IRE Ablation for Atrial Fibrillation Treatment: Research Status and Prospect

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1. ATRIAL FIBRILLATION AND ITS TREATMENT USING RF ABLATION

Atrial fibrillation (AFib) is the most common arrythmia characterized by rapid and irregular beating of the atrial chambers of the heart. This disrupted rhythm occurs because of the irregular and unpredictable conduction of disordered impulses originated in the atria, which lead to the uncoordinated contractions between atria and ventricles. The ineffectual contractions result in lower blood output from atria into ventricles, and vulnerability to blood clot (thrombus) formation due to blood pooling in atria. This clot can then travel to the brain and block the flow of blood to part of the brain which can result in a stroke. As a matter of fact, AFib is a major risk factor for ischemic stroke and associated with a 5-fold higher risk of stroke [1]. It is estimated that AFib affects more than 3 million people in the US [2], and affects more than 33 million people worldwide [3]. The prevalence of AFib increases to more than 1 in 10 in the elderly [4], and this prevalence of AFib is rising in the near future [5], because of the aging of population globally. Besides, another factor related to this prevalence is due to the increasing survival from underlying conditions closely associated with AFib, such as hypertension, coronary heart disease, and heart failure [6]–[8]. With such a prevalence, AFib is predicted to affect 6–12 million people in the US by 2050 and 17.9 million in Europe by 2060 [9]. The treatments of AFib constitutes serious medical burden globally: it is estimated for 1% of the National Health Service budget in the United Kingdom [10] and $16–26 billion of annual US expenses[11], [12].

Besides medication, thermal ablation of myocardial tissue is currently the “gold standard” of clinical treatment of AFib, which works by destroying local myocardial tissue to isolate faulty electrical signals causing the arrhythmia within the local area. It is usually performed by heating local tissue with radio-frequency (RF) electrical current, creating conduction-blocking lesions that stop AFib. The introduction of RF catheter ablation in cardiac electrophysiology can be traced back to 1987. However, problems associated with RF ablation are: **1)** high recurrence rate (due to tissue cooling by arterial flow may limit transmural and continuous lesion formation)[13], **2)** tissue loss beyond the targeted tissue (non-uniform ablated region [14]), **3)** charring that hinders heat conduction[15], [16], **4)** steam pop that associated with cardiac perforation[17], and **5)** long duration of the ablation procedure [18]. Besides, there are small chances that RF ablation may lead to several complications due to the thermal side effects. In a US nationwide study involving more than 93801 RF ablation procedures from 2000 to 2010, the overall incidence of complications in the hospital was 6.29%, with 0.46% of mortality [19] [20]. The most frequent and severe complications of RF ablations are **1)** stroke (due to thrombosis formation[21]), **2)** pulmonary vein stenosis [22], **3)** cardiac tamponade [23], coronary artery injury [24], **4)** phrenic nerve injury [25], and **5)** esophageal fistula [26] ,etc. **Fig. 1 A-G** illustrate detailed problems RF ablation (RFA) may create, based on reported clinical data from AFib patients.

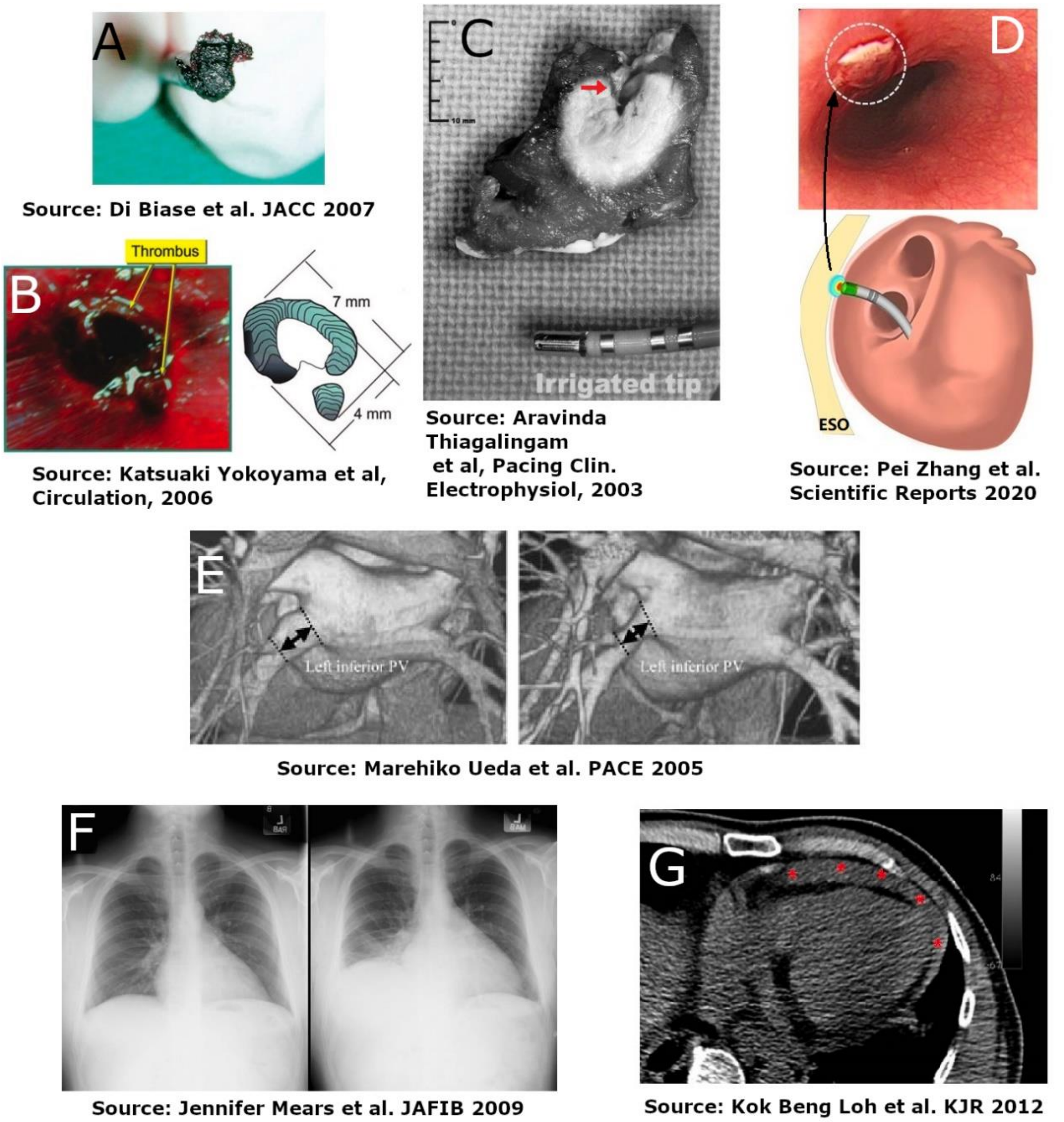

Figure 1. Listed problems associated with RFA. **A:** Charring occurs on the catheter tip after ablation, which hinders the heat conduction between the tip and contacted tissue, thus only the outer layer of tissue may be ablated, which means the lesion won't be transmural. **B:** Thrombus (or called blood clot) formed at the electrode-tissue interface produced by the RF in low blood flow environment. The extracted size of the thrombus was 7×4 mm (right). The clot may lead to stroke if falls out from the local region and flows into brain vessel. **C:** Representative elliptical ablated lesion obtained after RFA with typical RFA tip below. This non-uniform region leads to unwanted tissue loss in the near-end of catheter (pointed by red arrow), in order to reach the far-end to get transmural ablation from endocardium to

epicardium. **D:** Esophageal fistula (dot circled in white) complication post RFA shown by endoscopic ultrasonography. The anatomical relationship between esophagus (eso) and left atrium in vicinity is shown underneath pointed to fistula. The significant anatomical variability in the relationship between the esophagus and left atrium, places the esophagus in a vulnerable position, prone to injury, even in good hands of doctor during surgery. **E:** Volume rendering images of multi-slice CT of the left inferior pulmonary vein (PV) before (left panel) and after ablation (right panel), indicating PV stenosis (narrowing) due to thermal side effect of RFA. The trunk length of the PV shortened after ablation (23% reduction compared with that before ablation). **F:** Anteroposterior chest radiography of phrenic nerve injury (PNI) due to thermal side effect. Pre-ablation shows normal diaphragmatic height (left panel). When PNI occurs, patients are sometimes severely symptomatic with shortness of breath, which can be diagnosed with right hemidiaphragmatic elevation on routine chest radiography (right panel). **G:** Hemorrhagic cardiac tamponade secondary to RFA imaging from plain CT at lower thorax. Cardiac tamponade (with red asterisk indicated) is noted in post-RFA CT when patient developed hypotension.

