Mapping the maze: advancing atrial fibrillation models and therapies

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Chapter 1

General introduction

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Atrial fibrillation in the clinic

Atrial fibrillation (AF) is the most common cardiac arrhythmia and an important contributor of morbidity, mortality and healthcare costs in the western world. The current prevalence of AF is estimated to be 2-4% in the adult population (1) and is expected to more than double in the coming decades as the result of population ageing (2, 3). It was recently estimated that 1 in 3 Europeans will develop AF in their lifetimes (4, 5). Individuals developing AF can (initially) remain asymptomatic, however symptoms including palpitations, dyspnoea and fatigue are common, frequently resulting in impaired quality of life (6). Additionally, the presence of AF is associated with serious complications, including a 4- to 5-fold increased risk of stroke (7, 8), increased risk of heart failure (9, 10) and 1.5- to 3.5-fold increase in all-cause mortality (5, 11, 12). Overall, it is estimated that up to 2.6% of total healthcare costs in European countries can be attributed to the management of AF and its complications (13, 14).

Presence of AF is characterised by rapid and chaotic electrical activation of the atria and is the result of a complex interaction of triggers, perpetuators and an atrial substrate (15). The most important risk factor for developing AF is age, however other factors include hypertension, heart failure, diabetes mellitus, coronary artery disease, obesity, smoking, valve disease, chronic kidney disease and more (6, 16). The pathophysiology behind the manifestation of AF remains a heavily studied topic, as the understanding of the multiform mechanisms leading to its initiation, maintenance and termination remains incomplete (17). Generally, initiation of AF is the result of rapid electrical activation that originates from the pulmonary veins, although such ectopic activity can also arise from other locations within the atria (15). This rapid electrical activation can result in the induction of re-entrant electrical activity, sustaining the arrhythmia. The stability and longevity of this re-entrant activity is in turn influenced by the severity of the atrial substrate. In clinical practise, AF is classified based on its duration, being paroxysmal when lasting <7 days, persistent when continuously present >7 days, and long-standing persistent when continuing >1 year (6). In tandem with the chronicity, the underlying mechanism driving AF generally changes, shifting from an incidental trigger-based tachyarrhythmia, to stable re-entry sustained by heavy structural and electrical remodelling of the atria (15). The progression of AF can be driven by progressive atrial remodelling due to (untreated) comorbidities, however the

presence of AF itself can also lead to remodelling in a phenomenon called "AF begets AF" (18).

The current treatment strategy for individuals with AF, based on the AF-CARE pathway, comprises a combination of stroke risk reduction, symptom management and comorbidity treatment optimalization (6). Stroke prevention in the form of anticoagulation therapy is applied in individuals with an increased risk of stroke based on additional risk factors besides AF, commonly calculated using the CHA2DS2-VA score (6, 19). This has shown to be effective in reducing thromboembolic events, however comes with an increased risk of (major) bleeding (20, 21). In addition to stroke risk reduction, pharmacological "rate control" is frequently applied to limit the ventricular rate, with the aim of preventing tachycardia-induced cardiomyopathy and to reduce symptoms. When symptoms persist, "rhythm control" can be pursued to restore and maintain sinus rhythm, typically performed in conjunction with rate control and anticoagulation therapy. Acute restoration of sinus rhythm through electrical or pharmacological cardioversion is very effective, although limited by the need for a brief hospital admission each time and it having minimal effect on preventing AF recurrences (22, 23). Antiarrhythmic drug therapy can be used to maintain sinus rhythm, however, the efficacy is limited and is associated with significant side effects (24). Electrical isolation of the pulmonary veins, which is possible through various endovascular and surgical techniques, has shown to be effective in reducing the occurrence of AF (25), and has become an important modality in the treatment of symptomatic AF. Overall, the efficacy of these rhythm control therapies is higher in paroxysmal AF and have the benefit of slowing progression in this population (26). Additionally, there is a growing body of evidence supporting early rhythm control, suggesting a lower risk of adverse cardiovascular outcomes. Unfortunately, these treatment strategies are not effective in all patients and a large portion of patients will still progress to the persistent or long-standing persistent form of AF where restoration of sinus rhythm becomes more challenging.

