PeAF-BOX feasibility

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List of Abbreviations:

AAD	Anti Arrhythmic Drugs
AE	Adverse events
AF	Atrial fibrillation
Afl	Atrial flutter
AT	Atrial Tachycardia
CF	Contact Force
CFAE	Complex Fractionated Atrial Electrograms
CTIB	Cavo Tricuspidal Isthmus Block
Echo	Echocardiogram
EP Lab	Electrophysiology laboratory
FTI	Force-Time- integral
LA	Left Atrium
LAPW	Left Atrial Posterior Wall
LAPWI	Left Atrial Posterior Wall Isolation
MIB	Mitral isthmus Block
PAF	Paroxystic Atrial Fibrillation
PeAF	Persistent Atrial Fibrillation
PV	Pulmonary Vein
PVI	Pulmonary vein isolation
QOL	Quality of life
RA	Right Atrium
SR	Sinus Rhythm
WACA	Wide Area Circumferential Ablation

Content

PeAF-BOX Feasibility

A Study to Clarify the Safety and Feasibility of Isolating the Left Atrial Posterior Wall Adjunctive to Pulmonary Vein Isolation - the "Box Lesion" as First-line Therapy in Ablation for Persistent Atrial Fibrillation using SF-Smarttouch ® technology.

1 Background

1.1 Defining the Problem

Favorable outcomes in terms of quality of life, decreased symptom burden and freedom from atrial fibrillation (AF) are well established in patients with paroxysmal AF (PAF) who undergo pulmonary vein isolation (PVI). In more than 90 % of these patients electrical triggers and/or the electrical interplay between the left atrial body (LA) and the pulmonary veins are paramount for the initiation and maintenance of atrial fibrillation. Therefore efficient and durable PVI - when achieved - cures PAF in most patients.

Clinical experience and scientific studies however also agree that outcomes after PVI in patients with persistent atrial fibrillation (PeAF) are less favorable [1] and PVI in these patients carries a much higher rate of recurrent AF related arrhythmias. This lower success rate has been attributed to the assumption that atria that persist fibrillating for prolonged time periods harbor more complex anatomical and physiological disease with myofibrillar remodeling and disarray, increased wall strain, fibrosis etc. This translates into a complex and insufficiently understood electrophysiological behavior of the atrial wall - e.g. extrapulmonary triggers and rotors[2, 3].

The relatively poor outcome after PVI alone in PeAF in comparison to PAF has been interpreted as owing to a shift in the relative pathophysiological importance of pulmonary vein triggers versus the above mentioned complex behavior of the LA - the "substrate" to maintain atrial fibrillation. Accordingly different approaches and concepts have been developed to modify or eradicate the extrapulmonary arrhythmogenic atrial substrate as an adjunct to pulmonary vein isolation. Different techniques include supplementary lines in both the left and the right atrium (RA), mapping and ablating presumptive rotating electrical wave fronts (rotors), targeting the ganglionic plexi in close proximity to the LA posterior wall [4] as well as specific targeting of complex fractionated electrograms (CFAE). In a comprehensive meta-analysis of available data on the relative efficacy of different ablation strategies in PeAF Wynn and coworkers recently found that there are no data available which clearly point to the best first-line ablation strategy in patients with PeAF [5]. Their findings concur with another recent Cochrane analysis [6] which concluded, *first* that PVI remains the well documented baseline treatment, and *second* that supplementary lesions in the left atrium may increase the success rate in PeAF - however there is no scientific evidence to support one adjunctive ablation strategy added to PVI (e.g. PVI + roof line; PVI + CFAE ablations ; PVI + mitral isthmus line etc.) over the other. Current status is that ablating a limited number of supplementary lines in the left atrium is beneficial in PeAF. Which lesion set is better however still remains unsettled.

1.2 Demand and Opportunities

Due to the *demographic development* the population of patients with atrial fibrillation – both PAF and PeAF is expected to increase substantially over the next decades [7] and so is the need and expected demand for a safe evidence-based interventional treatment. In the area of AF ablation at least two important steps forward have been taken over the last couple of years: First *Technological development* such as reliable force sensing capabilities in the ablation catheters, precision and reliability of electro-anatomic mapping systems etc. Second, due to increased ablation activity the *operator experience* combined with optimization over time of *the entire EP-lab workflow* such as efficient and safe perioperative antithrombotic treatment have increased markedly.

