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC Jr, et al. 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation; a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. J Am Coll Cardiol. 2014;64(21):e1–76.
119. Zhang J, Goode KM, Rigby A, Balk AH, Cleland JG. Identifying patients at risk of death or hospitalisation due to worsening heart failure using decision tree analysis: evidence from Trans-European Network-Home Care Management System (TEN-HMS) study. Int J Cardiol 2013;163:149-56.
120. Biggs D, De Ville B, Suen E. A method of choosing multiway partitions for classification and decision trees. J Appl Stat 1991;18:49-62.
121. Tran DT, Perry JJ, Dupuis JY, Elmestekawy E, Wells GA. Predicting new-onset postoperative atrial fibrillation in cardiac surgery patients. J Cardiothorac Vasc Anesth 2015;29(5):1117-26.
122. Luo W, Huaibin W, Wenjun Z, Jie T, Xiaokang O, Zi W, Yujian M. Predictors of Postoperative Atrial Fibrillation after Isolated On-Pump Coronary Artery Bypass Grafting in Patients ≥60 Years Old. Heart Surg Forum 2017;20(1):E038-E042.
123. Sabzi F, Zokaei AH, Moloudi AR. Predictors of atrial fibrillation following coronary artery bypass grafting. Clin Med Insights Cardiol 2011;5:67-67.
124. El-Chami MF, Kilgo PD, Elfstrom KM, Halkos M, Thourani V, Lattouf OM, et al. Prediction of new onset atrial fibrillation after cardiac revascularization surgery. Am J Cardiol 2012;110:649-54.
125. Mariscalco G, Engstrom KG. Atrial fibrillation after cardiac surgery: Risk factors and their temporal relashionship in prophylactic drug strategy decision. Int J of Cardiol 2008;129:354-62.
126. Amar D, Shi W, Hogue CW, Zhang H, Passman RS, Thomas B, et al. Clinical prediction rule for atrial fibrillation after coronary artery bypass grafting. J Am Coll Cardiol 2004;44(6):1248-53.
127. Mathew JP, Fontes ML, Tudor IC, Ramsey J, Duke P, Mazer CD, et al. A multicenter risk index for atrial fibrillation after cardiac surgery. JAMA. 2004;291:1720-29.
128. Mariscalco G, Biancari F, Zanobini M, Cottini M, Piffaretti G, Saccocci M, et al. Bedside tool for predicting the risk of postoperative atrial fibrillation after cardiac surgery: The POAF Score. J Am Heart Assoc 2014;3:e000752.
129. Haghjoo M, Basiri H, Salek M, Sadr-Ameli MA, Kargar F, Raissi K, et al. Predictors of postoperative atrial fibrillation after coronary artery bypass graft surgery. Indian Pacing Electrophysiol J 2008;8:94-101.
130. Hashemzadeh K, Dehdilan M, Dehdilan M. Postoperative Atrial Fibrillation following Open Cardiac Surgery: Predisposing Factors and Complications. J Cardiovasc Thorac Res 2013;5:101-7.

131. Burgess DC, Kilbom MJ, Keech AC. Interventions for prevention of post-operative atrial fibrillation and its complications after cardiac surgery: a meta-analysis. Eur Heart J 2006;27:2846-57.
132. Mehaffey JH, Hawkins RB, Byler M, Smith J, Kern JA, Kron I, et al. Amiodarone Protocol Provides Cost Effective Reduction in Postoperative Atrial Fibrillation. Ann Thorac Surg 2018 Jan 24. pii: S0003-4975(18)30048-1.
133. De Keulenaer, G.W.; Brutsaert, D.L. Systolic and diastolic heart failure are overlapping phenotypes within the heart failure spectrum. Circulation 2011; 123, doi:10.1161/CIRCULATIONAHA.110.981431.
134. Aronow, W.S. Epidemiology, pathophysiology, prognosis, and treatment of systolic and diastolic heart failure in elderly patients. Heart Dis 2003; 5: 279–294.
135. Ponikowski P, Voors A.A, Anker S.D, 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 European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2016; 18: 891–975.
136. Brunner-La Rocca, H.P.; Sanders-van Wijk, S. Natriuretic Peptides in Chronic Heart Failure. Card Fail Rev 2019; 5: 44–49.
137. Fu S, Ping P, Wang F, Luo L. Synthesis, secretion, function, metabolism and application of natriuretic peptides in heart failure. J Biol Eng 2018; 12: doi:10.1186/s13036-017-0093-0.
138. Oikonomou E, Vogiatzi G, Tsalamandris, S, et al. Non-natriuretic peptide biomarkers in heart failure with preserved and reduced ejection fraction. Biomark Med 2018; 12: 783–797.
139. Meijers WC, van der Velde A.R, de Boer R.A. Biomarkers in heart failure with preserved ejection fraction. Neth. Heart J 2016; 24: 252–258.
140. D'Elia E, Vaduganathan M, Gori M, Gavazzi A, Butler J, Senni M. Role of biomarkers in cardiac structure phenotyping in heart failure with preserved ejection fraction: Critical appraisal and practical use. Eur J Heart Fail 2015; 17: 1231–1239.
141. Nadar S.K, Shaikh M.M. Biomarkers in Routine Heart Failure Clinical Care. Card Fail Rev 2019; 5: 50–56.
142. de Boer R.A, Nayor M, deFilippi C.R, et al. Association of Cardiovascular Biomarkers with Incident Heart Failure with Preserved and Reduced Ejection Fraction. JAMA Cardiol 2018; 3: 215–224.
143. Gruson D., Favvresse J. Peptides natriurétiques: Dégradation, formes circulantes, dosages et nouvelles approches thérapeutiques. Ann Biol Clin 2017; 75 : 259–267.
144. Maisel A.S, Duran J.M, Wettersten N. Natriuretic Peptides in Heart Failure: Atrial and B-type Natriuretic Peptides. Heart Fail Clin 2018; 14: 13–25.
145. Nishikimi T, Kuwahara K, Nakao K. Current biochemistry, molecular biology, and clinical relevance of natriuretic peptides. J Cardiol 2011; 57: 131–140.
146. Rahmutula D, Zhang H, Wilson E, Olgin J.E. Absence of natriuretic peptide clearance receptor attenuates TGF-b1-induced selective atrial fibrosis and atrial fibrillation. Cardiovasc Res 2019; 115: 357–372.
147. Calderone A, Thaik C.M, Takahashi N, Chang D.L, Colucci W.S. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. J Clin Investig 1998; 101: 812–818.
148. Volpe M, Carnovali M, Mastromarino V. The natriuretic peptides system in the pathophysiology of heart failure: From molecular basis to treatment. Clin Sci 2016; 130:57–77.

149. Matsuo A, Nagai-Okatani C, Nishigori M, Kangawa K, Minamino N. Natriuretic peptides in human heart: Novel insight into their molecular forms, functions, and diagnostic use. Peptides 2019; 111: 3–17.