Ablation of symptomatic long-standing persistent atrial fibrillation

Patients who have progressed to long-standing persistent AF (LSPAF) are particularly difficult to treat due to the extensive arrhythmogenic substrate present. Where in paroxysmal AF the majority of triggers can be localised to the pulmonary veins, triggers in LSPAF are also frequently located in other parts of the left and right atrium, particularly in the left atrial posterior wall and left atrial

appendage (15). As a result, minimally invasive catheter ablation of the pulmonary veins alone has shown poor outcomes in LSPAF, resulting in around 20-24% AF freedom after a single intervention and 41-47% AF freedom after multiple (up to 5) interventions at 5-year follow-up (27, 28). Performing more extensive endocardial ablation in this population, by for instance targeting complex fractionated electrograms and/or adding linear lesions to isolate the left atrial posterior wall or left atrial appendage, has thus far not yielded improved outcomes (29). This likely results from lesion transmurality and contiguity issues associated with the current point-by-point endocardial ablation techniques and technologies applied for linear lesions (30).

Conversely, surgical ablation using the Cox-maze IV technique, widely considered the gold standard for AF ablation, has shown to be effective in the LSPAF population with rates of AF freedom as high as 67% at 7-year follow-up (31). This technique, introduced in its current form in 2002, comprises an extensive set of lesions in the left and right atrium, including amputation of the left atrial appendage. The aim of this lesion set is prohibiting AF to establish and perpetuate, while allowing impulses from the sinus node to reach the atrioventricular node (32). The advantages of this surgical approach include better access to both the endo- and epicardial side of the atrial myocardium, elimination of the blood cooling effect and use of linear ablation tools and clamps, all aiding better lesion transmurality and contiguity. Despite the high efficacy, the applicability of the stand-alone Cox-maze IV is severely limited by its invasiveness, required expertise and need for cardiopulmonary bypass. As a result, the Cox-maze IV has a more prominent place as a concomitant procedure during cardiac surgery of patients with AF (33).

For stand-alone surgical ablation to serve as an alternative to catheter ablation in LSPAF, minimal invasiveness, ease of performing the procedure and high efficacy are important. With the advent of thoracoscopic surgery techniques, minimally invasive thoracoscopic ablation on the beating heart has become commonplace over the past decade. This has resulted in a wide array of minimally invasive thoracoscopic ablation devices and techniques applied in clinical practice to treat AF. The efficacy of these thoracoscopic ablation procedures, however, has (thus far) mostly been studied in patients with paroxysmal and early-persistent AF and not in the more difficult to treat LSPAF patients. As a result, literature on thoracoscopic ablation efficacy in LSPAF is sparse and has mostly been limited to short- to medium-term follow-up studies (34-36). In addition to the variability in devices and techniques in these studies, the modality of rhythm follow-up differs frequently, ranging from

incidental electrocardiography up to continuous rhythm analysis through implantable loop recorders. To provide more clarity on the applicability of thoracoscopic ablation as alternative to catheter ablation in LSPAF patients, more (preferably long-term) comparative data is needed on the different ablation techniques and rhythm follow-up strategies.

Preclinical research and models of atrial fibrillation

On the other side of the spectrum, development of novel therapeutics effectively targeting the underlying pathophysiology of AF has been arduous. In the last decade, no new antiarrhythmic drugs have been approved, and only a few compounds are currently in the pipeline (37, 38). One of the factors limiting preclinical safety and efficacy studies has been the lack of sufficient numbers of well-differentiated human atrial cardiomyocytes. Together with the (associated) absence of clinically relevant human-based in vitro models of AF, preclinical research into AF mechanisms and novel therapeutic strategies has been mainly limited to animal studies. Given the principal differences in (patho)physiology between humans and animals, these new therapeutics frequently fail due to limited efficacy or strong adverse effects in patients (39).

Replacing the animal-derived cells in the *in vitro* models of AF with human cells, aiming to increase the translational value, has been difficult. Despite good accessibility of the human heart during routine cardiac surgery, (atrial) cardiomyocytes isolated from surgical waste material rapidly dedifferentiate in culture and vary in terms of quality (40). Attempts to prolong the lifespan of these cells by saving the extracellular matrix and culturing cardiac tissue slices are promising (41), although still only applied on small scale, given that they are most commonly derived from scarcely available explanted human hearts.

