Catheter Ablation of Atrial Fibrillation and Atrial Flutter

A Comparison of Cryo and Radiofrequency Techniques

HELENA MALMBORG
Atrial fibrillation (AF) and atrial flutter (AFl) are two of the most common arrhythmias encountered in the population. Catheter ablation has emerged as a useful tool in the treatment of these arrhythmias. Although radiofrequency (RF) is the most commonly used energy source for ablation, cryoenergy may have some advantageous properties. The purpose of these studies was to evaluate and compare ablation with novel ablation catheters using either of these energy sources.

When used for AFl ablation, cryoenergy was associated with less perceived pain than radiofrequency. However, the acute success rate was significantly lower for cryoablation (56%) compared with RF ablation (100%) in our study.

Being one of the first centres to use a new so-called “one-shot” device for pulmonary vein isolation (PVI), the cryoballoon, we described our initial experience with this catheter in 40 patients undergoing AF ablation. A high rate of PVI could be achieved although an additional cryocatheter was needed in 44% of the procedures. Freedom from arrhythmia-related symptoms was seen in 53% after a mean follow-up of 8.9 months.

Comparing the cryoballoon and a RF-based device intended for PVI, the pulmonary vein ablation catheter (PVAC), both catheters proved comparably effective (≥93%) and safe in achieving PVI with comparable procedure times. After 12 months only 46% versus 34% (ns) in the cryoballoon- and the PVAC group, respectively, were regarded as free from AF without antiarrhythmic drugs, after one ablation procedure, whereas 60% versus 54% reported clinical success. Quality of life was significantly increased in both groups, to a level comparable with the general Swedish population.

We tested the hypothesis that RF ablation would be accompanied by a higher activation of the coagulation and inflammatory systems, measured by biomarkers. Such a difference could not be supported in our study, which showed a comparable response with either technique, even though the cryoballoon caused more pronounced myocardial damage.

Two different energy settings with different ratios of bipolar-to-unipolar energy were tested with the PVAC under the hypothesis that ablation with a higher proportion of unipolar energy would require fewer applications in order to achieve PVI. However, this study failed to show any difference between the groups.
To my beloved mother and father
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


V Lönnerholm S., Malmborg H., Blomström P., Blomström-Lundqvist C. Efficacy and safety of different energy settings for atrial fibrillation ablation using the duty-cycled radiofrequency ablation catheter (PVAC). Submitted.

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<td>ACT</td>
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<td>Pulmonary Vein Ablation Catheter</td>
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<td>pulmonary vein isolation</td>
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Introduction

Atrial fibrillation (AF) and atrial flutter (AFl) are two of the most common arrhythmias in the population, affecting 1–2%. Epidemiological studies have shown that the prevalence is likely to increase. Although asymptomatic in some, the majority of patients with AF and AFl report symptoms such as palpitations, dyspnoea and fatigue. Beyond generating symptoms, these arrhythmias are associated with an increased mortality, morbidity including the risk of thromboembolic complications as well as an impaired quality of life. They also account for high costs of care.

The primary goal when treating AF and AFl is to eliminate symptoms and thereby improve quality of life. Antiarrhythmic drugs (AAD) are often used for this purpose. Previous studies have, however, shown that AADs have a moderate effect in preventing arrhythmic episodes in patients over time, and are furthermore associated with a not inconsiderable risk of adverse side effects. Consequently, alternative methods have been developed in order to try to eliminate these arrhythmias. Catheter-based ablation is one such method. Different sources of energy can be used to create the lesions needed for ablation. These are radiofrequency (RF), cryothermy (cryo), laser, microwave and ultrasound. Since RF was first introduced and is considered safe and effective, it is often regarded as the standard choice. There are however potential theoretical advantages with cryoablation such as reduced perceived pain by patients, a potentially lower thrombogenicity and, in AF ablation, a reduced risk of PV stenosis, but the technique has been limited by longer procedure times and doubts concerning achieved transmural lesions.
Background

Atrial flutter and atrial fibrillation

Atrial flutter is a macro-reentrant circuit in the right atrium (RA). The classic type of AFl (i.e. typical flutter or isthmus-dependent flutter) is a circuit with a rotation around the tricuspid annulus using the atrial septum, the roof of the RA, the lateral wall and the cavo-tricuspid isthmus (CTI). The slow conduction in the CTI and the anatomical or functional block along the crista terminalis and Eustachian ridge forms the electrophysiological substrate for the arrhythmia.\textsuperscript{21, 22} The inferior vena cava (IVC) and the tricuspid valve limit the isthmus. If counter-clockwise, this type of AFl is characterized by dominant negative flutter waves in the inferior leads and positive flutter deflections in lead V1 on the 12-lead electrocardiogram (ECG). The atrial cycle length typically ranges from 200 to 260 ms.

Atrial flutter can occur as an isolated arrhythmia, but concomitant AF is frequently recognized in the AFl population.\textsuperscript{4, 23, 24}

Atrial fibrillation is a supraventricular arrhythmia with a more or less uncoordinated or chaotic electrical activity within the atrium, characterized by an irregular baseline and an irregular ventricular rhythm on the ECG. The atrial cycle length is variable and often less than 200 ms. The rate of the ventricular response is dependent on the atrioventricular (AV) junction that serves as a filter. Depending on the pattern of arrhythmia occurrence, AF, as well as AFl, is referred to as paroxysmal, persistent, longstanding persistent, or permanent (Fig 1).

AF is dependent on both a trigger for its onset as well as a substrate for its perpetuation. In 1998, Haïssaguerre et al. showed that paroxysmal AF (PAF) was triggered by ectopic beats originating from the pulmonary veins (PV).\textsuperscript{25} Sleeves of myocardium extending from the LA into the PVs constitute the arrhythmogenic substrate. Multiple sites inside one PV or multiple PVs can harbour the ectopic activity responsible for AF initiation in an individual.\textsuperscript{26} Experimental evidence supports automaticity, trigged activity and re-entry as the underlying mechanisms of arrhythmogenesis in PVs.\textsuperscript{27-29} Besides being triggers, PVs have also been described to play a part in the perpetuation of AF.\textsuperscript{30, 31} Although triggers in the PVs account for the majority of PAF, additional non-PV foci for AF initiation have been shown to be present in 14–28% of patients.\textsuperscript{32, 33}
The exact mechanism for maintenance of AF is not clear but seems to be related mainly to electrical and structural remodelling of the atrium. Patchy fibrosis, amyloid deposits and inflammatory changes have been shown to be present in the atrial myocardium of AF patients.

The “multiple-wavelet” hypothesis, proposed by Moe et al. in 1964, postulated that wave fronts that fractionate as they are divided by islets of refractory tissue, giving rise to new “daughter wavelets”, act as the mechanism for perpetuation. This hypothesis gained wide acceptance and was later reinforced by multielectrode mappings in the human heart. The multiple wavelets will sustain the arrhythmia as long as the number of wave fronts does not fall below a critical level. The mass of atrial tissue, or the left atrial size therefore seems to be important and atrial size has been shown to be predictive of the risk of developing AF as well as progressing to more sustained AF patterns. AF itself further promotes enlargement of the atrium, and sustained high rates promote remodelling of the electrophysiologic substrate, contributing to AF permanence.

Recent mapping studies have shown the presence of more organized, atrial rotors in patients with persistent AF, and that ablation of such rotors may result in termination of the arrhythmia.

Figure 1. Different patterns of arrhythmia. When the arrhythmia is first detected (central circle) it may belong to any of the four groups. Pattern may vary in an individual over time and tends to progress towards the more sustained forms.
Catheter ablation

Historical perspective

Ablation, derived from the Latin *ablatio*, means “to remove something.” Catheter ablation of cardiac tissue for the purpose of eliminating arrhythmias resulted from a combination of observations made from electrophysiological studies and experience from arrhythmia surgery. The first described catheter ablation was performed coincidentally in 1979 when Vedel et al. observed an AV block in a patient undergoing defibrillation while a defibrillator electrode was in contact with a catheter positioned at the bundle of His. In 1982, Gallagher et al. and Scheinman et al. introduced a technique to use high voltage direct current shocks for AV node ablation, which was then followed by treatment of other arrhythmias, using the same technique. Although effective, direct current ablation was accompanied by severe complications, which led to the search for alternative energy sources. Catheter-based RF ablation was first introduced in 1987, pioneered by Borggrefe et al. and Kuck et al.

Although cryoenergy has been used in surgical procedures for more than 30 years, the first series of transvenous cryoablation in humans was described by Dubuc et al. in 2001.

The first RF ablation of the CTI for the treatment of AF was described by Cosio et al. in 1993. The initial attempts to treat AF with catheter technique, presented in mid-1990, were based on the successful outcome of surgical Maze procedures and aimed at creating linear lesions to modify the AF substrate. Although feasible, the procedures were very complicated, with long procedure times and complication rates up to 30%. Following the landmark observation by Haïssaguerre et al. in 1998, attention shifted from maze-like linear lesions to electrical isolation of pulmonary veins.

Catheter ablation of typical atrial flutter

Catheter ablation of the CTI, using a combined electrophysiological and fluoroscopy-guided approach, is a technique where lesions are placed from the tricuspid annulus to the IVC in order to create a complete blocking line across the CTI for the atrial flutter circuit. This technique is considered a highly effective and safe method to permanently cure patients with typical, isthmus-dependent flutter. Catheter ablation of the CTI is currently considered first-line therapy for the treatment of typical AF. Comparisons of different RF catheters have established that large-tip (8–10 mm) RF catheters and irrigated-tip catheters are superior to conventional 4-mm-tip RF catheters for this purpose. Using these, bidirectional isthmus-block
can be achieved in 80–95% of cases with a low risk of recurrence.\textsuperscript{60-63} The complication rate is low\textsuperscript{59} and mainly related to vascular access.

In 2002, cryoenergy for CTI ablation was demonstrated to produce permanent bidirectional block in dogs,\textsuperscript{64} followed by the first report in humans a year later, comparing cryo- and RF energy in 14 patients\textsuperscript{15} with a special emphasis on pain perception, but also reporting comparable rates of bidirectional block with either energy source.

Concomitant AF is common in the AFL population.\textsuperscript{4, 23} Studies have shown that the occurrence of AF is reduced after AFL ablation in patients where AFL is the predominant arrhythmia prior to ablation or in patients where AFL has become the predominant arrhythmia due to treatment with a Vaughan-Williams class IC drug, provided the drug is continued after ablation.\textsuperscript{23, 65, 66}

Catheter ablation of paroxysmal and persistent atrial fibrillation

Electrical isolation of the pulmonary veins in order to block the ectopic beats from conducting to the atrium, and thereby eliminating the trigger for AF episodes, is the cornerstone of an AF ablation.\textsuperscript{67} Different approaches to encircle the PVs, which can be done either in pairs or separately for each vein, are used for this purpose. In the early era of AF ablation, a segmental ostial ablation was used, targeting only sites where PV signals were detected. This technique was followed by complete electrical isolation\textsuperscript{68, 69} of the pulmonary veins by circular lesions. Due to the risk of PV stenosis when ablatting in the PV ostium, the current approach is to deploy the lesions in the venous antrum.\textsuperscript{69, 70} The most widely used and assessed technique is that of point-by-point technique with an irrigated-tip RF catheter guided by 3-dimensional (3D) mapping. Early attempts to use cryoablation for PVI were performed in similar manners as RF, but resulted in extremely long procedure times\textsuperscript{18} and a disappointing success rate in some studies.\textsuperscript{71}

For the purpose of simplifying the AF ablation procedure, various so-called “one-shot” technologies for circumferential ablation of the PVI have been developed, including the cryoballoon and multipolar circular RF ablation catheters.

PVI has resulted in variable long-term efficacy rates, partly depending on the patient selection and the AF type studied.\textsuperscript{72-76} Different methods used for follow-up and reporting of outcomes make studies difficult to compare. A meta-analysis of RF AF ablation shows that the mean success rate after a single procedure, off AAD, was 57% after a follow-up of mean 14 months, and increased to 71% after repeat procedures.\textsuperscript{76} Different parameters have been suggested to be associated with a lower long-term success rate, including persistent AF, increased left atrial size, obesity, sleep apnoea and structural heart disease. Nevertheless, findings have not been consistent.\textsuperscript{77, 78}
To date, no consensus exists for ablation of non-paroxysmal AF. Many centres prefer to perform PVI as the initial procedure, whereas additional, substrate-modifying ablation, including linear lesions, ablation of complex fractionated atrial electrograms (CFAE) and ganglionated plexi, is advocated by some for initial treatment of non-paroxysmal AF.

Recognized complications of the AF procedure include tamponade, PV stenosis, thromboembolic episodes, phrenic nerve injury and left atrioesophageal fistulae formation and are attributable to catheter manipulation, direct effects of the energy delivery, as well as collateral damage to adjacent structures. The rate of major complications after AF ablation varies between reports and also mainly reflects high-volume centres. The first worldwide survey of AF ablation reported that at least one major complication was seen in ~6% of patients and in an updated version in 2010 the complication rate was 4.5%. Current guidelines recommend catheter ablation as a second-line therapy for symptomatic AF, after failure or intolerance to AAD, although they also state that it may be considered as a first-line treatment in some subjects.

