Università Campus Bio-Medico di Roma

Corso di dottorato di ricerca in Scienze Biomediche Integrate e Bioetica
XXXIII ciclo a.a. 2017-2018

Cardiac Adiposity and Arrhythmogenesis in Patients with Type-2 Diabetes Mellitus

Antonio Creta

Primary Supervisor

Prof. Paolo Pozzilli

Secondary Supervisor

Dr. M. Finlay

April 2021

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CHAPTER I

CARDIAC ADIPOSE TISSUE AND ARRHYTHMOGENESIS

Cardiac adiposity

Under physiological condition, the heart and great vessels are surrounded by adipose tissue [1-3]. Furthermore, cardiomyocytes contain triglyceride droplets which overall constitute the so-called *intramyocardial fat* [1-4]. The layers of adipose tissue surrounding the heart can be divided into several components. The *epicardial fat* is usually defined as the adipose tissue localised "between the myocardium and the visceral layer of the pericardium" [2]. [1] The *paracardial fat (*or *extrapericardial fat)* constitutes the "adipose tissue external to the parietal pericardium", while the *pericardial fat* is commonly defined as "the paracardial fat plus all the adipose tissue located internal to the parietal pericardium" [1]. The *pericoronary* or *perivascular epicardial fat* represents a layer of adipose tissue directly surrounding the coronary arteries [3]. However, it should be acknowledged that a standardised classification is missing, and the above terms have used interchangeably by different authors [1-2].

From an embryological point of view, the epicardial and paracardial fat originate from the splanchnopleuric mesoderm and primitive thoracic mesenchyme, respectively [1-3]. The epicardial fat is in direct contact with the myocardium, without interposition of a fascia, and receives blood supply from the coronary arteries [3]. Conversely, the pericardium separates the myocardium from the paracardial fat , which is perfused by branches of the internal mammary artery [1]. The epicardial fat covers approximately 80% of the heart and is mainly localised in the atrioventricular and interventricular grooves, along the major branches of the coronary arteries, and at a less extent "around the atria, right ventricular free wall, and left ventricular apex" [2].

Several physiological functions of the cardiac adipose tissue have been proposed. The cardiac fat is a metabolically active organ, with an important role in lipid and energy homeostasis [2]. The epicardial fat facilitates coronary artery remodelling and provides mechanical support by reducing vascular tension and torsion [2, 5], through the paracrine secretion of adiponectin and other molecules which can reduce the coronary response to vasoconstrictive stimuli [6]; adipokines such as adiponectin and

adrenomedullin have also anti-inflammatory properties, and adiponectin has been demonstrated to increase insulin sensitivity [2]. In addition, the epicardial fat represents an energetic reserve for the myocardium in case of increased physiologic demand and can also act as buffer to protect the cardiac tissue against excessive levels of circulating free fatty acids [2, 5]. A possible role of epicardial fat in the heart thermogenesis has also been proposed [8]. Compared to other visceral adipose tissue depots, the epicardial tissue has a higher fatty acid synthesis and breakdown, as well as lower rate of glucose utilization [2, 5, 7], smaller adipocytes [9], different fatty acid composition, and higher protein content [5, 10]. Despite these important physiological functions, the accumulation of epicardial fat can promote inflammation and oxidative stress, creating a substrate for arrhythmias [5].

Abnormal or excess cardiac adipose tissue and arrhythmias

Obesity represents the most important condition associated with excess cardiac adiposity, with several imaging studies showing a strong direct correlation between abdominal visceral adiposity and epicardial adipose tissue. Furthermore, obesity is associated with intramyocardial accumulation of triglyceride, a condition called *cardiac steatosis*. Type-2 diabetes mellitus is another well-established cause of cardiac steatosis. Epicardial fat accumulation may also represent a "compensatory mechanism developed in response to chronic insults of the myocardial tissue" [5]. In the presence of obesity, the adipocytes become larger with an increased expression of proinflammatory cytokines and reduced release of adiponectin. The adipose tissue contains also immune cells such as lymphocyte and macrophages, which can release a number of molecules involved in the inflammation process. Of note, obesity is typically associated with a low-grade chronic systemic inflammation. An increased number of macrophages and T-cells have been demonstrated within the epicardial fat in patients with coronary artery disease. The release of proinflammatory molecules has been shown to have pro-fibrotic effects, which contribute to create a substrate for arrhythmia development [5]. Oxidative stress in the adipose tissue is also involved in the pro-inflammatory and pro-fibrotic processes which can favour arrhythmia onset. Increased lipid levels, type-2 diabetes mellitus and hyperglycaemia can lead to adipocyte mitochondrial dysfunction with production of reactive oxygen species.

Pathological accumulation of intramuscular adipose tissue can occur in several cardiac diseases and lead to arrhythmias [2]. Fibrofatty replacement of cardiomyocyte is the typical histological change of the arrhythmogenic right ventricular cardiomyopathy, which is a heritable disease associated with heart failure, ventricular arrhythmias and sudden cardiac death [2]. The surviving myocardial bundles within the fibrofatty tissue create areas of slow conduction providing an electrophysiological substrate for reentrant ventricular tachycardia. However, ventricular arrhythmias can also develop in the concealed phase of the disease before the development of structural remodelling, suggesting that electrical abnormalities at the cellular level (desmosome, gap junction, voltage-gated sodium channels) play a role in the genesis of arrhythmia in these patients [2]. Infiltration of adipocyte within post-ischemic myocardial scar (so-called lipomatous metaplasia) [11] has been proposed as a possible mechanism favouring the electrophysiological changes leading to ischaemic ventricular tachycardia [2]. Epicardial fat thickness has been linked to increased risk of ventricular arrhythmias and long-term mortality in subjects with heart failure [12]. Fatty infiltration of the myocardium has been described in patients with myotonic dystrophy and has been associated with a higher risk of inducible ventricular arrhythmias [13].

Cardiac steatosis

Myocardial fat constitutes less than "1% of the organ mass in healthy lean individuals" [3, 4]. The overstorage of triglyceride droplet within the cardiomyocyte is called *cardiac steatosis*. Myocardial triglyceride content can be quantified in humans noninvasively using proton magnetic resonance spectroscopy, or invasively using myocardial biopsy [3]. The level of circulating free fatty acid contributes to regulate the myocardial fat depots [3]. Lamb et al [14] showed that progressive caloric restriction in healthy lean subjects, which results in an increased level of circulating free fatty acid, increases the myocardial fat content and decreases the diastolic left ventricular function; however, a short term high-fat diet does not influence the amount of intramyocardial fat, suggesting that the latter has buffering capacities [3]. The myocardial triglyceride content increases with age and is associated with the "age-related decline in diastolic function" in males [15]. Male gender is independently associated with cardiac steatosis [16].

Clinical conditions associated with excessive free fatty acid delivery, such as obesity and type-2 diabetes mellitus, can lead to an increased fatty acid uptake by cardiomyocytes with subsequent saturation of their mitochondrial oxidative capacity and accumulation of intramyocardial lipid [17]. Indeed, cardiac steatosis appears to be a hallmark of obesity and type-2 diabetes mellitus [3], which are characterised by two- to four-fold increase in the myocardial fat compared to healthy controls [3, 16]. In patients with type-2 diabetes mellitus, an increase of the free fatty acid level induced by a short-term (3 days) very-low calory diet led to accumulation of myocardial fat and reduction of diastolic function; however, after decreasing free fatty acid level with administration of acipimox the myocardial fat content and diastolic function remained similar to baseline values [18]. A prolongation of a very-low calory diet has been shown to reduce the myocardial triglyceride content and improve cardiac function in both diabetics and nondiabetic obese subjects [19-20]. With regards to the influence of anti-diabetes drugs, in a study including 74 participants with type-2 diabetes assigned to metformin or pioglitazone or placebo, no changes in the myocardial triglyceride content was demonstrated after 24 weeks [21].

In humans, cardiac steatosis has been associated with deterioration of left ventricular diastolic function, increase of left ventricular mass [3], and also with coronary artery disease [21]. Mazzali et al [21] compared the amount of myocardial triglyceride in patients with or without coronary artery disease; a total of 41 patients undergoing coronary-artery bypass or surgical valve replacement were included, and immunohistochemistry for perilipin 1 and 2 was used to characterise lipid droplets in myocardial biopsies from the right atrial appendage. The authors found a higher myocardial fat accumulation in subjects with coronary artery disease, and myocardial steatosis was shown to positively correlate with waist circumference and adiponectin level.

From a physiopathological perspective, the accumulation of intramyocardial triglyceride (secondary to an elevation of the free fatty acid) causes a hyperactivation of the beta-oxidation with subsequent excess formation of reactive oxygen species, which mediate lipotoxicity [22]; reactive oxygen species cause a modification of the sarcoplasmic reticulum Ca2+-ATPase (SERCA), which is "an early contributor of diastolic dysfunction in the insulin-resistant myocardium and of myocardial fibrosis

and hypertrophy" [3]. Nonetheless, it remains unclear whether cardiac steatosis is directly involved in the pathogenesis of some heart diseases, although there are clear evidence suggesting that it represents "at least an indirect marker of early cardiac dysfunction" [3].

Cardiac adiposity and atrial fibrillation

The possible role of cardiac adiposity in the pathogenesis of atrial arrhythmias was first suggested by necropsy studies performed in the 1960s and 1970s [23-26], which demonstrated excess adipose tissue in the interatrial septum and in epicardium in subjects with atrial fibrillation (AF). It was hypothesized that a "lipomatous hypertrophy" of the interatrial septum may promote atrial arrhythmias by interrupting electrical pathways [1, 27].

More recent studies have provided evidence supporting the association between epicardial fat and AF. In a subanalysis of the Framinghan Heart Study, CT-derived volume of pericardial fat (but not intrathoracic or visceral abdominal fat) was an independent predictor of AF in a multivariable-adjusted model (odds ratio 1.28, CI95%1.03-1.58) [28]. Similar results were found by Al Chekakie et al in a study including 273 patients [29]. Batal et al demonstrated that left atrial epicardial fat was an independent predictor of AF burden [30]. The indexed left atrial epicardial fat has been shown to predict outcomes of catheter ablation of AF [31]. Pericardial fat measured with cardiac magnetic resonance has been shown to independently predict the presence of AF, AF severity and outcomes of AF ablation [32]. In a metanalysis including a total of 352 275 individuals [33], Wong et al showed that a "1-standard deviation higher epicardial fat volume was associated with 2.6-fold higher odds of AF (odds ratio, 2.61; 95% confidence interval [CI], 1.89–3.60), 2.1-fold higher odds of paroxysmal AF (odds ratio, 2.14; 95% CI, 1.45–3.16) and 5.4-fold higher odds of persistent AF (odds ratio, 5.43; 95% CI, 3.24–9.12)"; conversely, abdominal and overall adiposity were much weaker predictors of AF. Interestingly, these findings suggest the presence of a biological gradient between AF severity and amount of epicardial fat, with increasing level of epicardial fat in subjects with persistent or permanent vs. paroxysmal forms of AF [1].

Haemers and co-authors identified a higher degree of subepicardial fibrofatty remodelling in patients with AF versus no-AF; fibrofatty infiltrates were more prominent in those with permanent vs. paroxysmal AF [34]. Another recent systematic review and metanalysis including 12 studies concluded that total epicardial fat, left atrial epicardial fat and epicardial fat thickness predict the risk of recurrence post AF ablation, although quality of the evidence was low [35]. An association between epicardial fat and post-surgery AF has also been proposed [36].