Based on these developments ablation for **PAF** has become a relatively fast and safe procedure. PVI using wide area circumferential ablation (WACA) to obtain durable bidirectional block of electrical conduction between the pulmonary veins and the LA body is thus routinely achieved in most electrophysiology labs in Denmark. Success rates in terms of freedom from recurrence of symptoms or signs of AF, atrial tachycardia (AT) or atrial flutter (Afl) is - depending on the quality and quantity of post-procedure follow-up (FU) - currently perceived to be in the range of 60-70 % [1] one year after one ablation for PAF with some late recurrences over the following years.

However, after one ablation for **PeAF** the one-year arrhythmia free rate is probably 30% [1, 8] although recent studies may point to arrhythmia free rates closer to 50 %. It must however be stressed that available data are heterogeneous and dependent on the follow-up scheme and the characteristics of the studied patient cohorts. For instance the duration of PeAF, the size of the LA, the presence of mitral valve disease, heart failure or other comorbidities contribute to the complexity of the single AF case and thus inversely to success rates after PVI.

It is widely accepted that success rates after PVI alone in PeAF are unsatisfactory. Until present time only relatively weak evidence point to positive effects of intervening with the electrical properties of the LA posterior wall (LAPW) by ablating one or two (thus isolating the LAPW) lines anchored to the isolated PVs.

<u>Putative effects of "add on" LAPW isolation (LAPWI) may include</u>:

1) The basic goal of isolating the pulmonary veins from the remaining LA is fortified at the posterior aspect where the box lesion will serve as a "second line of defense".

2) Posterior wall ablation may “inadvertently” damage or modify potentially pathophysiological important ganglionic plexi situated in close proximity to LAPW [9, 10].

3) Posterior wall fibrotic and potentially arrhythmogenic areas [11] will be isolated from the remaining LA

4) Macro reentry tachycardias involving LAPW are hampered – given the lines remain intact.

5) Insufficient lines with gaps/slow conduction predispose to atrial flutters.

Thus: Effective LAPW-isolation added to PVI might carry significant benefits as first-line treatment in PeAF while in-effective LAPW isolation may worsen procedural outcomes. We therefore examined the currently available literature and ongoing studies which more or less directly address the putative antiarrhythmic effects of electrical isolation of LAPW. The findings are presented below.

2 Studies addressing adjunctive linear lesions in LA in PeAF.

2.1 Published studies

1993 Cox et al [12] published their 5 year outcomes in 65 patients (~50 % PeAF) whom they were able to follow up after the original surgical MAZE procedure which included several "cut and sew" lesions in both atria in order to conduct the sinus node impulse through a maze to the AV node. The core lesion in the LA was isolating the LAPW "en bloc" with the PVs although the concept of "PV triggers" was not known at that time. The procedure had a very high success rate but several serious complications including the need for permanent pacing and death.

First generation ablation technology (solid tip)

2003 Ernst et al. [13] studied the feasibility and effect of different lesion sets in the LA in 84 patients on freedom from AF. Electroanatomic mapping (EAM) using Carto® and solid-tip (non-cooled) ablation catheters were used. The different lesion sets were intended to combine PVI with other lesions and were often impossible to achieve as was the ability to achieve and maintain electrical conduction block with the technology used. One of the designs, the "A" type lesion set attempted to mimic the "core" line of the surgical Cox procedure. This design consist of one long continuous encircling line that isolates the paired PV ostia together with the posterior LA wall from the rest of the LA. In only one (of 20) patients this design led to complete (isolating) lines. This one patient was arrhythmia free at 3-years F-U. All other patients experienced early recurrences. The remaining lesion sets were all equally difficult to complete and inefficient. In retrospect the technology of those days was not sufficient to obtain durable isolating lines in the LA.