Biophysics of radiofrequency and cryothermal lesion formation

Radiofrequency current is an alternating current that is delivered at cycle lengths of 300 to 750, typically 500 kHz, when used for catheter ablation. RF energy is most often used in an unmodulated, unipolar fashion between the tip electrode and an indifferent electrode patch placed on the skin. The mechanism by which RF heats tissue is resistive heating of a narrow rim of tissue that is in direct contact with the electrode. Deeper tissue planes are then heated by conduction from this area. Convective cooling from the circulating blood opposes this process. The lesion size is proportional to the temperature at the electrode-tissue interface and to the size of the electrode. Irreversible tissue destruction requires a tissue temperature of approximately 50°C. If the temperature at the electrode–tissue interface reaches 100°C, coagulated plasma and desiccated tissue may form on the electrode (charcoal), preventing effective delivery of the current and predisposing the patient to thromboembolic complications. To reduce the risk of high temperatures, RF catheters are now temperature-controlled. Cooling of the electrode-tissue interface with saline-irrigated tips allows for higher power delivery, which promotes the formation of larger and deeper lesions but also reduces the risk of thrombus formation. Other determinants for lesion size are tissue contact pressure and a parallel orientation of the catheter, which promotes lesion size for standard RF but reduces lesion size for irrigated-tip catheters.

Forty to sixty seconds of energy delivery is required to achieve a stable lesion with RF.
Duty-cycled RF is a mode where energy is delivered in a modulated “on-off” pattern, allowing the electrodes to cool during the off period, and may therefore increase power delivery. Duty-cycled RF has been described to result in deeper and more homogenous linear lesions than continuous RF with a multipolar ablation catheter.\textsuperscript{92}

Phased RF (Figure 2) refers to when RF is delivered out of phase by adjacent electrodes on a multi-electrode array, creating a voltage difference between adjacent poles as well as between the electrode and the indifferent patch, and hereby allows for simultaneous bipolar and unipolar energy delivery intended to result in continuous ablation lines. Studies in vitro and in vivo have shown that a higher proportion of unipolar RF energy increases the depth of the lesion.\textsuperscript{93}

\textbf{Figure 2.} A. RF delivered in duty cycles. B. Energy delivered in phase by the adjacent electrodes (blue and red), resulting in current flow (arrows) between each electrode and the indifferent electrode (grey); unipolar energy only. C. RF delivery out of phase, “phased RF”, leading to a voltage difference between the adjacent electrodes, which results in a current flow between the electrodes; bipolar energy, in addition to unipolar energy between each electrode and the indifferent electrode.

The primary mechanism of tissue destruction by RF current is hyperthermic cellular injury through a combination of coagulation and tissue necrosis,\textsuperscript{82} which results in a lesion displaying a central, necrotic zone, surrounded by a haemorrhagic region. Due to the beating heart, the contact between the ablation catheter and the tissue is not completely stable, making the rim of
the lesion less precise than what is seen with a cryocatheter that is fixed to the tissue during energy delivery.\textsuperscript{17} A slight deformation of the endocardium at the point of electrode contact can often be seen, due to shrinkage of the underlying tissue. Fibrinous material is frequently adherent to the surface, coating the area of endothelial injury, and occasionally thrombi are noted.\textsuperscript{17,94} After 4–5 days, the transition zone at the lesion border is lost and the lesion becomes more sharply demarcated. By 8 weeks, the necrotic zone is replaced by fatty tissue, cartilage and fibrosis, surrounded by chronic inflammation.\textsuperscript{95} The chronic lesion evolves to a uniform scar.

\textbf{Cryothermal energy}

Cooling of the tissue to -20ºC induces extracellular ice formation, osmotic shift and a transportation of water from the intracellular to the extracellular space.\textsuperscript{96} Lesions produced at -30ºC or warmer are most often reversible and can be used for mapping when in a critical position close to the normal conduction system (cryomapping).\textsuperscript{97} At -40ºC intracellular ice formation starts to destroy organelles and the plasma membrane.\textsuperscript{98} Continued cryoablation and ablation of adjacent tissue further leads to vascular stasis and damage to the blood vessels, resulting in an impaired regional blood flow.\textsuperscript{96}

Re-warming of the tissue leads to further cell destruction. During this, so-called “thaw phase”, mitochondria are irreversibly damaged due to increased membrane permeability.\textsuperscript{99}

The second phase, occurring within the first 48 h, is characterized by the development of haemorrhage, oedema and inflammation. After 1 week, the lesion is homogenous and sharply demarcated.\textsuperscript{17,100} The final phase of lesion formation, the replacement fibrosis phase, takes place within 2–4 weeks. At this time, the lesion consists of dense collagen and is infiltrated by fatty tissue. At its border, many small blood vessels are found. At 1 month, the lesion is composed of dense fibrosis.\textsuperscript{101,102} In comparison with standard RF lesions, cryoenergy lesions have been shown to be more homogenous and associated with substantially less endothelial disruption, reducing the risk of thrombus formation\textsuperscript{17} (Fig 3).

Little is known about the optimal duration of each cryoapplication in order to achieve a stable lesion in humans. Preclinical studies have demonstrated that lesions increase in size during the first 2–3 minutes and reach a plateau thereafter.\textsuperscript{97} Four to five minutes of energy delivery is recommended, although some studies suggest that shorter applications may be sufficient.\textsuperscript{103,104} Repeated freeze-thaw cycles have been shown to increase lesion size\textsuperscript{105,106} and to improve outcome in animal studies.\textsuperscript{97} Lesion size may further be enhanced by a lower temperature, an optimal tissue contact pressure, a higher refrigerant flow, a larger electrode tip size and a parallel electrode-tissue orientation, and is opposed by convective warming.\textsuperscript{107}
Several studies on cryothermal energy compared to RF energy in ablation for supraventricular tachycardias have reported comparable acute results\textsuperscript{108-110} even though in some reports the recurrence rate seems to be higher for cryo- than for RF energy sources.\textsuperscript{108, 109, 111} The potential advantages with cryoablation are thus the possibility of using cryomapping as a reversible injury to test at high-risk locations close to the normal conducting system, stability during energy delivery in these areas due to cryoadhesion, a potentially lower propensity for thrombus formation,\textsuperscript{17} a reduced risk for pulmonary vein stenosis after PV ablation,\textsuperscript{18} and less discomfort and pain\textsuperscript{15} during energy delivery.

**Catheters**

*Pulmonary Vein Ablation Catheter, PVAC (Ablation Frontiers, Medtronic)* is a 9 French (F), 10-pole circular catheter with a predefined diameter of 25 mm. The catheter allows delivery of bipolar and/or unipolar RF energy through the 3 mm long and 1.5 mm wide platinum electrodes placed 3 mm apart along the circle. The electrodes can also be used for recording of local electrograms and for pacing. Each electrode contains a thermocouple positioned on the surface of the tissue contact side of the electrode. The catheter has two control handles, one to allow bidirectional deflection of the shaft and the other handle is used to move the distal tip forward along the guidewire, which allows a change of the catheter from its circular shape to a spiral configuration and, finally, a longitudinal shape.

The generator (GENius) is a multichannel, duty-cycled RF generator by which RF energy can be delivered in unipolar (between an ablation electrode and a reference patch) and bipolar (between two adjacent ablation electrodes) configurations. Pairs of electrodes can be selected independently. The generator offers five energy settings: bipolar only, unipolar only and
4:1, 2:1, and 1:1 where the ratios refer to bipolar-to-unipolar energy. For AF ablation, the settings 4:1 and 2:1 and 60 s of energy delivery are recommended by the manufacturer. The energy delivery is controlled by a software algorithm that modulates power to reach the target temperature (60°C) with a maximum of 10W per electrode. (Fig 4)

Cryoballoon catheter (Arctic Front, Medtronic)
This 10.5 F catheter has an inflatable double-lumen polyurethane balloon at the distal end. The catheter is inserted over the wire into the left atrium and inflated with pressurized liquid nitrous oxide (N₂O), delivered from a tank in the CryoConsole. The refrigerant N₂O is delivered to the inner balloon where it undergoes a liquid to gas phase change, resulting in an inner balloon cooling to approximately -80°C. The catheter has a central lumen for insertion of the guidewire and for injection of contrast medium. The balloon is available in two sizes: 23 or 28 mm in diameter. The catheter itself is bidirectional through a pull wire mechanism, but is intended to be used together with a steerable sheath that enhances the possibility of reaching the PV ostia. A 4–5 min energy delivery/application is recommended. (Fig 4)

Freezor Max (CryoCath, Medtronic) is a 9 F unidirectional catheter with an 8-mm-tip electrode and three other proximal recording electrodes. A thermocouple is integrated into the tip for temperature monitoring. Liquid N₂O flows in the central lumen of the catheter and evaporates at the tip, which reaches a temperature of -80°C.

Contactr (Medtronic) is a 7 F steerable unidirectional RF catheter with an 8-mm deflectable tip and an additional three electrodes for recording of intracardiac electrograms.

Figure 4. PVAC (left) and cryoballoon catheter (right). Reproduced with permission from Medtronic Inc.
Coagulation and inflammatory activity

Thromboembolic events are uncommon but one of the most feared complications of AF ablation, occurring in about 0.3–1.5% of cases.\textsuperscript{80, 112, 113} The plausible causes of emboli are pre-existing thrombi in the left atrial appendage, thrombus formation in the sheaths\textsuperscript{114} or on the catheters,\textsuperscript{115} or local thrombus formation at the ablation site where the endothelium is disrupted due to energy delivery.\textsuperscript{17} Precautions are taken in order to minimize these events by careful monitoring of anticoagulation pre-, per- and post procedure, imaging of the atrial appendage, flushing of the sheaths, and technical solutions to minimize charcoal formation on the RF catheter tip.

Cryoenergy is thought to be safer in this aspect, partly due to the absence of char formation but also based on an animal study comparing a conventional non-irrigated RF catheter and a focal cryocatheter, showing a higher incidence of thrombus formation and a greater thrombus volume after RF ablation.\textsuperscript{17} (Fig 3)

Activation of the coagulation system, measured by an increase in biomarkers, has previously been demonstrated during both electrophysiological procedures and RF catheter ablation procedures of arrhythmias other than AF.\textsuperscript{116, 117} Subsequent studies on AF ablation have shown significant increases of vWF and D-dimer after ablation with RF\textsuperscript{118} and, in a randomized study, a more persistent platelet activation was demonstrated, measured by flow cytometry, after ablation with a non-irrigated RF catheter compared with a conventional focal cryocatheter.\textsuperscript{119}

Increased concentrations of biomarkers reflecting coagulation activity have further been related to left atrial appendage thrombi and predictive of thromboembolic events in atrial fibrillation.\textsuperscript{120, 121}

Haemostasis and coagulation is a complex process including endothelium, platelets and circulating coagulation factors. In summary, the first step is the adhesion of platelets to the injured vessel wall followed by their activation and aggregation (primary haemostasis). The coagulation cascade becomes activated and generates thrombin and fibrin that strengthens the platelet plug (secondary haemostasis) and the formed thrombus is degraded by the proteolytic system.

Von Willebrand factor (vWF) is predominately located in vascular endothelial cells but also present in platelets.\textsuperscript{122} Active secretion is stimulated by, for example, thrombin and fibrin. vWF mediates platelet adhesion to the damaged arterial wall and platelet aggregation. Increased vWF levels are believed to reflect endothelial damage and perturbation.\textsuperscript{123} P-selectin is a component of the platelet α-granulae and, upon platelet activation, it is expressed on the platelet surface membrane and shed into the plasma as soluble P-selectin.\textsuperscript{124} P-selectin is also found in the Weibel-Palade
bodies in the endothelial cells, but the bulk of sP-selectin appears to be platelet-derived.  

Upon exposure of tissue factor, the coagulation cascade is initiated. Prothrombin factor 1+2 (F1+2) is formed when prothrombin is cleaved to thrombin, and is consequently a marker of thrombin generation, which is the central part of the coagulation cascade. D-dimer is a fibrin degeneration product and thus reflects the fibrin turnover, one of the final stages of coagulation (Fig 5).

Previous studies have shown that AF itself causes local cardiac platelet activation within minutes of onset as reflected by a rise in sP-selectin and F1+2.

Heparin, which is used during the ablation procedure, produces its major anticoagulant effect by inactivating thrombin and activated factor X (Xa) after binding to antithrombin. By inactivating thrombin, it also inhibits thrombin-induced activation of platelets and endothelial cells.

A small study demonstrated that early administration of heparin (after insertion of sheaths compared with just before energy delivery) markedly limited the increase of biomarkers in RF ablation procedures for accessory pathways.

Figure 5. Simplified scheme of the final steps of the coagulation cascade outlining the generation of the measured biomarkers F1+2 and D-dimer.

An inflammatory response is a common finding after cardiac damage such as myocardial infarction. Inflammatory reactions around the injury site have been shown in histopathological studies of RF and cryolesions. Furthermore, systemic inflammatory changes after PV RF ablation were demonstrated. Elevated CRP levels after ablation have been shown to predict early but not late AF recurrences.
Tolerability

Patients may perceive pain and discomfort during RF energy delivery, which results from direct stimulation of cardiac sensory nerves or collateral visceral irritation. Although not systematically studied, pain is noted particularly when ablating the posterior LA wall, coronary sinus or CTI region. Cryoenergy has been shown to be associated with a reduced perceived pain in ablation of supraventricular arrhythmia and AFI\textsuperscript{15, 16} and may therefore theoretically result in lower anaesthetic and analgesic requirements in selected procedures.
Aims

During the last decade, there has been technological development in order to find new types of catheters to facilitate the ablation procedure in atrial flutter- and atrial fibrillation ablation. This is of interest to both the patient as well as the electrophysiologist since AF ablation in particular, is still a challenging and time-consuming procedure. However, the most important issue is whether the newer techniques are effective and safe.