150. Seewöster T, Büttner P, Nedios S, et al. Association Between Cardiovascular Magnetic Resonance-Derived Left Atrial Dimensions, Electroanatomical Substrate and NT-proANP Levels in Atrial Fibrillation. J Am Heart Assoc 2018; 7, e009427, doi:10.1161/JAHA.118.009427.
151. Nattel S. Natriuretic peptide receptors and atrial-selective fibrosis: Potential role in atrial fibrillation. Cardiovasc Res 2019;115: 258–260.
152. Wu C.K, Su M.M, Wu Y.F, Hwang J.J, Lin L.Y. Combination of Plasma Biomarkers and Clinical Data for the Detection of Myocardial Fibrosis or Aggravation of Heart Failure Symptoms in Heart Failure with Preserved Ejection Fraction Patients. J Clin Med 2018; 7: 427.
153. Zakeri R, Cowie M.R. Heart failure with preserved ejection fraction: Controversies, challenges and future directions. Heart 2018; 104: 377–384.
154. Reddy Y.N.V, Carter R.E, Obokata M, Redfield M.M, Borlaug B.A. A Simple, Evidence-Based Approach to Help Guide Diagnosis of Heart Failure with Preserved Ejection Fraction. Circulation 2018; 138: 861–870.
155. Michalska-Kasiczak M, Bielecka-Dabrowa A, von Haehling S, Anker S.D, Rysz J, Banach M. Biomarkers, myocardial fibrosis and co-morbidities in heart failure with preserved ejection fraction: An overview. Arch Med Sci 2018; 14: 890–909.
156. Cui K, Huang W, Fan J, Lei H. Midregional pro-atrial natriuretic peptide is a superior biomarker to N-terminal pro-B-type natriuretic peptide in the diagnosis of heart failure patients with preserved ejection fraction. Medicine 2018; 97: e12277, doi:10.1097/MD.0000000000012277.
157. Paulus W.J, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: Comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol 2013; 62: 263–271.
158. van Heerebeek L, Hamdani N, Falcão-Pires I, et al. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation 2012; 126: 830–839.
159. Maisel A, Mueller C, Nowak R.M, et al. Midregion prohormone adrenomedullin and prognosis in patients presenting with acute dyspnea: Results from the BACH (Biomarkers in Acute Heart Failure) trial. J Am Coll Cardiol 2011; 58: 1057–1067.
160. Zheng S.L, Chan F.T, Nabeebaccus A.A, et al. Drug treatment effects on outcomes in heart failure with preserved ejection fraction: A systematic review and meta-analysis. Heart 2018; 104: 407–415.
161. Salo P.P, Havulinna A.S, Tukiainen T, et al. Genome-Wide Association Study Implicates Atrial Natriuretic Peptide Rather Than B-Type Natriuretic Peptide in the Regulation of Blood Pressure in the General Population. Circ Cardiovasc Genet 2017; 10, e001713, doi:10.1161/CIRCGENETICS.117.001713.
162. Hausfater P, Claessens Y.E, Martinage A, et al. Prognostic value of PCT, copeptin, MR-proADM, MR-proANP and CT-proET-1 for severe acute dyspnea in the emergency department: The BIODINER study. Biomarkers 2017; 22: 28–34.
163. Kang S.H, Park J.J, Choi D.J, et al. Prognostic value of NT-proBNP in heart failure with preserved versus reduced EF. Heart 2015; 101: 1881–1888.
164. Richards A.M, Januzzi J.L., Jr, Troughton R.W. Natriuretic peptides in heart failure with preserved ejection fraction. Heart Fail Clin 2014; 10: 453–470.
165. Anjan V.Y, Loftus T.M, Burke M.A, et al. Prevalence, clinical phenotype, and outcomes associated with normal B-type natriuretic peptide levels in heart failure with preserved ejection fraction. Am J Cardiol 2012; 110: 870–876.

166. Anand I.S, Rector T.S, Cleland J.G, et al. Prognostic value of baseline plasma amino-terminal pro-brain natriuretic peptide and its interactions with irbesartan treatment effects in patients with heart failure and preserved ejection fraction: Findings from the I-PRESERVE trial. Circ Heart Fail 2011; 4: 569–577.
167. Jaubert M.P, Armero S, Bonello L, et al. Predictors of B-type natriuretic peptide and left atrial volume index in patients with preserved left ventricular systolic function: An echocardiographic-catheterization study. Arch Cardiovasc Dis 2010; 103: 3–9.
168. Maisel A.S, McCord J, Nowak R.M, et al. Bedside B-Type natriuretic peptide in the emergency diagnosis of heart failure with reduced or preserved ejection fraction. Results from the Breathing Not Properly Multinational Study. J Am Coll Cardiol 2003; 41: 2010–2017.
169. Lam C.S, Rienstra M, Tay W.T, et al. 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.
170. Kraigher-Krainer E, Shah A.M, Gupta D.K, et al. Impaired systolic function by strain imaging in heart failure with preserved ejection fraction. J Am Coll Cardiol 2014; 63; 447–456.
171. Pieske B, Butler J, Filippatos G, et al. Rationale and design of the SOluble guanylate Cyclase stimulatoR in heArT failurE Studies (SOCRATES). Eur J Heart Fail 2014; 16: 1026–1038.
172. Solomon S.D, Rizkala A.R, Gong J, et al. Angiotensin Receptor Neprilysin Inhibition in Heart Failure with Preserved Ejection Fraction: Rationale and Design of the PARAGON-HF Trial. JACC Heart Fail. 2017; 5: 471–482.
173. Iwanaga Y, Nishi I, Furuichi S, et al. B-type natriuretic peptide strongly reflects diastolic wall stress in patients with chronic heart failure comparison between systolic and diastolic heart failure. J Am Coll Cardiol 2006; 47: 742–748.
174. Ledwidge M, Gallagher J, Conlon C, et al. Natriuretic peptide-based screening and collaborative care for heart failure: The STOP-HF randomized trial. JAMA 2013; 310: 66–74.
175. Obineche E.N, Pathan J.Y, Fisher S, et al. Natriuretic peptide and adrenomedullin levels in chronic renal failure and effects of peritoneal dialysis. Kidney Int 2006; 69: 152–156.
176. Santos-Araújo C, Leite-Moreira A, Pestana M. Clinical value of natriuretic peptides in chronic kidney disease. Nefrologia 2015; 35: 227–233.
177. Singh J.S.S, Burrell L.M, Cherif M, Squire I.B, Clark A.L, Lang C.C. Sacubitril/valsartan: Beyond natriuretic peptides. Heart 2017; 103: 1569–1577.
178. Brunner-La Rocca H.P, Eurlings L, et al. Which heart failure patients profit from natriuretic peptide guided therapy? A meta-analysis from individual patient data of randomized trials. Eur J Heart Fail 2015; 17:1252–1261.
179. Bishu K, Redfield M.M. Acute heart failure with preserved ejection fraction: Unique patient characteristics and targets for therapy. Curr Heart Fail Rep 2013; 10: 190–197.
180. Dandamudi S, Chen H.H. The ASCEND-HF trial: An acute study of clinical effectiveness of nesiritide and decompensated heart failure. Expert Rev Cardiovasc Ther 2012; 10: 557–563.