Several mechanisms have been proposed to explain the correlation between epicardial fat and AF. Epicardial fat releases pro-inflammatory adipokines [37] which can cause structural and electrical atrial remodelling, hence promoting AF [38]. Local inflammation appears to have a physiopathological role in AF, indeed several inflammation markers such as "C-reactive protein, IL-6, IL-8, IL-1b, and TNF-alfa have been associated with incidence, severity and recurrence of AF" [1, 39]. Furthermore, epicardial fat has been shown to have "greater 18-fluorodeoxyglucose uptake on positron emission tomography" [1] compared to other adipose tissues, possibly suggesting a local inflammatory activity [39]. In addition, systemic anti-inflammatory drugs such as corticosteroids have been shown to reduce atrial remodelling and AF incidence [1, 40-41]. Inflammation mediated by epicardial fat has also been proposed as possible pathogenetic mechanism of post-operative AF [42], however surgical removal of fat pad in subjects undergoing coronary artery bypass has shown contradictory results with regards of the maintenance of sinus rhythm post-surgery [43-44]. Another postulated mechanism correlating AF and epicardial fat is the direct infiltration of adipose tissue in the underlying myocardium. Myocardial infiltration by adipocytes has been shown in animal models [45] and may induce development of conduction abnormalities and anisotropy similarly to microfibrosis [1]. In addition, epicardial fat appears to be associated with atrial structural remodelling, which has a well-established role in the onset and perpetuation of AF. Indeed, epicardial fat positively correlates with left atrial size [46] which is a key substrate of AF and has also been associated with diastolic dysfunction. Epicardial fat is likely to promote atrial fibrosis via paracrine effects [1], a mechanism supported by a study on animal models [47] and is also a source of reactive oxygen species (lipotoxicity). Salgado-Somoza et al demonstrated a higher concentration of proteins

involved in oxidative stress in the epicardial vs. subcutaneous adipose tissue [48]. A study by Gaborit et al has shown that epicardial fat tissue has specific transcriptomic signature depending on its anatomical location, with peri-atrial adipocytes "expressing genes implicated in cardiac muscle contraction and intracellular calcium signalling pathway" [49] which might be involved in AF pathogenesis. Some recent evidences demonstrate that both epicardial fat and paracardial adipose tissue express the enzyme aromatase [50], which has a key role in the synthesis of estrogen and has been associated with an increased incidence and duration of AF [51].

The epicardial fat contains and might influence the ganglionated plexi, which are implicated in AF pathogenesis [1]. This is supported by data showing that botulin injection in the epicardial fat results in a reduction of post-operative AF and AF burden at 3 years in patients undergoing cardiac surgery [52]. Furthermore, there is a large body of evidence showing that autonomic cardiac denervation improved outcomes of catheter ablation for AF [53].

The epicardial fat might represent a source of trigger activity, which has a key role in the pathogenic model of AF. Indeed, areas of left atrial epicardial fat accumulation has been shown to correlate with sites of high dominant frequency identified with 3-D mapping, suggesting that epicardial fat may influence AF triggers [54]. Finally, epicardial fat might also cause an electrical atrial remodelling via paracrine effects. Epicardial adipocyte-incubated left atrial myocytes were shown to have a longer action potential duration and a more-positive resting membrane potential [55].

CHAPTER II

ATRIAL FIBRILLATION

Definition

Atrial fibrillation (AF) is a supraventricular arrythmia characterised by a rapid and irregular atrial activity [57-58]. On electrocardiogram (ECG), diagnostic criteria for AF include absence of distinct P waves with irregular atrial activity and irregularly irregular R-R intervals (in the presence of a preserved atrioventricular conduction). The atrial cycle length during AF is typically lower than 200ms, with an atrial activation rate ranging from 300 to 600 beats per minute (bpm) [57-58]. The diagnosis requires rhythm documentation of AF on an ECG tracing and, by convention, clinical AF is defined by the presence of an episode lasting for at least 30 seconds [59].

Epidemiology

It has been estimated that about 44 million individuals worldwide were suffering from AF in 2016 [58]. Prevalence of AF in adults ≥20-year-old is about 3%, and increases up to 10% in those aged ≥80 [60] and 17.8% in subjects ≥85-year-old according to the Rotterdam Study [61]. The lifetime risk of AF in Europe is currently estimated at 1 in 3 subjects at the age of 55 [58]. The overall incidence of AF is expected to increase over the next decades given the progressively extended longevity [58]. Males have a higher risk of developing AF compared to females, and prevalence and incidence of AF appears to be higher in Caucasians vs. non-Caucasians [58, 62-63].

Pathogenesis

According to the prevailing hypothesis, the pathogenesis of AF involves an interaction between an initiating *trigger* and a vulnerable atrial *substrate* [63]. As the substrate remodels and progresses over time, the role of the triggers in initiating AF might become less important as AF becomes more stabilised. Factors triggering AF include "sympathetic or parasympathetic stimulation, bradycardia, premature atrial complex", "supraventricular tachycardias" such as atrial flutter or atrio-ventricular

reentrant tachycardia, and "acute atrial stretch" [63]. It is now well established that premature atrial complexes originating from the myocardial sleeves within the pulmonary veins represent the source of triggers in the large majority of patients; Haïssaguerre et al first demonstrated in a seminal study the role of atrial ectopic beats arising from the pulmonary veins in the genesis of AF and showed a reduction of AF burden following catheter ablation of such foci [64]. The complex fiber architecture and electrical properties of the pulmonary veins can lead to rapid atrial firing and also reentry activity [64, 65]. Abnormalities in the Ca^{2+} intracellular metabolism have a key role in the genesis of the triggered activity in the pulmonary veins [63], with a diastolic leak of Ca2+ from the sarcoplasmic reticulum causing spontaneous depolarisation (early- or after-depolarisation). AF-triggering foci have also been identified in non-pulmonary vein structures, including superior vena cava, coronary sinus, ligament of Marshall, posterior left atrial wall, left atrial appendage, and crista terminalis [66].

The mechanisms of maintenance of AF remain controversial [63, 67]. One factor is the persistence of the triggers ("focal drivers"), with AF being maintained by a continuous firing of the triggering focus. However, perpetuation of AF can occur "even in the absence of focal drivers" [63] as result of structural and electrical remodelling, including shortening of the atrial refractoriness and left atrial dilatation. Such atrial abnormalities promote the maintenance of AF by stabilizing reentry. Two dominant theories have been proposed to explain the reentrant mechanism underlying AF, including the *multiple independent wave reentry* or the *reentrant rotor* hypothesis [63, 67]. According to the former, AF is "sustained by multiple randomly wandering wavelets" [63] colliding with each other and extinguishing themselves or creating "new daughter wavelets" [63]. As result of this process, the atria are continually reexcited and AF is perpetuated. The short cycle length of these circuits results in atrial stimulation during local refractoriness, with subsequent development of functional block, slow conduction, and multiple wavefronts. The maintenance of a "multiple-circuit reentry" relies on the capacity of the atrial tissue to perpetuate enough simultaneous reentrant wavefronts [63]. There is an increasing body of evidence supporting the *reentrant rotor* hypothesis, which implies the presence of localized source drivers [63, 67]. This hypothesis suggests that "AF is intermittently maintained" by "localized (spatially stable) high-frequency sources with periods of

self-sustaining disorganization" [63]. Rotors (or spiral wave) are characterized by a core and peripheral spiral arms propagating in the surrounding tissue (fibrillatory conduction). Rotors may be fixed or alternatively precess in small areas [63, 67].

The development of a vulnerable substrate is a key element for the maintenance of AF and is characterised by structural and electrical changes which occur mainly in the left atrium (atrial remodelling). Such changes are also induced by the arrhythmia itself and favour its perpetuation ("AF begets AF").

Electrical remodelling

Electrical remodelling is caused by "the high rate of electrical activation" [63], which promotes changes in refractoriness caused by AF including shortening of the action potential duration and atrial refractory period, as well as reduction in the amplitude of the action potential plateau [63, 67]. In addition, "the refractory period fails to lengthen appropriately at slow rates (e.g., with return to sinus rhythm)" [63] in patients with AF and changes in refractoriness are "spatially nonuniform", leading to increased differences in refractoriness among different atrial regions [63]. The mechanisms underlying electrical remodelling are not completely clear and may involve downregulation of the L-type Ca^{2+} current, increase in the outward K^{+} currents, and altered expression and localisation of connexins that connect atrial myocytes. Atrial ischemia may contribute to the electrical remodelling and shortening of the atrial refractory period by activating the sodium/hydrogen exchanger [63].

Structural remodelling

Structural changes linked to AF include cardiomyocyte hypertrophy, necrotic and apoptotic cell loss, fibrosis, impaired atrial contractility, as well as atrial dilatation and stretch [63]. Atrial dilatation is one the most prominent aspect of structural remodelling and is associated with an increased electrical instability secondary to slowing of atrial conduction velocity and shortening of the refractoriness. Furthermore, atrial dilatation is also strongly correlated to the amount of fibrosis. Fibrosis causes heterogeneity of electrical conduction and predisposes to the creation of reentrant circuits, as such it appears to have a central role in the development and maintenance of AF. Atrial fibrosis can be related to underlying risk factors for AF such

as heart failure, hypertension, diabetes mellitus, obesity, or to AF itself [63]. The molecular mechanisms underlying this pro-fibrotic process involve "pro-inflammatory cytokines, oxidative stress, transforming growth factor-β_1, connective tissue growth factor, renin–angiotensin–aldosterone system, calcium-dependent proteases/phosphatases, extracellular matrix regulatory proteins, hypoxia-inducible factor-1α, and endothelin-1 system" [69]. AF can also cause relevant contractile and cellular remodelling including apoptosis, "loss of myofibrils, accumulation of glycogen, changes in mitochondrial shape and size, fragmentation of sarcoplasmic reticulum, and dispersion of nuclear chromatin" [63].

Inflammation and oxidative stress

There is a growing body of evidence suggesting a critical role of inflammation in the pathogenesis of AF. Inflammation has been linked to several pathological processes including apoptosis, fibrosis and oxidative stress [63]. Excessive generation of reactive oxygen species has been shown in patients with AF compared to controls and various major enzymatic sources of reactive oxygen species have been implicated in this process, including NADPH oxidase (NOX), uncoupled nitric oxide synthase and xanthine oxidase [63, 70]. The increased generation of reactive oxygen species may have a role in favouring AF onset as well as promoting its maintenance [63]. Inflammation and oxidative stress have been proposed as possible therapeutical targets for preventing AF, however studies investigating use of statins, vitamin C, glucocorticoids, statins, and angiotensin II inhibitors for this purpose have shown somehow contradictory results [63].

Role of the autonomic nervous system

Both the extrinsic and intrinsic autonomic nervous system is involved in initiation and perpetuation of AF [63]. The atrial innervation is provided by both the sympathetic and parasympathetic elements of the extrinsic ganglia [63], as well as the network of ganglionated plexi localized in the epicardial fat and in the ligament of Marshal [66, 68]. Increased sympathetic activity shortens the atrial refractory period and promotes calcium release and subsequent after-depolarisation activity which can initiate AF [63]. Enhanced vagal tone is involved in the onset of AF in young patients in the

absence of structural cardiac abnormalities [63]. Increased parasympathetic activity can cause a heterogeneous shortening of the atrial refractory period, and as such can promote reentry and subsequent AF onset and maintenance. Furthermore, parasympathetic stimulation can result in the emergence of focal atrial triggers [63].