The overall aim of the studies included in this thesis was to evaluate and compare ablation with novel ablation catheters using two different energy sources; radiofrequency- or cryothermal energy, with regard to efficacy and safety.

The more specific aims are described for each study.

**Study I**
Being one of the first centres in Europe to use the cryoballoon for AF ablation, the aim of this study was to evaluate and report on our initial experience with this novel ablation catheter in patients with paroxysmal and persistent AF with regard to acute success, procedural data, safety and clinical outcome.

**Study II**
Related to the recognized pain frequently experienced during RF catheter ablation of AFI, the purpose of this study was to compare cryo- and RF energy for AFI ablation with regard to tolerability as the primary endpoint, as well as efficacy and safety.

**Study III**
The desire to facilitate the AF ablation procedure has led to the development of new ablation catheters specifically designed as “one-shot tools” for PVI. The purpose of this study was to compare the efficacy, with freedom from AF 12 months after ablation set as the primary endpoint, safety and procedure times for two such catheters using different energy sources, the cryoballoon and the RF-based PVAC. We also aimed to evaluate quality of life and arrhythmia-related symptoms prior to and up to 12 months after treatment.
Study IV
Thromboembolic events are one of the most feared complications related to AF ablation. Since RF energy is thought to be associated with a higher risk of thrombus formation than cryoenergy, the hypothesis for this explorative study was that ablation procedures performed with the RF energy-based PVAC would result in a higher degree of activation of coagulation and inflammation, measured by biomarkers, as compared with procedures performed with the cryoballoon catheter.

Study V
The purpose of this study was to compare two different energy settings, with different ratios of bipolar-to-unipolar energy, for the PVAC under the hypothesis that a higher proportion of unipolar energy with the PVAC would result in deeper and more transmural lesions, leading to fewer applications in order to achieve PVI (primary endpoint) and thus shorter procedure times.
Materials and methods

Patient selection

The study population in all studies consisted of patients who were already scheduled for ablation of their arrhythmia, based on clinical indication, i.e. paroxysmal or persistent, symptomatic AF (Study I, III-V) or AFl (Study II) that was documented and verified on 12-lead ECG. For AF ablation, patients should have failed treatment with at least one (Study III-V) or two (Study I) antiarrhythmic drugs, of Vaughan-Williams class I or III.

In Study I, the first 40 consecutive patients who underwent AF ablation with the cryoballoon catheter at our centre were included, beginning in May 2006. Exclusion criteria were advanced valvular heart disease, congestive heart failure and age >70 years.

In Study II, patients scheduled for ablation of AFl were asked to participate. Between November 2003 and August 2005, 40 patients with ECG-verified, typical AFl were included in the study. Patients with previous AFl ablation were excluded.

In Study III, 110 patients planned for AF ablation were enrolled between March 2009 and December 2011. Exclusion criteria were longstanding persistent or permanent AF, previous AF ablation, left atrial diameter >6 cm, left ventricular ejection fraction (LVEF) <30% and congestive heart disease with a New York Heart Association class >III, unless judged due to tachycardiomyopathy.

The study population in Study IV (30 patients) and Study V (35 patients) were subgroups of patients included in study III. Patients who were on treatment with acetylsalicylic acid or who were given vitamin K were excluded from Study IV.

Demographic data was collected at inclusion.

Definition of AF and AFl type

The AF and AFl type was defined as paroxysmal if episodes converted spontaneously to sinus rhythm within 7 days, or were sometimes terminated by electrical or pharmacological cardioversion within 48 hours. Persistent AF was defined as present if episodes sustained more than 7 days or required cardioversion to terminate. Mixed pattern was a combination of paroxysmal as well as persistent episodes in the same patient. In paper III and V, data
was converted to match definitions suggested in the consensus document from 2012, which states that if patients display both paroxysmal and persistent AF, the type should be defined as the most frequent type experienced in the 6 months prior to ablation.

**Study design**

Apart from Study I, which was a descriptive study of our initial experience using a novel catheter, the studies were randomized studies. After inclusion, patients were randomized 1:1 to treatment with cryoablation or RF ablation. Starting in June 2010, patients who were included in Study III were asked to also participate in Study IV and, after randomization in the main study, were included until the quota was filled for each group (15 patients allocated to cryo- and 15 to RF ablation). In Study V, all patients allocated to the PVAC arm of Study III from May 2010 were further randomized to treatment with one of the two energy settings: bipolar-to-unipolar ratio 4:1 or 2:1.

**Pre-ablation procedure**

Before the ablation, all patients underwent echocardiography transthoracically to assess LA size (parasternal long axis view), LVEF and potential structural disorders, and for AF ablation, transesophageally to exclude LA thrombi. Left atrium and pulmonary vein anatomy was visualized by a CT scan and reconstructed into a 3D model using CartoMerge software (Biosense Webster) in studies I and III -V.

**Anticoagulation treatment**

Patients scheduled for AF ablation were treated with oral anticoagulation (warfarin) with an international normalized ratio (INR) level $\geq 2$ for at least 3 weeks prior to the procedure. Warfarin was withdrawn 2-3 days before the ablation in order to reach an INR level below 1.8 on the day of the procedure. Low-molecular-weight heparin (dalteparin) 100 units (U)/kg, was given twice daily when the INR level dropped below 2, with the last dose administered in the morning of the day before the procedure, when it was replaced by intravenous heparin infusion from 8 a.m. Heparin was stopped 4 hours before the procedure, administered again as a bolus (100 U/kg) immediately after the transseptal puncture and then titrated to achieve an activated clotting time (ACT) between 250–350 seconds. The ACT level was measured by HEMOCHRON Jr. (Stago) before the procedure and then every
20 minutes after the first heparin dose. Heparin infusion was restarted within 4 hours post ablation and continued for 20 hours, followed by dalteparin at a dose of 200 U/kg daily until INR was ≥2. Warfarin was withdrawn at the earliest one month, but in general three months after the ablation at the discretion of the physician and based on CHADS scores.

For AFI ablation, warfarin was stopped 2–3 days before the ablation and only in cases with ongoing AFI or in patients at high risk of thromboembolism, bridged by low-molecular-weight heparin and heparin. A heparin bolus dose of 50 U/kg was given to all patients before energy delivery.

Ablation procedure

The procedure was performed with the patient awake. Diazepam and paracetamol were given as premedication before the AF ablation procedure. Before AFI ablation, diazepam was offered to the patient but was optional. Ketobemidone was given as analgesics during both types of procedures. The procedure time was defined as the time from local anaesthetic infiltration to removal of all catheters. The ablation time was defined as the time from first to last ablation application. The total time of energy delivery was defined as the ablation application time or energy delivery duration. The fluoroscopy time and dose was measured using an automatic fluoroscopy counter during the procedure.

Atrial flutter ablation

A 7F, 20-pole Halo catheter, (Cordis) was placed around the tricuspid annulus, a 6F, 4-pole catheter in the proximal coronary sinus and an ablation catheter was advanced to the right atrium. If the patient was in sinus rhythm, the atrium was paced from the proximal coronary sinus (CS) and the lower right atrial free wall to evaluate bidirectional conduction over the tricuspid isthmus. If the patient had ongoing atrial flutter, isthmus dependency was determined by confirmation of a high to low sequence of activation along the Halo catheter (or vice versa).

RF ablation was performed with the Contactr catheter. Linear lesions were created by use of a point-by-point technique with gradual pull back of the catheter from the tricuspid ring to the IVC. RF energy was delivered for approximately 40 seconds at each point whereafter the catheter was moved. One continuous RF application had a maximum length of 4 minutes. If block was not achieved after these continuous applications, the ablation line was mapped to find remaining gaps and additional applications were given to that site. The output was pre-set at 65W with a maximum temperature of 60ºC.

Cryoablation was performed with the Freezor Max. Applications were made as separate 4-minute (min) point-by-point applications from the
tricuspid ring to the IVC. After a primary linear lesion was made, extra applications were delivered to remaining gaps, which were prematurely stopped after 2 min if block was not achieved.

Acute (procedural) success was defined as bidirectional isthmus block confirmed by pacing from the CS and the lower right atrial free wall. Patients were observed in the laboratory for 30 min after bidirectional block was achieved in order to identify early recurrences of conduction. If bidirectional block was not achieved despite attempts to close conduction gaps with extra applications, cross-over to the other ablation catheter was permitted. It was at the discretion of the electrophysiologist to decide when this point was reached. A cross-over to ablation with the other source of energy was defined as a failure.

**Atrial fibrillation ablation**

After vascular access in the right femoral vein, a bipolar catheter was positioned in the right ventricular apex and a 10-pole catheter in the CS. The single transseptal puncture was performed with a Brockenbrough needle (St. Jude Medical) guided by bi-plane fluoroscopy and intracardiac pressures. Selective angiography was performed of the left and right superior PVs. The 8F transseptal sheath (SL0, St. Jude Medical) was then exchanged for a steerable sheath, 12F or 10F (FlexCath, Medtronic), for the cryoballoon catheter and the PVAC, respectively.

The endpoint of the procedure was complete electrical isolation of all PVs, evaluated by entrance block that was confirmed with an additional 10- or 20-pole circular mapping catheter during sinus rhythm for the right PVs and during CS pacing for the left PVs. Pacing manoeuvres were used in selected cases when there were difficulties in the differentiation of far-field atrial signals from PV signals. A maximum number of ablation applications was not defined.

**Cryoballoon** was performed with the cryoballoon catheter. Apart from the first cases in study I, the 28-mm cryoballoon was chosen primarily unless the PV diameters were small and judged more suitable for the 23-mm balloon. Different positions were tried with the cryoballoon and were evaluated by contrast injection distal to the balloon, to ensure the highest occlusion rate possible before energy delivery. In Study I, a 4-grade scale was used to score the degree of occlusion, with a score of 4 indicating total occlusion. Two 5-minute applications were routinely given per vein before conduction block was evaluated. If PV potentials were still present, 1 or 2 extra cryoballoon applications were delivered to that vein before re-evaluation. If an acceptable occlusion or position could not be achieved during the first two applications or if PVI was not achieved after additional cryoballoon ablation attempts, a conventional cryocatheter (Freezor Max) was used for touch-up ablation until complete PV isolation was achieved. The phrenic nerve function was checked during ablation of the right superior
PV by visual inspection of the diaphragmatic movement under fluoroscopy every 20 seconds.

The RF ablation procedure was performed with the PVAC. The PVAC was carefully positioned in the antrum of the veins under fluoroscopic guidance. Radiofrequency was delivered in the 2:1 or 4:1 bipolar-to-unipolar energy setting for 60 seconds per application to electrodes indicating good tissue contact, based on local electrograms. Electrode pairs were deselected if the temperature did not reach more than 50°C. Energy delivery was prematurely switched off in the case of catheter dislocation during the application or if the patient experienced intolerable pain. In cases where PV isolation could not be achieved with the PVAC, a 4-mm-tip RF ablation catheter (Biosense Webster) was used for touch-ups when judged to be necessary.

Evaluation of pain (Study II)

In Study II, perceived pain was evaluated by a Visual Analogue Scale (VAS) that was translated to a number from 0–10, where 0 corresponded to “no pain at all” and 10 to “worst possible”. The VAS score was determined at the end of each ablation application. Patients were also asked to score the perceived pain related to the injection of local anaesthetics in the groin and the total pain perception during the entire procedure.

Blood sampling and analysis of biomarkers (Study IV)

The biomarkers used in our study were chosen to cover the different parts of the coagulation cascade: Von Willebrand factor antigen and sP-selectin were used as markers of endothelial damage and platelet activation, respectively. Coagulation activity was reflected by F1+2 and D-dimer.

Blood samples for the coagulation biomarkers were collected at 5 pre-specified time points during the procedure:

- from a peripheral vein (cubital vein) after insertion of the first introducer in the femoral vein (v baseline)
- from the left atrium after transseptal puncture but before heparin was administered (LA baseline)
- from LA before the start of ablation (LA pre ablation)
- from LA when ablation was completed (LA post ablation)
- from the peripheral vein before the withdrawal of introduces (v post ablation)
Blood was drawn into vacutainer tubes containing 3.8% citrate. The first 10 ml of blood was discarded before sample collection. The samples were immediately centrifuged and frozen at -70°C until analysis. Methods used for analysis, normal reference intervals and inter-assay coefficients of variance (CV) are shown in Table 1. To reflect the damage of cardiomyocytes, Troponin I (Trop I) was taken prior to, and 6 and 24 hours after the procedure and analysed using the Architect System (Abbott). Levels <0.04 µg/L were considered normal. Inflammatory response was reflected by CRP, analysed with a lower detection rate of 0.2 mg/L (Architect, Abbot) and was measured prior to and 24 hours after the procedure. A ratio was calculated between the concentration of the biomarker (LA post ablation) and the Trop I level (6 hours post ablation) to reflect the coagulation activity in relation to the size of the lesion (myocyte damage).