181. Wan S.H, Stevens S.R, Borlaug B.A, et al. Differential Response to Low-Dose Dopamine or Low-Dose Nesiritide in Acute Heart Failure with Reduced or Preserved Ejection Fraction: Results from the ROSE AHF Trial (Renal Optimization Strategies Evaluation in Acute Heart Failure). Circ Heart Fail 2016; 9: e002593, doi:10.1161/CIRCHEARTFAILURE.115.002593.

182. Chen H.H, Glockne J.F, Schirger J.A, et al. Novel protein therapeutics for systolic heart failure: Chronic subcutaneous B-type natriuretic peptide. J Am Coll Cardiol 2012; 60: 2305–2312.
183. Travessa A.M, Meneze Falcão L. Vasodilators in acute heart failure—Evidence based on new studies. Eur. J. Intern. Med. 2018; 51: doi:10.1016/j.ejim.2018.02.020.
184. Packer M, O'Connor C, McMurray J.J.V, et al. Effect of Ularitide on Cardiovascular Mortality in Acute Heart Failure. N. Engl. J. Med. 2017; 376: 1956–1964.
185. Lisy O, Huntley B.K, McCormick D.J, Kurlansky P.A, Burnett J.C. Jr. Design, synthesis, and actions of a novel chimeric natriuretic peptide: CDNP. J Am Coll Cardiol 2008; 52: 60–68.
186. McKie P.M, Sangaralingham S.J, Burnett J.C Jr. CD-NP: An innovative designer natriuretic peptide activator of particulate guanylyl cyclase receptors for cardiorenal disease. Curr Heart Fail Rep 2010; 7:93–99.
187. Lee C.Y, Huntley B.K, McCormick D.J, et al. Cenderitide: Structural requirements for the creation of a novel dual particulate guanylyl cyclase receptor agonist with renal-enhancing in vivo and ex vivo actions. Eur. Heart J. Cardiovasc. Pharmacother. 2016; 2: 98–105.
188. Martin F.L, Sangaralingham S.J, Huntley B.K, et al. CD-NP: A novel engineered dual guanylylcyclase activator with anti-fibrotic actions in the heart. PLoS ONE 2012, 7, e52422, doi:10.1371/journal.pone.0052422.
189. von Lueder T.G, Sangaralingham S.J, Wang B.H, et al. Renin-angiotensin blockade combined with natriuretic peptide system augmentation: Novel therapeutic concepts to combat heart failure. Circ Heart Fail 2013; 6: 594–605.
190. McKie P.M, Cataliotti A, Ichiki T, et al. M-atrial natriuretic peptide and nytroglycerin in a canine model of experimental acute hypertensive heart failure: A differential actions of 2 cGMP activating therapeutics. J Am Heart Assoc 2014; 3: e000206, doi:10.1161/JAHA.113.000206.
191. Pan S, Chen H.H, Dickey D.M, et al. Biodesign of a renal- protective peptide based on alternative splicing of B-type natriuretic peptide. Proc Natl Acad Sci USA 2009; 106: 11282–11287.
192. Meems L.M.G, Burnett J.C Jr. Innovative Therapeutics: Designer Natriuretic Peptides. JACC Basic Transl Sci 2016; 1: 557–567.
193. Kelesidis I, Mazurek J, Khullar P et al. The Effect of Nesiritide on Renal Function and Other Clinical Parameters in Patients with Decompensated Heart Failure and Preserved Ejection Fraction. Congest. Heart Fail 2012; 18: 158–164.
194. Bijkerk R, Aleksinskaya M.A, Duijs J.M.G, et al. Neutral endopeptidase inhibitors blunt kidney fibrosis by reducing myofibroblast formation. Clin Sci 2019; 133: 239–252.
195. Grodin J.L, Philips S, Mullens W, et al. Prognostic implications of plasma volume status estimates in heart failure with preserved ejection fraction: Insights from TOPCAT. Eur J Heart Fail 2019 doi:10.1002/ejhf.1407.
196. Myhre P.L, Vaduganathan M, Claggett B.L, et al. Association of Natriuretic Peptides with Cardiovascular Prognosis in Heart Failure with Preserved Ejection Fraction: Secondary Analysis of the TOPCAT Randomized Clinical Trial. JAMA Cardiol 2018; 3: 1000–1005.
197. Anand I.S, Claggett B, Liu J, et al. Interaction between spironolactone and natriuretic peptides in patients with heart failure and preserved ejection fraction: From the TOPCAT trial. JACC Heart Fail 2017; 5: 241–252.
198. Zile M.R, Baicu C.F, Ikonomidis J.S, et al. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: Contributions of collagen and titin. Circulation 2015; 131: 1247–1259.

199. Chen Y, Wang H, Lu Y, et al. Effects of mineralocorticoid receptor antagonists in patients with preserved ejection fraction: A meta-analysis of randomized clinical trials. BMC Med. 2015; 13: 10.
200. Seidelmann S.B, Vardeny O, Claggett B, et al. An NPPB promoter polymorphism associated with elevated n-terminal pro-B-type natriuretic peptide and lower blood pressure, hypertension, and mortality. J. Am. Heart Assoc. 2017; 6, e005257.
201. Packer, M. Epicardial adipose tissue may mediate deleterious effects of obesity and inflammation on the myocardium. J Am Coll Cardiol 2018; 71: 2360–2372.
202. Hsich E.M, Grau-Sepulveda M.V, Hernandez A.F, et al. Relationship between sex.; ejection fraction.; and B-type natriuretic peptide levels in patients hospitalized with heart failure and associations with inhospital outcomes: Findings from the Get with the Guideline-Heart Failure Registry. Am Heart J 2013 166.
203. Berry C, Doughty R.N, Granger C, et al. The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: An individual patient data meta-analysis. Eur Heart J 2012; 33 1750–1757.
204. Lam C.S, Gamble G.D, Ling L.H, et al. Mortality associated with heart failure with preserved vs. reduced ejection fraction in a prospective international multi-ethnic cohort study. Eur Heart J 2018; 39:1770–1780.
205. Levy W.C, Anand I.S. Heart failure risk prediction models: What have we learned? JACC Heart Fail 2014; 2:437–439.
206. van Veldhuisen D.J, Linssen G.C, Jaarsma T, et al. B-type natriuretic peptide and prognosis in heart failure patients with preserved and reduced ejection fraction. J Am Coll Cardiol 2013; 61:1498–1506.
207. Salah K, Stienen S, Pinto Y.M, et al. Prognosis and NT-proBNP in heart failure patients with preserved versus reduced ejection fraction. Heart 2019, doi:10.1136/heartjnl-2018-314173.
208. Wang T.J, Larson M.G, Levy D, et al. Heritability and genetic linkage of plasma natriuretic peptide levels. Circulation 2003; 108: 13–16.
209. Richards A.M. Do the Natriuretic Peptides Cause Atrial Fibrillation or is it Not So Black and White? J. Am Heart Assoc 2019; 8: e012242, doi:10.1161/JAHA.119.012242.