Epicardial fat

As discussed in Chapter 1, there is a growing body of evidence suggesting that epicardial fat plays an important role in the development of the AF substrate [69]. Several studies have demonstrated a correlation between epicardial fat and AF, and these results were confirmed in a metanalysis by Wong et al [33] including a total of 352 275 individuals; in this study, "a 1-standard deviation higher epicardial fat volume was associated with a 2.6-fold higher odds of AF (odds ratio, 2.61; 95% confidence interval [CI], 1.89–3.60), 2.1-fold higher odds of paroxysmal AF (odds ratio, 2.14; 95% CI, 1.45–3.16) and 5.4-fold higher odds of persistent AF (odds ratio, 5.43; 95% CI, 3.24–9.12) compared with sinus rhythm". Furthermore, previous studies have demonstrated an association atrial pericardial fat and "the severity of AF and post ablation recurrence, which persisted after adjusting for body weight" [1]. Of note, the correlation between pericardial fat and AF appears to be independent from other measures of obesity [1]. Several pathophysiological mechanisms could contribute to the association between epicardial fat and AF [1], including: 1) fatty infiltration of the underlying myocardium, which could result in conduction abnormalities similarly to microfibrosis; 2) stimulation of atrial fibrosis via paracrine effects; 3) inflammation; 4) oxidative stress, which results from production of reactive oxygen species in the epicardial fat; 5) changes in the cardiac muscle activity; 6) adipocyte-related gene expression; 7) autonomic dysfunction; 8) diastolic dysfunction; 9) trigger activity.

Classification

According to the Guidelines of the European Society of Cardiology (ESC) [58, 60], based on clinical presentation, duration and spontaneous termination, AF can be classified as per following:

- *"First diagnosed*: AF not diagnosed before, irrespective of its duration or the presence/severity of AF-related symptoms.

- *Paroxysmal*: AF that terminates spontaneously or with intervention within 7 days of onset.
- *Persistent*: AF that is continuously sustained beyond 7 days, including episodes terminated by cardioversion (drugs or electrical cardioversion) after 7 days
- *Long-standing persistent*: continuous AF of >12 months' duration when decided to adopt a rhythm control strategy.
- *Permanent*: AF that is accepted by the patient and physician, and no further attempts to restore/maintain sinus rhythm will be undertaken"

Early persistent AF is defined as AF lasting for ≥7 days and less than 3 months according to the guidelines of the American Heart Association/American College of Cardiology/Heart Rhythm Society (AHA/ACC/HRS) [71].

Based on the different clinical subtypes, a further classification of AF has been proposed [60]. This includes:

- AF secondary to structural heart disease
- Focal AF, which typically occurs in young subjects who have frequent and short paroxysms of AF caused by trigger generally localized in the pulmonary veins
- Polygenic AF, which occurs in patients with multiple genetic variants associated with early onset of AF
- Post-operatory AF, which occurs post major surgery in subjects previously in sinus rhythm and with no background of AF
- AF in subjects with mitral stenosis or valvular prosthesis
- AF in the athletes
- Monogenic AF, which typically occurs in subjects with inherited cardiomyopathy, including channelopathies.

Predisposing factors for AF

Several cardiovascular and non-cardiovascular conditions increase the risk of developing AF. Unmodifiable risk factors include:

- *Age*: the risk of AF increases with advancing age, which represents the most important risk factor. The prevalence of AF is less than 1% in subjects younger than 65 and increases to more than 10% in those older than 80 [58, 60, 67].
- *Gender*: AF is more common in men, in both developed and developing countries. In the Framinghan Heart Study, the age-adjusted incidence of AF was 3.8 in men and 1.6 in women [72]. Gender-related differences are likely to be caused by other underlying risk factors. A study by the CHARGE-AF group did not identify sex-related differences after adjusting for AF-related risk factors [73]. The lifetime risk for AF does not differ between men and women despite higher AF incidence in men, this is likely related to the shorter life expectancy in men [60, 63].
- *Race*: the risk of AF adjusted for age is "higher in whites as compared to blacks, Asians, and Hispanics" [63].
- *Genetics*: a family history of AF is associated with "a 40% increased risk in first-degree relatives" [63, 58]. A genetic predisposition appears to be particularly relevant for early-onset AF. Rare form of familiar AF caused by disease-causing gene mutation have been described. Furthermore, at least 14 genetic variants have been identified as risk factors for AF [62, 66-67].

Modifiable risk factors for AF include [58, 63]:

- Hypertension
- Heart failure
- Valvular heart disease
- Coronary artery disease
- Congenital heart disease
- Obstructive sleep apnoea
- Obesity
- Diabetes mellitus
- Hyperthyroidism
- Pulmonary embolism

- Chronic kidney disease
- Physical activity and sedentary lifestyle
- Alcohol
- Smoking
- Recreational drugs

Clinical presentation and Diagnosis

Patients with AF can experience a variety of symptoms including palpitation, dyspnoea, fatigue, reduced exercise tolerance, lethargy, chest pain, sleep disorder, anxiety, polyuria, syncope and presyncope [63]. Syncope is an uncommon presentation and can occur on termination of the arrhythmia in patients with sinus node disease or because of fast ventricular response especially in subjects with hypertrophic cardiomyopathy, aortic stenosis or accessory pathway with antidromic conduction [74].

Up to 25% of patients with AF can be asymptomatic, more commonly the elderly and those with persistent AF [60]. Between 25 and 40% of AF patients have minimal or no symptoms, while 15-30% experience severe or disabling symptoms [58]. Symptomatic as well as asymptomatic episodes of AF can occur even in the same patient [63]. More than 60% of patients with AF report impaired quality of life, and 16-20% report depression [58]. AF-related complications such as stroke/TIA or acute heart failure can be the first clinical manifestation of AF in some patients. The European Heart Rhythm Association (EHRA) recommend the use of the EHRA symptoms scale to characterise the symptom status and severity in AF patients (class of recommendation I, level of evidence C) [58, 60].

Diagnosis

According to the ESC guidelines, "the diagnosis of AF requires rhythm documentation using an electrocardiogram (ECG) showing the typical pattern of AF" [58]. Given the non-negligible rate of asymptomatic AF, "opportunistic screening by pulse taking or ECG rhythm strip is recommended" in subjects ≥65 years (class of recommendation I, level of evidence B) [68]. In patients with clinical suspicion of AF and only sporadic symptoms, diagnosis can be achieved with ECG

Holter, event recorder or loop recorder. In addition, over the last few years several technologies have been developed for AF detection including smartwatches, dedicated connectable devices, dedicated smartphone apps, oscillometric blood pressure cap etc. Patients with AF should have diagnostic assessement including full medical history and evaluation of concomitant conditions, 12-lead ECG, echocardiogram, blood test (including full blood count, thyroid, kidney and liver function) [58]. Further investigations can be necessary based on patient's characteristics.

Natural history

"AF begets AF" is a commonly used expression to describe the natural history of this arrhythmia. A seminal study on animal models have shown that AF causes an electrophysiological atrial remodelling with subsequent perpetuation of the arrhythmia [75]. An analysis of the Framinghan Heart Study has shown that only 10% of AF patients remained free from AF relapses after 2 years from the initial diagnosis, while 26% and 34% developed recurrent or persistent AF, respectively [76]. However, these findings have not been confirmed by other studies. In the CARAF registry, AF progression from paroxysmal to permanent forms occurred in only 8.6% of the participants at one year and 24.7% at 5 years [77]; nonetheless, the 10-year follow up of that registry showed a >50% progression from paroxysmal to persistent AF [78]. In the Euro Heart Survey, 80% of the patients with paroxysmal AF continued to suffer from paroxysmal episodes after one year [79]. Studies in subjects with permanent pacemaker, which allows an accurate measurement of the AF burden, have shown that 54-76% of the paroxysmal forms of AF do not progress to persistent or permanent forms [80-81]. There are evidences that "progression to persistent/permanent AF is associated with adverse clinical outcomes" [82], but "whether AF progression is a determinant of adverse prognosis or rather a marker of an underlying progressive disease/substrate" remains unclear [58].

Prognosis

AF is independently associated with a 1.5-3.5 increased risk of all-cause mortality [58]. Stroke and heart failure represent the main causes for morbidity and mortality. It is estimated that 20-30% of all ischaemic strokes and 10% of cryptogenic strokes are caused by AF. 20-30% of AF patients develop left ventricular dysfunction or heart failure, which can be secondary to fast ventricular rate, irregular ventricular contraction or underlying cause of AF. Several studies have also shown an 1.4-1.6 increased risk of cognitive decline/vascular dementia in AF patients, irrespectively of stroke history; micro-embolism and hypoperfusion have been proposed as possible physiopathological mechanisms causing brain white matter lesions. More than 60% of subjects with AF reports an impaired quality of life, and 17% report disabling symptoms [83]. Decreased quality of life can lead to depression in 16-20%. Patients with AF have a 10-40% rate of annual hospitalization [58].

Clinical management and treatment

The implementation of the "Atrial Fibrillation Better Care (ABC) pathway" ("A" Anticoagulation/Avoid stroke; "B" Better symptom management; "C" Cardiovascular and Comorbidity optimization) for guiding clinic management of AF has been shown to improve clinical outcomes and healthcare costs compared to usual care [58, 84].

"A"- Anticoagulation and thromboembolic risk

AF represents an important risk factor for thromboembolism and is associated with a 5-fold increase of the risk of stroke; however, such risk varies among the AF population and depends on the presence of specific predisposing factors. Thromboembolic strokes secondary to AF are particularly severe; they tend to be large and multiple, often involving bilateral infarcts, and are associated with the worst outcomes in terms of mortality and permanent disability [85]. Use of oral anticoagulation has been shown to reduce the risk of stroke and other thromboembolic events by approximately two-thirds compared to placebo [86]. Nonetheless, anticoagulation is associated with an increased bleeding risk and as

such it should be initiated only after careful risk stratification and assessment of its pro and cons.

Use of the CHA_2DS_2VASc score is recommended by current guidelines for stratifying the thromboembolic risk in subjects with AF (class of recommendation I, level of evidence A) [58]. This score includes the following risk factors: congestive heart failure (1 point), hypertension (1 point), age ≥75 years (2 points), diabetes mellitus (1 point), stroke (2 points), vascular disease (1 point), age ≥65-74 years (1 point), and female sex (1 point). Males with CHA_2DS_2VASc score of zero and females with a score of 1 have a low risk of stroke and do not require anticoagulation (class of recommendation I, level of evidence A) [58]. Oral anticoagulation is recommended in patients with CHA_2DS_2VASc score ≥2 in men and ≥3 in women (class of recommendation I, level of evidence A) [58]. There are less definite evidence supporting use of anticoagulation in subjects with CHA_2DS_2VASc score of 1 in men and 2 in women; based on current guidelines anticoagulation should be considered in this sub-group, and "treatment should be individualized" according to the "net clinical benefit" and take into account "patient's values and preferences" (class of recommendation IIa, level of evidence B) [58].

Of note, the CHA_2DS_2VASc score has been validated only in patients with "non-valvular" AF and should not be used in subjects with at least moderate mitral stenosis, who benefit from anticoagulation given the high risk of stroke [58, 60]. In addition, there is a large body of evidence showing that patients with concomitant hypertrophic and AF benefit from anticoagulation [79] regardless of the CHA_2DS_2VASc.