### Table 1. Methods for analysis, reference intervals and inter-assay coefficients of variance for biomarkers

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Method</th>
<th>Ref interval</th>
<th>Inter-assay CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF</td>
<td>STA-Liatest vWF, Stago</td>
<td>0.60 – 1.60 kIU/L</td>
<td>4.4%</td>
</tr>
<tr>
<td>sP-Selectin</td>
<td>P-Selectin, R&amp;D Systems (ELISA)</td>
<td>18 – 40 µg/L</td>
<td>9.9%</td>
</tr>
<tr>
<td>F1+2</td>
<td>Enzygnost F1+2, Siemens (ELISA)</td>
<td>69 – 229 pmol/L</td>
<td>11.2%</td>
</tr>
<tr>
<td>D-dimer</td>
<td>Asserachrom D-DI, Stago (ELISA)</td>
<td>&lt; 500 µg/L</td>
<td>5.6%</td>
</tr>
</tbody>
</table>

### Follow-up

In Study I, patients were followed at outpatient visits with regard to arrhythmia-related symptoms and complications. In the case of arrhythmia symptoms, patients were asked to seek medical attention for ECG verification. A 24-hour Holter ECG recording was performed at the earliest 6 months after the ablation procedure unless recurrence of AF had already been documented on a 12-lead ECG due to symptoms or from the pacemaker diagnostics in patients with a permanent pacemaker.

Antiarrhythmic drugs were continued for at least 3 months after the ablation procedure and thereafter withdrawn if the patients were free from arrhythmia-related symptoms. A recurrence of AF was defined to be present if the duration exceeded 30 seconds.

A blanking time for recurrence of AF of 3 months after the ablation was used.

In Study II, antiarrhythmic drugs were withdrawn after the procedure if bidirectional isthmus block was achieved, provided the patient had no history of atrial fibrillation. Patients were then followed at outpatient visits. For patients referred from other areas, the follow-up was obtained at the referring hospital. In the case of arrhythmia-related symptoms, patients were
encouraged to seek medical attention for ECG verification. After a minimum of 6 months, all patients received a questionnaire concerning symptoms of arrhythmia and current medication. Medical records were reviewed for patients who reported symptoms of arrhythmia after the ablation. A recurrence of AFI was defined to be present if confirmed by an ECG tracing.

The study scheme for Study III (IV-V) is outlined in Figure 6

![Figure 6. Study scheme for AF-COR study.](image)

A recurrence of AF was defined to be present if the duration exceeded 30 seconds.

A blanking time of 3 months after the ablation was used. A repeat CT scan was performed after 6 months or prior to a redo procedure to exclude PV stenosis. A reduction of the PV diameter by >50% was defined as a significant stenosis.

The primary endpoint was complete freedom from AF without AAD at 12 months after one ablation procedure. Asymptomatic patients included those who only displayed asymptomatic episodes on Holter and those who were free from AF on AAD that was tried ineffective prior to the ablation. Patients with clinical success were defined as those who, after one procedure, were free from symptomatic AF with or without AADs or had a symptomatic improvement to the extent that a redo procedure was not desired.
Quality of life and symptom severity questionnaires (Study III)

Quality of life (QoL) was assessed using the Short Form 36-item (SF-36) questionnaire. SF-36 is a generic, validated instrument\textsuperscript{131} that has been widely used, including in a number of studies on AF\textsuperscript{8, 132} and that has been translated into Swedish. The eight variables measured by the SF-36 – physical functioning, role limitation due to physical problems, bodily pain, general health, vitality, social functioning, role limitation due to emotional problems, and mental health – were converted according to the manual\textsuperscript{133} to a scale ranging from 0 to 100, with a higher score representing better quality of life. Standard rules were used to record the items and handle missing data.\textsuperscript{133}

Normative data for the general Swedish population, by age group, is available\textsuperscript{133} and data for an age group matching our study population as close as possible was plotted in the QoL figure for a rough comparison. Symptoms were assessed by five variables in a symptom severity questionnaire (SSQ)\textsuperscript{134} (palpitations, fatigue, dizziness, lack of energy, dyspnoea) and were each scored with regard to severity on a 5-point scale with a higher value representing a more pronounced symptom. The questionnaires were answered by the patients at the time of inclusion in the study and at the follow-up visits 6 and 12 months after the ablation. All patients, even those who had undergone a redo procedure, were included in the analysis.

Complications

All complications during the ablation procedure and during the hospital stay were recorded. Furthermore, patients were asked for symptoms that could reflect a complication, such as dyspnoea, difficulty swallowing, or symptoms that could indicate thromboembolic complications, at the follow-up visits.

Statistics

Continuous variables were given as mean values ±1 standard deviation (SD) and compared using the Student’s \textit{t}-test or Mann-Whitney U test. Categorical variables were compared using Chi-square test or Fisher’s exact test. A p-value <0.05 was considered statistically significant.
Specific:
Study III: The sample size was calculated to detect a difference of 25% in outcomes between the two treatment groups with a power of 80% and a type I error of 5%. Calculation was based on early reports of more than 80% success for PVAC after 6 months follow-up\textsuperscript{135} compared to 53% for the cryoballoon from our previous study. QoL variables and the sum of the five SSQ items were expressed as mean values with confidence intervals. Repeated measures analysis of variance (ANOVA) was used for within- and between-group comparisons.

Study IV: Continuous variables were given as means and standard deviations or as medians and interquartile range (25\textsuperscript{th}-75\textsuperscript{th}) as appropriate. For variables not normally distributed, Wilcoxon signed-rank test was used for within-group comparisons and Mann-Whitney U test for between-group comparisons. For evaluation of correlations between biomarkers, Spearman’s correlation coefficient was used.
Main results

Study I

Patients
For baseline patient characteristics: see Table 2.

Table 2. Patient characteristics at baseline

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Gender (male/female)</th>
<th>Age (y, range)</th>
<th>BMI (kg/m²)</th>
<th>History of AF (y, range)</th>
<th>Hypertension (%)</th>
<th>LA size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>36/4</td>
<td>56±9 (35-68)</td>
<td>26±3</td>
<td>9±6 (0.8-30)</td>
<td>33%</td>
<td>44±6</td>
</tr>
</tbody>
</table>

Abbreviation: y; years

Paroxysmal AF was seen in 32/40 (80%) of the patients, combined paroxysmal and persistent AF in 7 (17.5%) and persistent AF in one (2.5%) patient.

In the 40 patients included in the study 43 ablation procedures were performed. Three patients thus underwent two procedures with the balloon catheter during the study period. Reconnection was observed in 2 veins in each of these patients. Six patients had failed either one (4 patients) or two (2 patients) previous attempts of AF ablation with other techniques. In those patients, recurrence of PV–left atrial conduction was confirmed prior to the cryoablation procedure and was verified in all four pulmonary veins in five patients and in two veins in one patient. Two patients had a left sided common ostium. Thus, the total number of targeted veins in the 40 patients was 156, plus an additional 6 veins in the redo procedures in the study.

Acute success and procedure
Acute success, defined as complete PVI, was achieved in 39/43 (91%) of the procedures. In two cases the procedure was terminated prematurely due to complications and in two other cases complete PVI could not be achieved. All targeted PVs were completely isolated with the cryoballoon catheter in 24 (56%) of the 43 procedures, while in the remaining 19 procedures (44%) a standard cryoablation catheter was also used.

The 28-mm balloon was used in 28 procedures and the 23-mm balloon in 11, while both balloon sizes were used in the remaining 4 procedures. The
mean number of balloon applications per procedure was 9.6±1.6 (range 7–13) among the patients who had all veins ablated. The number of applications per vein was significantly lower for the right superior PV (2.1±0.6) when compared with the other veins (2.6±0.9 for left superior PV, 2.6±1.0 for left inferior PV, and 2.4±0.8 for right inferior PV). In procedures requiring an additional catheter, the mean number of applications with the conventional cryocatheter was 4.7±1.7 (range 1–11).

The proportion of successful PVIs after the first two applications was significantly higher for veins that could be totally occluded (occlusion score 4 for both applications) (47 of 56 veins, 83.9%) than for those with occlusion score 3 during one or both applications (scores 3 and 4, or 3 and 3) (49 of 77 veins, 63.6%, p=0.01), which in turn was significantly higher than for veins with even lower occlusion scores (8 of 21 veins, 38.1%, p=0.04).

Procedure time, ablation time, energy delivery and fluoroscopy time is shown in Table 3.

An analysis of the learning curve showed that the mean procedure time was reduced by 50 minutes (from 267±49 to 217±45 minutes, p=0.03) when the first 10 procedures and the last 10 procedures were compared.

<table>
<thead>
<tr>
<th>Table 3. Procedural data for study I and III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Study I cryoballoon</td>
</tr>
<tr>
<td>Study III cryoballoon</td>
</tr>
<tr>
<td>PVAC</td>
</tr>
</tbody>
</table>

**Safety**

Eight adverse events were seen in 7/43 (17.5%) of the procedures. Two cases of right phrenic nerve paralysis (PNP) occurred (4.7%) during one procedure with each balloon size. One of the PNPs resolved within an hour, while the other persisted for over 6 months. A local haematoma in the left groin resulting in prolonged hospitalization was seen in one patient. One patient had a minor peroperative haematemesis requiring premature termination of the procedure. This patient had a clinical history of
gastroesophageal reflux and the gastroscopy performed one day later showed a mucosal redness in the stomach. A minor pericardial effusion was observed in one patient the day after the procedure. One patient suffered from coughing and minor haemoptysis during the first weeks after ablation. A CT scan showed a mild oedema surrounding a narrowing of the left inferior PV but without significant stenosis. The CT scan 6 months later showed the same degree of non-significant stenosis but without oedema. In this patient the balloon had been inflated while it was partly inside the vein. There was no PV stenosis among the remaining 17 patients who underwent a post ablation CT scan.

Two patients suffered from dysphagia, which appeared a few weeks after the ablation procedure. The gastroscopy and oesophageal cinegraphy, performed in one of these patients, were both normal. The other patient, who did not complain until 8 months after ablation, was not yet evaluated at the time of data analysis.

Clinical outcome
Patients were followed for a mean of 8.9 months (range 6–22 months). At follow-up, 21/40 (52.5%) of the patients were free from arrhythmia-related symptoms and 18 (86%) of these were off AAD (Vaughan-Williams class I or III). Another 7 patients (17.5%) experienced a clear reduction of arrhythmia-related symptoms after ablation, while the remaining 12 patients reported no or limited effect on clinical symptoms. Two of the patients who were free from arrhythmia-related symptoms had asymptomatic sustained AF recorded during the 24-hour Holter monitoring. No apparent difference regarding freedom from symptomatic AF after ablation was seen between patients with paroxysmal AF alone and those with combined paroxysmal/persistent AF nor between patients in whom only the balloon was used and patients in whom combined procedures were required.
Study II

Patients
The study groups were comparable at baseline (Table 4). Concomitant AF was seen in 48% of the study population.

Table 4. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>RF (n=20)</th>
<th>Cryo (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (male/ female)</td>
<td>18/2</td>
<td>17/3</td>
</tr>
<tr>
<td>Age, years, mean (range)</td>
<td>60±11 (29-71)</td>
<td>57±9 (29-70)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25±5</td>
<td>27±6</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Ischemic heart disease, n</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>LVEF&lt;0.5, n</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Enlarged right atrium, n</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>AFl pattern, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td>Persistent/permanent</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Isolated AFl</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Combined AFl/AF</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Class 1C AFl</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

Pain perception
Cryoablation was associated with significantly less average and peak pain perception compared to RF ablation. The pain scores during infiltration of local anaesthetics did not differ significantly between the groups (Figure 7).

Figure 7. Perceived pain assessed by VAS score. Puncture site refers to scored VAS during infiltration of local anaesthetics in the groin. Chest mean refers to the mean of VAS scores estimated after applications and peak, the highest estimated VAS score during the ablation. RF applications were excluded for patients who crossed over from cryo to RF in chest mean and peak VAS scores. Values are means ± SD * = significant difference between the groups.
**Procedure**

**Table 5 Procedural data**

<table>
<thead>
<tr>
<th></th>
<th>RF (n=20)</th>
<th>Cryo (n=20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure time (min)</td>
<td>111.4±28.6</td>
<td>136.6±34.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Ablation time (min)</td>
<td>47.6±30.1</td>
<td>81.0±39.8</td>
<td>0.007</td>
</tr>
<tr>
<td>Number of applications (n)</td>
<td>11.3±6.1</td>
<td>15.7±10.7*</td>
<td>-</td>
</tr>
<tr>
<td>Energy delivery duration (min)</td>
<td>13.1±7.1</td>
<td>39.2±17.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fluoroscopy time (min)</td>
<td>30.2±18.5</td>
<td>25.8±13.8</td>
<td>0.41</td>
</tr>
<tr>
<td>Fluoroscopy dose (uGy/m²)</td>
<td>4219±3325</td>
<td>4088±2589</td>
<td>0.89</td>
</tr>
</tbody>
</table>

Data includes procedures with cross-over to RF. *= figure denotes the total number of applications, of which 4.4±6.4 were RF applications due to cross-over.

The differences in procedure time and ablation time were no longer present when all patients crossing over from cryo- to RF ablation were excluded. Under these circumstances, the fluoroscopy time was shorter in the cryo group but no difference in dose could be seen.

**Acute success**

Bidirectional isthmus block was achieved in 34 out of the 40 patients (85%) and unidirectional block in 3 patients (7.5%). In 3 patients (7.5%) no block could be accomplished.

Bidirectional block was more frequently achieved by RF ablation (20/20 patients (100%)) than by cryoablation (10/18 patients (56%), p=0.001).

In the RF group, the RF ablation catheter was exchanged to another type of RF catheter in 2 patients due to failure to reach the most distal part of the isthmus.