2004 Kottkamp et al. [14] followed patients one year after attempted WACA + roof line + mitral isthmus line in 100 AF patients (20 % with PeAF). They used solid tip ablation catheters and did not measure if PVI or electric conduction block of lines were achieved. At one year F-U 65 % of the PeAF pts - some on AAD had recurrence on 7 day ECG monitoring.

Second generation ablation technology (cooled tip)

2005 Fassini et al. [15] randomized 61 patients with PeAF to PVI with or without additional mitral isthmus block (PVI + MIB). At 18 months (50 % still on AAD) F-U only 36% in the PVI only group were recurrence free while 74% in the PVI + MI group were recurrence free.

2006 Willems et al. [16] randomized 62 patients with PeAF to PVI + CTI block or PVI + CTI block + roof line + MIB. They found that it was difficult to achieve electrical conduction block of both roof

line and mitral isthmus line. At long term F-U (70 weeks) only 20 % in the PVI + CTI group were in SR while 69 % of the patients with additional lines in the LA were in SR

2007 Sanders et al. [17] reported their results in 27 patients with "chronic" AF. Patients had a median continuous AF duration of 24 months at the time of the procedure and the atria were moderate-severely enlarged (AP diameter ~ 49 mm). Procedures started with patients in AF. First PVI, then CTI block and then LAPWI were performed. In five patients (19%) the AF terminated during LAPWI – either to SR or Afl. The patients had clinical follow up every three months for one year. Further F-U was outside protocol. 50 % of the patients developed atrial arrhythmias at an average of 10 months after the index procedure. Four of these patients were successfully re-ablated to SR off AAD while 1 patient achieved SR on AAD without further ablation. Using this strategy they achieved a 68% SR success rate at two years F-U.

2008 Gaita el al. [18] randomized 79 patients with PeAF in a 1:2 fashion to either PVI + CTI block (26 pts.) or PVI + Lines in LA + CTI block (53 pts.). Electrical conduction block to the pulmonary veins (PVI) was not formally tested and the persistence of the electrically blocking lines in the LA (roof line and mitral isthmus line) were not formally tested either. F-U continued for at least 3 years with 24 h Holter monitoring at intervals. At one year F-U after one procedure 27% of the PVI alone patients and 45 % in the PVI + lines experienced freedom from arrhythmia. After 3 years and additional ablations, if needed, 39 % and 75 % were free from AF.

2009 Tamborero et al. [19] studied 120 consecutive patients with AF (40% with PeAF) who underwent "baseline ablation" with PVI + MIB and randomized them to either additional roof line or roof line and inferior line that is: LAPW isolation. They used a single transseptal technique (no "lasso" catheter) and did not rigorously prove uni- or bidirectional PVI or electrical conduction block over the ablation lines. However in some of the patients offered LAPW isolation they demonstrated disappearance of signals in the LAPW box. At 10 months F-U the overall recurrence rate was 55% in both groups. No data were given pertaining to PeAF patients alone.

2010 Mikhaylov et al. [20] Randomized 34 patients with longstanding PeAF to either a "baseline" lesion set consisting of PVI + roof line + MIB vs. "baseline" + septal line in LA anchored between the right superior pulmonary vein and the mitral annulus. PVI with electrical conduction block was shown using the lasso technique but conduction block over the other lines was not rigorously tested. At two year follow-up after one ablation the recurrence rate on AAD (Amiodarone or D-sotalol) was 60 % in both groups.

2011 Pak et al. [21] Investigated 200 patients with PeAF who in a non-randomized fashion were offered either PVI + roof line + MIB or PVI + roof + left atrial anterior wall line (LAAW). PVI and conduction block over the roof line were documented by pacing maneuvers in all patients. Ablation line conduction block was provable by differential pacing in 69% in the LAAW group whereas line durability was only shown in 32 % in the LLMI group. The one year AF free survival on AAD was 73% in the LAAW group compared to 63 % in the LLMI group.

2011 Estner et al. [22] randomized 116 patients with PeAF to either PVI using WACA and addition of at least one but up to all three of the following lines: LA roof, LA anterior (RSPV to mitral valve) or CTI line in the right atrium (group 1) or ostial single vein PVI using spot lesions + elimination of all CFAE potentials in both atria (group 2). Cooled tip ablation. At one year F-U freedom from atrial tachyarrhythmia was 37 % in group 1 and 39% in group 2 after one single procedure.