Current guidelines recommend the use of a "structured risk-score-based bleeding risk assessment" (such as the HASBLED score) in order to detect "non-modifiable and address modifiable bleeding risk factors", and also to identify patients with high risk of bleeding who might benefit from more frequent follow-up (class of recommendation I, level of evidence B) [58]. Importantly use of bleeding risk scores should not in itself guide decision of whether or not commencing thromboprophylaxis with oral anticoagulation (class of recommendation III, level of evidence A) [58].

Although some recent evidences suggest that patients with non-paroxysmal forms of AF might have a higher thromboembolic risk compared to those with paroxysmal AF [88], "clinical pattern of AF (i.e. first detected, paroxysmal, persistent, long-standing persistent, permanent) should not condition the indication to thromboprophylaxis" (class of recommendation III, level of evidence B) [58].

In patients eligible for oral anticoagulation, non-vitamin K anticoagulants are recommended in preference to vitamin K-antagonist, with the exception of subjects with at least moderate mitral stenosis or mechanical prosthetic valve (class of recommendation I, level of evidence A) [58].

In patients with absolute contraindication to oral anticoagulation (such as recent intracranial haemorrhage), non-drug options such as left atrial appendage occlusion might be considered (class of recommendation IIb, level of evidence B) [58].

"B"- Better Symptom management

A rate or rhythm control strategy can be adopted to reduce symptoms. Several randomised studies have compared these two approaches, overall demonstrating similar cardiovascular outcomes [89-90]. However, in the recently published EAST-AFNET 4 [91] a rhythm control strategy, compare to rate control, was associated with a significant reduction of the primary endpoint of cardiovascular death, stroke, or serious adverse events related to rhythm-control therapy (hazard ratio 0.79, 95% CI 0.66-0.94) after a median follow-up of 5.1 years. This trial included 2789 patients at high risk of cardiovascular complications, i.e. age >75 years of age, prior transient ischemic attack or stroke, or at least two of the following criteria: age >65 years, female sex, heart failure, hypertension, diabetes, severe coronary artery disease, chronic kidney disease, and left ventricular hypertrophy. Unlike the older RACE and AFFIRM trials [89-90], the EAST-AFNET 4 [91] study included catheter ablation in the rhythm control arm; in addition, enrolled patients had a much shorter duration of AF from the first clinical presentation (median of 36 days) compared to the older studies. Further evidences are required to clarify whether a rhythm control strategy offers clinical benefit compared to rate control. To the date, the decision between these two strategies should be mainly guided by patients' symptoms and preference. According to the

ESC/EHRA guidelines, "a rhythm control therapy is recommended for symptom and quality of life improvement" in subjects with symptomatic AF (class of recommendation I, level of evidence A) [58].

Several drugs can be adopted for implementing a rate control strategy, including beta-blockers, non-dihydropyridine calcium antagonist, digoxin, or rarely amiodarone; combination of these drugs is sometimes necessary in selected cases when the targeted heart rate is not achieved. In subjects elected to rate control, a ventricular response at rest <110bpm should be targeted in the first instance (class of recommendation IIa, level of evidence B). However, a lower heart rate (<80 bpm) should be targeted in case of persistent symptoms or deterioration of the left ventricular function. In selected patients who are unresponsive or intolerant to rate or rhythm control therapy, implantation of a permanent pacemaker followed by atrioventricular node ablation should be considered (class of recommendation IIa, level of evidence B) [58].

A long-term rhythm control strategy can be achieved with antiarrhythmic drugs or catheter ablation. Most commonly used antiarrhythmic drugs include class Ic (flecanide or propafenone), and class III (such as amiodarone, sotalol, dronedarone).

Catheter ablation of AF

Catheter ablation is a well-established treatment for AF and has been shown to be superior compared to antiarrhythmic drugs for maintaining sinus rhythm and preventing AF recurrence [92]. The cornerstone of AF ablation is the pulmonary vein isolation (PVI), which can be achieved by creating linear lesions around the pulmonary vein antrum (*circumferential antral* ablation) with the use of either point-to-point radiofrequency or single-shot technology such as the cryoballoon. The main indication for AF ablation is symptom relief, as there are no definite evidences showing an improvement of cardiovascular outcomes compared with medical therapy. The CABANA randomized controlled trial, which enrolled 2204 patients, failed to prove a reduction of the primary composite outcome of death, stroke, major bleeding or cardiac arrest with AF ablation versus medical therapy on the pre-specified intention-to-treat analysis (8.0% vs. 9.2%, p=0.30); however, the

results of this study were biased by the lower-than-expected event rates as well as treatment cross-over, with 27.5% of the patients ultimately receiving AF ablation despite being randomized in the drug arm. Indeed, on both the received treatment and per-protocol analyses catheter ablation was significantly superior to medical therapy [93]. The recent EAST-AFNET 4 trial [91] has demonstrated the superiority of a rhythm control strategy (including catheter ablation) versus rate control, however this study was not designed to compare catheter ablation versus antiarrhythmic drugs. To the date, PVI is recommended to reduce AF symptoms after one failed or not tolerated antiarrhythmic drug (Vaughan-Williams class I or III) in patients with both paroxysmal and persistent AF (class of recommendation I, level of evidence A/B) [58]. In addition, AF ablation as first-line therapy should be considered in patients with paroxysmal AF (class of recommendation IIa, level of evidence B) and may be considered for those with "persistent AF without major risk factors for recurrence" (class of recommendation IIb, level of evidence C) [58].

There are evidences supporting use of catheter ablation of AF for improving cardiovascular outcomes in patients with heart failure. The CASTLE-AF trial [94] randomized 363 patients with heart failure with reduced ejection fraction, primary prevention dual chamber implantable cardioverter defibrillator and symptomatic paroxysmal or persistent AF to catheter ablation vs. medical therapy. After a median follow-up of 37.8 months, the primary composite endpoint of any-cause death and hospitalization for heart failure occurred in fewer participants undergoing AF ablation compared to those in the medical therapy group (28.5% vs. 44.6%; hazard ratio, 0.62; 95% CI, 0.43-0.87; P=0.007). According to the recent ESC/EHRA guidelines, AF ablation should be considered in selected patients with heart failure with reduced ejection fraction to improve survival and reduce hospitalization (class of recommendation IIa, level of evidence B) [58].

In AF patients with tachycardia-induced cardiomyopathy, catheter ablation is recommended to reverse left ventricular dysfunction regardless of the symptom status (class of recommendation I, level of evidence B) [58].

The long-term success rate of AF ablation depends on the type and duration of AF, degree of cardiac structural remodelling, ablation technique adopted and definition of success. Overall, freedom from AF-recurrence at 12-month post

ablation is achieved in approximately 70% of patients with paroxysmal AF and 50% of those with persistent AF [56]. Rate of periprocedural complication is low, with vascular complications at the femoral access site being the most frequent (2-4%). Cardiac tamponade can occur in about 1% of the procedures, thromboembolic events in <1%, and oesophageal perforation/fistula in approximately 0.5% [58]. Other severe complications include phrenic nerve palsy (1%) and pulmonary vein stenosis, which has become extremely rare with the current ablation technique (<1%); the mortality rate is 0.1% [58].

"C"- Cardiovascular risk factors and concomitant diseases: detection and management

Cardiovascular risk factors and diseases as well as unhealthy lifestyle have an important role in the onset and perpetuation of AF, contributing to the development of atrial remodelling and substrate. Interventions targeting such predisposing conditions have been shown to improve rhythm control in AF patients [95]. Lifestyle interventions include weight loss particularly in obese patients, regular moderate physical activity/exercise, and reduction of alcohol intake [96]. Adequate treatment of specific comorbidities such as hypertension, obstructive sleep apnoea and diabetes mellitus is of particular importance and is recommended by current guidelines [58].

CHAPTER III

EXPERIMENTAL SECTION

Background: atrial fibrillation and type-2 diabetes mellitus

Type-2 diabetes mellitus (DM) is a complex chronic disease. Its prevalence among adult population was 6.4% in 2010 and is expected to increase up to 7.7% by 2030 [97]. There is a large body of evidence suggesting that DM is an independent risk factor for AF. In the Framinghan Heart Study, which included a total of 4731 patients, DM was associated with a 40% increased risk of AF in men and 60% in women during a follow-up of 38 years [98]. A metanalysis including a total population of 8,037,756 indicated that "patients with DM had approximately 49% greater risk of developing AF compared with individuals without DM"; however, after adjusting for other risk factors the relative risk was estimated at 23% [99]. Furthermore, the development of AF in diabetic patients appears to be a strong marker of poor outcomes. In the ADVANCE trials, including 11140 patients with DM, AF was associated with a 61% greater risk of any-cause death and a comparable higher risk of cardiovascular events [100]. Similarly, in a sub-analysis of the ORBIT-AF registry, which enrolled 9749 patients, among AF patients DM was associated with "worse AF symptoms and lower quality of life", as well as "increased risk of death and hospitalization" [101].

Pathophysiological mechanisms of atrial fibrillation in diabetes mellitus

The pathogenesis of AF in DM patients has not been completely understood, and several mechanisms have been hypothesized. Atrial structural remodelling has been described in diabetic hearts, typically manifesting as interstitial fibrosis which contribute to left atrial dilatation and left ventricular hypertrophy [102-104]. DM has been shown to promote atrial fibrosis in both animal and human studies through different mechanisms [102]. Segwick et al showed that atrial cardiac fibroblasts in DM patients have an enhanced level of collagen synthesis [105]. In addition, DM is associated with "enhanced angiotensin II, TGF-β signalling, and increased production of reactive oxygen species" (oxidative stress) [102], which are all well-known pro-

fibrotic pathways [102-108]. Oxidative stress is a hallmark of diabetic cardiomyopathy. Several mechanisms that generate reactive oxygen species in the diabetic heart have been described, including mitochondrial electron leakage, "enzymes such as NADPH oxidase, xanthine oxidase and 12/15 lipoxygenase, uncoupling of nitric oxide synthase, accumulation of AGEs and activation of PKC" [109]. The upregulation of reactive oxygen species induced by DM stimulates cardiac fibroblast to produce collagen, with subsequent development of fibrosis. The reactive oxygen species production is increased in diabetic patients with poor glycaemic control [110]. In addition, advanced glycation end product (AGE) and receptor for AGE (RAGE) activation also appears to be an important contributor in the development of atrial fibrosis in DM [102, 111]. AGEs promote the production of reactive oxygen species, expression of connective tissue growth factor (CTGF) [112], and inflammation of the heart via activation of myeloid differentiation 2 which is a coreceptor of toll-receptor 4 (a component of the innate immune system) [113]. Indeed, inflammation is an important mediator of atrial structural remodelling in DM. A recent study by Wu et al demonstrated that "NLRP3-inflammasome/caspase-1/Gal-3 signalling pathway is related to the pathogenesis of AF" in the diabetic hearts of rabbits, and inhibition of NLRP3 by glibenclamide reduced atrial remodelling and AF inducibility [114].

Increased cardiac adiposity is a hallmark of both DM and obesity and can promote atrial fibrosis via paracrine effects. Adipokines such as leptin have been shown to play a key role in the development of atrial fibrosis in animal models [115]. Overall, there are evidences suggesting the role of increased cardiac adiposity (particularly epicardial fat) in the genesis of AF, as discussed in the previous Chapters.