Two patients in the cryoablation group were prematurely switched to RF ablation due to technical problems with the cryoablation catheter, despite one exchange attempt, or the CryoConsole.

Eight patients allocated to cryoablation crossed over to RF because bidirectional block was not achieved with cryoablation. A mean of 16±1 applications were given before cross-over occurred. In these 8 patients, bidirectional block was achieved in 2 patients and unidirectional block in 3 patients, while no block could be achieved in the remaining 3 patients after cross-over (Figure 8).
**Figure 8.** Flow chart of outcome after atrial flutter ablation with RF or cryoenergy. The right panel displays outcome in patients after cross-over from cryo to RF.

**Clinical outcome**
Atrial flutter, verified by an ECG, recurred in 5 of 34 patients (15%) in whom primary bidirectional block was achieved. These recurrences occurred in 2/10 patients (20%) treated successfully with cryoablation only and in 3/20 (15%) patients ablated with RF only (p=0.45) (Fig 8). In 1 of 3 patients with achieved unidirectional block and in 2 of 3 patients who failed isthmus ablation, no further symptomatic arrhythmia had occurred by the time of follow-up.

Among the patients who were free from recurrence of AFl at the time of follow-up, 8 patients (5 in RF-only group and 3 in cryo-only group) were still on antiarrhythmic drug treatment (Vaughan-Williams class I and III) due to AF.

**Safety**
There were no complications during or immediately after the procedure.

**Study III**

**Patients**
Baseline characteristics are shown in Table 6. Due to technical problems with the cryoballoon (despite one catheter exchange) or the CryoConsole, a switch to another ablation strategy was required in 4 patients during the procedure. These patients were excluded from further analysis. A left common PV ostium was seen in 4 cryoballoon patients and in 7 PVAC
patients. Additional PVs were isolated in 2 cryoballoon patients and in 1 PVAC patient.

Table 6. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Cryoballoon (n=54)</th>
<th>PVAC (n=56)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/ female), n</td>
<td>43/11</td>
<td>40/16</td>
</tr>
<tr>
<td>Age, years</td>
<td>59±9</td>
<td>62±7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28±4</td>
<td>27±3</td>
</tr>
<tr>
<td>Atrial size, mm</td>
<td>40±6</td>
<td>42±5</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>22</td>
<td>35</td>
</tr>
<tr>
<td>CHADS² score</td>
<td>0.6±0.9</td>
<td>0.9±0.9</td>
</tr>
<tr>
<td>AF types, n:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>Persistent</td>
<td>15</td>
<td>19</td>
</tr>
<tr>
<td>History of AF, years (range)</td>
<td>8±7(1-32)</td>
<td>8±8(1-40)</td>
</tr>
<tr>
<td>Antiarrhythmic drugs tried, n</td>
<td>2.4±1.0</td>
<td>2.5±1.0</td>
</tr>
<tr>
<td>Ongoing Amiodarone</td>
<td>15</td>
<td>9</td>
</tr>
</tbody>
</table>

Figures are mean values ± standard deviation unless otherwise stated

Procedure
For procedural data see Table 3, page 36. A cavo-tricuspid isthmus ablation was performed in 2 PVAC patients with a documented isthmus-dependent atrial flutter. One cryoballoon patient displayed a typical AV-nodal reentry tachycardia, induced by catheter manipulation, which was ablated during the same procedure. Procedure time, ablation time and energy delivery duration was adjusted for these extra ablations.

Efficacy
Acute success
Complete PVI was achieved in 98% (49/50) of cryoballoon-treated patients and in 93% (52/56) of PVAC-treated patients (p=0.37). The 28-mm cryoballoon was used for 46 procedures and the 23-mm balloon for 4 procedures. The number of cryoballoon applications was 9.0±1.7 per patient with a temperature of -43.5±4.7°C, and the number of PVAC applications was 41.5±12.7 per patient. An additional ablation catheter, using the same allocated energy source, was more frequently required in the cryoballoon group, 24/50 procedures (48%), than in the PVAC group, 11/56 procedures (20%) (p=0.002).
Clinical outcome

Freedom from AF, without symptoms and with no AF episodes documented on 7-day Holter monitoring or 12-lead ECG and without AAD treatment after one procedure was achieved in 52% (26/50) of the patients in the cryoballoon group and 38% (21/56) of the patients in the PVAC group, p=0.13 at six months after ablation, and by 46% (23/50) versus 34% (19/56) of the patients, p=0.21 at 12 months (primary endpoint). Complete freedom from arrhythmia-related symptoms was reported by 56% versus 50% (p=0.54) at 6 months, and by 54% versus 38%, p=0.09 at 12 months. The predefined clinical success was reached by 62% (31/50) in the cryoballoon group compared to 61% (34/56) in the PVAC group, p=0.89 at 6 months and by 60% (30/50) versus 54% (30/56), p=0.5 at 12 months (Figure 9).

One patient, defined as failure in the cryoballoon group, suffered from AF at 6 months but had developed amiodarone-related thyreotoxicosis and was therefore difficult to evaluate.

No apparent difference was seen in outcome between patients with PAF and with persistent AF.

At 12 months, 1.2 procedures per patient had been performed in each group, while 7 cryo patients and 10 PVAC patients were scheduled for a redo procedure.

Figure 9. Different measures of outcome at 6 and 12 months after ablation (one procedure).

Quality of life and severity of symptoms

The questionnaires were responded by at least 95 % of patients at each time point.

All QoL variables in the SF-36 questionnaire increased after ablation except for bodily pain, which remained at the same level over time. After 6 months, the increase was significant for all parameters as compared with
baseline except for the general health variable, which did not reach statistical significance in the cryoballoon group (p=0.08) until after 12 months. No further significant changes were seen between 6 and 12 months. The groups were comparable with respect to absolute score values at the different time points and in the change of QoL from baseline to after ablation (Figure 10). According to the SSQ, the symptom scores decreased significantly between baseline and 6 months, whereafter they remained unchanged (Figure 11), without any differences between the groups.

**Figure 10.** Comparison of quality of life SF-36 scores between baseline and 6 and 12 months after AF ablation within cryoballoon and PVAC groups, respectively. Mean values are presented for each variable with 95% confidence interval, depicted by vertical bars, given for baseline and 6 months follow-up. Dotted line represents a general Swedish population in the age group 55-64 years. PF, physical functioning; RP, role limitation due to physical problems; BP, bodily pain; GH, general health; VT, vitality; SF, social functioning; RE, role limitation due to emotional problems; MH, mental health
Figure 11. Comparison of symptom severity score at 3 different time points. Bars denote mean value with confidence interval for the sum of five symptom variables (palpitations, fatigue, dizziness, lack of energy, dyspnea), each scored with 1-5 with a higher value representing a more pronounced symptom.

Safety
Five periprocedural adverse events occurred, of which 4 were related to a cryoballoon- and one to a PVAC procedure, p=0.19. In the cryoballoon group, a major groin haematoma requiring intervention or prolonged hospitalization occurred in two patients. A suspected phrenic nerve paralysis was seen related to 2 procedures, one with each balloon size. Both resolved, one within minutes and the other within 24 hours. One PVAC patient had a major groin haematoma. A repeat CT scan, performed after 6 months in all but 5 patients (2 in the cryoballoon and 3 in PVAC group), did not show any PV stenoses. A less than 50% narrowing of the PV was seen in 1 cryoballoon patient and in 5 PVAC patients, p=0.21.
Study IV

Patients
The baseline characteristics are presented in Table 7. One patient in each group was treated with dalteparin 200 U/kg instead of warfarin prior to ablation due to unstable INR levels.

Table 7. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Cryoballoon (n=15)</th>
<th>PVAC (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/ female)</td>
<td>11/4</td>
<td>8/7</td>
</tr>
<tr>
<td>Age, y</td>
<td>60±8</td>
<td>62±9</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28±4</td>
<td>27±3</td>
</tr>
<tr>
<td>Hypertension, n</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>CHADS2 score</td>
<td>0.7±1.2</td>
<td>0.5±0.5</td>
</tr>
<tr>
<td>Atrial fibrillation type, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Persistent</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Combined</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>81±16</td>
<td>81±18</td>
</tr>
<tr>
<td>White blood cell count, 10⁹/L</td>
<td>5.7±1.5</td>
<td>6.6±1.5</td>
</tr>
<tr>
<td>Platelets, 10⁹/L</td>
<td>267±56</td>
<td>262±45</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>147±12</td>
<td>136±8</td>
</tr>
</tbody>
</table>

Procedure
Complete PVI was achieved in 14/15 (93%) of the patients in the cryo group and 13/15 (87%) in the RF group. Procedural data and INR and ACT levels are shown in Table 8. Two patients randomized to cryoablation had a DC cardioversion the day before the ablation due to ongoing persistent AF. Four patients arrived to the electrophysiology (EP) lab in AF (2 in each group) and another 7 patients had episodes of AF during the procedure (3 in the cryoballoon group and 4 in PVAC group). The time in AF during the procedure was 27±52 min and 28±42 min for the cryoballoon group and the PVAC group, respectively (p=1.0). In each group, DC cardioversion was required in two patients and pharmacological conversion in two patients before the evaluation of PV conduction block.

Complications related to cryoablation procedures included a major haematoma in the groin in one patient and a transient phrenic nerve paralysis, which had resolved the day after, in another patient. No signs of thromboembolic events were seen during the hospital stay and no symptoms correlated to thromboembolic events were reported by the patients at follow-up.
Table 8. Procedural data

<table>
<thead>
<tr>
<th></th>
<th>Cryoballoon procedures (n=15)</th>
<th>PVAC procedures (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedure time (min)</td>
<td>157±32</td>
<td>164±39</td>
<td>0.64</td>
</tr>
<tr>
<td>Ablation time (min)</td>
<td>96±33</td>
<td>122±35</td>
<td>0.04</td>
</tr>
<tr>
<td>Energy delivery duration (min)</td>
<td>49±12</td>
<td>44±14</td>
<td>0.34</td>
</tr>
<tr>
<td>Procedures with add catheter (n)</td>
<td>5</td>
<td>5</td>
<td>1.00</td>
</tr>
<tr>
<td>Baseline ACT (s)</td>
<td>127±22</td>
<td>125±21</td>
<td>0.80</td>
</tr>
<tr>
<td>Mean procedure ACT (s)</td>
<td>268±24</td>
<td>263±26</td>
<td>0.59</td>
</tr>
<tr>
<td>Baseline INR level</td>
<td>1.5±0.2</td>
<td>1.5±0.2</td>
<td>0.94</td>
</tr>
</tbody>
</table>

**Endothelial damage and platelet activation**

As seen in Figure 12 A the levels and pattern of vWF antigen during the procedure were comparable in the two groups. The left atrial vWF antigen levels increased significantly after the ablation (pre- to post ablation) in both groups. The vWF antigen levels measured in the peripheral vein after the procedure were comparable to those levels obtained at baseline in both groups. No significant differences in vWF antigen concentrations were seen between the groups at any time point (p=0.3–0.7).

The sP-selectin concentrations were generally slightly lower in the cryo group compared to the RF group, and the difference was significant at all time points except for at the v baseline, where the difference did not reach statistical significance (p=0.06). (Figure 12 B) In the cryo group, there was a modest but significant increase in sP-selectin levels from LA pre ablation to LA post ablation and a similar pattern of change was observed in the RF group (p=0.06) (Figure 12 B). This change in sP-selectin levels from pre- to post ablation was of comparable magnitude between the cryo group, median 9% (-2 to +24%) and the RF group, median 8% (-6 to +18%) (p=0.4).

**Markers of coagulation activity**

The F1+2 concentrations increased significantly from v baseline to after the transseptal puncture in both the cryo group and the RF group (Figure 13 A). The LA concentrations then decreased progressively throughout the procedure to reach similar levels as v baseline in both groups. The significant reduction in concentrations seen from LA baseline to LA post ablation was comparable between the groups (p=0.4). There was no difference in levels of F1+2 between the cryo group and the RF group at any time point (p=0.1–0.9).

The concentrations of D-dimer were within the normal range at v baseline and remained at a similar level after the transseptal puncture both in the cryo group and the RF group. The major changes in D-dimer levels occurred during the time of ablation, from LA pre- to post ablation, when they
increased by 24% (10–63%) in the cryo group and by 47% (26–54%) in the RF group. The increase was comparable between the groups (p=0.2) (Figure 13B). There was no difference in the concentrations of D-dimer between the groups at the different time points (p=0.5–1.0).

**Figure 12.** Concentrations of (A) vWF and (B) sP-selectin at the different time points during the procedure. Figures are median values with interquartile range. P values are given for comparison within the group between different time points.

**Figure 13.** Concentrations of (A) F 1 + 2 and (B) D-dimer at the different time points during the procedure. Figures are median values with interquartile range. P values are given for comparison within the group between different time points.
The Trop I level was normal in all patients pre ablation and increased in all patients after the procedure. The levels at 6 hours post ablation were significantly higher in the cryoballoon group (median 7.95 (4.23–11.00) µg/L) than in the RF group (2.60 (1.80–3.10) µg/L) (p<0.001) (Figure 14 A). The CRP levels increased significantly with comparable magnitudes in both groups to similar levels at 24 hours post ablation (median 7.40 (3.40–20.00) mg/L in the cryo group and 5.95 (2.92–15.75) mg/L in the RF group) (p=0.7) (Figure 14 B). The patient who developed a major haematoma with concomitant rise in CRP after ablation was excluded from this analysis. There was a significant correlation between the F1+2 and Trop I (r=0.52, p=0.01). The correlation between D-dimer and Trop I did not reach statistical significance (r=0.41, p=0.06).