2011 Chilukuri et al. [23] randomized 29 consecutive patients with AF (7 with PeAF) to either PVI or a "BOX" ablation much like Cox´ original surgical "core line". They documented unidirectional electrical conduction block to the LAPW (but not the PVs *per se*) in the "BOX" group and the PVs in the PVI group. Follow-up was based on a portable leadless Omron® monitor with patients instructed to record 30 sec/day and during symptomatic episodes. At 10 months follow up the recurrence free survival was 25% in the "BOX" group and 15 % in the PVI group (P = 0,52). These were fairly poor results in both groups. Larger sample sizes, rigorous testing and documenting electrical conduction block over lines might have proven interesting.

2012 Lim et al. [24] randomized 220 AF patients (38% with PeAF) to receive either single ring ablation which attempts to isolate the pulmonary veins and LAPW in one continuous ring ablation (SRI) similar to the Cox core line OR a very wide WACA connected with a roof line (WAI). Both these groups were further randomized to MIB or not (2x2 factorial design). Most of the patients in all groups also had a CTI ablation. At two-year follow-up among the PeAF patients the AF free survival was 68 % in the SRI and 53 % in the WAI group (NS). Data on AF related (AF + Afl + AT) recurrence was not given for PeAF but overall was around 50% at 2 year follow-up. Attempted MIB was only successful in 54% of the patients randomized to that treatment. Sample size was obviously too small for comparison within the PeAF group.

2014 Saad and Slater [25] published a non-randomized series of 25 patients with both short- and longstanding PeAF lasting on average 11 months. At the initial procedure all patients had PVI + LAPWI + CTIB. Further, dormant conduction was induced with adenosine and ablated. AAD were continued for one month and ambulatory F-U was conducted at 1, 3, 6 and 12 months and 7 day Holter monitors were done when appropriate. On average F-U at 16 months 20 pts. (80%) were free from arrhythmia (two of these in AADs).

2014 Very recently Kim et al. [26] randomized 120 patients to PVI + several lines in the LA and RA (control) or the same lesion set as control + a lower posterior line thus leading to LAPW isolation. At 12 months F-U the AF recurrence rate was significantly lower in the LAPW group (17%) compared to control (37%).

Cool tip technology comparing "lines" and CFAE ablation

Dixit et al [27] randomized 156 patients with PeAF to 3 arms: 1. PVI + ablation of non-PV triggers identified using a stimulation protocol (standard approach); 2. Standard approach + empirical ablation at common non-PV AF trigger sites (mitral annulus, fossa ovalis, Eustachian ridge, crista ter-

minalis, and superior vena cava); or 3. Standard approach + ablation at sites of left atrial CFAE´s. At one year follow up after one procedure on AAD´s 49%, 58% and 29% of patients were free of atrial arrhythmias in arms 1, 2 and 3 respectively.

In the STAR AF II trial[28] a total of 549 PeAF patients were randomized in a 1:4:4 fashion in an international trial spanning four continents and 35 ablation centers to PVI, PVI + CFAE or PVI + Lines (roof + MIB). Surprisingly in the PVI only group freedom from AF and any AF related tachycardia after one procedure was 59 % (!) and 49 % respectively at 18 months follow-up. Another important finding was that in the "lines" group electrical block was only achieved in 74% of patients. Compared to PVI alone the outcomes at 18 months F-U tended to worsen in the PVI + lines and the PVI + CFAE groups (Freedom from AF 44% and 37 % respectively) !

Taken together these recent studies speak against the use of CFAE ablations and the proposed supplementary lesion sets in patients with PEAF.

2.2 Ongoing Studies (source: www.clinicaltrials.org)

A) *Outcome of atrial fibrillation ablation after permanent pulmonary vein antrum isolation with or without proven left atrial posterior wall isolation.* [29]

A study initiated in August 2012 as collaboration between Texas Cardiac Arrhythmia Research Foundation, a center in Milan and two centers in China: Beijing and Wuhan. They started recruiting patients in 2013 for the study which randomizes patients with Atrial fibrillation PAF and "non-PAF" (short term and long term persistent) to either WACA or WACA + proven isolation of the left atrium posterior wall (LAPW). Recruitment appears to be multi-center. The technique of isolating the LAPW and schedule F-U is not depicted. A repeat procedure to prove and ensure, by further ablation if necessary, PVI and LAPW isolation will be mandatory three months after the index procedure. Estimated primary completion date was august 2014 and estimated publication is 2016. An estimated 400 patients will be recruited.