Beyond atrial structural changes, electrical atrial remodelling in DM has been implicated in the genesis of AF. A number of studies in animal model with DM have shown a prolonged action potential duration [116-117], reduction of the conduction velocity and repetitive ectopic focal ectopy discharge [118], as well as increased AF susceptibility [116-117]. Such "alterations in atrial action potential morphology may involve Na+ and K+ channels, as well as Ca2+ homeostasis" [102]. There are evidences suggesting a reduction of Na+ and K+ current in DM animal models [119-120]. These currents participate in the atrial repolarisation and their abnormalities

might promote AF. The "calcium/calmodulin-dependent protein kinase II (CaMKII) is upregulated in DM" [121] and together with other mechanisms contribute to the abnormalities in Ca2+ homeostasis which can lead to AF in diabetic patients [121]. Beyond these ion channels abnormalities, there is evidence of changes at the level of gap junctions, which are composed by connexin and have a key role in the myocardial electromechanical coupling [102]. For example, Li and co-authors showed a lateralization of the connexin 43 in the left atrium of type-2 diabetic rats, this would increase conduction heterogeneity and favour arrhythmias [122].

Finally, autonomic dysfunction system may play a role in the pathophysiology of AF [102]. Autonomic neuropathy is a common sequalae of DM; however, there is paucity of data of whether this is associated with AF. Russo et al demonstrated a strong correlation between autonomic neuropathy measured as heart rate variability and AF in DM patients [123]. Neural remodelling in DM rats has been shown to increase the heterogeneity of atrial effective refractive period and susceptibility to AF [124].

Impact of type-2 diabetes mellitus in the outcomes of catheter ablation of atrial fibrillation

Rationale of the study

Catheter ablation is a well-established treatment for AF, however data regarding efficacy and safety in the DM population are mainly restricted to small size and single centre reports, with conflicting results [125] and limited use of the cryoballoon technique. The aim of this study was to further investigate implication of DM on the outcomes of catheter ablation of AF.

Methods

This was an observational non-randomised study conducted in 7 European centres. We included all patients aged over 18 undergoing a left atrial ablation procedure during a 24 months' time interval, with AF refractory to at least one class I or class III antiarrhythmic drug. All patients provided written informed consent prior to the procedure. We assessed DM as a potential independent predictor of AF/atrial tachycardia relapse. The study complied with the Declaration of Helsinki and the research protocol was approved by the local ethics committees. Demographics, and admission day anthropometric data were collected. Patients' notes and electronic records were systematically assessed to identify relevant comorbidities. Data from the referral transthoracic echocardiogram was analysed and a multislice computed tomography scan imaging of the left atrium was systematically collected pre-procedure. Procedures were performed under sedation or general anaesthesia, according to each institution's protocol. Venous access was obtained via the femoral vein, with use of vascular ultrasound at operator's discretion. In the absence of patent foramen ovale, a single or dual transseptal puncture was performed under fluoroscopic guidance. Use of transoesophageal echocardiography was left at operator preference. Intravenous heparin was administered to maintain an activated clotting time of 300–350 seconds. Pulmonary vein isolation was the main procedural endpoint and was performed as a first step in all procedures. If the patient was in AF at the start of the procedure and the arrhythmia organized into an atrial tachycardia this was mapped and ablated. In patients undergoing cryoballoon ablation, if the patient remained in AF

after isolation of all four pulmonary veins, direct-current cardioversion to sinus rhythm was performed and no further ablation undertaken. In patients undergoing radiofrequency ablation of persistent AF and not cardioverting to sinus rhythm or not organizing to atrial tachycardia during ablation we mapped and ablated areas of complex fractionated atrial electrograms in both atria and the coronary sinus and subsequently DC cardioverted the patient if AF persisted. Patients were evaluated at 3, 6, and 12 months after the procedure. A 12-lead electrocardiogram (ECG) and 24-hour ECG Holter were performed at each follow-up. Additional patient visits and further testing were allowed in case of symptoms. Annual follow-up was performed after the first year. Antiarrhythmic drugs at discharge were prescribed only in selected patients (i.e., for those suffering from longstanding persistent AF, or having relapse during the admission, needing for cardioversion, etc.) and at the operator's discretion, and were discontinued after the first 3 months in the absence of recurrence. Blanking period was defined as the first 3 months post-procedure. Recurrence was defined as any symptomatic or asymptomatic atrial arrhythmia lasting >30 seconds following the blanking period. In case of arrhythmia recurrence during the blanking period not responding to cardioversion (pharmacologic or electrical), patients were classified as having a relapse.

The main efficacy endpoint was freedom from atrial arrhythmias following a blanking period of three months. AF or atrial tachycardia relapse during the initial 3-month blanking period was also documented. The following complications were systematically recorded in order to explore safety: vascular complications (if requiring intervention or prolongation of admission), thromboembolism (transient ischemic attack, stroke and/or systemic embolism during or in the first month after the procedure), phrenic nerve palsy, pericardial effusion (if causing haemodynamic instability and/or requiring pericardiocentesis or prolonged monitoring), oesophageal fistula, and procedure-related death. Other complications were reported at the discretion of the operator.

The chi-square test was used for categorical and Student's t-test for continuous variables. Levene's test was used to check the homogeneity of variance; equivalent non-parametric tests were used when Kolmogorov–Smirnov was in favour of the absence of normal distribution. Results with $P < 0.05$ were regarded as significant.

Kaplan-Meier curves were traced for illustrating freedom from AF or atrial tachycardia among patients with or without DM, and the log rank P test was used for assessing existing differences. Independent predictors of sinus rhythm maintenance after a single ablation procedure were assessed through Cox regression (Method: Forward Likelihood Ratio, Probability for Stepwise 0.05). A propensity score matching was performed to adjust for differences in baseline clinical characteristics. A propensity score was obtained through binary logistic regression: diagnosis of DM (yes or no) was the binary outcome, and all baseline variables were used as covariates for estimating a probability (the propensity score). Then, probabilities in the DM group were matched 1:2 to the closest non-DM patient fulfilling inclusion criteria using the nearest neighbour matching approach. The propensity score was matched to 5 decimals whenever possible. If this was not possible, we subsequently attempted 4, 3 and then 2 decimal matching. If a DM patient could not be matched to any non-DM patient on the second digit of the propensity score, then the DM subject was discarded from the matched analysis. Comparisons between DM and no-DM were performed. Based on Stuart [126], analyses were performed using the groups as a whole, rather than using the individual matched pairs. PASW Statistics version 18.0 was used for descriptive and inferential statistical analysis.

Results

A total of 2504 patients (mean age 61.1±10.2, 29.4% female) underwent catheter ablation of AF. As many as 234 patients (9.3%) suffered from DM. Most patients had paroxysmal AF (57.5%) at baseline, and mean AF duration was 5.0±5.4 years. The cryoballoon technique was adopted in 29.4% of the patients. The baseline population characteristics before and after propensity matching are reported in Tables 2 and 3.

Pulmonary vein isolation was achieved at the end of the procedure in almost all the patients (99.0%), with no significant differences between the two groups. Use of the cryoballoon was comparable among patients with or without DM. Rate of relapse during blanking was significantly more frequent in subjects with DM (24.3% vs. 32.8%, p=0.012). Similarly, relapses at 12 months occurred more frequently in the DM group (25.3 vs. 32.0%, p=0.031) (Table 4). After adjusting for type of AF (i.e., paroxysmal vs. persistent), during a median follow-up of 17±16 months, atrial

arrhythmia free-survival was lower in the diabetics vs. non-diabetics after ablation of persistent AF (log-rank p=0.003), and comparable after ablation of paroxysmal AF (log-rank p=0.554). These findings were confirmed after comparing the DM patients vs. a propensity-matched group of non-diabetics (log-rank p=0.038 for persistent AF). These results are shown in Figures 2 and 3.

Assessment of independent predictors of AF or arrhythmia relapse is illustrated in Table 5. On multivariate Cox regression, DM, BMI, AF duration and LA volume were independent predictors of relapse.

The rate of peri-procedural complications was similar among DM and non-DM patients (3.8% vs. 6.4%, p=0.128) (Table 4). The incidence of cardiac tamponade, other bleeds, major vascular complications, phrenic nerve palsy, and stroke, transient ischemic attack or systemic embolism was very low and comparable.

Efficacy and safety of cryoballoon ablation were comparable to radiofrequency ablation in both DM and no-DM groups (log-rank p=0.437 for persistent AF and p=0.531 for paroxysmal AF).

Discussion

The main finding of this multicentre study is that DM is associated with a higher incidence of atrial arrhythmia relapses at 12 months in patients undergoing catheter ablation of AF. On the Kaplan-Meier analysis, after adjusting for type of AF (i.e., paroxysmal vs. persistent), arrhythmia-free survival at a median follow-up of 17±16 months was lower in diabetic patients with persistent AF compared to those with no DM; however, relapse rates were similar in DM vs. non-DM subjects undergoing ablation for paroxysmal AF. In our series, patients with DM have a higher prevalence of comorbidities such as hypertension, obstructive sleep apnoea, vascular disease and congestive heart failure, and more commonly suffer from non-paroxysmal forms of AF. However, on a multivariate analysis, after adjusting for confounding factors, DM remains an independent predictor of atrial arrhythmia relapses (HR1.39; $CI_{95\%}$1.07-1.88; p=0.016). The higher rate of post-ablation relapses in the DM population was also confirmed in a propensity-matched analysis. Finally, catheter ablation of AF appears to be safe in DM patients, with no significant difference in the complication rate compared to the non-diabetics. Notably, despite DM being a risk factor for

thromboembolism in the AF population, we have found no differences in the number of peri-procedure thromboembolic events between diabetics and non-diabetics. Efficacy and safety of the cryoballoon ablation was comparable to radiofrequency ablation in both DM and no-DM group.

Our findings are clinically relevant, considering the high prevalence of DM and its strong association with AF. Subjects with DM have not only an increased risk of developing AF but are also more prone to AF-related complications such as thromboembolism and heart failure. For these reasons, DM patients might warrant the greatest benefit from an effective treatment of this arrhythmia, with the potential aim not only to improve quality of life, but also prevent its relevant clinical sequalae. The present study confirms that AF ablation is effective and safe in the DM population, despite this traditionally representing a higher risk sub-group with more frequent comorbidities. In fact, among DM patients, as much as 80.2% of those with paroxysmal AF and 57.6% with persistent AF were free from atrial arrhythmia at the 12 months' follow-up. However, an important finding of our analysis is that DM is associated with higher long-term relapse rate after catheter ablation of persistent AF, while outcomes for paroxysmal AF are similar among diabetics and non-diabetics. DM is known to cause significant myocardial remodelling (i.e., diabetic cardiomyopathy) and can promote AF through several physiopathological mechanisms, as discussed above. It is conceivable that in diabetic patients, compared to the non-diabetic, persistent forms of AF are associated with a more severe degree of atrial myopathy and a more complex and multifactorial substrate, resulting in a lower long-term efficacy of catheter ablation. Indeed, DM has been independently associated with left atrial enlargement, regardless of concomitant hypertension and diastolic dysfunction [127]. Our finding could have relevant clinical implications, as an early ablative strategy might be particularly valuable in subjects with DM, in order to prevent the progression from paroxysmal to persistent forms of AF, as the latter appear to be more aggressive and difficult to treat.