The ratio of endothelial damage and platelet activation to myocardial damage showed that the vWF antigen (LA post ablation)/ Trop I (6 hours post ablation) ratio was lower in the cryo group versus the RF group, as was the sP-selectin/Trop I ratio. The same pattern was seen for the coagulation activity to myocardial damage ratio, where the cryo group showed a lower D-dimer/Trop I ratio than the RF group (Table 9).

Figure 14. Median concentrations of (A) Trop I with interquartile ranges prior to, 6 and 24 h after the procedure and (B) CRP prior to and 24 h after the procedure.
Table 9. Ratios of biomarkers to Troponin I

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Cryoballoon</th>
<th>PVAC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>vWF antigen</td>
<td>0.2 (0.1-0.2)</td>
<td>0.5 (0.4-0.7)</td>
<td>0.001</td>
</tr>
<tr>
<td>sP-selectin</td>
<td>3.3 (1.9-5.0)</td>
<td>12.3 (9.1-20.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D-dimer</td>
<td>48.4 (38.7-56.9)</td>
<td>127.9 (79.0-236.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Calculated ratio between biomarker sample from LA post ablation and Trop I 6 h post ablation.

Study V

Patients

Baseline characteristics are shown in Table 10

Table 10. Baseline patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>RF 4:1 group (n=17)</th>
<th>RF 2:1 group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male</td>
<td>5/12</td>
<td>7/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61±8</td>
<td>63±8</td>
</tr>
<tr>
<td>AF duration (years)</td>
<td>10±10</td>
<td>7±6</td>
</tr>
<tr>
<td>Paroxysmal AF/persistent AF (n,%)</td>
<td>7/10 (41/59%)</td>
<td>12/6 (67/33%)</td>
</tr>
<tr>
<td>Hypertension (n, %)</td>
<td>12 (71%)</td>
<td>10 (56%)</td>
</tr>
<tr>
<td>Diabetes (n, %)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Coronary artery disease (n, %)</td>
<td>2 (12%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Left ventricular dysfunction (n, %)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>27±3</td>
<td>27±3</td>
</tr>
</tbody>
</table>

Procedure and acute success

The mean number of applications with the PVAC catheter to isolate all veins or before switching to another catheter was 41±10 and 51±15 (p=0.3) respectively, with the 4:1 and 2:1 setting. The number of successful pulmonary vein isolations with the 4:1 and 2:1 setting, respectively, is shown in Table 11. An additional RF ablation catheter was required in 3 patients with the 4:1 setting and in 7 patients with the 2:1 setting. Complete PV isolation of all veins could be achieved in 16 of 17 patients in the 4:1 group as compared with 16 of 18 patients in the 2:1 group.

One patient in each group also had isthmus-dependent atrial flutter and an isthmus ablation in the right atrium was also performed. The total procedure time and fluoroscopy time includes these procedures as well.
Procedure times, energy delivery durations, fluoroscopy times and the mean number of active RF electrode pairs per application are shown in Table 11.

**Safety**

There were no major complications during the procedure, such as death, tamponade, TIA/stroke or phrenic nerve palsy.

At 6-months follow-up no patients had experienced any late complications and no pulmonary vein stenosis was observed at CT scan.

Table 11. *Procedural data and results*

<table>
<thead>
<tr>
<th>Energy setting</th>
<th>4:1 (n=17)</th>
<th>2:1 (n=18)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated veins, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSPV</td>
<td>15/17 (88)</td>
<td>16/18 (89)</td>
<td>ns</td>
</tr>
<tr>
<td>LIPV</td>
<td>15/17 (88)</td>
<td>14/18 (78)</td>
<td>ns</td>
</tr>
<tr>
<td>RSPV</td>
<td>15/17 (88)</td>
<td>17/18 (94)</td>
<td>ns</td>
</tr>
<tr>
<td>RIPV</td>
<td>15/17 (88)</td>
<td>12/18 (64)</td>
<td>ns</td>
</tr>
<tr>
<td>Mean number of electrode pairs used</td>
<td>2.2±0.3</td>
<td>1.9±0.4</td>
<td>ns</td>
</tr>
<tr>
<td>Procedure time, min</td>
<td>155±35</td>
<td>174±41</td>
<td>ns</td>
</tr>
<tr>
<td>Total RF delivery, min</td>
<td>40.8±12</td>
<td>44.3±13</td>
<td>ns</td>
</tr>
<tr>
<td>Fluoroscopy time, min</td>
<td>42±14</td>
<td>50±17</td>
<td>ns</td>
</tr>
</tbody>
</table>

Table displays the number of vein that could be isolated with PVAC alone, mean electrode pairs activated per application, procedure time, total RF time and fluoroscopy time with the different RF settings. In the 6 cases (2 in the 4:1 group and 4 in the 4:1) with a left common ostium a successful/ unsuccessful isolation is presented as successful/ unsuccessful for both LSPV and LIPV. Abbreviations: LSPV = left superior pulmonary vein; LIPV = left inferior pulmonary vein; RSPV = right superior pulmonary vein; RIPV = right inferior pulmonary vein.
Catheter ablation is a valuable treatment for patients with symptomatic atrial fibrillation and atrial flutter. Ablation has been shown to be superior to treatment with AAD in order to reduce the risk of further arrhythmia.\textsuperscript{9, 136-138} It has furthermore been proven to increase quality of life and reduce arrhythmia-related symptoms.\textsuperscript{9, 134, 139-141}

Ablation of typical AFl by creating a conduction block over the cavo-tricuspid isthmus has been accepted as an effective and safe method to cure this arrhythmia, and is now recommended as first-line therapy for AFl.\textsuperscript{57}

During the last decade, AF ablation has rapidly evolved from an investigational procedure to a widely used method all over the world. Different techniques and approaches have been and are being used to reach the goal for the ablation. This includes the use of different energy sources, among which RF and cryoenergy are the two most commonly used. The present thesis focuses on the evaluation and comparison of novel ablation catheters using one or the other of these energy sources.

Atrial flutter ablation (Paper II)

\textit{Tolerability}

Although not systematically studied, it is recognized that patients often report pain and discomfort during RF catheter ablation of AFl. Our study was initiated following a small randomized study of 14 patients, showing reduced perceived pain with the use of cryoenergy.\textsuperscript{15} In the meantime, this was further reported by another group.\textsuperscript{142} These previous observations\textsuperscript{15, 142} were reinforced by our study showing significantly lower pain scores during ablation application using cryo- as compared to RF ablation. Furthermore, our study showed that the overall perception of the procedure was also significantly better for cryoablation, which was not studied earlier. This was the case even though the cryoablation procedures were longer and even when the cross-overs to RF ablation were included.

\textit{Procedure}

The longer procedure- and ablation duration observed with cryoablation seemed related to the longer application times at each point. The finding that there was no significant difference in fluoroscopy time and dose was
unexpected since fluoroscopy may be avoided during cryoablation while the catheter adheres to the tissue during the application. It was, however, probably explained by the lower success rates for cryoablation, necessitating longer procedures and also cross-over to RF ablation in order to achieve isthmus block.

**Efficacy**

Previous non-randomized studies have reported high (87–100%) acute and long-term (81–100%) success rates using cryoablation.\textsuperscript{143-146} The lower acute success rate of 56% achieved by cryoablation compared with 100% for RF ablation in our study is divergent to previous, and now also more recent randomized studies, which have presented comparable success rates using either technique\textsuperscript{15, 142, 147-149} although in one of these studies, the trend was in favour of RF with a reported 83% success rate compared with 69% for cryo ablation.\textsuperscript{148}

The type of cryocatheter used was the same in all but one of these studies. The RF- and cryo groups were comparable at baseline. The RF catheter design with a flexible tip may be an advantage due to the variable anatomy of the isthmus region, making it easier to reach into valleys and apply firmer pressure on the tissue. Moreover, complete linear lesions may be easier to achieve with the RF ablation technique with the possibility to move the catheter during energy delivery compared with the contiguous focal lesions applied with cryoablation.

The difference in outcome between our study and others is, however, difficult to explain. In a later study, published in 2009, Kuniss et al. elegantly showed that persistence of bidirectional block, assessed by a repeat electrophysiological study 3 months after ablation was significantly inferior after cryo- than after RF ablation, 66% versus 85%, although the majority of these patients were clinically free from recurrent arrhythmia.\textsuperscript{149} This finding may however indicate that complete and/or durable ablation lines may be more difficult to achieve with the cryocatheter.

Our study protocol did not pre-specify an absolute cut-off point for when to cross over from one technique to the other, which is a limitation to this study.

A comparable recurrence rate of AFl was seen in both groups. The many patients with concomitant AF in the AFI population, as reflected in our and other studies,\textsuperscript{147, 148} may, however, underestimate the recurrence rate of AFI since the symptoms are similar.
Atrial fibrillation ablation (Paper I, III-V)

Efficacy

Acute success and procedure

Our studies (I and III) show that a high, more than 90%, and comparable acute success rate, defined as complete PVI, can be achieved with both cryoenergy, represented by the cryoballoon and RF, represented by the PVAC, which is in line with other reports.\textsuperscript{150-153}

The two ablation catheters used in our studies were developed for the purpose of facilitating the AF procedure, making the procedures less time-consuming and shortening the learning curve.

In our first study on the cryoballoon, conducted in 2006–2007, the procedure time and fluoroscopy time were long (mean 239±48 min and 57±21 min, respectively). This must be seen in light of these being our very first patients treated with this novel catheter, but also suggests that it is indeed not a simple procedure. Notably, the procedure time was reduced by 50 minutes between the first 10 and last 10 procedures in the study, indicative of a learning curve, but also further reduced to 165±40 minutes and an almost halved fluoroscopy time in the AF-COR study (2009–2011) (Study III), suggesting the learning curve continued beyond the first 40 patients. A recent report presented a reduction in procedure time over more than 200 procedures.\textsuperscript{154}

Both catheter types have now been described to be associated with shorter procedure times compared to the point-by-point technique.\textsuperscript{153, 155} In our randomized study comparing the cryoballoon and the PVAC, the AF-COR study, the procedure time was comparable between the groups although spent differently, with a longer time from first to last application, ablation time, for the PVAC, and a longer energy delivery time for the cryoballoon. In this study, our procedure- and fluoroscopy times for the cryoballoon procedures were comparable to others\textsuperscript{150, 156} but among the higher reported for PVAC ablations.\textsuperscript{153, 157} The higher fluoroscopy exposure observed for the PVAC compared with the cryoballoon procedures is a disadvantage and may be explained by the many repositionings, and the need for fluoroscopy during energy delivery to ensure catheter stability as opposed to the cryoballoon with its property of adhering to the tissue and thus can be left unattended.

An additional ablation catheter in order to achieve PVI was more frequently used during the cryoballoon procedures than during the PVAC procedures, and seems to be consistent over time if compared with Study I. The fact that less than 60% of the procedures could be completed with the balloon only is a limitation to this technique and makes the procedure more costly. This is, however, not an uncommon finding\textsuperscript{151, 158} and may partly be explained by a possible mismatch between the shape of the balloon and some of the PVs with their individual anatomy and size.\textsuperscript{159} This may
correspond with the finding in Study I, that total occlusions of the PVs by
the balloon were associated with a higher rate of electrical isolation than
incomplete occlusions. Furthermore, the catheter design, with its zone of
optimal cooling as a circumference just in front of the equator of the balloon,
with less effective cooling along the distal pole, requires a perfect angulation
towards the PV ostium to ensure an optimal lesion.\textsuperscript{159, 160} A second
generation cryoballoon, trying to address these issues, has been developed
and is now in clinical use.

The need for an additional touch-up catheter in cryoballoon ablation
however varies greatly in other reports.\textsuperscript{150, 158, 161} The use of the smaller (23
mm) balloon size has been described to be associated with a higher rate of
electrical isolation than the 28-mm cryoballoon catheter,\textsuperscript{162} which was
mainly used in our study. In some reports both balloon sizes were used in the
same procedure in a high proportion,\textsuperscript{150, 154} which is seldom the case at our
center. Different cut-off points for switching to an additional catheter may
further explain differences seen between studies. In a series of 27 patients,
Chun et al. show that it is possible to achieve PVI with only the big
balloon, through the use of different catheter maneuvers.\textsuperscript{161} This was, on
the other hand, the purpose of that study and required up to 13 balloon
applications in some veins. Some of these maneuvers have been
implemented in our lab and were used in the AF-COR study. The less
frequent need for an additional catheter for touch-ups during PVAC
procedures in our study may be related to the option of delivering energy
focally through selected electrodes with this catheter.

\textit{The effect of different energy settings}

In study V, which was a sub-study to the AF-COR study, we hypothesized
that the use of a higher proportion of unipolar energy (2:1 bipolar-to-
unipolar energy) with the PVAC would result in deeper and more transmural
lesions, leading to fewer applications in order to achieve PVI and thus
shorter procedure times as compared to the 4:1 setting.

This study did, however, not show any statistically significant difference
between ablation with the 4:1 and 2:1 bipolar-to-unipolar energy settings
with regard to the number of applications needed to create PVI. There was
further no significant difference in the number of patients in which PVI
could be achieved without touch-up applications, the procedure time or the
fluoroscopy time between the two energy settings.