B) *Substrate Ablation and Remodeling in Non-paroxysmal Atrial Fibrillation (SMAAN-PAF)* [30]

A study sponsored by The Liverpool Heart and Chest Hospital NHS foundation Trust. In a single center design 130 patients with “non-paroxysmal” atrial fibrillation are randomized and treated single blinded with PVI + amiodarone vs. PVI + amiodarone + additional lines (LA roof + mitral isthmus + CTI) Enrollment started 2011 and has stopped recruiting patients. Primary outcome measure is freedom from atrial fibrillation/ atrial tachycardia at 6 months following a single procedure.

2.3 Current status and ideas for further research

Status on linear lesions in PeAF

As discussed there is presently no known "best practice" of supplementary lesions added to PVI in PeAF. Several studies were hampered by the ambition of investigating multiple hypotheses in one study resulting in comparison of small patient groups. Further, utilizing second generation technology with cool tip ablation the original idea from Cox´ surgical "core line" with one single line encircling the PV´s and the LAPW has been addressed in three relatively recent studies [19, 23, 24] but the

effect in PeAF was impossible to assess due to technical and methodological problems. Recently the Star AF study appears to unambiguously negate any putative advantages of the alternative strategies other than PVI-only in PeAF used in that particular study.

However there is an unmet need to clarify if patients with PeAF will benefit from a combination of proper PVI with rigorous proof of bidirectional electrical conduction block AND electrical isolation of the LAPW with equally rigorous proof of durable bidirectional block. To our knowledge only one group worldwide currently conducts a randomized study to elucidate that problem [29].

It is probably impossible to obtain 100% long term electrical conduction block in one procedure in all patients. However, in our EP-laboratory at Gentofte Hospital we frequently do add LAPWI to the lesion set on an empirical basis when, during the procedure, this option is perceived to be rational. With the current state of the art (not including SF catheters) we experience *first* that establishing proven LAPWI is relatively fast in most patients and *second* at re-do procedures the preliminary experience is that these BOX lesions tend to persist (i.e. still be blocked). Thus we believe that a combination of the latest technological developments and procedural techniques will allow a sufficiently high share of permanently isolated PVs and LAPWs during the index procedure to enable us to show putative advantages of that lesion set.

Important technological improvements and developments of procedures

- Third generation technological developments to optimize ablation lesions using contact force (CF) and *time-force-temperature-impedance* integration have become an integral part of AF- related procedures in our EP lab. Moreover, recently the *surround flow technology* (SF) offering even more efficient cooling than seen before has been integrated with the Smarttouch ® technology.

- Rigorously proving bidirectional electric block over all ablation lines to ensure PVI and LAPW isolation.

- Allowing proper intra-procedural waiting time supplied with adenosine testing for dormant conduction to detect and ablate early reconduction.

Caveats - collateral damage

With larger lesion sets (such as PVI + LAPWI) and more efficient lesions - e.g. with SF Technology - the risk of damaging tissue surrounding the LA might increase. Most importantly the esophagus is notoriously prone to heat related lesions, ulcerations and eventually fatal fistulae. 2010 Halm et al. from Leipzig [31] published their data on 185 patients monitored with an esophageal temperature probe during LA ablation procedures. At esophagoscopy 1 day post procedure they found RFA related lesions in 14,5 % of the patients ranging from small superficial ulcer-like lesions to larger hemorrhagic lesions. Maximal esophageal temperature averaged 41,4 º C in patients without lesions and 42,6 ºC in patients with lesions. No lesions were found if max temperature was ≤ 41 º C. Later Knopp et al [32] found superficial thermal esophageal lesions in 11 % of 425 patients undergoing LA ablation using a cooled tip catheter in a steerable sheath. However, recently a Japanese group, per-

forming PVI for AF, allowed esophagus temperatures up to 42 º C before pausing ablation. They found a range of lesions from erythema to frank ulcers in 25% of patients [33].