210. Cheng V, Kazanagra R, Garcia A, et al. A rapid bedside test for B-type peptide predicts treatment outcomes in patients admitted for decompensated heart failure: A pilot study. J Am Coll Cardiol 2001; 37: 386–391.
211. Wang T.J, Larson M.G, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med 2004; 350: 655–663.
212. Cypen J, Ahmad T, Testani J.M, DeVore A.D. Novel Biomarkers for the Risk Stratification of Heart Failure with Preserved Ejection Fraction. Curr Heart Fail Rep 2017; 14: 434–443, doi:10.1007/s11897-017-0358-4.
213. Gegenhuber A, Struck J, Dieplinger B, et al. Comparative evaluation of B-type natriuretic peptide.; mid-regional pro-A-type natriuretic peptide.; mid-regional pro-adrenomedullin.; and copeptin to predict 1-year mortality in patients with acute destabilized heart failure. J Card Fail 2007; 13:42–49.
214. Miller W.L, Hartman K.A, Grill D.E, et al. Serial measurements of midregion proANP and copeptin in ambulatory patients with heart failure: Incremental prognostic value of novel biomarkers in heart failure. Heart 2012; 98; 389–394.
215. Shah R.V, Truong Q.A, Gaggin H.K, et al. Mid-regional pro-atrial natriuretic peptide and pro-adrenomedullin testing for the diagnostic and prognostic evaluation of patients with acute dyspnoea. Eur. Heart J. 2012; 33: 2197–2205.
216. Kristensen S.L, Jhund P.S, Mogensen U.M, et al. N-Terminal Pro-B-Type Natriuretic Peptide Levels for Risk Prediction in Patients with Heart Failure and Preserved Ejection

Fraction According to Atrial Fibrillation Status. Circ Heart Fail 2019; 12: e005766, doi:10.1161/CIRCHEARTFAILURE.117.004409.
217. Alehagen U, Dahlström U, Rehfeld J.F, Goetze J.P. Pro-A-type na- triuretic peptide.; proadrenomedullin.; and N-terminal pro-B-type natriuretic peptide used in a multimarker strategy in primary health care in risk assessment of patients with symptoms of heart failure. J Card Fail 2013;19: 31–39.
218. Zabarovskaja S, Hage C, Linde C, et al. Adaptive cardiovascular hormones in a spectrum of heart failure phenotypes. Int J Cardiol 2015; 189: 6–11.
219. Smith J.G, Newton-Cheh C, Almgren P, et al. Assessment of conventional cardiovascular risk factors and multiple biomarkers for the prediction of incident heart failure and atrial fibrillation. J Am Coll Cardiol 2010; 56:1712–1719.
220. Crozier I, Richards A.M, Foy S.G, Ikram H. Electrophysiological effects of atrial natriuretic peptide on the cardiac conduction system in man. Pacing Clin Electrophysiol 1993; 16:738–742.
221. Arakawa M, Miwa H, Noda T, et al. Alternations in atrial natriuretic peptide release after DC cardioversion of nonvalvular chronic atrial fibrillation. Eur Heart J 1995; 16: 977–985.
222. Latini R, Masson S, Pirelli S, et al. Circulating cardiovascular biomarkers in recurrent atrial fibrillation: Data from the GISSI-atrial fibrillation trial. J Intern Med 2011; 269:160–171.
223. Zografos T.A, Katritsis D.G. Natriuretic Peptides as Predictors of Atrial Fibrillation Recurrences Following Electrical Cardioversion. Arrhythm Electrophysiol Rev 2013; 2: 109–114.
224. Khan M.S, Siddiqi T.J, Usman M.S, et al. Does natriuretic peptide monitoring improve outcomes in heart failure patients? A systematic review and meta-analysis. Int J Cardiol 2018; 263: 80–87.
225. Sanders-van Wijk S, van Asselt AD, Rickli H, et al. Cost- effectiveness of N-terminal pro-B-type natriuretic-guided therapy in elderly heart failure patients: Results from TIME- CHF (Trial of Intensified versus Standard Medical Therapy in Elderly Patients with Congestive Heart Failure). JACC Heart Fail 2013; 1: 64–71.
226. Schou M, Gustafsson F, Videbaek L, et al. Extended heart failure clinic follow-up in low-risk patients: A randomized clinical trial (NorthStar). Eur Heart J 2013; 34:432–442.
227. Maeder M.T, Rickenbacher P, Rickli H, et al. N-terminal pro brain natriuretic peptide-guided management in patients with heart failure and preserved ejection fraction: Findings from the Trial of Intensified versus standard medical therapy in elderly patients with congestive heart failure (TIME-CHF). Eur J Heart Fail 2013; 15: 1148–1156.
228. Troughton R.W, Frampton C.M, Brunner-La Rocca, et al. Effect of B-type natriuretic peptide-guided treatment of chronic heart failure on total mortality and hospitalization: An individual patient meta-analysis. Eur Heart J 2014;35: 1559–1567.
229. Mohiuddin S, Reeves B, Pufulete M, et al. Model-based cost- effectiveness analysis of B-type natriuretic peptide-guided care in patients with heart failure. BMJ Open 2016; 6: e014010, doi:10.1136/bmjopen-2016-014010.
230. Eurlings L.W, van Pol P.E, Kok W.E, et al. Management of chronic heart failure guided by individual N-terminal pro-B- type natriuretic peptide targets: Results of the PRIMA (Can PRo-brain-natriuretic peptide guided therapy of chronic heart failure IMprove heart failure morbidity and mortality?) study. J Am Coll Cardiol 2010; 56: 2090–2100.
231. Ibrahim N.E, Januzzi J.L Jr. The Future of Biomarker-Guided Therapy for Heart Failure After the Guiding Evidence-Based Therapy Using Biomarker Intensified Treatment in Heart Failure (GUIDE-IT) Study. Curr Heart Fail Rep 2018;15: 37–43.

232. Shah M.R, Califf R.M, Nohria A, et al. The STARBRITE trial: A randomized.; pilot study of B-type natriuretic peptide- guided therapy in patients with advanced heart failure. J Card Fail 2011;17: 613–621.
233. Sabatine M.S, Morrow D.A, de Lemos J.A, et al. Evaluation of multiple biomarkers of cardiovascular stress for risk prediction and guiding medical therapy in patients with stable coronary disease. Circulation 2012; 125: 233–240.
234. Arrigo M, Truong Q.A, Szymonifka J, et al. Mid- regional pro-atrial natriuretic peptide to predict clinical course in heart failure patients undergoing cardiac resynchronization therapy. Europace 2017; 19:1848–1854.
235. Wu C.K, Su M.Y, Lee J.K, et al. Galectin-3 level and the severity of cardiac diastolic dysfunction using cellular and animal models and clinical indices. Sci. Rep. 2015; 5: 17007, doi:10.1038/srep17007.
236. Circulating NEP and NEP Inhibition Study in Heart Failure with Preserved Ejection Fraction (CNEPi). ClinicalTrials.gov Identifier:NCT03506412. Available online: https://clinicaltrials.gov/ct2/show/NCT03506412?cond=heart+failure+preserved+ejection+fraction&draw=2&rank=18 (accessed on 2 April 2019).