Since the early 2010s, an alternative source of human atrial cardiomyocytes is provided by human pluripotent stem cells (hPSCs), where the development of differentiation protocols utilizing retinoic acid have allowed the creation of cardiomyocytes with atrial features (42, 43). However, in spite of certain attractive features and unique applications in personalized disease modelling, current hPSC-derived atrial cardiomyocytes (hPSC-AMs) are associated with laborious workflows, high phenotypic variation, and overall immaturity. Attempts to create in vitro models of human AF using hPSC-AMs have been reported (44, 45), however they were unable to represent the rapid activation frequencies of 6-8 Hz (46-48) seen during human AF.

The creation of permanent cardiac cell lines from primary material to create a standardised and unlimited source of cardiomyocytes has been pursued on several occasions. Currently, three cell lines are commercially available and frequently cited in literature (49). These include the H9c2 cell line, generated in 1976 through selective serial passage of embryonic BDIX rat heart tissue (50). These cells have a myoblast origin and display properties of both skeletal muscle and, to some extent, cardiac muscle (51). Two decades later, the HL-1 cell line was established (52), originating from adult mice where the expression of simian virus 40 large T antigen (SV40-LT) was targeted to atrial cardiomyocytes via the atrial natriuretic factor promoter. Later, the AC16 line was generated through the fusion of primary human ventricular cardiomyocytes with SV40-transformed human fibroblasts (53). Despite their widespread use and popularity in literature, these three cell lines show limited resemblance to mature cardiac cells (49), likely due to the fact that continuing proliferation inhibits differentiation in most cell types (54). To address this limitation, efforts to generate conditionally immortalized cardiomyocyte cell lines that can switch between proliferative and differentiated phenotypes have been undertaken (55-59). Among these conditionally immortalized lines, the iAM-1 line (59) demonstrated the most effective transition between a proliferative state and a differentiated phenotype. This cell line, functioning through doxycycline-induced expression of SV40-LT in neonatal rat atrial cardiomyocytes, could even be applied to model atrial arrhythmias in vitro. Although the aforementioned lines are of great value in reducing the need for animals in cardiovascular research, their differences with respect to human (patho)physiology remain significant, reducing their clinical relevance as previously mentioned. Successfully applying this technology to human atrial cardiomyocytes could lead to more accurate models for human heart diseases and reduce reliance on animal testing, ultimately accelerating the development of effective therapies, not just for atrial fibrillation, but for cardiovascular conditions in general.

Aims and outline of this thesis

Part One. Outcomes of thoracoscopic ablation in long-standing persistent atrial fibrillation

The first part of this thesis, is aimed at investigating the efficacy of thoracoscopic ablation in the LSPAF population as an intermediate option between minimally invasive (but limited efficacious) catheter ablation and highly effective (but highly invasive) surgical ablation. In chapter 2, the efficacy of the two most common ablation tools used for isolating the posterior left atrium during thoracoscopic ablation are compared on perioperative performance and short-term follow-up. In chapter 3, the long-term efficacy of thoracoscopic ablation in LSPAF is studied, including the role of catheter ablation in patients with recurrences. Also, recurrence rates characterized by incidental 24h-Holter monitoring and continuous rhythm monitoring using implantable loop recorders are compared.

Part Two. Development of clinically relevant human in vitro models of atrial fibrillation

The second part of this thesis is aimed at investigating the improvement of access to well-differentiated human atrial cardiomyocytes and to develop a clinically relevant in vitro model of AF. In chapter 4, the electrophysiological properties of excised human atrial tissue are studied, and the feasibility of creating culturable atrial tissue slices is assessed. In chapter 5, the conditional immortalization of human atrial cardiomyocytes is explored, including analysis of the success rate, extensive characterisation of the phenotype of these cells and their applicability for modelling AF in vitro.

Finally, in chapter 6 the main conclusions and their implications, as well as future perspectives, are discussed.

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