2. IRE ABLATION IN CARDIOLOGY

Electroporation is a biophysical phenomenon in which cell membrane permeability to ions and molecules increases significantly in response to externally applied electric field. When the electric field applied is of relatively strong intensity, it may induce persistent change in membrane permeability, leading to an irreversible breakdown of membrane structure and function and ultimately to cellular death. This process is called irreversible electroporation (IRE) and is an emerging non-thermal modality, approved by the US FDA that successfully used for clinical tumor ablation. Due to this non-thermal mechanism, there are many advantages of IRE ablation, compared to conventional RF ablation. The pilot study of IRE ablation in cardiac research is reported by Lavee et al. in 2007, that transmural lesions are obtained by the open chest surgery on pigs using bipolar 40 mm parallel clamp, revealing a sharp demarcation between ablated and normal tissue. [27]. In **Table 1,** we detail the significant historical IRE ablation events that pushes this innovative modality towards a safer and more effective clinical application, avoiding severe complications found in RF ablation, and summaries these remarkable discoveries in **Figure 2** using the timeline from 2007 to 2020.

2.1 IRE MODALITIES

Depending on the protocols, IRE can be achieved with various modalities: direct current (DC), alternative current (AC), pulsed electric field (PEF), or any combination of these [28]. DC catheter ablation was routinely applied between 1980 and 1990, for ablation of the bundle of His and ventricular tachycardia, specifically [29]. Despite high success rates, DC ablation was quickly replaced by the more elegant RF ablation technique appeared around 1990 [30]. The main disadvantages of DC ablation were the required complete anesthesia, serious complications supposedly related to barotrauma, generations of arcing and bubbles, and proarrhythmia in the first few days after ablation [29], [31]–[33]. In 2011 Wittkampf et al. successfully applied the low-energy DC ablation to create myocardial lesions, avoiding harmful side effects of standard DC ablation such as the generation of sparking and explosion [29]. The DC shocks (pulse duration of a few milliseconds) were applied between a catheter

Study (Year)	Experiment model (number)	Electrode configuration and delivery method	IRE parameters of protocol	Effect	Follow-up	Significance
Lavee J (2007) [27]	Porcine (n=5), open chest	Bipolar 40 mm parallel clamp electrodes, Epi_Delv	PN=8, 16, 32, PD=100 µs, PRI=200 ms, PA=1.5-2 kV	Transmural lesions (n=10) with mean 0.9 cm depth, 3-3.5 cm length, created in 1-4 seconds	24h	First reported successful using IRE atrial transmural ablation in vivo, with clear ablated/unablated boundary.
Hong J (2009) [34]	Ovine (n=4), open chest	Bipolar 53 mm linear clamp electrodes, Epi_Delv	3 or 5 trains of pulses, each train with PN=10 to 40, PD=100-400 µs, PRI=200-1000 ms, PA=0.78 kV	100% transmural lesions in SVC and IVC (n=14), and high degree of transmural in RAA, LAA and PVs (n=19)	Not Mentioned	High degree of transmural lesions in different locations in atria. Besides, esophageal lesions are created showing this IRE ablation is selective that epithelial tissue are kept alive while targeted muscular tissue are destroyed.
Wittkampf (2011) [29]	Porcine (n=10), closed chest	Circular-20mm-diameter with 10-poles 2mm electrodes, Endo_Delv	200 J DC pulses, PD=a few ms, the DC is applied between a skin patch and the multipolar circular electrode	Sharp lesions (maximal depth=3.5mm) were created inside PV ostia with reduced PV potentials	3 weeks	First reported successful low energy IRE ablation in a sensitive environment like PV ostia safely. PV angiograms did not show any stenosis during this short follow-up.
Wittkampf (2012) [35]	Porcine (n=10), open chest	Two devices: Circular-20mm-diameter with 10-poles 2mm electrodes, and Circular-20-mm-diameter single ring electrode, Epi_Delv	50/100/200 J DC pulse, the DC is applied between a skin patch and the multipolar circular electrode	Continuous lesions (n=5) were created with up to median width 5.3 ± 3.0 mm and median depth 5.2 ± 1.2 mm	3 weeks	Proved lesion depth increased with the magnitude of the pulses in a blood-myocardial tissue environment. Continuous and deep lesions for PV isolation, can be created by a single 200 J pulse of a few ms in duration.
Neven (2014) [36]	Porcine (n=5), open chest	Linear electrode in suction cup, Epi_Delv	30/100/300 J DC pulses, PD=6 ms, the DC is applied between a skin patch and the linear electrode	Lesions of mean depth was 3.2±0.7, 6.3±1.8 and 8.0±1.5 mm, mean width was 10.1±0.8, 15.1±1.5 and 17.1±1.3 mm, transmural was 25%, 100% and 100% for 30, 100, and 300 J epicardial ablation, respectively	3 months	No permanent coronary arteries damage. No luminal arterial narrowing was observed after 3 months.

Study (Year)	Experiment model (number)	Electrode configuration and delivery method	IRE parameters of protocol	Effect	Follow-up	Significance
Van Driel (2015) [37]	Porcine(n=20), open and closed chest	Circular-20mm-diameter with 8-poles 4 mm electrodes, and Circular-18mm-diameter with 8-poles 2mm electrodes, Endo_Delv	200 J DC pulses, the DC is applied between a skin patch and the multipolar circular electrode	Transmural lesions were created in firm contact with the inner superior caval vein wall	3–13 weeks	No phrenic nerve damage.
Xie (2015) [38]	Rabbit (n=12), Isolated heart	Parallel penetrating needle electrodes spaced 2-4mm, Epi_Delv	PN=6, 20 PD=350 ns, PRI=300 ms, 1s, PA=2.3 kV	Transmural lesions were verified from histological and optical mapping data	No	First reported case that nanosecond pulsed electric field can create the transmural lesions.
Neven (2017) [39]	Porcine(n=8), open chest	Suction cup containing single 35 × 6 mm linear electrode, Epi_Delv	100/200 J DC pulses, PD=6 ms, the DC is applied between a skin patch and the linear electrode	IRE ablation was tested purposely on anterior esophageal adventitia	2 months	No esophageal ulceration or fistula found.
Reddy (2018) [40]	Human (n=22), open and closed chest	Bipolar 12-F over-the-wire catheter with 5 splines each containing 4 separate electrodes for Endo_Delv, and bipolar linear closed ring catheter with multiple electrodes for Epi_Delv	Mean 78J per ablation for Endo_Delv and 1146 J per ablation for Epi_Delv, over a few seconds train of multiple ms pulses, PA=0.9-2.5 kV	15 patients out of 15 are PV isolated with 3.26 ± 0.5 mm lesions from endovascular delivery, and 6 patients out of 7 from epicardial delivery	1 month	The first-in-human clinical application of IRE ablation for acute PV isolation, for both cardiac surgery and catheter-based ablation.
Van Es (2019) [41]	Porcine(n=5), open chest	Suction cup containing single 35 × 6 mm linear electrode, Epi_Delv	200 J pulses, PN=10, PD=2ms, PRI=400 ms, Frequency=167kHZ	Only one transmural lesion was obtained with asymmetric high-frequency (aHF), but not for symmetric high-frequency (sHF)	3 weeks	aHF IRE ablation avoids skeletal muscle contractions and gas bubble formation. aHF pulses can also create deeper lesions than symmetric of same energy in cardiac tissue.
Reddy (2019) [42]	Human (n=81), closed chest	Bipolar 12-F over-the-wire catheter with 5 splines each containing 4 separate electrodes, Endo_Delv	Over a few seconds train of multiple ms pulses, PA=0.9-2kV	All PVs were isolated by monophasic (n=15) or biphasic (n=66). With successive IRE refinement, durability improved from 18% to 100%	120 days	In 81 patients, 100% of all PVs were acutely isolated and no additional primary complications such as stroke, phrenic nerve injury, PV stenosis, and esophageal injury over the 120-day median follow-up.