The impact of DM on the outcomes of AF ablation has been previously evaluated by other authors, with conflicting results [125]. In the absence of randomised trials, to the best of our knowledge, the largest available controlled study included 339 DM patients from the German Ablation Registry [128]; in this series, after a median follow-

up of 460 days, no differences were found between subjects with or without DM in terms of arrhythmia-free survival. However, these results included diabetic patients with both paroxysmal and persistent AF, and as such no separate outcomes were provided for subjects with different forms of AF; in addition, patients with persistent AF were underrepresented, and results were based on telephone follow-up only. A systematic review and metanalysis by Anselmino et al [129] showed similar outcomes of AF ablation in DM patients compared to the general population, although with relatively frequent need of redo procedure in the diabetics. However, data from a metanalysis including 886 individuals should be interpreted very carefully, especially in the context of relevant methodological bias such as the absence of a direct comparison with a control group.

Another relevant finding is that cryoballoon ablation appears to be effective and safe in DM patients, showing comparable results with the radiofrequency technique. These findings are of interest, as the diabetic population was underrepresented in most of the studies evaluating cryoballoon AF ablation; as such, the cornerstone FIRE and ICE trial included only 22 and 37 diabetic patients in the radiofrequency and cryoballoon group, respectively [130].

Finally, the results of the present study might suggest a potential benefit of an adequate treatment of DM to counteract its deleterious effect on the long-term outcomes of AF catheter ablation. The ARREST-AF study demonstrated that an aggressive risk factor management, including better glycaemic control, significantly improves arrhythmia-free survival after catheter ablation of AF [131]. However, although promising, these data should be confirmed in a prospective randomised fashion.

Several limitations should be acknowledged. First, no data regarding glycaemic control (e.g. HbA1c), DM duration and therapy were available. In addition, this was a multicentre study including experienced large volume centres and might not represent the type of ablation activity performed in other centres with lower caseloads. Obstructive sleep apnoea has emerged as an important predisposing factor for AF, however patients in the present study were not systematically screened for this condition; therefore, the prevalence of obstructive sleep apnoea in our population

could be underestimated. Finally, systematic use of implantable loop recorders may have allowed to document a higher rate of asymptomatic recurrence.

Cardiac steatosis and atrial fibrillation in patients with type-2 diabetes mellitus

Rationale

In recent years, oxidative stress and inflammation have been shown to be central mediators of AF in diabetic and obese patients [132]. Antioxidants agents have been shown to reduce atrial remodelling in animal models [19]. Furthermore, there are evidences of increased level of reactive oxygen species in "mitochondria isolated from atrial tissues of diabetic patients and animal models" [102]. Additional sources of reactive oxygen species in the diabetic heart include "Xanthine oxidase, NADPH oxidase, Monoamine oxidase, Protein Kinase C, Nitric oxide synthase (NOS), and Advanced glycation end-products (AGE)" [102]. Moreover, "antioxidant defence systems such as glutathione are depleted in the atria of diabetic hearts" and "mismatch between reactive oxygen species scavenging and generation promotes oxidative stress and inflammation" [102]. Cardiac steatosis can cause lipotoxicity, which is a central pathway of oxidative stress [3]. There are evidences suggesting a correlation between epicardial fat and AF, however the role of cardiac steatosis (i.e., intramyocardial fat infiltration) in the pathophysiology of AF in DM patients has not been investigated. The aims of this study were: 1) to compare the amount of in vivo cardiac steatosis between DM patients with or without background of AF and 2) to characterize oxidative stress and its correlation with in vivo cardiac steatosis in DM patients with or without background of AF.

Methods

Patients with type-2 DM and coronary artery disease undergoing elective coronary artery bypass surgery (CABG) were included in the study. The study complied with the Declaration of Helsinki and the research protocol was approved by the Barts BioResource review committee (registration ID number 77). All the patients provided written informed consent. In the AF group, participants had a previous established

diagnosis of AF with confirmed rhythm documentation using an electrocardiogram (ECG). ECG, transthoracic echocardiogram, and routine blood test (including glycated haemoglobin and fasting blood sugar) were routinely performed pre-surgery in each patient. Anthropometric variables including height and weight were collected, and body mass index was calculated. Exclusion criteria included age <18, history of congestive heart failure with reduced ejection fraction, cardiomyopathy, hemodynamically significant valvular heart disease, valvular AF, previous cardiac surgery, thyrotoxicosis, and inability to provide informed consent. To avoid confounding factors, women were not included given previous evidence of sex dimorphism in the cardiac adiposity distribution and function [3, 21]. During cardiac surgery, myocardial specimen from the right and/or left atrial appendage were obtained in the operating theatre. The samples were collected and stored at -80 degrees. Primary rabbit polyclonal anti 4-hydroxynonenal and rabbit polyclonal anti-ADFP (perilipin 2-PLP2) were used at dilution of 1:100. The 4-hydroxynonenal is a highly reactive product of lipoperoxidation and was used as a marker of oxidative stress [133]. The PLP2 was used to quantify the amount of cardiac steatosis [21]. The immunohistochemistry was performed using the Ventana DabMap Horseradish Peroxidase Kit. The expression of PLP2 and 4-hydroxinonenal was assessed using a semiquantitative scoring (0: absent, 1: slight, 2: moderate, 3: intense) on 10 randomly selected areas. Based on a previous study which quantified a PLP2 optical density of 0.24 ± 0.05 [21] and assuming a 30% higher density in patients with background of AF, a sample size of 16 patients (8 in both groups) was estimated with alfa 0.05 and 80% power. The chi-square test or Fisher's exact test for categorical variables and Student's t-test for comparison of means. Levene's test was used to check the homogeneity of variance; equivalent non-parametric tests were used when Kolmogorov–Smirnov was in favour of the absence of normal distribution. Pearson's coefficient correlation was implemented for testing association between the variables. Results with $P < 0.05$ were considered significant. SPSS version 26.0 was used for statistical analysis.

Results

A total of 16 patients (68.9±9.5, 100% men) were enrolled, 8 with history of AF and 8 with no previously diagnosed AF. In the AF group, 7 subjects (87.5%) had persistent AF and one paroxysmal AF. All patients in both groups suffered from hypertension and hypercholesterolemia. The mean BMI was 28.4±13.3 kg/m^2, with no significant difference between the two groups. The mean left ventricular systolic function was 47.2±12.4 vs. 52.5±13% in the AF and non-AF group, respectively (p=0.21). As many as 6 patients in the AF group were on metformin, one was on insulin only, and one on no medical therapy for DM. Among those with no AF, 6 were on metformin, one on metformin and gliclazide, and one on sitagliptin. Detailed baseline population characteristics are shown in Table 2.1.

PLIN2 was significantly higher in patients with history of AF compared to those with no AF (1.9±0.3 vs. 1.4±0.5, p=0.04). 4-hydroxynonenal was slightly higher in patients with AF vs. no AF, however the difference was not statistically significant (2.0±0.5 vs. 1.5±0.5, p=0.08). These results are shown in Figure 2.1 and 2.2. A Pearson's regression analysis demonstrated a moderate correlation of PLIN2 expression with BMI (r=0.53) and 4-hydroxynonenal expression (r=0.35), and a low correlation with LA size (r=0.22). There was a negative correlation of PLIN2 expression with age (r=-0.2) and left ventricular ejection fraction (r=-0.12).

Discussion

The main finding of the present study is that intramyocardial fat (i.e., cardiac steatosis) appears to be higher in diabetic patients with history of AF compared to a control group with no AF. To the best of our knowledge, this is the first study to investigate the correlation between cardiac steatosis and AF.

There is a growing body of evidence showing a role of cardiac adiposity in the pathogenesis of AF, however previous reports have mainly focused on the study of epicardial fat. Cardiac steatosis has more recently emerged as a possible contributor to several myocardial diseases [3, 21-22]. A recent study by Mazzali et al [21] has shown a higher concentration of intramyocardial fat in patients with vs. without coronary artery disease. Furthermore, cardiac steatosis has been associated with deterioration of left ventricular diastolic function and increase of left ventricular mass [21-22].

From a physiopathological perspective, we hypothesize that cardiac steatosis might contribute to the development and perpetuation of AF by promoting oxidative stress. Previous studies have implicated increased oxidative stress within the atrial tissue in the pathogenesis of AF [134-138]. Our data seem to corroborate this hypothesis by showing a trend toward a higher concentration of 4-hydroxynonenal in DM subjects with AF vs. no AF, although the difference was not statistically significant; the small sample size might account for the lack of significant difference. Intramyocardial fat accumulation causes a hyperactivation of the beta-oxidation with subsequent excess formation of reactive oxygen species, which mediate lipotoxicity. Mihm et al first demonstrated an increase of nitrotyrosine and protein carbonyl formation in the right atrial appendage of patients undergoing cardiac surgery with vs. without AF, providing evidence of oxidative damage in human AF [134]. In keeping with these findings, Corradi et al demonstrated increased atrial tissue levels of hemeoxygenase-1 and 3-nitrotyrosine in patients with persistent AF compared with controls [135]. Oxidative stress can promote arrhythmogenesis by several mechanisms [136]. Excessive reactive oxygen species have been shown to modify "several ionic currents in cardiomyocytes, cardiomyocyte coupling, and extracellular matrix" [136-137]. Specifically, oxidative stress prolongs the duration of the action potential and favours triggered activity (early after-depolarisation) by promoting late Na+ current [136-137]. Furthermore, reactive oxygen species reduce total Na+ channels causing a delay in the cardiac conduction and therefore promoting reentry [136-137]. In addition, oxidative stress can stimulate L-type Ca2+ currents with subsequent prolongation of the action potential duration and reduction of the repolarization reserve [136-137]. Reactive oxygen species can induce inflammation and promote cardiac fibrosis and also interfere with connexin proteins in the gap junction prolonging cardiac conduction [136-137]. Moreover, the Ca+2/calmodulin-dependent protein kinase II has emerged as a reactive oxygen species-activated proarrhythmic signal, which can trigger AF via Ca+2 leak through ryanodine receptor [136, 138].

The findings from the present study might have relevant clinical implications. Non-invasive quantification of cardiac steatosis using proton magnetic resonance spectroscopy might be adopted to predict the risk of developing AF in DM patients. AF is associated with worse cardiovascular outcomes and increased risk of death and

hospitalization in the DM population [100-101], hence identification of subjects at higher risk of AF could prompt a more aggressive management of DM and other risk factors. In addition, measuring cardiac steatosis in DM patients might potentially guide AF treatment and predict outcomes of catheter ablation. Indeed, our data suggest that cardiac steatosis is an important element of the atrial substrate remodelling in the DM population, and such structural changes might contribute to explain the lower efficacy of AF ablation in diabetics vs. non-diabetics, as demonstrated in our previous study. For these reasons, the presence of cardiac steatosis might potentially help to identify DM subjects with a more advanced atrial substrate who are therefore less likely to benefit from catheter ablation. However, further studies with larger sample size are required to confirm this hypothesis.