237. A Randomized, Double-blind Controlled Study Comparing LCZ696 to Medical Therapy for Comorbidities in HFpEF Patients (PARALLAX). ClinicalTrials.gov Identifier: NCT03066804. Available online: https://clinicaltrials.gov/ct2/show/NCT03066804?cond=heart+failure+preserved+ejection+fraction&draw=3&rank=27 (accessed on 2 April 2019).
238. De Keulenaer, G.W. Brutsaert, D.L. Systolic and diastolic heart failure are overlapping phenotypes within the heart failure spectrum. Circulation 2011;123 (18):1996-2004.
239. Aronow, W.S. Epidemiology, pathophysiology, prognosis, and treatment of systolic and diastolic heart failure in elderly patients. Heart Dis 2003; 5:279–294.
240. Ponikowski P, Voors A.A, Anker S.D, Bueno H, Cleland J.G, Coats A.J, Falk V, González-Juanatey J.R, Harjola V.P, Jankowska E.A. 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 European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail 2016; 18: 891–975.
241. Brunner-La Rocca, H.P.; Sanders-van Wijk, S. Natriuretic Peptides in Chronic Heart Failure. Card. Fail. Rev. 2019; 5: 44–49.
242. Fu S, Ping P, Wang F, Luo L. Synthesis, secretion, function, metabolism and application of natriuretic peptides in heart failure. J Biol Eng 2018; 12:2. doi: 10.1186/s13036-017-0093-0.
243. Oikonomou E, Vogiatzi G. Tsalamandris S, et al. Non-natriuretic peptide biomarkers in heart failure with preserved and reduced ejection fraction. Biomark Med 2018; 12: 783–797.
244. Meijers W.C, van der Velde A.R, de Boer R.A. Biomarkers in heart failure with preserved ejection fraction. Neth Heart J 2016; 24: 252–258.
245. D'Elia E, Vaduganathan M, Gori M, Gavazzi A, Butler J, Senni M. Role of biomarkers in cardiac structure phenotyping in heart failure with preserved ejection fraction: Critical appraisal and practical use. Eur J Heart Fail 2015; 17:1231–1239.
246. Nadar S.K, Shaikh M.M. Biomarkers in Routine Heart Failure Clinical Care. Card Fail Rev 2019; 5: 50–56.
247. de Boer R.A, Nayor M, deFilippi C.R, et al. Association of Cardiovascular Biomarkers with Incident Heart Failure with Preserved and Reduced Ejection Fraction. JAMA Cardiol 2018; 3: 215–224.

248. Gruson D, Favvresse J. Peptides natriurétiques: Dégradation, formes circulantes, dosages et nouvelles approches thérapeutiques. Ann Biol Clin 2017; 75: 259–267.
249. Maisel A.S, Duran J.M, Wettersten N. Natriuretic Peptides in Heart Failure: Atrial and B-type Natriuretic Peptides. Heart Fail Clin 2018; 14: 13–25.
250. Nishikimi T, Kuwahara K, Nakao K. Current biochemistry, molecular biology, and clinical relevance of natriuretic peptides. J Cardiol 2011; 57: 131–140.
251. Rahmutula D, Zhang H, Wilson E, Olgin J.E. Absence of natriuretic peptide clearance receptor attenuates TGF-b1-induced selective atrial fibrosis and atrial fibrillation. Cardiovasc Res 2019; 115:357–372.
252. Calderone A, Thaik C.M, Takahashi N, Chang D.L, Colucci W.S. Nitric oxide, atrial natriuretic peptide, and cyclic GMP inhibit the growth-promoting effects of norepinephrine in cardiac myocytes and fibroblasts. J. Clin. Investig 1998; 101: 812–818.
253. Volpe M, Carnovali M, Mastromarino V. The natriuretic peptides system in the pathophysiology of heart failure: From molecular basis to treatment. Clin Sci 2016; 130: 57–77.
254. Matsuo A, Nagai-Okatani C, Nishigori M, Kangawa K, Minamino N. Natriuretic peptides in human heart: Novel insight into their molecular forms, functions, and diagnostic use. Peptides 2019; 111: 3–17.
255. Seewöster T, Büttner P, Nedios S, et al. Association Between Cardiovascular Magnetic Resonance-Derived Left Atrial Dimensions, Electroanatomical Substrate and NT-proANP Levels in Atrial Fibrillation. J Am Heart Assoc 2018; 7: e009427.
256. Nattel, S. Natriuretic peptide receptors and atrial-selective fibrosis: Potential role in atrial fibrillation. Cardiovasc Res 2019; 115: 258–260.
257. Wu C.K, Su M.M, Wu Y.F, Hwang J.J, Lin L.Y. Combination of Plasma Biomarkers and Clinical Data for the Detection of Myocardial Fibrosis or Aggravation of Heart Failure Symptoms in Heart Failure with Preserved Ejection Fraction Patients. J Clin Med 2018; 7: 427.
258. Zakeri R, Cowie M.R. Heart failure with preserved ejection fraction: Controversies, challenges and future directions. Heart 2018; 104: 377–384.
259. Reddy Y.N.V, Carter R.E, Obokata M, Redfield M.M, Borlaug B.A. A Simple, Evidence-Based Approach to Help Guide Diagnosis of Heart Failure with Preserved Ejection Fraction. Circulation 2018; 138: 861–870.
260. Michalska-Kasiczak M, Bielecka-Dabrowa A, von Haehling S, Anker S.D, Rysz J, Banach M. Biomarkers, myocardial fibrosis and co-morbidities in heart failure with preserved ejection fraction: An overview. Arch Med Sci 2018; 14: 890–909.
261. Cui K, Huang W, Fan J, Lei H. Midregional pro-atrial natriuretic peptide is a superior biomarker to N-terminal pro-B-type natriuretic peptide in the diagnosis of heart failure patients with preserved ejection fraction. Medicine 2018; 97: e12277.
262. Paulus W.J, Tschöpe C. A novel paradigm for heart failure with preserved ejection fraction: Comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol 2013; 62: 263–271.
263. van Heerebeek L, Hamdani N, Falcão-Pires I, et al. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation 2012; 126:830–839.
264. Tsoupras A. B., C. Iatrou, C. Frangia, C. A. Demopoulos, Te implication of platelet activating factor in cancer growth and metastasis: potent benefcial role of PAF-inhibitors and antioxidants. Infectious Disorders - Drug Targets 2009; 9(4): 390–399.
265. Kelesidis T., V. Papakonstantinou, P. Detopoulou, et al. Te role of platelet-activating factor in chronic infammation, immune activation, and comorbidities associated with HIV infection. AIDS Reviews 2015;17(4):191–201.

266. Sager H. B, Nahrendorf M. Infammation: a trigger for acute coronary syndrome. European Journal of Nuclear Medicine and Molecular Imaging 2016; 60(2):185–193.
267. Ponikowski P, Voors A, Anker S, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur J Heart Fail 2016; 18: 891–975.