Table 1. Selected historical events using IRE cardiac ablation since 2007. For each study, only the first author is

mentioned. Abbreviations: PD=Pulse Duration, PRI=Pulse Repetition Interval (or called Pulse Cycle Length), PN=Pulse Number, Pulse Amplitude=PA, Direct Current=DC, Epi_Delv=epicardial IRE delivery, Endo_Delv= endovascular IRE delivery.

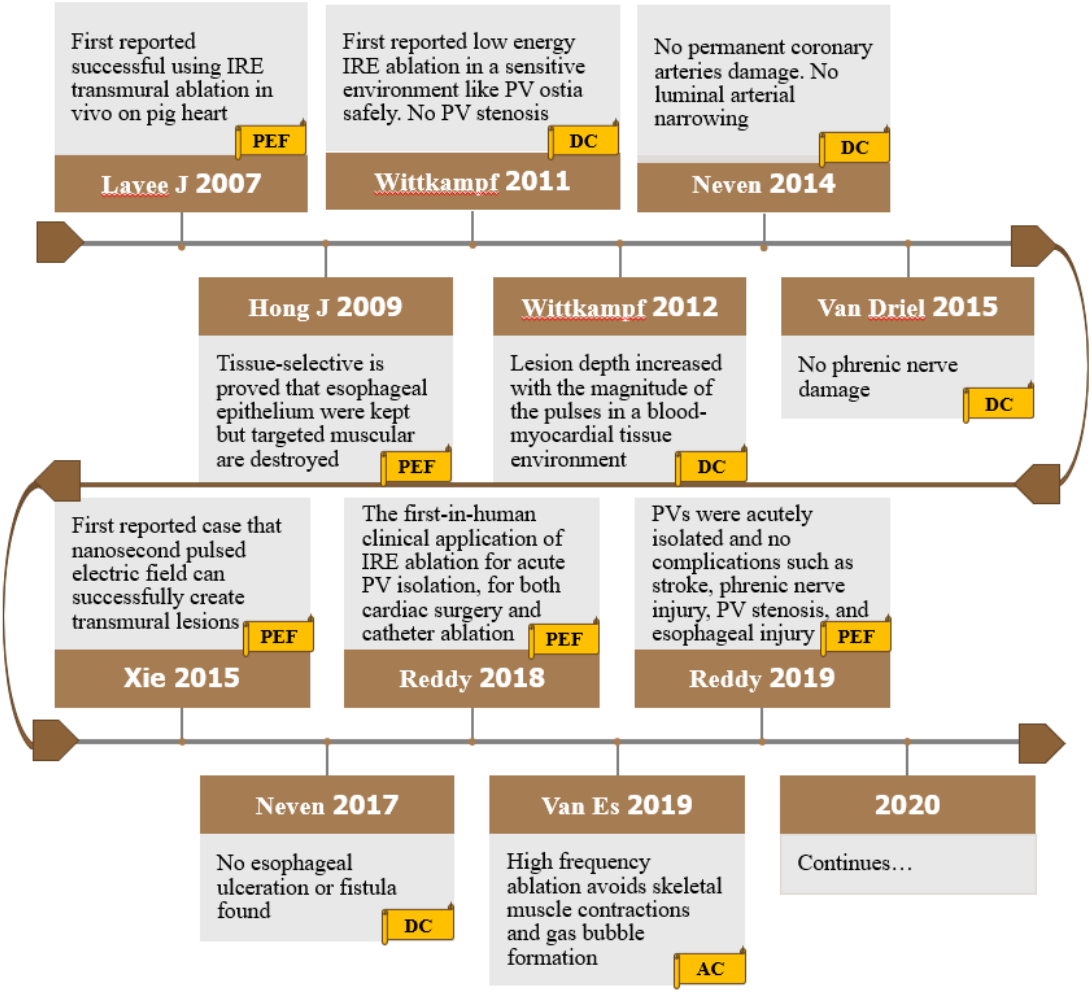

Figure 2. Timeline summarizing Table 1 of the remarkable discoveries, including the first author, publication year and historical events using IRE ablation for the treatment of AFib, IRE modalities are highlighted in yellow including direct current (DC), alternative current (AC), and pulsed electric field (PEF) ablation.

The electrode positioned onto the target myocardium and an external patch as ground. The second IRE modality of AC ablation is not reported as much as DC or PEF, and the pilot research of using high frequency AC ablation is reported by Van Es in 2019, that asymmetric high frequency ablation successfully created myocadiac lesions without skeletal muscle contractions and gas bubble formation on pig hearts [41]. The third IRE modality, PEF ablation is an adaptation of DC, however, a more controlled low energy form, delivered over a few seconds across multiple electrodes without the use of an external ground patch [42]. PEF consists of a series of multiple, short DC pulses with pulse duration in the scale of microseconds or nanoseconds). The created local electric field in bipolar fashion by PEF, lead to the reorganization of the lipid structure of cardiomyocytes membrane, resulting in nanoscale pores formation. Those pores increase cell membrane permeability and thus the leakage of cell contents, finally leading to cell death. This phenomenon subsequently results in either immediate necrosis or delayed apoptotic cell death [43]–[46]

2.2 IRE PARAMETERS OF PROTOCOLS

Electroporation protocols include many parameters that can be modified. These parameters include biphasic or monophasic pulses, voltage amplitude (hundreds to thousands of volts), pulse duration (nanoseconds to milliseconds), pulse repetition interval, interphase interval, number of pulses in the train (single pulse to hundreds), and number of trains delivered, and bipolar or unipolar electrodes delivery. The characteristics of each pulse that can vary include pulse rise time, pulse fall time, overshoot and ringing, and undershoot [47]. Each of these parameters, combined with others, can have a significant influence on the efficiency of IRE treatment and result in more/less tissue destructions.