On the other side, pharmacological treatments targeting intramyocardial fat infiltration and/or oxidative stress might have an important role in the management of AF, particularly in the DM population. Vitamin C has strong antioxidative capacity and has been shown to reduce the risk of postoperative AF in patients undergoing cardiac surgery [139]. However, data appear to be contradictory overall [140] and evidences beyond the setting of postoperative AF are limited. N-3 polyunsaturated fatty acids (omega 3) have been proposed as indirect antioxidant agents; indeed, by inducing a low-to-moderate increase in radical oxygen species levels they could decrease the vulnerability of myocardial tissue to a subsequent oxidative challenge [141]. A combination therapy with omega-3 fatty acid, vitamin C and E has been shown to reduce postoperative AF in a recent metanalysis, while omega 3 alone did not show any significant effect [142]. Statins have been extensively investigated for reduction of AF, mainly in the postoperative setting. Beyond the lipid lowering action, statins are well known to have pleiotropic effects, including antioxidant [143-144]. A recent study has found that statins reduce the amount of epicardial fat [143], but whether they have a similar effect on cardiac steatosis is currently unknown. The role of statins in the prevention of AF remains unclear. Although statins reduce the incidence of postoperative AF in patients undergoing cardiac surgery [145], a recent metanalysis has not identified any impact of statin in the incidence of AF recurrence post catheter ablation [144]. Of note, to the best of our knowledge there are no studies specifically designed to evaluate the impact of statins or antioxidant drugs in the DM

population with AF. Given the important role of cardiac adiposity and oxidative stress in DM patients with AF, it is conceivable that this population might particularly benefit from antioxidant and lipid lowering therapy. In the absence of available data, further research is required to address this relevant topic. With regards to the antidiabetic drugs, in a study including 74 participants with type-2 diabetes assigned to metformin or pioglitazone or placebo, no changes in the myocardial triglyceride content was demonstrated after 24 weeks [21]. Sulfonylureas and insulin have been shown to have a pro-AF effect through induction of hypoglycaemia [146]. A possible anti-arrhythmic effect of metformin and GLP-1 receptor agonists has been suggested, but further studies are required to confirm these findings [146-147]. It remains unclear whether thiazolidinedione and DPP-4 inhibitors might have any anti-arrhythmic property [146]. SGLT2 inhibitors appear as the most promising drugs for improvement of cardiovascular outcomes and have been shown to reduce the risk of AF in the DM population, as confirmed by a recent metanalysis [148]; however, the underlying mechanisms have not been entirely understood. A recent study on animal models found that empaglifozin can reduce the late sodium current-induced calcium overload, and also attenuate the level of oxidative stress [149]; however, further research is required to confirm these findings.

Conclusions

Cardiac steatosis appears to be higher in diabetic patients with history of AF compared to a control group with no AF. Intramyocardial fat infiltration might contribute to the onset and perpetuation of AF in DM patients by promoting oxidative stress. Non-invasive quantification of cardiac steatosis in the DM population might allow to identify subjects at higher risk of developing AF, who might benefit from a more aggressive treatment for preventing cardiovascular sequelae.

Future directions

Despite the growing clinical and epidemiological evidence demonstrating an association between DM, cardiac adiposity and AF, further research is required to clarify the underlying mechanisms and cause-effect relationship. Comorbidities such as obesity, coronary artery disease, obstructive sleep apnoea or hypertension

predispose to AF and are common in the DM population, therefore prospective studies with large sample size are warranted to avoid potential bias derived by such confounding factors. The association between cardiac steatosis and AF in the DM population should be further explored in larger studies including a control cohort of non-diabetics. Further studies are also required to explore the association between cardiac adiposity and other visceral deposits. More work is necessary to understand the relationship between glycaemic control, cardiac adiposity and AF in the diabetic patients, and whether antidiabetic drugs might reduce intramyocardial fat infiltration and cardiac lipotoxicity. In addition, further research should investigate whether cardiac adiposity may predict outcomes of catheter ablation of AF in the DM population, and whether antidiabetic drugs (particularly SGLT2 inhibitors) may improve ablation outcomes. Finally, improved imaging techniques are necessary to allow an easier, cheaper and more accurate quantification of the cardiac adipose tissue, particularly the intramyocardial deposits.

TABLES

Table 1.1. Baseline characteristics of the study population

Variable	Total sample (n=2504)	Diabetes mellitus		Overall P
		NO (n=2270)	YES (n=234)	
Age (years)	61.1±10.2	60.8±10.4	63.9±7.5	<0.001
Women	29.4% (736)	29.3% (665)	30.3% (71)	0.738
AF duration (years)	5.0±5.4	5.0±5.4	4.6±4.2	0.405
Paroxysmal AF	57.5% (1441)	58.6% (1330)	47.4% (111)	<0.001
Persistent AF	42.5% (1063)	41.4% (940)	52.6% (123)	
Mean N of Procedures	1.2±0.5	1.2±0.5	1.2±0.5	0.729
CHA_2DS_2-VASc	1.6±1.4	1.5±1.2	3.0±1.2	<0.001
Congestive heart failure	8.1% (202)	7.4% (168)	14.5% (34)	<0.001
Hypertension	45.8% (1,148)	43% (976)	73.5% (172)	<0.001
BMI (Kg/m^2)	28.4 ±13.3	28.1±13.0	31.6±15.5	<0.001
Stroke or TIA	7.5% (188)	7.4% (167)	9.0% (21)	0.371
Vascular disease	8.5% (213)	7.4% (167)	19.7% (46)	<0.001
Obstructive Sleep apnea	7.0% (176)	6.4% (145)	13.2% (31)	<0.001
eGFR (ml/min)	75.1±18.4	75.5±18.0	71.1±21.3	0.009
Indexed LA volume (mL/m^2)	48.6±18.6	48.3±18.7	51.2±17.4	0.043
LVEF (%)	61.4±9	61.9±8.6	59.7±9.1	0.002
Cryoballoon ablation	29.4% (736)	29.4% (668)	29.1% (68)	0.906
Use of General Anaesthesia	67.6% (1,692)	67.7% (1536)	66.7% (156)	0.746
Procedure Duration (min)	136±58	134±57	141±59	0.094
Fluoroscopy Duration (min)	23±13	23±13	23±13	0.406
Class I or III AADs on discharge	21.6% (542)	25.5% (483)	31.2% (59)	0.089
CFAE ablation	14.2% (356)	13.8% (313)	18.4% (43)	0.056
LA lines	23% (576)	22.4% (508)	29.1% (68)	0.201
CTI	21.8% (546)	21.7% (492)	23.1% (54)	0.621
NYHA	1.1±0.4	1.1±0.4	1.2±0.5	0.008

Legend: AF - atrial fibrillation; N -number; BMI- body mass index; TIA - transitory ischemic attack; CHA_2DS_2-VASc - cardiac failure or dysfunction, hypertension, age ≥75 years [doubled], diabetes, stroke [doubled] - vascular disease, age 65–74 years, sex category [female]; LA - left atrium; LVEF - left ventricular ejection fraction; AADs – anti-arrhythmic drugs; CFAE – complex and fragmented electrograms; CTI- cavotricuspid isthmus line; NYHA- New York Heart Association class.

Table 1.2. Baseline characteristics of the DM cohort and Propensity-Matched Controls.

Variable	PM controls (n=468)	Type-2 diabetes (n=234)	Overall P
Age (years)	64.4±8.2	63.9±7.5	0.489
Women	31.8% (149)	30.3% (71)	0.687
AF duration (years)	4.8±5.1	4.6±4.2	0.656
Paroxysmal AF	46.6% (218)	47.4% (111)	<0.831
Persistent AF	53.4% (250)	52.6% (123)	
Mean N of Procedures	1.3±0.5	1.2±0.5	0.525
Congestive heart failure	14.5% (68)	14.5% (34)	1.000
Hypertension	72.4% (339)	73.5% (172)	0.764
BMI	29.6±16.1	31.6±15.5	0.116
Stroke or TIA	9.2% (43)	9.0% (21)	0.926
Vascular disease	20.3% (95)	19.7% (46)	0.842
Obstructive Sleep apnea	13.9% (65)	13.2% (31)	0.816
eGFR (ml/min)	71.8±17.9	71.1±21.3	0.687
Indexed LA volume (mL/m^2)	49.0±17.1	51.2±17.4	0.145
LVEF (%)	60.8±10.1	59.7±9.1	0.228
Procedure Duration (min)	139±61	141±59	0.635
Fluoroscopy Duration (min)	24±13	23±13	0.645
Class I or III AADs on discharge	26.1% (111)	31.2% (59)	0.187
CFAE ablation	17.5% (82)	18.4% (43)	0.780
LA lines	31.8% (149)	29.1% (68)	0.453
CTI	25.0% (117)	23.1% (54)	0.576
NYHA	1.3±0.5	1.2±0.5	0.949

Legend: PM – propensity match; AF - atrial fibrillation; N -number; BMI- body mass index; TIA - transitory ischemic attack; CHA_2DS_2-VASc - cardiac failure or dysfunction, hypertension, age ≥75 years [doubled], diabetes, stroke [doubled] - vascular disease, age 65–74 years, sex category [female]; LA - left atrium; LVEF - left ventricular ejection fraction; AADs – anti-arrhythmic drugs; CFAE – complex and fragmented electrograms; CTI- cavotricuspid isthmus line; NYHA- New York Heart Association class.

Table 1.3. Efficacy and Safety Endpoints

	Variable	Total sample (n=2504)	Diabetes mellitus		Overall P
			NO (n=2270)	YES (n=234)	
Efficacy	**Pulmonary Vein Isolation**	99.0% (2,479)	99.0% (2248)	98.7% (231)	0.647
	Relapse during blanking	20.2% (506)	24.3% (446)	32.8% (60)	0.012
	Relapse during first 12 months	28.9% (623)	25.3% (553)	32.0% (70)	0.031
Safety	**Peri-procedural complications**	6.1% (152)	6.3% (143)	3.8% (9)	0.128
	Cardiac tamponade	0.7% (18)	0.7% (15)	1.3% (3)	0.284
	TIA	0.2% (4)	0.2% (4)	0% (0)	0.520
	Stroke	0.2% (6)	0.3% (6)	0% (0)	0.431
	Transient phrenic nerve palsy	1.5% (37)	1.5% (35)	0.9% (2)	0.407
	Major vascular complications	2.6% (65)	2.7% (62)	1.3% (3)	0.184
	Procedure-related death*	0.1% (1)	0% (1)	0% (0)	0.748
	Other complications	0.8% (21)	0.9% (20)	0.4% (1)	0.469
Other Complications	**Oesophageal fistula**	0.1% (2)	0.1% (2)	0% (0)	0.640
	Gastroparesis	0.1% (2)	0.1% (2)	0% ()	0.650
	Oesophageal ulcer	0.1% (1)	0% (1)	0% (0)	0.748
	Non-Access related bleeds	0.2% (6)	0.2% (5)	0.4% (1)	0.537
	Bradyarrhythmia complications	0.2% (5)	0.2% (5)	0% (0)	0.472
	Anaphylaxis	0.1% (1)	0% (1)	0% (0)	0.748
	Transient myocardial stunning	0.1% (1)	0% (1)	0% (0)	0.748
	PV stenosis	0.1% (1)	0% (1)	0% (0)	0.748
	Air embolism	0.1% (1)	0% (1)	0% (0)	0.748
	Acute pulmonary oedema	0.1% (1)	0% (1)	0% (0)	0.748

Table 1.4. Predictors of Post-blanking atrial arrhythmia relapse after an ablation procedure