268. Haththotuwa R.. Cardiovascular complications of diabetes. InnovAiT: Education and inspiration for general practice 2016; 9(11):694–701.
269. Fox C.S,Golden S. H, Anderson C, et al. Update on prevention of cardiovascular disease in adults with type 2 diabetes mellitus in light of recent evidence: a scientific statement from the American Heart Association and the American Diabetes Association. Diabetes Care 2015; 38 (91):777–1803.
270. Bonapace S, Valbusa F, Bertolini L, et al. Nonalcoholic fatty liver disease is associated with aortic valve sclerosis in patients with type 2 diabetes mellitus. PloS One 2014; 9: e88371.
271. Ballestri S, Lonardo A, Bonapace S, Byrne CD, Loria P, Targher G. Risk of cardiovascular, cardiac and arrhythmic complications in patients with non-alcoholic fatty liver disease. World J Gastroenterol 2014; 20: 1724-45.
272. Y. Dogan, A. Soylu, G. A. Eren, et al. Evaluation of QT and P wave dispersion and mean platelet volume among inflammatory bowel disease patients. International Journal of Medical Sciences 2011; 8(7): 540–546.
273. T. T. Issac, H. Dokainish, and N. M. Lakkis. Role of inflammation in initiation and perpetuation of atrial fibrillation: a systematic review of the published data. J Am Coll Cardiol 2007; 50 (21):2021–2028.
274. Kirchhof P, Benussi S, Kotecha D, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J 2016, 37(38):2893-2962. doi: 10.1093/eurheartj/ehw210.
275. Thomas L, Abhayaratna W.P. Left Atrial Reverse Remodeling: Mechanisms, Evaluation, and Clinical Significance. JACC Cardiovasc Imaging 2017; 10(1):65-77.
276. Habibi M, Lima J.A, Khurram I.M, et al. Association of Left Atrial Function and Left Atrial Enhancement in Patients With Atrial Fibrillation: Cardiac Magnetic Resonance Study. Circ Cardiovasc Imaging 2015; 8(2):e002769. doi:10.1161/CIRCIMAGING.114.002769.
277. Marrouche N.F, Wilber D, Hindricks G, et al. Association of atrial tissue fibrosis identified by delayed enhancement MRI and atrial fibrillation catheter ablation: the DECAAF study. JAMA 2014, 311(5):498-506. doi: 10.1001/jama.2014.3.
278. Tandon K, Tirschwell D, Longstreth W.T. Jr, Smith B. Akoum, N. Embolic stroke of undetermined source correlates to atrial fibrosis without atrial fibrillation. Neurology 2019, 93(4):e381-e387. doi: 10.1212/WNL.0000000000007827.
279. Goette A, Kalman J.M, Aguinaga L, et al. EHRA/HRS/APHRS/SOLAECE expert consensus on Atrial cardiomyopathies: Definition, characterisation, and clinical implication. J Arrhythm 2016 : 32(4):247-78.
280. Benussi S, de Maat G.E. Atrial remodelling and function: implications for atrial fibrillation surgery. Eur J Cardiothorac Surg 2018; 53(suppl_1):i2-i8. doi: 10.1093/ejcts/ezx340.
281. Zghaib T, Nazarian S. New Insights into the Use of Cardiac Magnetic Resonance Imaging to Guide Decision-Making in AF Management. Can J Cardiol. 2018, 34(11):1461-147.
282. Bisbal F, Baranchuk A, Braunwald E, Bayés de Luna A, Bayés-Genís A. Atrial Failure as a Clinical Entity: JACC Review Topic of the Week. J Am Coll Cardiol 2020; 75(2):222-232.

283. Guichard J.B, Nattel S. Atrial Cardiomyopathy: A Useful Notion in Cardiac Disease Management or a Passing Fad? J Am Coll Cardiol 2017; 70(6):756-765.
284. Maron B.J, Towbin J.A, Thiene G, et al. American Heart Association; Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; Council on Epidemiology and Prevention. Contemporary definitions and classification of the cardiomyopathies: an American Heart Association Scientific Statement from the Council on Clinical Cardiology, Heart Failure and Transplantation Committee; Quality of Care and Outcomes Research and Functional Genomics and Translational Biology Interdisciplinary Working Groups; and Council on Epidemiology and Prevention. Circulation. 2006, 113(14):1807-16.
285. Shen M.J, Arora R, Jalife J. Atrial Myopathy. JACC Basic Transl Sci 2019; 4(5):640-654. doi: 10.1016/j.jacbts.2019.05.005.
286. Hirsh B.J, Copeland-Halperin R.S, Halperin J.L. Fibrotic atrial cardiomyopathy, atrial fibrillation, and thromboembolism: mechanistic links and clinical inferences. J Am Coll Cardiol 2015; 65(20): 2239-51.
287. Habibi M, Samiei S, Ambale Venkatesh B, et al. Cardiac Magnetic Resonance-Measured Left Atrial Volume and Function and Incident Atrial Fibrillation: Results From MESA (Multi-Ethnic Study of Atherosclerosis). Circ Cardiovasc Imaging. 2016, 9(8):10.1161/CIRCIMAGING.115.004299 e004299. doi: 10.1161/CIRCIMAGING.115.004299.
288. Lang R.M, Badano L.P, Mor-Avi V, 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. J Am Soc Echocardiog 2015; 28(1): 1-39.e14. doi:10.1016/j.echo.2014.10.003.
289. Nakamori S, Ngo L.H, Tugal D, Manning W.J, Nezafat R. Incremental Value of Left Atrial Geometric Remodeling in Predicting Late Atrial Fibrillation Recurrence After Pulmonary Vein Isolation: A Cardiovascular Magnetic Resonance Study. J Am Heart Assoc 2018; 7(19): e009793. doi: 10.1161/JAHA.118.009793.
290. Bisbal F, Guiu E, Calvo N, et al. Left atrial sphericity: a new method to assess atrial remodeling. Impact on the outcome of atrial fibrillation ablation. J Cardiovasc Electrophysiol 2013; 24(7): 752-9.
291. Bisbal F, Guiu E, Cabanas P, et al. Reversal of spherical remodelling of the left atrium after pulmonary vein isolation: incidence and predictors. Europace. 2014, 16(6), 840-7. doi: 10.1093/europace/eut385.
292. Moon J, Lee H.J, Yu J, et al. Prognostic implication of left atrial sphericity in atrial fibrillation patients undergoing radiofrequency catheter ablation. Pacing Clin Electrophysiol 2017; 40(6): 713-720. doi: 10.1111/pace.13088.
293. Njoku A, Kannabhiran M, Arora R, et al. Left atrial volume predicts atrial fibrillation recurrence after radiofrequency ablation: a meta-analysis. Europace 2018; 20(1): 33-42.
294. Bisbal F, Fernández-Armenta J, Berruezo A, et al. Use of MRI to guide electrophysiology procedures. Heart 2014; 100(24):1975-84.