2.3 TISSUE SPECIFICITY

All thermal energies, including RF, cryothermy, laser, ultrasound, microwave, and even x-ray, is their feature of ablating all tissues indiscriminately. IRE (either DC or PEF), on the other hand, due to its nonthermal ablation modality nature, can ablate the tissue with its unique tissue selectivity. This is because myocardium have the lowest characteristic threshold value of 400 V/cm than any surrounding tissues, and will first induce cardiomyocytes necrosis [40]. This feature potentially limits collateral damage of periatrial nontarget tissue, such as the esophagus and phrenic nerves, coronary arteries, PVs, and other proteins, if IRE protocols are precisely controlled (evidence from Wittkampf in 2011, Neven in 2014, Van Driel in 2015, Neven in 2017, Reddy in 2018, and Reddy in 2019, **Table 1**). This underlying mechanism of IRE treatment offers a degree of tissue-specific safety, sparing supra-threshold tissues within an ablative zone, avoiding clinical complications as RF inherently holds (refer to **Table 2** listed all aspects for comparison).

<u>*NO EVIDENCE FOR PV STENOSIS*</u>

Pulmonary vein stenosis is a serious and can be a life-threatening complication of RF ablation, caused by hyperthermia to induce contraction of the vein wall. Because of the nature of nonthermal mechanism of IRE, the propensity for collateral damage appears to be much lower than with thermal energy sources. It is noted the diameter of PV kept consistent post DC ablation, while RF ablation lead to shrinking of PV in the controlled group from animal model, both after 3 weeks (Wittkampf in 2011 in **Table 1**). It is also confirmed from patients there are no PV stenosis observed, after 1-month follow-up visit (Reddy in 2018 in **Table 1**).

<u>*NO EVIDENCE FOR CORONARY ARTERIES DAMAGE*</u>

Thermal ablation induces heat damage to all tissue near the ablation site, so does coronary arteries nearby, which may have serious risk. The heat may not only coagulate blood inside the vessel causing thrombus, but also causes intimal hyperplasia and shrinkage of the collagen fibers in the arterial wall, which may lead to vessel stenosis and subsequent infarction of the perfused territory [13]. In contrast, IRE ablates the myocardium but selectively saves arteries themselves entirely. The selective ablation feature is applied in oncological IRE treatment at the beginning: solid tumors are ablated, but vital structures of close proximity such as blood vessels and nerves remain relatively spared, as these structures are relatively resistant to pulsed electric fields [13]. For the cardiac ablation, the potential coronary damage was further investigated by histological analysis of arteries that happened to be present in the lesions, and also after intentionally targeting main coronary arteries via a epicardial circular catheter in the pericardial space (Neven in 2014 in **Table 1,** and du Pre in 2013 [13], [36], [48], [49]). Both studies demonstrated that coronary arteries remained free of significant damage, up to 3 months follow up.

<u>NO EVIDENCE FOR PHRENIC NERVE DAMAGE</u>

The locations phrenic nerves are in close proximity to the superior vena cava, right pulmonary veins, and left atrial appendage. Phrenic nerve palsy is another complication of pulmonary vein isolation (PVI) procedure with thermal ablation. The report by Van Driel in 2015 (refer to **Table 1**), found no evidence to support right phrenic nerve damage, by intentionally targeted ablation using a circular 200 J DC with good tissue contact inside the superior caval vein wall. Occasionally, nerve palsy was transiently induced, but normal pacing was monitored from the superior caval vein 30 min and 3-13 weeks after the ablation.

<u>NO EVIDENCE FOR ESOPHAGEAL FISTULA</u>

The esophageal fistula is another rare but devastating complication of thermal left atrial ablation due to the anatomical left atrium in vicinity to esophagus. When the overheat conduct to esophagus from LA, the esophageal tissue is over burned and can lead to fistula. However, IRE ablation is characterized by tissue-specific thresholds, thereby facilitating preferential ablation of only myocardium, with relative sparing of collateral tissue like esophagus. From Neven's report in 2017 (refer to **Table 1**), architecture of the esophageal tissue remained chronically intact after 2 months.

<u>NO EVIDENCE FOR STROKE (DUE TO THROMBOSIS)</u>

Thermal delivery in very close proximity to a coronary artery can cause thrombosis and occlusion, as the heat may coagulate blood inside the vessel. If the blood clot falls and flows into the brain, it will be the lethal complication causing stroke, even it is rare of happing. Because of the absence of obvious temperature rising of IRE, no evidence of thrombus formation is documented, which can lead to stroke.

2.4 PROCEDURE TIME

Due to the time-dependent conductive heating/cooling, thermal ablation techniques require several seconds to achieve measurable effects and minutes to achieve steady-state temperature gradients. The effects of IRE, in contrast, due to its nonthermal modality nature, are almost instantaneous (in the scale of a few seconds maximum, from previous report in **Table 1**) in one spot ablation. The cumulative time cost to create continuous lesions, extending to the overall time cost for all the lesions created in the heart, can be dramatically reduced. It is reported by Reddy in 2018 (refer to **Table 1**) that the IRE catheter ablation mean procedure time was 67±10.5 minutes, over all 57 PVs in 15 patients. In contrast, the mean procedure time for RF energy using multielectrode catheter is 96±36 minutes (61 patients), and 166±46 minutes using standard focal irrigated catheter (59 patients), both for PV isolation (PVI), from the report by Lucas Boersma et al in 2016 [50]--- it is obvious the procedure time drops dramatically using IRE modality.

2.5 RECURRENCE RATE

Durable efficacy of successful acute thermal ablation for AFib still remains a major challenge in long terms: success rates range from 60% to 80% for paroxysmal AFib, depending on ablation strategies, and between 50% to 60% for persistent AFib [51]. Among different ablation strategies, PVI constitutes the cornerstone of thermal catheter ablation strategy of therapy, by creating a circumferential ring of nonconductive lesions around the individual PV from left atrium [52]. The reasons of AFib recurrences in long term after ablation, can be classified into 4 possible groups: (1) recovery of PV-left atrial (LA) conduction that was previously ablated, (2) the presence of triggers ostial to the site of PV disconnection, (3) the development of new triggers in nonablated PVs, or (4) the development of new triggers outside the PVs [53]. Among these four possibilities, the first reason is the main mechanism leading to AFib recurrence, i.e. recurrence of PV-LA electrical conduction, is almost universal in patients with recurrent AF after ablation. This is found independently by different clinical research from Cappato et al [54], Callans et al [55], and Verma et al [56]: resumption and delay of PV conduction in at least 1 previously ablated PV occurred, in almost all patients with AF recurrence. This recurrence of PV-LA conduction is linked with the presence of gaps, within the unfavorable incomplete lesions created by RF energy, between the PV and LA.

Generally, In short-term, PVI can be achieved even with incomplete lesion formations that causes temporary electric uncoupling but not irreversible cell death ⩽3.5 mm away from the lesion boundary, after a transient phase of reversible tissue injury [57]. After ablation, local conduction block appears despite a gap of ⩽1.4 mm, and in abnormal tissue with a gap as large as 4 mm [58]. These incomplete lesions with gaps, “mask” the need of permanent complete lesion formation, for permanent PVI. Formation of lesion gaps are due to insufficient heat, which is carried away by convective cooling from the surrounding arterial blood flow. With time, undead tissue at the boundary of the transmural lesion recovers normal conduction during the course of 1 to 4 weeks [57], and PV-LA conduction gradually exhibits in several weeks or months thereafter since tissue injury heals. Therefore, the achievement of permanent PV isolation should be considered the main goal of AFib treatment in order to avoid recurrences.

The verification of recurrence rate of IRE ablation, however, is still in early stage, due to its short application history. Reddy et all are the pioneers of using the PEF for AFib patients: it is confirmed progressive improvement of durable PV isolation from initial monophasic waveform of 18%, to biphasic waveform and successive “waveform refinement” of 100% of patients with all PVs isolated, in 3 months [42]. The details of successive waveform modifications were not reported, but it appears bipolar basket electrode configuration without skin patch is an effective delivery way for PEF ablation.