Variable	Univariate Cox Regression			Multivariate Cox Regression		
	HR	**95%CI**	**P**	**HR**	**95%CI**	**P**
Age (per year)	**1.01**	**1.00-1.01**	**0.027**	-	-	-
Female gender	1.10	0.96-1.28	0.146	-	-	-
AF duration (per year)	**1.02**	**1.01-1.03**	**<0.001**	**1.02**	**1.01-1.04**	**<0.001**
Paroxysmal AF	**0.54**	**0.47-0.61**	**<0.001**	**0.55**	**0.46-0.65**	**<0.001**
Congestive heart failure	**1.74**	**1.42-2.13**	**<0.001**	-	-	-
Hypertension	**1.18**	**1.04-1.34**	**0.013**	-	-	-
Diabetes mellitus	**1.39**	**1.13-1.71**	**0.002**	**1.39**	**1.07-1.82**	**0.016**
Stroke or TIA	1.24	0.98-1.55	0.071	-	-	-
Vascular disease	**1.27**	**1.03-1.58**	**0.026**	-	-	-
Obstructive Sleep Apnoea	**1.34**	**1.06-1.68**	**0.013**	**-**	**-**	**-**
CHA_2DS_2-VASc	**1.12**	**1.07-1.17**	**<0.001**	-	-	-
BMI (per Kg/m^2)	**1.01**	**1.00-1.01**	**0.024**	**1.03**	**1.02-1.05**	**<0.001**
eGFR (per ml/min)	0.99	0.99-1.00	0.149	-	-	-
Indexed LA volume (per mL/m^2)	**1.01**	**1.01-1.02**	**<0.001**	**1.01**	**1.00-1.01**	**<0.001**
LVEF (per %)	**0.99**	**0.98-0.99**	**<0.001**	-	-	-
Cryoballoon ablation	0.92	0.80-1.06	0.274	-	-	-
NYHA	**1.57**	**1.36-1.83**	**<0.001**	-	-	-
CTI	0.97	0.83-1.13	0.682	-	-	-

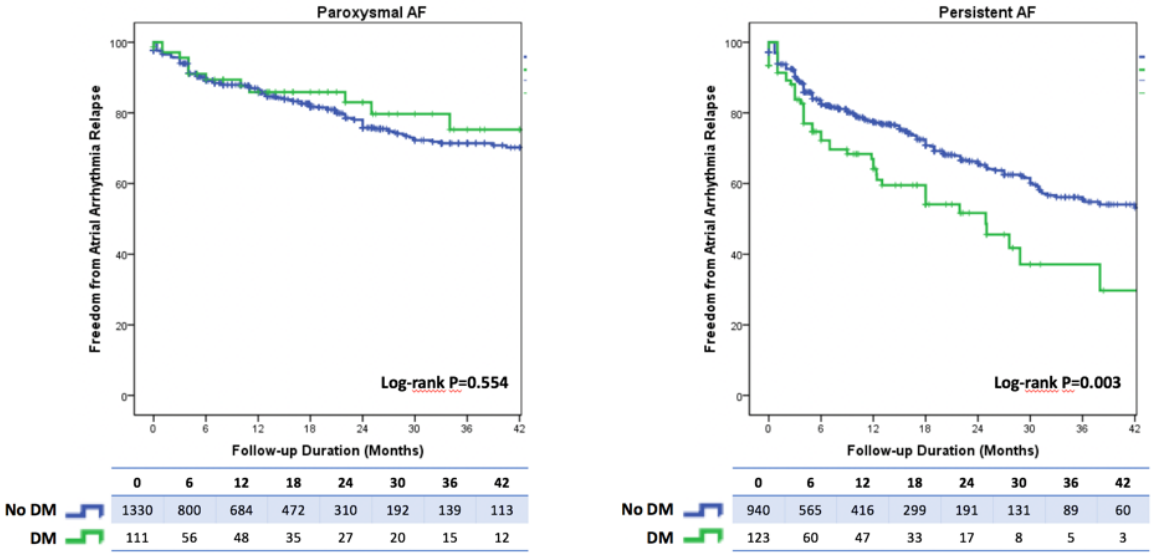
Legend: HR – hazard ratio; CI – confidence interval; AF - atrial fibrillation; TIA - transitory ischemic attack; CHA_2DS_2-VASc - cardiac failure or dysfunction, hypertension, age ≥75 years [doubled], diabetes, stroke [doubled] - vascular disease, age 65–74 years, sex category [female]; BMI - body mass index; LA - left atrium; LVEF - left ventricular ejection fraction; AAD – anti-arrhythmic drugs.

Table 2.1. Baseline characteristics of the study population

Variable	Total sample (n=16)	Atrial fibrillation		Overall P
		NO (n=8)	YES (n=8)	
Age (years)	68.0±9.5	66.8±10.6	69.2±8.9	0.31
Men	100% (16)	100%	100%	1.0
AF duration (years)	-	N/A	4.6±4.2	-
Paroxysmal AF	-	-	1	-
Persistent AF	-	-	7	
Hypertension	100%	100%	100%	1.0
BMI (Kg/m²)	28.4 ±13.3	29.0±3.5	30.3±6.1	0.20
Obstructive Sleep apnoea	0%	0%	0%	1.0
Diabetic neuropathy	0%	0%	0%	1.0
LA diameter	40.3±3.9	38.6±3.4	42.0±4.2	0.52
LVEF (%)	49.9±12.6	52.5±13.0	47.2±12.4	0.21
NYHA	1.3±0.5	1.3±0.5	1.4±0.5	0.31
Statin	100%	100%	100%	1.0
Oral antidiabetics	87.5% (14)	100% (8)	75% (6)	0.47
Insulin	6.3% (1)	0%	12.5% (1)	1.0

FIGURES

Figure 1.1



	0	6	12	18	24	30	36	42
No DM	1330	800	684	472	310	192	139	113
DM	111	56	48	35	27	20	15	12

	0	6	12	18	24	30	36	42
No DM	940	565	416	299	191	131	89	60
DM	123	60	47	33	17	8	5	3

Figure 2.1 Immunohistochemical staining showing expression of PLIN2 in a subject with background of atrial fibrillation

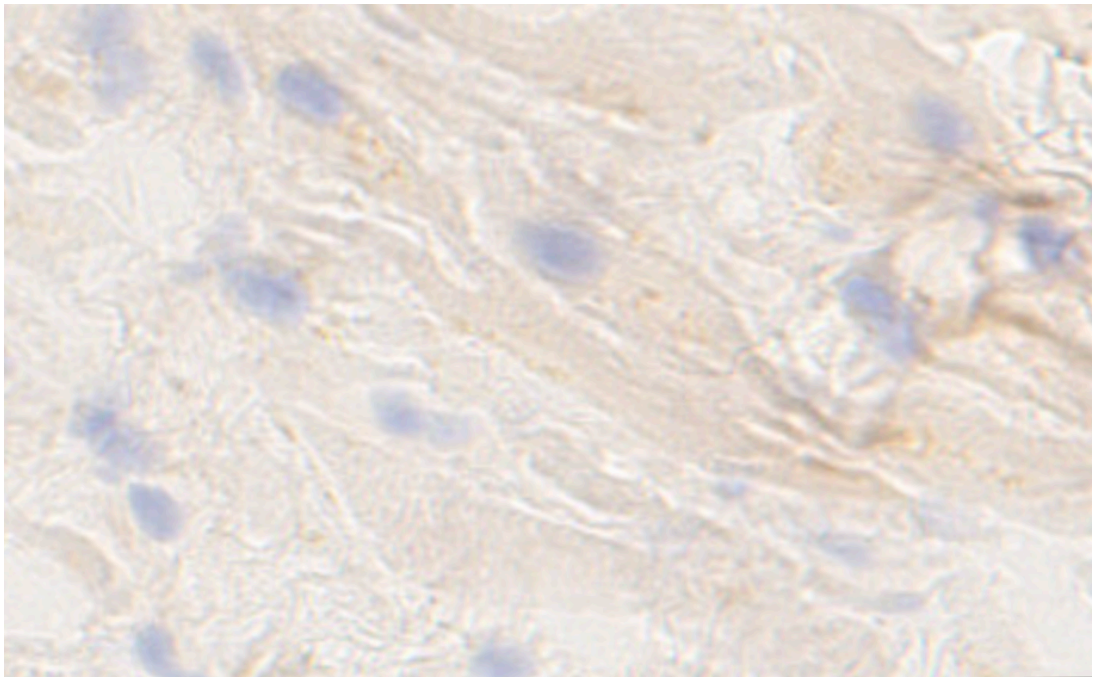


Figure 2.2. Histogram showing expression of PLIN2 on immunohistochemical staining in diabetic patients with vs. without atrial fibrillation

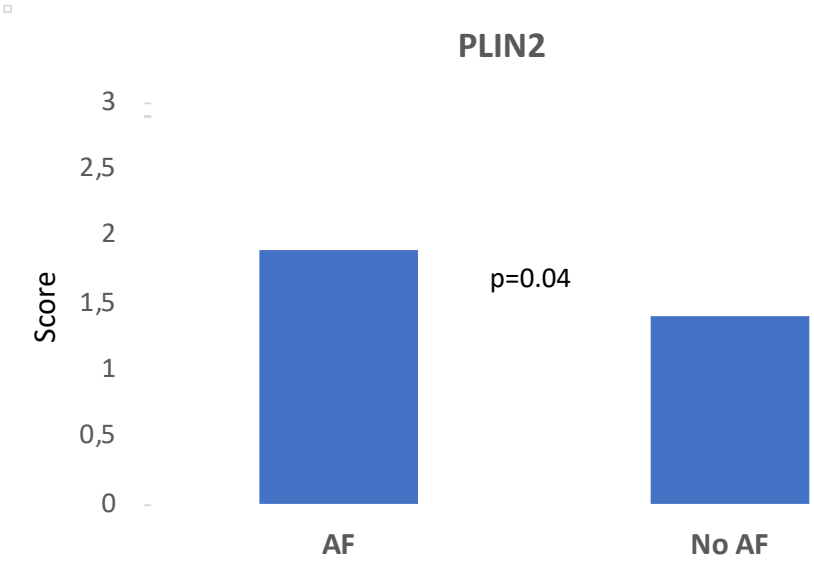
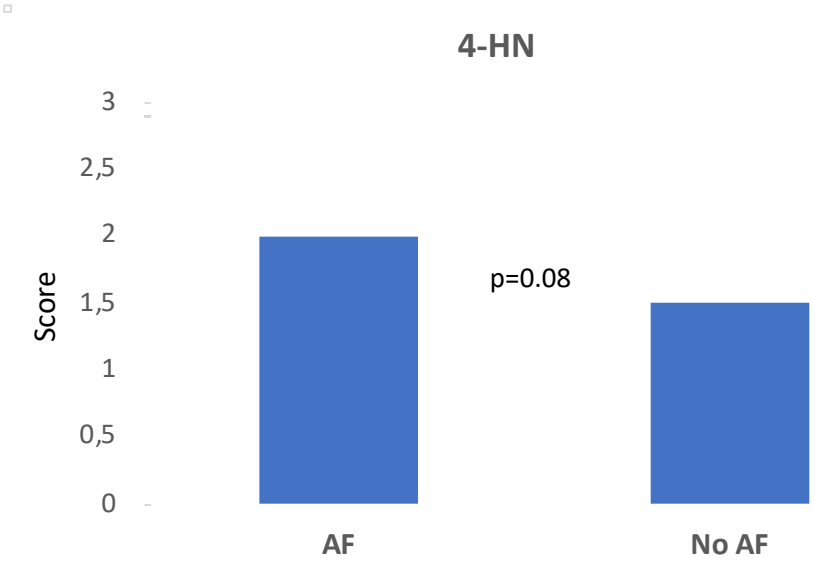


Figure 2.3. Histogram showing expression of 4-hydroxynonenal on immunohistochemical staining in diabetic patients with vs. without atrial fibrillation



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