Several studies have reported a gradual increase in lesion depth by
increasing the ratio of unipolar energy delivered by the PVAC.\textsuperscript{93, 163} Wijffels
et al.\textsuperscript{93} showed that all energy settings (unipolar, 1:1, 2:1 and 4:1
bipolar/unipolar ratio) produce transmural lesions. However, this was
studied in pigs with ablation applications in the superior caval vein, which
may not be anatomically equal to the human left atrium. Presumably,
applications with the 4:1 setting yield transmural lesions in the human heart
as well, and increasing the lesion depth by ablating with the 2:1 setting do
not add any clinical benefit. A recently published randomized study,
comparing the 4:1 and 2:1 ablation modes with the PVAC in patients
undergoing a redo procedure, reported shorter procedure- and fluoroscopy
times with the 2:1 setting. This was due to less risk of reconnection within a
30-minute waiting time in the 2:1 group,\textsuperscript{164} indicating that the deeper lesion
created by the 2:1 setting may be needed in some areas. Our study protocol
did not include a waiting time. Another possible reason that we did not see
any benefit with the 2:1 setting could be that this setting seemed to be more
painful for the patients. Pain was by far the most common cause for not
giving full length applications of 60 s, which was more often the case with
the 2:1 setting. Furthermore, due to pain, often no more than three electrode
pairs could be activated at each application, leading to a higher number of
applications and longer procedure times.

\textbf{Clinical outcome}

AF ablation is associated with an important risk of recurrence, with the
highest risk within the first 6–12 months, although there does not seem to be
a certain point beyond which no further recurrences occur.\textsuperscript{165, 166} Repeat
procedures are thus often required to achieve complete freedom from AF.\textsuperscript{154, 167}
Atrial-PV reconduction is an almost universal finding in redo
procedures.\textsuperscript{164, 167-169}

In Study III freedom from AF without symptoms and with no AF
episodes documented on 7-day Holter monitoring or 12-lead ECG, without
AAD treatment after one procedure, was reached by 52\% versus 38\% at 6
months and 46\% versus 34\% at 12 months with the cryoballoon and the
PVAC, respectively. For the cryoballoon, the rate seems to be in line with
what we reported after mean 8.9 months follow-up in Study I, although the
two are not entirely comparable due to the different inclusion criteria,
follow-up strategy and endpoint used in that study where we reported
freedom from arrhythmia-related symptoms rather than actual freedom from
AF. These outcome figures, however, have to be regarded as moderate or
even low. Nevertheless, it should be emphasized that using this harsh
endpoint may underestimate the true clinical value of ablation since, for
example, patients with only one or sporadic episodes of AF after ablation
will be defined as failures. We therefore also used the definition of clinical
success, to include those who after a single procedure were free from
symptomatic AF on previously failed AAD, displayed asymptomatic AF on
Holter monitoring or reported a symptomatic improvement to the extent that
a redo procedure was not desired. When using this endpoint, the
corresponding figures were 60\% versus 54\% for the groups at 12 months.
The measured outcome rates did not reach statistical difference between the
groups at any time.
The early reports of more than 80% arrhythmia-free survival with the PVAC at 6 months\textsuperscript{135} formed the basis for our hypothesis that AF ablation with the PVAC would be superior to the cryoballoon, which, in our first study, showed a lower (53%) efficacy rate. The AF-COR study, however, could not support such superiority and, on the contrary, there was a trend in favour of the cryoballoon.

Success rates reported by others, which are mainly prospective, non-randomized case series of AF ablations with 6 and 12 months follow-ups, vary greatly, between 49–89% for cryoballoon\textsuperscript{150, 151, 170} and 52–85% for PVAC ablation.\textsuperscript{153, 171, 172} Varying AF populations, follow-up strategies and definitions of success, however, make comparisons between studies difficult. In the AF-COR study, we used the recommended definition of success according to the recently published consensus statement.\textsuperscript{67} Van Belle et al., who used the same primary endpoint, reported a 49% success rate with the cryoballoon in patients with PAF followed for 12 months,\textsuperscript{151} which is comparable to our study even though we also included patients with persistent AF. Higher rates of freedom from arrhythmias after AF ablation with the cryoballoon were reported in patients with PAF (74%) than in those with persistent AF (42%).\textsuperscript{150} One report on AF ablation with the PVAC in patients with PAF showed a 55% success rate after 12 months, decreasing to 49% after 24 months,\textsuperscript{152} as compared with a 61% arrhythmia-free survival in a mixed AF population after 12 months,\textsuperscript{157} using the same primary endpoint. The highest efficacy rates seem to be the early reports with smaller patient series and shorter follow ups.\textsuperscript{135, 156, 172}

Several factors have been discussed to be predictive of recurrence after AF ablation although data is inconsistent on this matter.\textsuperscript{77} A relatively long clinical history of AF, mean 9 years (range 1–30) in Study I and 8 years (1–40) in Study III, as compared with others,\textsuperscript{150, 153, 158, 161} may possibly partly explain the apparently low efficacy rates seen in our study. A recent small cryoballoon study showed that, when used as a first-line treatment in patients with lone PAF, the success rate was as high as 89% after 14 months,\textsuperscript{170} which may support the idea that duration of AF history may be important for the outcome. Other studies of the PVAC with AF durations similar to those in our study have reported similar or even better outcomes, though,\textsuperscript{152, 157} and in one of these studies\textsuperscript{152} the AF burden rather than duration was more predictive of the outcome.

In our studies, the 28-mm cryoballoon was used in the majority of cases. Even though the bigger, 28-mm balloon is a more appealing choice, based on its lower risk for phrenic nerve injury\textsuperscript{150} and its larger surface contact area,\textsuperscript{173} a sub-analysis in a recent long-term follow-up study after cryoballoon ablation showed that patients treated with the larger balloon had a higher recurrence rate than those treated with the 23-mm balloon.\textsuperscript{154}
Both ablation catheters used in this study were constructed for the purpose of simultaneous energy delivery around the PV, a one-shot ablation tool. The different catheter designs, however, possibly makes this concept more true for the cryoballoon with its continuous cryoablation surfaces than for the PVAC with its multipolar circular non-continuous electrode design, resulting more in simultaneously applied point-by-point lesions bound together by bipolar energy between the electrodes. Theoretically, these inherent differences may partly explain the potentially better outcome with the cryoballoon versus the PVAC. Furthermore, in our study, RF energy was rarely delivered through all 5 pairs of the PVAC electrodes simultaneously, because energy was not switched on to electrodes indicating poor tissue contact, and also, as mentioned earlier, since patients despite analgesics, tended to experience intolerable pain when energy was delivered through all electrode pairs simultaneously. One may therefore speculate if deeper sedation, enabling the use of more electrode pairs simultaneously, potentially would have reduced the risk of conduction gaps and thereby would have improved the outcome figures for the PVAC. This limitation may also explain the relatively high number of RF applications needed to achieve PVI and, consequently, the higher exposure to fluoroscopy. In contrast to our study, a deeper sedation or even general anaesthesia seems to have been used in many studies\cite{153, 157, 172, 174} and in some studies all electrode pairs of the PVAC were expected to be used simultaneously until only local gaps were remaining.\cite{153, 155, 175} However, we strongly believe that patients should be treated while awake to be able to detect possible complications at an early stage.

Our study did not include a waiting time or provocation with adenosine to reveal early reconnection after complete PVI, which has been shown to occur in a substantial number of veins, especially after point-by-point RF ablation\cite{176-178}. This was however not implemented in our clinical praxis at that time, but if used, may have affected our results. Limited data exists on the usefulness of such manoeuvres for revealing reconnection\cite{179-183} and for the clinical outcome\cite{181, 183} after PVI with the cryoballoon and the PVAC, why it is difficult to draw any definite conclusions about their individual or combined role for each of these new catheters.

**Tolerability**

The AF-COR study did not include assessment of pain, which would have been interesting with regard to the previous finding on AFL ablation as well as our experience in the study, that RF energy delivery was often prematurely switched off due to pain. Furthermore, we noticed that a higher proportion of unipolar energy in the 2:1 setting for PVAC seemed to cause more pain than the 4:1 setting, which was unexpected. It is, however, not obvious that the finding, that cryoenergy is associated with less pain than RF energy for AFL ablation, can be extrapolated to include ablation in the LA

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with the different catheters used in the AF-COR study. A retrospective study showed that patients who had undergone cryoballoon ablation required less analgesics than patients treated with irrigated-tip point-by-point ablation\(^{184}\) but, to date, there are no randomized prospective studies dealing with this subject. In our experience, the modality of pain also seems to differ between the cryoballoon and the PVAC as cryoballoon-treated patients report referred pain rather than the more direct pain reported by PVAC-treated patients. This subject, however, warrants further studies.

**Quality of life and arrhythmia-related symptoms**

To date, the primary justification for an AF or AFl ablation is to improve quality of life. Although there are data suggesting that mortality and risk of stroke following an AF ablation may be reduced,\(^{140, 185}\) this has not yet been evaluated in large randomized trials and must therefore be seen as unproven.

Despite the moderate outcome figures according to the definition of primary efficacy, our study showed that patients reported a substantial reduction in symptoms and an improvement in quality of life after AF ablation, which is in accordance with other reports.\(^{134, 139, 140}\) In our study, quality of life was increased to a level comparable to that of a general Swedish population, aged 55-64 years.

The SF-36 questionnaire used for assessment of quality of life is a generic, validated instrument\(^{131}\) that covers a broad range of quality of life dimensions, physical as well as psychological. As such, it is not specific for AF but rather reflects the general quality of life. It has, however been used in several AF studies.\(^{8, 139, 140}\) The symptom severity scale, created by the group of Pappone,\(^ {134}\) was designed for AF studies and includes questions on symptoms typical for AF.

**Safety**

Being one of the most complex interventional electrophysiological procedures, AF ablation is associated with a risk of complications. The overall complication rate seen in the AF-COR study is in line with the 4.5% rate reported in an updated worldwide survey of AF ablation.\(^ {80}\) The higher rate seen in our first report on cryoballoon ablation probably reflects the limited experience at that time. In that paper, we reported all adverse events related to the procedure although causality was not always clear. One of the reported complications was a mild pericardial effusion, visualized by echo the day after the procedure. Chierchia et al. demonstrated that pericardial effusion was seen related to 11% of cryoballoon procedures and may probably reflect the transmurality of the lesions rather than being a complication of the procedure.\(^ {186}\)

The most frequently observed complications were haematomas at the site of vascular access and phrenic nerve paralysis. All cases of PNP occurred related to the use of the cryoballoon and is a known complication, especially

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to this modality, occurring in ~6% (range 3–11%)\textsuperscript{187, 188} of cases when ablating the right superior PV. Following, at the time of the first study, unpublished reports of more frequently occurring PNP with the 23-mm balloon,\textsuperscript{150} we decided to primarily use the 28-mm balloon. Fortunately, the majority of PNP has been shown to be transient,\textsuperscript{187} which was also the case in our study. In the worldwide survey, only permanent PNP was reported as a complication.\textsuperscript{80}

Dysphagia occurred in two patients in Study I, and was at that time only recently described after ablation with RF. Pyloric spasm, gastric hypomobility and prolonged gastric half-emptying time were reported as consequences of RF ablation and were postulated to be related to collateral damage of the peri-oesophageal vagal plexi, when ablating the posterior wall.\textsuperscript{189} We discussed that, if the dysphagia observed in our series was of the same postulated mechanism, it may indicate that similar transmural lesions could be achieved with the cryoballoon. This complication has since been further described to occur after cryoballoon ablation.\textsuperscript{190, 191}

No significant PV stenoses were seen after ablation in either study, nor were there any signs of thromboembolic complications.

\textbf{Comparison of effects on coagulation and inflammatory markers}

One of the most feared complications related to AF ablation is the risk of thromboembolic events. Early bench research showed a higher risk of thrombus formation on the ablated surface after RF than after cryoablation.\textsuperscript{17} In Study IV we sought to evaluate whether such a difference could be reflected in a higher coagulation- and/or inflammatory activity response measured by biomarkers. Because both catheters were one-shot tools designed to for PVI, and given the similarities between the procedures with comparable numbers and types of diagnostic catheters and sheaths, we argued that these devices were suited to compare the two energy sources.

In our study, no major differences in the overall activation of the coagulation system or inflammation between the two ablation groups could be seen even though the cryoballoon caused more pronounced myocardial damage than the PVAC, as indicated by the higher Trop I level post ablation. The higher Trop I levels seen are supported by another study, comparing the same ablation catheters,\textsuperscript{118} and may reflect the larger contact surface between the cryoballoon catheter and the endocardial tissue than what is obtained with the PVAC catheter.

In our study, the measured levels of the biomarkers and the pattern of change during the procedure were comparable between the cryoballoon- and the PVAC group, except for sP-selectin levels, which were higher from the start and throughout the procedure in the PVAC group, although they displayed a similar pattern in the two groups with, in general, a relatively constant level during the procedure.

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Prothrombin 1+2 increased from the start of the procedure to after the transseptal puncture, when heparin was given, and then decreased throughout the procedure, while the other biomarkers mainly increased during the time of ablation. These patterns of change are consistent with previous reports.119, 126, 192

The overall relatively limited effects on the coagulation system throughout the procedure suggest a satisfactory anticoagulating effect of heparin despite the relatively low ACT levels in our study.