Burning questions in ablation for PeAF - Aims of study:

There needs to be done a sufficiently powered randomized multicenter trial utilizing the latest technology to clarify if LAPWI as an adjunct to PVI is beneficial as a first procedure strategy in PeAF.

However, because of the substantial economical and logistical burden of such a study we suggest to first conduct a minor non-randomized, single center study. The aims of such as study is to clarify:

1) Utilizing SF-Smarttouch technology is it feasible to obtain PVI + LAPWI within a reasonable procedure- and fluoroscopy time ?
2) Is PVI + LAPWI with this potentially efficient technology safe ?
3) Is PVI + LAPWI durable ?
4) To uncover trends regarding the intermediate term effect on arrhythmia recurrence of this treatment ?

Depending on the outcomes of this study the obtained data will aid in the design of a randomized, study comparing the effects of PVI vs. PVI + LAPWI.

3 BOX - PeAF – feasibility study

3.1. Proposal of study

It is clinically and scientifically important to clarify if LAPW isolation as an adjunct to PVI in PeAF as a first line therapy is feasible and safe when utilizing the latest generation of ablation technology.

3.2 Primary aims

In patients who undergo first ablation for PeAF with WACA-PVI the addition of anchored lines connecting the zeniths of the superior pulmonary veins (roof) and the nadirs of the inferior pulmonary veins with proven isolation of the LA posterior wall (LAPW-isolation, LAPWI) is feasible, safe and durable.

Feasibility: **Descriptive**: In patients with PeAF and established WACA-PVI it is possible to establish complete electrical isolation (bidirectional conduction block) of the LAPW by ablating a superior and inferior line connecting the PVs within a reasonable prolongation / increase of procedure-, ablation- and fluoroscopy time.

Safety: **Hypothesis to be tested (see 3.4)**. Guided by a temperature probe in the esophagus WACA-PVI+LAPWI can be achieved without increasing the incidence of inflammation/ injury to the esophageal epithelial lining as proven by endoscopy on the day after the procedure and compared to reported incidences of up to 30% in recently published studies.

Descriptive: WACA-PVI+LAPWI can be achieved without increasing the risk of pericardial effusion and tamponade as proven by post-procedure TTE.

Durability: **Descriptive**: At a *per protocol* interventional control procedure (independent of symptoms) 3 months after the index procedure a large proportion of LAPWs of PV´s will remain durably isolated.

3.3 Secondary aims

1) To monitor and report the aggregate **signs** of AF related arrhythmias as measured by an implanted ILR during months 0 to 6 at follow-up after WACA-PVI + LAPWI.

   To monitor and report the **symptoms** of arrhythmia with validated questionnaires during the first 6 months F-U post procedure. QOL studies.

2) Repeat the above for 6 - 12 months post procedure.

3.4 Hypothesis (see paragraph 3.2, safety)

H_1: By esophagoscopy the day after successful WACA-PVI + LAPWI the proportion of inflammation or visible injury in the esophagus induced by the ablation procedure is less than 0.3

H_0: By esophagoscopy the day after successful WACA-PVI + LAPWI the proportion of inflammation or visible injury in the esophagus induced by the ablation procedure is more than or equal to 0.3

3.5 Flow sheet

See appendix A

3.6 Methods

A non-randomized single center study with consented participation of 23 eligible consecutive patients referred for radio frequency ablation for PeAF

3.6.1 Inclusion criteria:

- Symptomatic PeAF (EHRA Symptom class II or more).
- Failing at least one AAD class I-IV.
- Aggregate duration of persistent AF more than 3 months and less than 12 months. during any time period prior to study entry.
- Age between 18 years and 80 years.

3.6.2 Exclusion criteria:

- Former ablation or surgery in the left atrium.
- Former valve surgery.
- LA diameter (TTE-PLAX) > 52 mm in males and > 47 mm in females
- Severe mitral valve disease (stenosis or regurgitation) .
- LVEF < 35 %.
- Implanted pacemaker or ICD leads.
- Pregnancy or susceptibility to that.
- Severe other disease that may hamper adherence to study protocol.
- Intolerance to amiodarone.
- Participation in other clinical research studies.