2.6 TISSUE LOSS BEYOND TARGET

Due to the RF time-dependent conductive thermal mechanism, in order to effectively create transmural lesions, targeted zone temperature relies on 1) reaching the peak temperature on the tissue-electrode interface in the proximal, and 2) spreading of the peak temperature from proximal to distal to destroy the tissue transmurally from endocardium to epicardium, for catheter ablation. In general, the cross-section area of lesions is in elliptical shape: bigger area in proximal and smaller area in distal. In order to reach enough distal for transmural lesion, proximal will be sacrificed more. Another extra loss of tissue is due to the convective cooling by the arterial blood flow that will carry certain amount of heat away, leading to limited formation of enough lesion in the distal. In order to create adequate lesions in the distal, extra tissue in proximal will be lost. However, IRE ablation is not based on time-dependent thermal mechanism. By selecting optimized IRE protocol parameters and electrode configuration, well controlled ablation region can be expected with clear ablated/unablated boundary.

2.7 CHARRING AND STEAM POP

During RF procedure, the charring hinders heat conduction thus affect the efficacy of lesion formation. And if tissue temperatures exceed catheter tip temperature and go beyond 100°C, steam explosions can occur, which sound as “steam pops” [59]. Pops may cause superficial craters or deep tissue tears , associated with the potential development of cardiac perforation and tamponade [59]. Luckily, due to the nature of PEF energy, its nonthermal ablative mechanism eliminates high temperature phenomena like catheter tip charring and steam pops, as RF holds.

2.8 TISSUE-CATHETER CONTACT DEPENDENT

For RF ablation, achieving good tissue-catheter contact is critical to produce adequate lesion depth and width. Insufficient electrode tissue contact may lead to shallow and discontinuous lesions that may not be recognized acutely [28]. Insufficient contact force (between electrode tip and myocardium wall) can result in inadequate lesion formation and higher rates of PV reconnection, but excessive contact force can result in complications, such as perforation [60]. Therefore, extra need of continuous monitoring contact force would be expected to maximize ablation efficacy and improve safety, for RF ablation.

In contrast, IRE relies upon tissue proximity and are not solely contact dependent, because they are depending on lethal electric field strength. Although the relationship between electric field and the applied external voltage is complicated in the heterogeneous tissue, the local electric field still equals local voltage divided by distance at specific point. If local voltage increases, local electric field increases, even distance is constant. The creation of an electric field, rather than contact-dependent lesions, is another attractive feature for cardiac tissue ablation, which does not require good contact of tissue and catheter tip, eliminating the extra dependency on contact force sensing and lethal tissue temperatures.

3 LIMITATIONS ASSOCIATED WITH IRE ABLATION

Due to its short history of development of IRE ablation in AFib treatment, IRE's clinical limitations are not fully exploded. Here is the list of a few most concerned with.

3.1 INVOLUNTARY MUSCLE CONTRACTION

The conventional direct current (DC), routinely applied between 1980 and 1990 for ablation of the bundle of His and ventricular tachycardia, can leads to significant involuntary skeletal muscle contractions and pain, necessitates the use of complete anesthesia [29]. The DC is delivered in a unipolar fashion, with an active electrode coupled with a reference dispersal electrode patched on the skin. Instead, bipolar energy delivered between local electrodes may confine the electric field, resulting in effective local tissue ablation without significant muscle contractions. Depending on the delivered energy, complete anesthesia may or may not required. According to Reddy et al in 2019 (refer to **Table 1**), 15 out of 81 patients undergoing monophasic PEF ablation, undertook general anesthesia and skeletal muscle contraction was not observed. Another group of 65 patients accepting biphasic procedures were performed with conscious sedation, and this resulted in only mild degrees of muscle activation that was tolerated well, and some occasionally experienced transient intraprocedural cough.

3.2 BUBBLE AND ARCING

The standard DC current causes electrolysis on the metal electrode surfaces in the blood pool, thus producing micro bubbles containing the gaseous products from the electrolysis. Arcing is another phenomenon that when the electric current density between the electrode and surrounding blood becomes critical, a flash or arc results which in turn, leads to a temperature rise as high as a thousand in transient within the bubble [61]. With the low-energy DC energy and modified electrode configuration, Wittkampf et al. in 2011 (refer to **Table 1**) created myocardial lesions without arcing and claimed the small gas bubbles that adhere to the metal surface of electrode and dissolve within a few seconds. It is also found with low energy PEF protocol by Reddy et al in 2019 (refer to **Table 1**), no arcing is found or significant bubble formation visible.

3.3 ORIENTATION-DEPENDENT

The local electric field distribution (electric current density divided by conductivity tensor) determines the effective ablated cardiac tissue region. Due to the anisotropic orientations of cardiac fibers, lethal electric field cannot create consistent lesions, according to the numerical model by Xie et al. in 2016 [62]. Besides anisotropic orientations of cardiac fibers, the existing of fibrosis and blood vessels can make myocardium conductivity even more heterogeneous. Plus, electrical conductivity of infarcted myocardium is more conductive than that of a healthy myocardium [63]. Therefore, electroporation protocols must be tailored according to the local target clinical cardiac tissue [64], to make the ablation lesion more consistent. Unfortunately, due to the complicated geometry of the heart, there are very few researches currently investigating how varying conductivity and protocol modification can differentiate the local heterogeneous ablated lesions.

3.4 AREAS OF REVERSIBILITY

The nonconductive lesion (including complete and incomplete) from RF ablation, originate from the combination of irreversible and reversible atrial thermal injury. The incomplete lesion from reversible damage can causes temporary electric uncoupling but not complete cell death $\leqslant$3.5 mm away from the lesion boundary, dependent on tissue temperature and tissue geometry, increases the risk of long-term AFib recurrence [57]. IRE ablation, due to its short application history, is lacking supportive evidence that irreversible injury could happen, and not clear that how far the length away from the lesion boundary can also lead to nonconductive lesion temporary.

		RF	IRE
Clinical Complications	PV Stenosis	😔	😃 🕐
	Coronary Arteries Damage	😔	😃 🕐
	Phrenic Nerve Injury	😔	😃 🕐
	Esophageal Fistula	😔	😃 🕐
	Stroke (due to thrombosis)	😔	😃
Problems Associated with Thermal Effect	Long Time Procedure	😔	😃
	High Recurrence Rate	😔	😃 🕐
	Tissue Loss Beyond Target	😔	😃
	Charring	😔	😃
	Steam pop	😔	😃
	Extra Tissue-Catheter Contact Force Sensing Required	😔	😃
Possible Complications	Bubble	✕	❔
	Arcing	✕	❔
	Muscle Twitching	✕	❔
Electrically-induced Permeability	Apoptosis	✕	☑
	Orientation-Dependent	✕	❔
	Areas of Reversibility	☑	❔
Both Safety and Efficacy can be achieved		😔	😃

😔 does have the problem 😃 does not have the problem ✕ does not apply
❔ maybe or maybe not, depend on the external conditions ☑ does apply
😃 🕐 the combined emoji means data here only shows short-term satisfaction, and long-term clinical data are not available at present

Table 2. Comparisons between RF and IRE in several aspects. In summary, for RF it is the zero-sum relationship between safety and efficacy (greater efficacy is achieved with more

ablation but at the expense of safety, and vice versa). However, for IRE the safety and efficacy relationship is win-win (it can be balanced and achieved at the same time).