The comparable response in biomarkers between the groups is consistent with the findings in another study comparing the cryoballoon with an irrigated-tip RF catheter used for the point-by-point technique,193 whereas Tse et al.119 described how a non-irrigated RF ablation catheter seemed to cause more persistent platelet activation, measured by flow cytometry, than a focal cryocatheter. The PVAC, having a duty-cycled, phased RF mode, may however differ from other RF energy catheters with regard to its effect on tissue and activation of coagulation and platelets.

In an attempt to estimate endothelial damage, platelet activation and coagulation activity per lesion size, we used biomarker ratios to Trop I, where Trop I was used as an indirect measure of lesion size. These ratios were higher for the PVAC. Although this is not a standardized measure, one may speculate from the differences seen as to whether the lesions produced by the PVAC were indeed more thrombogenic per se than the lesions produced by the cryoballoon, which would then be consistent with the previous morphological study by Khairy et al., comparing lesions produced by a standard RF- and a focal cryocatheter.17

Recently, concern was raised about the safety of the PVAC. This was based on non-randomized studies reporting a significantly higher incidence of silent cerebral emboli, assessed by magnetic resonance imaging (MRI), with the PVAC, with new lesions occurring in rates as high as ~38% compared with both the cryoballoon and irrigated-RF ablation catheters (4–8%) after AF ablation procedures.174, 194 Different theories, such as charring (charcoal formation of the catheter), air embolism or an energy-dependent risk of thrombus formation on the ablation site, were discussed as possible mechanisms behind these emboli.174, 194

Further investigations of the underlying cause of these emboli have since demonstrated that primarily gaseous-, but also small amounts of thrombus micro emboli are generated during ablation, especially when the distal and the proximal electrode on the PVAC are in close proximity or overlapping during bipolar energy delivery which results in excessive heating of tissue and blood.195, 196

Apart from the direct effect of ablation, several other factors may contribute to an increase of the biomarkers during a procedure, such as puncture of vessels and atrial septum, exposure to foreign materials, time, heparin and AF itself.125 Since the procedure was standardized with
comparable numbers and types of diagnostic catheters and sheaths and since similar procedure times, ACT levels and peroperative AF durations were seen in both groups in our study, one can anticipate that a difference in activation of the biomarkers would mainly reflect the different energy sources used for ablation.

This study was limited to an evaluation of the coagulation system during the ablation procedure. In fact, thromboembolic events do occur even weeks after an ablation\textsuperscript{112}. Even though no major differences were seen between the different energy sources used, it cannot be ruled out that such differences may appear during the healing phase. Lesions produced by cryo- and RF energy develop in different ways, why further sampling after the completed procedure may have added valuable information.

The size of this study was small and it was considered an explorative study and, as such, was not powered to show minor differences between the two ablation techniques.

**Limitations**

Beyond the limitations already discussed, it should be emphasized that these studies were all single-centre studies with limited sample sizes.
Conclusions

Based on the work presented in this thesis the following conclusions can be drawn:

Paper I
Describing our first experiences with a novel ablation tool for PVI, the cryoballoon was shown to be a feasible technique with a high acute success rate and moderate short-term outcome rate. The need for an additional conventional cryocatheter to achieve PVI in >40% of the procedures is, however, a limitation to the technique and makes the procedure more costly.

Paper II
Cryoablation was shown to be associated with significantly less pain and discomfort compared to RF ablation for treatment of typical atrial flutter. In our study, however, cryoablation resulted in a significantly lower acute success rate compared with RF ablation and, consequently, a need to switch to another catheter.

Paper III
The cryoballoon and the PVAC proved to be effective and safe in achieving acute PVI. This could be done with comparable procedure times but with a longer fluoroscopy time for PVAC procedures, and a higher need for an additional touch-up catheter for the cryoballoon procedures. The 12-month rates of freedom from AF, off AAD, after one ablation procedure of 46% and 34% for the cryoballoon and the PVAC group, respectively, were moderate and failed to reach statistical significance. Nevertheless, a comparable and significant improvement of arrhythmia-related symptoms and an increase in quality of life to a level similar to that of a general Swedish population was seen in both groups after ablation.

Paper IV
Even though the cryoballoon catheter caused more extensive myocardial damage than the PVAC, as indicated by a higher Troponin I, no major differences in the overall activation of the coagulation and inflammatory systems, measured by biomarkers, were shown in this study.
Paper V
There was no significant difference between the 4:1 and 2:1 bipolar-to-unipolar energy settings with the PVAC regarding the number of applications needed to achieve PVI, nor in the procedure- or fluoroscopy times.
Clinical implementation and future perspective

With an increasing prevalence of atrial fibrillation and atrial flutter in the population and with a more widespread use of catheter ablation, it is of great importance to identify what is the most effective and safe technique to treat patients with these arrhythmias.

Successful ablation depends upon achieving reliable transmural lesions. However, the aim to create these transmural lesions must be balanced against the risk of collateral damage if the lesions are too deep and the risk of other complications related to the technique or energy used.

Even though cryo and RF as energy sources for ablation have different inherent properties, our studies suggest that it does not seem to be necessary to rule out the use of either of these, as they prove to be comparable in many aspects.

Nevertheless, the clearly higher efficacy rate with the use of RF as compared to cryo in our study on AFL ablation has led us to primarily use RF catheters for this purpose, but with the possibility of switching to a cryocatheter if patients report difficulties tolerating the procedure due to pain.

The high acute PVI rate seen in our studies, with both the cryoballoon and the PVAC, apparently did not correspond to freedom from AF to the same extent, which indicates that the lesions produced are not reliably transmural and/or complete, continuous lines. Especially if AF ablation is to be seen as a curative treatment for AF, these studies highlight the need for further development of catheter design, or even technique, to improve the outcome of AF ablation.

There is, however, rapid technological development in this area. In the time that has passed since our studies were performed, a second generation of the cryoballoon, i.e. Artic Front Advance, has been developed and is now in use. Owing to technological improvements, the cooling zone has been optimized. In this second-generation device, the cooling zone comprises the entire front hemisphere of the balloon, which may allow for a more uniform and effective circumferential energy delivery around the PV.

Although still not in clinical use, the phased RF PVAC has also been further developed in order to reduce the risk of emboli. In the new catheter design the tenth electrode has been removed to avoid possible interaction between the most proximal and the distal electrode. Furthermore, the
material of the electrodes has been changed from platinum to gold, to achieve improved heat transfer properties.

The use of uninterrupted oral anticoagulation during the procedure has been implemented in clinical practice as it has been shown to be safe\textsuperscript{199} and may possibly reduce the risk of both bleeding and thromboembolic complications.\textsuperscript{200, 201}

Although our studies hopefully add a small piece to the puzzle, the answer to what is the optimal energy source, best catheter design or even the safest and most effective technique, especially for the AF ablation, remains to be seen.
Förmaksflimmer och förmaksfladder är två av de vanligast förekommande arytmierna i befolkningen. Utöver att de, hos en majoritet, ger upphov till symptom så som hjärtklappning, nedsatt fysisk ork, andfåddhet och trötthet, medför de också en ökad mortalitets- och morbiditetsrisk samt en nedsatt livskvalitet. Kateterablation har utvecklats till ett värdefullt redskap i behandlingen av dessa båda arytmier och rekommenderas idag som förstahandsbehandling av förmaksfladder och andrahandsbehandling av förmaksflimmer, då man inte har haft effekt av eller inte tolererat behandling med antiarytmiska läkemedel.

Olika energiformer kan användas vid ablationsbehandling. Radiofrekvensenergi är den mest använda energiformen, vilken har använts under lång tid och med goda resultat. Det finns dock potentiella fördelar med att använda s.k. kryoenergi som t ex en lägre upplevd smärta i samband med vissa ablationsingrepp, en möjlig minskad risk för tromboemboliska komplikationer och en lägre risk för lungvensstenos efter förmaksflimmerablation. Nackdelen har framför allt relaterats till att kryobehandlingen krävt långa procedurtider.

Vid ablation av förmaksfladder är målet att med punkter eller s k dragteknik skapa en kontinuerlig linje mellan vena cava inferior och tricuspidalklaffen för att förhindra konduktionen av den elektiska krets i höger förmak som är mekanismen bakom förmaksfladder.

Målet med förmaksflimmerablation är att elektriskt isolera lungvenerna från övriga förmaket då triggern som utlöser förmaksflimmerepisoder lokaliseras i lungvenerna i majoriteten av fall. Den mest utbredda metoden för att åstadkomma denna isolering är att med hjälp av punktformiga applikationer successivt skapa kontinuerliga ablationslinjer runt vardera lungvenen och i par om vardera de högra och vänstra lungvenerna.

Speciellt förmaksflimmerablation är ett komplicerat och tidskrävande ingrepp vilket kräver en lång upplämningsstid. För att förenkla proceduren har nya katetrar utvecklats, vars syfte är att energi skall kunna avges simultant runt lungvenens munning.

Det övergripande syftet med dessa studier var att beskriva och jämföra kateterablationer med nya katetrar som använder RF eller kryoenergi.
Arbete I
Då vi var ett av de första centra i Europa som fick möjlighet att använda en helt ny typ av kryokateter för lungvensisolering, den så kallade kryoballongen, ville vi i denna studie utvärdera och beskriva våra tidiga erfarenheter med denna ablationskateter, med avseende på effekt, säkerhet och procedurrelaterade parametrar som procedurtid samt stråltid. Fyrtio patienter med paroxysmalt eller persistierande förmaksflimmer, remitterade för kateterablation, abladerades med kryoballongen och följes kliniskt.

Studien visade att man med den nya ablationskatetern kan åstadkomma komplett lungvensisolering i hög grad med en acceptabel komplikationsrisk. En tilläggs kateter behövdes dock i 44 % av procedurerna för att uppnå detta. Procedurtiden var i genomsnitt 239±48 min men uppvisade en tydlig inlärningskurva med en ca 50 minuters förkortning av tiden vid jämförelse mellan de tio första och tio sista procedurerna i serien. Vid uppföljning efter genomsnittligen 8,9±4,6 mån uppvisade 53 % av patienterna symptomatisk flimmerfrihet och ytterligare 18 % hade förbättrats kliniskt.

Arbete II
Baserat på vetskapen om att kateterablation av förmaksfladder med radiofrekvensenergi ofta är förknippat med smärta och obehag för patienten, ville vi i denna studie, där patienter som remitterats för fladderablation randomiserades till behandling med radiofrekvens eller med kryoenergi, med hjälp av en skattningsskala jämföra den upplevda smärtan under ingreppet. Patienterna följes sedan under ett minimum av 6 månader avseende arytmisymptom.

Studien visar att förmaksfladderablation med kryoenergi är förenat med en signifikant lägre smärtupplevelse under ingreppet. Dock var lyckandefrekvensen betydligt lägre i kryogruppen, 56 % jämfört med 100 % för de som behandlades med RF vilket medförde att byte till RF var nödvändigt i hög utsträckning i kryogruppen. Bland dem som behandlats framgångsrikt med vardera energiformen primärt sågs ingen skillnad i återfall mellan grupperna.

Arbete III
följdes sedan under 12 månader efter ablationen med kliniska besök och upprepad EKG-monitorering.

Studien påvisar att men kan åstadkomma en hög (≥ 93 %) och jämförbar grad av lungvensisolering med båda teknikerna med en låg komplikationsrisk. Teknikerna har jämförbara procedurtider emedan en längre röntgenstråltid sågs vid behandling med PVAC och behovet av en tilläggskateter var mer frekvent i kryogruppen. Efter 12 månader kvarstod endast 46 % respektive 34 % (icke signifikant skillnad) i komplett arytmifrihet, emedan 60 % respektive 54 % för vardera kryo- och RF-gruppen, rapporterade en sådan förbättring att de inte var intresserade av ett ytterligare ingrepp.


Arbete IV

En av de allvarligaste komplikationerna som föreligger i samband med ablation av förmaksflimmer är risken för stroke eller annan embolisering. En djurexperimentell studie har påvisat en lägre grad av trombbildning vid användning av kryoenergi jämfört med radiofrekvensenergi. I denna randomiserade, explorativa studie, jämfördes aktiveringen av koagulationssystemet medelst fyra olika biomarkörer under förmaksflimmarytvätt (30 patienter) med antingen kryoballongkatetern eller PVAC. Vår hypotes var att en högre grad av aktivering skulle ses vid behandling med RF (PVAC). Ytterligare mättes också Troponin I samt CRP innan samt efter ingreppet i syfte att spegla graden av myokardskada samt inflammation. Sammanfattningsvis visar studien inga skillnader avseende aktivering av koagulationssystemet eller inflammation mellan de båda teknikerna. Kryoballongen gav dock upphov till en större myokardskada vilket sannolikt kan relateras till den större anläggningsytan mot vävnaden av denna kateter jämfört med PVAC.

Arbete V

PVAC är en cirkulär ablationskateter med 10 elektroder via vilka både bipolär och unipolär energi kan avges till vävnaden. Olika proportioner av bipolär-till-unipolär energi (enbart unipolär, 4:1, 2:1, 1:1 el enbart bipolär) kan väljas via generatorn. Leverantören rekommenderar behandling med inställningarna 4:1 och 2:1 för lungvensisolering. Unipolär energi har visats ge djupare lesioner än bipolär energi. I denna randomiserade studie jämfördes ablation med enbart 4:1 kontra 2:1 i syfte att utvärdera vår
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”Framför allt som skall bevaras, må du bevara ditt hjärta
– ty därför utgår livet.”

Ordspråksboken 4:23
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