295. Bisbal F, Alarcón F, Ferrero-de-Loma-Osorio A, et al. Left atrial geometry and outcome of atrial fibrillation ablation: results from the multicentre LAGO-AF study. Eur Heart J Cardiovasc Imaging 2018; 19(9): 1002-1009.
296. Oakes R.S, Badger T.J, Kholmovski E.G, et al. Detection and Quantification of Left Atrial Structural Remodeling With Delayed-Enhancement Magnetic Resonance Imaging in Patients With Atrial Fibrillation. Circulation 2009; 119(13): 1758-67.

297. Siebermair J, Kholmovski E.G, Marrouche N. Assessment of Left Atrial Fibrosis by Late Gadolinium Enhancement Magnetic Resonance Imaging: Methodology and Clinical Implications. JACC Clin Electrophysiol 2017; 3(8):791-802.
298. Navaravong L, Marrouche N. CMR Guidance of RFA to Atrial Arrhythmias. In: Cardiovascular Magnetic Resonance Imaging. Editors Kwong, R.Y.; Jerosch-Herold, M.; Heydari, B, 2nd ed.; Humana Press, New York, USA. 2019, pp. 407-18.
299. Sohns C, Marrouche N.F. Atrial fibrillation and cardiac fibrosis. Eur Heart J. 2019, ehz786. [Epub ahead of print]. doi: 10.1093/eurheartj/ehz786.
300. Gal P, Marrouche N.F. Magnetic resonance imaging of atrial fibrosis: redefining atrial fibrillation to a syndrome. Eur Heart J 2017; 38(1): 14-19.
301. Khurram I.M, Habibi M, Gucuk Ipek E, et al. Left Atrial LGE and Arrhythmia Recurrence Following Pulmonary Vein Isolation for Paroxysmal and Persistent AF. JACC Cardiovasc Imaging 2016; 9(2): 142-8.
302. Chubb H, Karim R, Mukherjee R, et al. A comprehensive multi-index cardiac magnetic resonance-guided assessment of atrial fibrillation substrate prior to ablation: Prediction of long-term outcomes. J Cardiovasc Electrophysiol 2019; 30(10): 1894-1903.
303. Chrispin J, Ipek E.G, Habibi M, et al. Clinical predictors of cardiac magnetic resonance late gadolinium enhancement in patients with atrial fibrillation. Europace 2017; 19(3): 371-377.
304. Gal P, Pacchia C, Morris A, Cates J, Kaur G, Elvan A, Marrouche N. P1304: Architecture of fibrosis predicts atrial fibrillation recurrences after ablation. Europace. 2015; 7(S3):iii176. doi: 10.1093/europace/euv154.
305. Higuchi K, Cates J, Gardner G, et al. The Spatial Distribution of Late Gadolinium Enhancement of Left Atrial Magnetic Resonance Imaging in Patients With Atrial Fibrillation. JACC Clin Electrophysiol 2018; 4(1): 49-58.
306. Benito E.M, Cabanelas N, Nuñez-Garcia M, et al. Preferential regional distribution of atrial fibrosis in posterior wall around left inferior pulmonary vein as identified by late gadolinium enhancement cardiac magnetic resonance in patients with atrial fibrillation. Europace 2018; 20(12): 1959-1965.
307. Spronk H.M, De Jong A.M, Verheule S, et al. Hypercoagulability causes atrial fibrosis and promotes atrial fibrillation. Eur Heart J 2017; 38(1): 38-50.
308. King J.B, Azadani P.N, Suksaranjit P, et al. Left Atrial Fibrosis and Risk of Cerebrovascular and Cardiovascular Events in Patients With Atrial Fibrillation. J Am Coll Cardiol 2017; 70(11): 1311-1321.
309. Daccarett M, Badger T.J, Akoum N, et al. Association of left atrial fibrosis detected by delayed-enhancement magnetic resonance imaging and the risk of stroke in patients with atrial fibrillation. J Am Coll Cardiol 2011; 57(7): 831-838.
310. Akoum N, Fernandez G, Wilson B, Mcgann C, Kholmovski E, Marrouche N. Association of atrial fibrosis quantified using LGE-MRI with atrial appendage thrombus and spontaneous contrast on transesophageal echocardiography in patients with atrial fibrillation. J Cardiovasc Electrophysiol 2013; 24(10): 1104-1109.
311. Inoue Y.Y, Alissa A, Khurram I.M, et al. Quantitative tissue-tracking cardiac magnetic resonance (CMR) of left atrial deformation and the risk of stroke in patients with atrial fibrillation. J Am Heart Assoc 2015; 4(4): e001844. doi: 10.1161/JAHA.115.001844.
312. Habibi M, Lima J.A.C, Gucuk Ipek E, et al. The association of baseline left atrial structure and function measured with cardiac magnetic resonance and pulmonary vein isolation outcome in patients with drug-refractory atrial fibrillation. Heart Rhythm 2016;13(5): 1037-1044.

313. Ciuffo L, Inoue Y.Y, Tao S, et al. Mechanical dyssynchrony of the left atrium during sinus rhythm is associated with history of stroke in patients with atrial fibrillation. Eur Heart J Cardiovasc Imaging 2018; 19(4): 433-441.
314. Akkaya M, Higuchi K, Koopmann M, et al. Higher degree of left atrial structural remodeling in patients with atrial fibrillation and left ventricular systolic dysfunction. J Cardiovasc Electrophysiol 2013; 24(5): 485-491.
315. Zhao L, Li S, Ma X, et al. Prognostic Significance of Left Ventricular Fibrosis Assessed by T1 Mapping in Patients with Atrial Fibrillation and Heart Failure. Sci Rep 2019; 9(1): 13374. doi: 10.1038/s41598-019-49793-8.
316. Marrouche N.F, Brachmann J, Andresen D, et al. CASTLE-AF Investigators. Catheter Ablation for Atrial Fibrillation with Heart Failure. N Engl J Med 2018; 378(5): 417-427.
317. Gal P, Marrouche NF. Magnetic resonance imaging of atrial fibrosis: redefining atrial fibrillation to a syndrome. Eur Heart J 2017;38(1):14-19.
318. Platonov PG. Atrial fibrosis: an obligatory component of arrhythmia mechanisms in atrial fibrillation? J Geriatr Cardiol 2017;14(3):174-178.
319. Njoku A, Kannabhiran M1, Arora R, et al. Left atrial volume predicts atrial fibrillation recurrence after radiofrequency ablation: a meta-analysis. Europace 2018;20(1):33-42.
320. Jordan K, Yaghi S, Poppas A. Left Atrial Volume Index Is Associated With Cardioembolic Stroke and Atrial Fibrillation Detection After Embolic Stroke of Undetermined Source. Stroke. 2019:STROKEAHA119025384. [Epub ahead of print].
321. Siebermair J, Kholmovski E, Marrouche N. Assessment of Left Atrial Fibrosis by Late Gadolinium Enhancement Magnetic Resonance Imaging. Methodology and Clinical Implication. JACC: Clinical Electrophysiology 2017; 3(8): 791-802.