4. CONCLUSION AND FUTURE PERSPECTIVES

IRE holds the significant potential to become an important ablation modality in both invasive and interventional cardiology. In the field of AFib treatment, the unique ability of IRE to induce a tissue selective and well controlled ablation zone with no thermal damage offers important advantages, since its non-thermal nature can be translated into reduced risk of complications, such as pulmonary vein stenosis, coronary damage, phrenic nerve injury, and esophageal fistula. The absence of a large thermal effect also benefits shortening ablation procedure, lower recurrence rate, avoiding char formation, and may allowing direct treatment of vessels, where the muscular cell layer is destroyed but the structural integrity of the vessel is preserved, as has been demonstrated in prior studies. A high level of safety was demonstrated by IRE's tissue selective capability which spares of nerves, coronary arteries, esophageal muscle, phrenic nerves and pulmonary veins, but destroys myocardium. However, the exact mechanism to explain why cardiac tissue possessing a lowest electric field strength threshold of irreversible electroporation other than surrounding tissue, is not completely clear. This may be relate to cell size, orientation of cardiac fibers, membrane characteristics, and sensitivity to nonspecific cation entry [47]. With axons connected, only parts of those collateral membrane in vicinity will be exposed to the electroporation field, but these parts may be too small to be lethal [28]. So far, it is confirmed IRE ablation does not have short-term complications as RF does from animal and patients. To be firmed with the safety, long-term follow up of the complications (such as PV stenosis, coronary arteries damage phrenic nerve injury, esophageal fistula) are required with computed tomography / magnetic resonance imaging, before further development to a clinically applicable technology.

In terms of efficacy, efforts should be made to develop strategies that achieve more durable lesions. Strategies to ensure permanent PV isolation from recurrent PV-LA conduction after ablation should have the greatest impact on reducing AF recurrence. Although it is reported PEF ablation with waveform refinement improves the durability of AFib treatment from 18% to 100% in 3 month by Reddy et al [42], and yet provocative testing with adenosine or isoproterenol was not performed, and longer-term survival research need to be addressed to ensure more patients will have permanent PVI that will result in fewer recurrences of AF.

Although these preclinical studies in **Table 1** and **Table 2**, indicate an excellent efficacy and safety profile for AFib ablation, however, the IRE approach may some disadvantages, such as the need for general anesthesia and neuromuscular paralysis to avoid skeletal muscle contraction, and arcing and bubble need to be avoided. Thus, an optimal IRE protocol that can produce bubble-free and arc-free, and free of muscle contraction and pain, is highly desired. Besides, the feature of orientation dependent and area of reversibility also need to be investigated, to fully understand the whole picture of how to exploit this promising technique as the replacement of conventional RF ablation.

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References:

[1] M. K. Son, N.-K. Lim, H. W. Kim, and H.-Y. Park, “Risk of ischemic stroke after atrial fibrillation diagnosis: A national sample cohort,” *PLoS ONE*, vol. 12, no. 6, Jun. 2017, doi: 10.1371/journal.pone.0179687.

[2] J. Y. Chyou, T. D. Hunter, S. A. Mollenkopf, M. P. Turakhia, and M. R. Reynolds, “Individual and Combined Risk Factors for Incident Atrial Fibrillation and Incident Stroke: An Analysis of 3 Million At-Risk US Patients,” *J. Am. Heart Assoc. Cardiovasc. Cerebrovasc. Dis.*, vol. 4, no. 7, Jul. 2015, doi: 10.1161/JAHA.114.001723.

[3] Chung Mina K. *et al.*, “Lifestyle and Risk Factor Modification for Reduction of Atrial Fibrillation: A Scientific Statement From the American Heart Association,” *Circulation*, vol. 0, no. 0, p. CIR.0000000000000748, doi: 10.1161/CIR.0000000000000748.

[4] Q. Chen, Z. Yi, and J. Cheng, “Atrial fibrillation in aging population,” *AGING Med.*, vol. 1, no. 1, pp. 67–74, 2018, doi: 10.1002/agm2.12015.

[5] S. Stewart, N. Murphy, A. Walker, A. McGuire, and J. J. V. McMurray, “Cost of an emerging epidemic: an economic analysis of atrial fibrillation in the UK,” *Heart*, vol. 90, no. 3, pp. 286–292, Mar. 2004, doi: 10.1136/hrt.2002.008748.

[6] K. MacIntyre *et al.*, “Evidence of improving prognosis in heart failure: trends in case fatality in 66 547 patients hospitalized between 1986 and 1995,” *Circulation*, vol. 102, no. 10, pp. 1126–1131, Sep. 2000, doi: 10.1161/01.cir.102.10.1126.

[7] S. Capewell *et al.*, “Age, sex, and social trends in out-of-hospital cardiac deaths in Scotland 1986-95: a retrospective cohort study,” *Lancet Lond. Engl.*, vol. 358, no. 9289, pp. 1213–1217, Oct. 2001, doi: 10.1016/S0140-6736(01)06343-7.

[8] T. H. Lee and M. H. Alderman, “Malignant hypertension. Declining mortality rate in New York City, 1958 to 1974,” *N. Y. State J. Med.*, vol. 78, no. 9, pp. 1389–1391, Aug. 1978.

[9] C. A. Morillo, A. Banerjee, P. Perel, D. Wood, and X. Jouven, “Atrial fibrillation: the current epidemic,” *J. Geriatr. Cardiol. JGC*, vol. 14, no. 3, pp. 195–203, Mar. 2017, doi: 10.11909/j.issn.1671-5411.2017.03.011.

[10] S. Stewart, N. F. Murphy, N. Murphy, A. Walker, A. McGuire, and J. J. V. McMurray, “Cost of an emerging epidemic: an economic analysis of atrial fibrillation in the UK,” *Heart Br. Card. Soc.*, vol. 90, no. 3, pp. 286–292, Mar. 2004, doi: 10.1136/hrt.2002.008748.

[11] W. C. Lee, G. A. Lamas, S. Balu, J. Spalding, Q. Wang, and C. L. Pashos, “Direct treatment cost of atrial fibrillation in the elderly American population: a Medicare perspective,” *J. Med. Econ.*, vol. 11, no. 2, pp. 281–298, 2008, doi: 10.3111/13696990802063425.

[12] M. H. Kim, S. S. Johnston, B.-C. Chu, M. R. Dalal, and K. L. Schulman, “Estimation of total incremental health care costs in patients with atrial fibrillation in the United States,” *Circ. Cardiovasc. Qual. Outcomes*, vol. 4, no. 3, pp. 313–320, May 2011, doi: 10.1161/CIRCOUTCOMES.110.958165.

[13] B. C. du Pré *et al.*, “Minimal coronary artery damage by myocardial electroporation ablation,” *EP Eur.*, vol. 15, no. 1, pp. 144–149, Jan. 2013, doi: 10.1093/europace/eus171.

[14] A. Thiagalingam, C. R. Campbell, A. Boyd, D. L. Ross, and P. Kovoor, "Catheter Intramural Needle Radiofrequency Ablation Creates Deeper Lesions than Irrigated Tip Catheter Ablation," *Pacing Clin. Electrophysiol.*, vol. 26, no. 11, pp. 2146–2150, 2003, doi: 10.1046/j.1460-9592.2003.00334.x.

[15] U. F. O. Themes, "Cardiac Ablative Technologies," *Thoracic Key*, Jul. 10, 2016. https://thoracickey.com/cardiac-ablative-technologies/ (accessed Apr. 25, 2020).

[16] L. D. Biase *et al.*, "Remote Magnetic Navigation: Human Experience in Pulmonary Vein Ablation," *J. Am. Coll. Cardiol.*, vol. 50, no. 9, pp. 868–874, Aug. 2007, doi: 10.1016/j.jacc.2007.05.023.

[17] Cochet Hubert, Sacher Frederic, Chaumeil Arnaud, and Jais Pierre, "Steam Pop During Radiofrequency Ablation," *Circ. Arrhythm. Electrophysiol.*, vol. 7, no. 3, pp. 559–560, Jun. 2014, doi: 10.1161/CIRCEP.113.001418.

[18] H. Haghshenas, P. Mansoori, S. Najafi, M. H. Nikoo, N. Zare, and M. Jonoobi, "The effect of changes in patients' body position on the back pain intensity and hemodynamic status during and after radiofrequency catheter ablation of cardiac dysrhythmias," *Iran. J. Nurs. Midwifery Res.*, vol. 18, no. 2, pp. 89–93, 2013.

[19] M. I. Scanavacca, "How to prevent, recognize and manage complications of AF ablation?," *Rev. Port. Cardiol. Engl. Ed.*, vol. 36, pp. 43–49, Nov. 2017, doi: 10.1016/j.repce.2017.09.012.

[20] Deshmukh Abhishek *et al.*, "In-Hospital Complications Associated With Catheter Ablation of Atrial Fibrillation in the United States Between 2000 and 2010," *Circulation*, vol. 128, no. 19, pp. 2104–2112, Nov. 2013, doi: 10.1161/CIRCULATIONAHA.113.003862.

[21] Yokoyama Katsuaki, Nakagawa Hiroshi, Wittkampf Fred H.M., Pitha Jan V., Lazzara Ralph, and Jackman Warren M., "Comparison of Electrode Cooling Between Internal and Open Irrigation in Radiofrequency Ablation Lesion Depth and Incidence of Thrombus and Steam Pop," *Circulation*, vol. 113, no. 1, pp. 11–19, Jan. 2006, doi: 10.1161/CIRCULATIONAHA.105.540062.

[22] M. Ueda *et al.*, "Pulmonary vein morphology before and after segmental isolation in patients with atrial fibrillation," *Pacing Clin. Electrophysiol. PACE*, vol. 28, no. 9, pp. 944–953, Sep. 2005, doi: 10.1111/j.1540-8159.2005.00214.x.

[23] K. B. Loh, S. I. Bux, B. J. J. Abdullah, R. A. R. Mokhtar, and R. B. Mohamed, "Hemorrhagic Cardiac Tamponade: Rare Complication of Radiofrequency Ablation of Hepatocellular Carcinoma," *Korean J. Radiol.*, 2012, doi: 10.3348/kjr.2012.13.5.643.

[24] A. Bhaskaran, W. Chik, S. Thomas, P. Kovoor, and A. Thiagalingam, "A review of the safety aspects of radio frequency ablation," *Int. J. Cardiol. Heart Vasc.*, vol. 8, pp. 147–153, Sep. 2015, doi: 10.1016/j.ijcha.2015.04.011.

[25] J. A. Mears, N. Lachman, K. Christensen, and S. J. Asirvatham, "The Phrenic Nerve And Atrial Fibrillation Ablation Procedures," *J. Atr. Fibrillation*, vol. 2, no. 1, Jun. 2009, doi: 10.4022/jafib.176.

[26] P. Zhang *et al.*, "Characteristics of Atrial Fibrillation Patients Suffering Esophageal Injury Caused by Ablation for Atrial Fibrillation," *Sci. Rep.*, vol. 10, no. 1, pp. 1–9, Feb. 2020, doi: 10.1038/s41598-020-59539-6.

[27] J. Lavee, G. Onik, P. Mikus, and B. Rubinsky, "A novel nonthermal energy source for surgical epicardial atrial ablation: irreversible electroporation," *Heart Surg. Forum*, vol. 10, no. 2, pp. E162-167, 2007, doi: 10.1532/HSF98.20061202.

[28] F. H. M. Wittkampf, R. van Es, and K. Neven, "Electroporation and its Relevance for Cardiac Catheter Ablation," *JACC Clin. Electrophysiol.*, vol. 4, no. 8, pp. 977–986, 2018, doi: 10.1016/j.jacep.2018.06.005.

[29] F. H. Wittkampf *et al.*, "Feasibility of electroporation for the creation of pulmonary vein ostial lesions," *J. Cardiovasc. Electrophysiol.*, vol. 22, no. 3, pp. 302–309, Mar. 2011, doi: 10.1111/j.1540-8167.2010.01863.x.

[30] J. J. Gallagher *et al.*, "Catheter technique for closed-chest ablation of the atrioventricular conduction system," *N. Engl. J. Med.*, vol. 306, no. 4, pp. 194–200, Jan. 1982, doi: 10.1056/NEJM198201283060402.

[31] R. N. Hauer, E. O. Robles de Medina, and C. Borst, "Proarrhythmic effects of ventricular electrical catheter ablation in dogs," *J. Am. Coll. Cardiol.*, vol. 10, no. 6, pp. 1350–1356, Dec. 1987, doi: 10.1016/s0735-1097(87)80140-7.

[32] M. Haissaguerre, P. Montserrat, J. F. Warin, J. P. Donzeau, P. Le Metayer, and J. P. Massiere, "Catheter ablation of left posteroseptal accessory pathways and of long RP' tachycardias with a right endocardial approach," *Eur. Heart J.*, vol. 12, no. 8, pp. 845–859, Aug. 1991, doi: 10.1093/eurheartj/12.8.845.

[33] G. H. Bardy, F. Coltorti, R. B. Stewart, H. L. Greene, and T. D. Ivey, "Catheter-mediated electrical ablation: the relation between current and pulse width on voltage breakdown and shock-wave generation," *Circ. Res.*, vol. 63, no. 2, pp. 409–414, Aug. 1988, doi: 10.1161/01.res.63.2.409.

[34] J. Hong, M. T. Stewart, D. S. Cheek, D. E. Francischelli, and N. Kirchhof, "Cardiac ablation via electroporation," *Conf. Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. IEEE Eng. Med. Biol. Soc. Annu. Conf.*, vol. 2009, pp. 3381–3384, 2009, doi: 10.1109/IEMBS.2009.5332816.

[35]Wittkampf Fred H.M. *et al.*, "Myocardial Lesion Depth With Circular Electroporation Ablation," *Circ. Arrhythm. Electrophysiol.*, vol. 5, no. 3, pp. 581–586, Jun. 2012, doi: 10.1161/CIRCEP.111.970079.

[36] K. Neven, V. van Driel, H. van Wessel, R. van Es, P. A. Doevendans, and F. Wittkampf, "Epicardial linear electroporation ablation and lesion size," *Heart Rhythm*, vol. 11, no. 8, pp. 1465–1470, Aug. 2014, doi: 10.1016/j.hrthm.2014.04.031.

[37] V. J. H. M. van Driel, K. Neven, H. van Wessel, A. Vink, P. A. F. M. Doevendans, and F. H. M. Wittkampf, "Low vulnerability of the right phrenic nerve to electroporation ablation," *Heart Rhythm*, vol. 12, no. 8, pp. 1838–1844, Aug. 2015, doi: 10.1016/j.hrthm.2015.05.012.

[38] F. Xie *et al.*, "Ablation of Myocardial Tissue With Nanosecond Pulsed Electric Fields," *PLoS ONE*, vol. 10, no. 12, Dec. 2015, doi: 10.1371/journal.pone.0144833.

[39] K. Neven *et al.*, "Acute and Long-Term Effects of Full-Power Electroporation Ablation Directly on the Porcine Esophagus," *Circ. Arrhythm. Electrophysiol.*, vol. 10, no. 5, May 2017, doi: 10.1161/CIRCEP.116.004672.

[40] V. Y. Reddy *et al.*, "Ablation of Atrial Fibrillation With Pulsed Electric Fields: An Ultra-Rapid, Tissue-Selective Modality for Cardiac Ablation," *JACC Clin. Electrophysiol.*, vol. 4, no. 8, pp. 987–995, Aug. 2018, doi: 10.1016/j.jacep.2018.04.005.