322. Chubb H, Karim R, Roujol S, et al. The reproducibility of late gadolinium enhancement cardiovascular magnetic resonance imaging of post-ablation atrial scar: a cross-over study. J Cardiovasc Magn Reson 2018; 20(1):21.
323. Marrouche NF, Wilber D, Hindricks G, et al. Association of atrial tissue fibrosis identified by delayed enhancement MRI and atrial fibrillation catheter ablation: the DECAAF study. JAMA 2014;311(5):498-506.
324. Vergara GR, Marrouche NF. Tailored management of atrial fibrillation using a LGE-MRI based model: from the clinic to the electrophysiology laboratory. J Cardiovasc Electrophysiol. 2011;22:481–487.
325. Tandon K, Tirschwell D, Longstreth WT Jr, et al. Embolic stroke of undetermined source correlates to atrial fibrosis without atrial fibrillation. Neurology. 2019. pii: 10.1212/WNL.0000000000007827.[Epub ahead of print].
326. Mahnkopf C, Badger TJ, Burgon NS, et al. Evaluation of the left atrial substrate in patients with lone atrial fibrillation using delayed-enhanced MRI: implications for disease progression and response to catheter ablation. Heart Rhythm. 2010;7:1475-81.
327. Yasuda R, Murata M, Roberts R, 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;16(9):1008-14.
328. Truong VT, Palmer C, Wolking S, et al. Normal left atrial strain and strain rate using cardiac magnetic resonance feature tracking in healthy volunteers. Eur Heart J Cardiovasc Imaging 2019; jez157. https://doi.org/10.1093/ehjci/jez157.
329. Hrynkiewicz-Szymanska A, Dluzniewski M, Platek AE, et al. Association of the CHADS2 and CHA2DS2-VASc scores with left atrial enlargement: a prospective cohort study of unselected atrial fibrillation patients. J Thromb Thrombolysis 2015;40(2):240-7.

330. Kashani RG, Sareh S, Genovese B, et al. Predicting postoperative atrial fibrillation using CHA2DS2-VASc scores. J Surg Res 2015;198(2):267-72.
331. Schmidt EU, Schneider R, Lauschke J, et al. The HATCH and CHA2DS2-VASc scores. Prognostic value in pulmonary vein isolation. Herz 2014;39(3):343-8.
332. Platonov PG, Mitrofanova LB, Orshanskaya V, Ho SY. Structural abnormalities in atrial walls are associated with presence and persistency of atrial fibrillation but not with age. J Am Coll Cardiol 2011;58(21):2225-2232.
333. Goldberger JJ, Arora R2, Green D, et al. Evaluating the Atrial Myopathy Underlying Atrial Fibrillation: Identifying the Arrhythmogenic and Thrombogenic Substrate. Circulation 2015;132(4):278-91.
334. Khurram IM, Habibi M, Gucuk Ipek E, et al. Left atrial LGE and arrhythmia recurrence following pulmonary vein isolation for paroxysmal and persistent AF. J Am Coll Cardiol Img 2016;9:142–8.
335. Lloyd-Jones DM, Wang TJ, Leip EP, et al. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. Circulation 2004;110:1042– 6.
336. Miyasaka Y, Barnes ME, Gersh BJ, et al. Secular trends in incidence of atrial fibrillation in Olmsted County, Minnesota, 1980 to 2000, and implications on the projections for future prevalence. Circulation 2006;114:119 –25.
337. Kainuma S, Masai T, Yoshitatsu M, et al. Advanced left-atrial fibrosis is associated with unsuccessful maze operation for valvular atrial fibrillation. Eur J Cardiothorac Surg 2011;40(1):61-69.
338. Kostin S, Klein G, Szalay Z, Hein S, Bauer EP, Schaper J. Structural correlate of atrial fibrillation in human patients. Cardiovasc Res 2002;54:361–79.
339. de Vos CB, Pisters R, Nieuwlaat R, et al. Progression from paroxysmal to persistent atrial fibrillation: clinical correlates and prognosis. J Am Coll Cardiol 2010;55:725–31.
340. Everett TH, Wilson EE, Verheule S, et al. Structural atrial remodeling alters the substrate and spatiotemporal organization of atrial fibrillation: a comparison in canine models of structural and electrical atrial remodeling. Am J Physiol Heart Circ Physiol 2006;291:H2911–23.
341. Corradi D, Callegari S, Benussi S, et al. Myocyte changes and their left atrial distribution in patients with chronic atrial fibrillation related to mitral valve disease. Hum Pathol 2005; 36: 1080 – 1089.
342. Burstein B, Nattel S. Atrial fibrosis: mechanisms and clinical relevance in atrial fibrillation. J Am Coll Cardiol 2008;51(8):802-9.
343. El-Serag HB, Sweet S, Winchester CC, Dent J. Update on the epidemiology of gastro-oesophageal reflux disease: a systematic review. Gut 2014; 63: 871–880.
344. Velagapudi P, Turagam MK, Leal MA, Kocheril AG. Atrial fibrillation and acid reflux disease. Clin Cardiol 2012;35:180-6.
345. Malik M, Bigger JT, Camm JA, et al. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Heart rate variability: Standards of measurement, physiological interpretation and clinical use. Eur Heart J 1996; 17: 354–381.
346. Milovanovic B, Filipovic B, Mutavdzin S, et al. Cardiac autonomic dysfunction in patients with gastroesophageal reflux disease. World J Gastroenterol 2015;21(22):6982-9.
347. Cho YK. How to Interpret Esophageal Impedance pH Monitoring. J Neurogastroenterol Motil 2010;16:327-330.
348. Reddy YM, Singh D, Nagarajan D, et al. Atrial fibrillation ablation in patients with gastroesophageal reflux disease or irritable bowel syndrome-the heart to gut connection! J Interv Card Electrophysiol 2013;37:259-65.

349. M. Rienstra, S. Cheng, M. G. Larson, et al. Vitamin D status is not related to development of atrial fibrillation in the community. The American Heart Journal 2011;162(3):538–541.
350. D. A. Hanafy, S. L. Chang, Y. Y. Lu, et al. Electromechanical effects of 1,25-dihydroxyvitamin D with antiatrial fibrillation activities. J Cardiovasc Electrophysiol 2014;25(3):317–323.
351. Canpolat U, Yayla Ç, Akboğa MK, et al. Effect of Vitamin D Replacement on Atrial Electromechanical Delay in Subjects with Vitamin D Deficiency. J Cardiovasc Electrophysiol 2015;26(6):649-55.
352. Chen WR, Liu ZY, Shi Y, et al. Relation of low vitamin D to nonvalvular persistent atrial fibrillation in Chinese patients. Ann Noninvasive Electrocardiol 2014;19(2):166-73.
353. Cardim N, Dalen H, Voigt JU, et al. The use of handheld ultrasound devices: a position statement of the European Association of Cardiovascular Imaging (2018 update). Eur Heart J Cardiovasc Imaging 2019;20(3):245–252.
354. Birrane J, Misran H, Creaney M, et al. A Scoping Review of Ultrasound Teaching in Undergraduate Medical Education. Med Sci Educ 2018;28-45.