[41] R. van Es *et al.*, "High-frequency irreversible electroporation for cardiac ablation using an asymmetrical waveform," *Biomed. Eng. OnLine*, vol. 18, no. 1, p. 75, Jun. 2019, doi: 10.1186/s12938-019-0693-7.

[42] V. Y. Reddy *et al.*, "Pulsed Field Ablation for Pulmonary Vein Isolation in Atrial Fibrillation," *J.*

Am. Coll. Cardiol., vol. 74, no. 3, pp. 315–326, Jul. 2019, doi: 10.1016/j.jacc.2019.04.021.

[43] T. Kotnik, P. Kramar, G. Pucihar, D. Miklavcic, and M. Tarek, “Cell membrane electroporation-Part 1: The phenomenon,” *IEEE Electr. Insul. Mag.*, vol. 28, no. 5, pp. 14–23, Sep. 2012, doi: 10.1109/MEI.2012.6268438.

[44] R. V. Davalos, I. L. M. Mir, and B. Rubinsky, “Tissue ablation with irreversible electroporation,” *Ann. Biomed. Eng.*, vol. 33, no. 2, pp. 223–231, Feb. 2005, doi: 10.1007/s10439-005-8981-8.

[45] B. Rubinsky, G. Onik, and P. Mikus, “Irreversible electroporation: a new ablation modality--clinical implications,” *Technol. Cancer Res. Treat.*, vol. 6, no. 1, pp. 37–48, Feb. 2007, doi: 10.1177/153303460700600106.

[46] J. F. Edd, L. Horowitz, R. V. Davalos, L. M. Mir, and B. Rubinsky, “In vivo results of a new focal tissue ablation technique: irreversible electroporation,” *IEEE Trans. Biomed. Eng.*, vol. 53, no. 7, pp. 1409–1415, Jul. 2006, doi: 10.1109/TBME.2006.873745.

[47] “The Promise of Pulsed Field Ablation | EP Lab Digest.” https://www.eplabdigest.com/promise-pulsed-field-ablation (accessed May 13, 2020).

[48] K. Neven *et al.*, “Safety and feasibility of closed chest epicardial catheter ablation using electroporation,” *Circ. Arrhythm. Electrophysiol.*, vol. 7, no. 5, pp. 913–919, Oct. 2014, doi: 10.1161/CIRCEP.114.001607.

[49] K. Neven, V. van Driel, H. van Wessel, R. van Es, P. A. Doevendans, and F. Wittkampf, “Myocardial lesion size after epicardial electroporation catheter ablation after subxiphoid puncture,” *Circ. Arrhythm. Electrophysiol.*, vol. 7, no. 4, pp. 728–733, Aug. 2014, doi: 10.1161/CIRCEP.114.001659.

[50] Boersma Lucas V. *et al.*, “Multielectrode Pulmonary Vein Isolation Versus Single Tip Wide Area Catheter Ablation for Paroxysmal Atrial Fibrillation,” *Circ. Arrhythm. Electrophysiol.*, vol. 9, no. 4, p. e003151, Apr. 2016, doi: 10.1161/CIRCEP.115.003151.

[51] A. Sultan *et al.*, “Predictors of Atrial Fibrillation Recurrence after Catheter Ablation: Data from the German Ablation Registry,” *Sci. Rep.*, vol. 7, no. 1, pp. 1–7, Nov. 2017, doi: 10.1038/s41598-017-16938-6.

[52] S. Kumar and G. F. Michaud, “Unipolar electrogram morphology to assess lesion formation during catheter ablation of atrial fibrillation: successful translation into clinical practice,” *Circ. Arrhythm. Electrophysiol.*, vol. 6, no. 6, pp. 1050–1052, Dec. 2013, doi: 10.1161/CIRCEP.113.001145.

[53] E. P. Gerstenfeld, D. J. Callans, S. Dixit, E. Zado, and F. E. Marchlinski, “Incidence and location of focal atrial fibrillation triggers in patients undergoing repeat pulmonary vein isolation: implications for ablation strategies,” *J. Cardiovasc. Electrophysiol.*, vol. 14, no. 7, pp. 685–690, Jul. 2003, doi: 10.1046/j.1540-8167.2003.03013.x.

[54] R. Cappato *et al.*, “Prospective assessment of late conduction recurrence across radiofrequency lesions producing electrical disconnection at the pulmonary vein ostium in patients with atrial fibrillation,” *Circulation*, vol. 108, no. 13, pp. 1599–1604, Sep. 2003, doi: 10.1161/01.CIR.0000091081.19465.F1.

[55] D. J. Callans *et al.*, “Efficacy of repeat pulmonary vein isolation procedures in patients with recurrent atrial fibrillation,” *J. Cardiovasc. Electrophysiol.*, vol. 15, no. 9, pp. 1050–1055, Sep. 2004, doi: 10.1046/j.1540-8167.2004.04052.x.

[56] A. Verma *et al.*, “Response of atrial fibrillation to pulmonary vein antrum isolation is directly related to resumption and delay of pulmonary vein conduction,” *Circulation*, vol. 112, no. 5,

pp. 627–635, Aug. 2005, doi: 10.1161/CIRCULATIONAHA.104.533190.

[57] M. A. Wood and I. A. Fuller, “Acute and Chronic Electrophysiologic Changes Surrounding Radiofrequency Lesions,” *J. Cardiovasc. Electrophysiol.*, vol. 13, no. 1, pp. 56–61, 2002, doi: 10.1046/j.1540-8167.2002.00056.x.

[58] “Gaps in the ablation line as a potential cause of recovery from electrical isolation and their visualization using MRI. - PubMed - NCBI.” https://www.ncbi.nlm.nih.gov/pubmed/21493875 (accessed May 18, 2020).

[59] J. Seiler, K. C. Roberts-Thomson, J.-M. Raymond, J. Vest, E. Delacretaz, and W. G. Stevenson, “Steam pops during irrigated radiofrequency ablation: Feasibility of impedance monitoring for prevention,” *Heart Rhythm*, vol. 5, no. 10, pp. 1411–1416, Oct. 2008, doi: 10.1016/j.hrthm.2008.07.011.

[60] Barnett Adam S., Bahnson Tristram D., and Piccini Jonathan P., “Recent Advances in Lesion Formation for Catheter Ablation of Atrial Fibrillation,” *Circ. Arrhythm. Electrophysiol.*, vol. 9, no. 5, p. e003299, May 2016, doi: 10.1161/CIRCEP.115.003299.

[61] G. H. Bardy and P. L. Sawyer, “Biophysical and anatomical considerations for safe and efficacious catheter ablation of arrhythmias,” *Clin. Cardiol.*, vol. 13, no. 6, pp. 425–433, Jun. 1990, doi: 10.1002/clc.4960130611.

[62] F. Xie and C. W. Zemlin, “Effect of Twisted Fiber Anisotropy in Cardiac Tissue on Ablation with Pulsed Electric Fields,” *PloS One*, vol. 11, no. 4, p. e0152262, 2016, doi: 10.1371/journal.pone.0152262.

[63] “Cardiac Ablation by Electroporation | JACC: Clinical Electrophysiology.” http://electrophysiology.onlinejacc.org/content/4/11/1481 (accessed May 14, 2020).

[64] Y. Zager, D. Kain, N. Landa, J. Leor, and E. Maor, “Optimization of Irreversible Electroporation Protocols for In-vivo Myocardial Decellularization,” *PLoS ONE*, vol. 11, no. 11, Nov. 2016, doi: 10.1371/journal.pone.0165475.