3.6.3 Data handling

The procedures will be conducted in compliance with our standards for AF ablations and as specified in appendices A and B. All data to be analyzed in this study will be generated within the framework of this study protocol. There will be no transfer of data from the patient´s hospital files to the study other than data generated as part of this study

Descriptive data:
Feasibility: Time consumption (min.), use of X-ray (mGy), ablation time (min.) and energy delivery (watt x sec.) are registered when WACA-PVI is completed and compared to the same values at the end of the procedure. An estimate of the percentage increase of the procedure parameters due to the added LAPWI will be calculated. In the event that LAPWI cannot be achieved within *20 min additional ablation time* after WACA-PVI then LAPWI will be considered *not achievable*.

Safety: The unlikely occurrence of pericardial effusion will be estimated and measured by TTE from subxiphoid and parasternal windows immediately after the procedure and compared to preprocedure echo measurements.

Durability: At the follow-up procedure after 3 months a single transseptal puncture will be done and the durability of bidirectional block of the PV´s and the LAPW will be checked with a soft 20 polar catheter (Pentarray ®). If electrical conduction gaps in the ablation lines are detected a second TS puncture will be performed and an ablation catheter inserted into the LA. Then the precise localization of gaps will be noted and related to the data from the index procedure (force-time integral, ablation index, esophagus temperature) for each point before gap closure with RFA. **Secondary aims (paragraph 3.3):** The patients will be asked to answer the AFEQT 20 item validated questionnaire [34] (see ref.; full questionnaire not append in protocol) on quality of life in patients with atrial fibrillation prior to the ablation and at 3, 6 and 12 months post ablation. The implanted ILR will be programmed to diagnose AF and AF related arrhythmias with the best achievable sensitivity and specificity. There will be a routine reading and clearing of device at 1, 3, 6, 9 and 12 months after the index procedure. Further the patients will be instructed to have the device read if they experience palpitations or other AF related symptoms.

Esophagus data (hypothesis testing, safety): Endoscopies will be performed the day after the procedure and video material from each endoscopy will be evaluated by two independent gastroenterologists. If any reaction or other pathology is encountered in the esophagus any decision on follow-up or treatment will be at the discretion of the gastroenterologist.

All data and calculations obtained in this study will be kept on storage for 5 years after the results have been published.

3.7 Statistical Considerations

There will be no randomization or direct between-groups comparisons. Although this study is primarily exploratory and descriptive in nature we will formally test the hypothesis given in paragraph 3.4. Thus the sample size calculation of this study hinges on the need to demonstrate - with a reasonable power - that putative esophageal reactions to the WACA-PVI + LAPWI procedure do not exceed earlier findings after PVI alone.

Calculation of sample size and power:

a) The outcome of esophagoscopy on day 1 after ablation in the LA is considered to be either positive (inflammation or other esophageal reaction is noted) or negative (no reaction). Therefore the probability distribution of outcomes of esophagoscopy will behave binomially.

b) Recent data indicate that esophageal reactions can be observed in the range of 10 % (proportion = 0.1) up to 30 % (proportion = 0.3) with the second generation ablation technology. We would be interested in showing that the proportion of esophageal reactions to WACA-PVI + LAPWI does not exceed proportions reported earlier (i.e. render probable that the proportion of any visible esophageal reaction to ablation is less than 0.3)

c) Setting as comparator that the underlying proportion (P) of patients subjected to this treatment that experience esophageal reactions is 0.3 or more AND assuming that the "true" proportion in our study is 0.1 then when:

- P = 0.3
- One-tailed α = 0.05
- "true" proportion = 0.1

- recruiting 23 patients will yield 80 % power to reject the (H_0) hypothesis that the proportion experiencing esophageal reactions is equal to or higher than the 0.3.

Based on these calculations we aim to recruit 23 patients for the study.

The above computations were aided by the software program (IBM/ SPSS - Sample power ® ver. 3.0.1)

Post - hoc analyses: Statistical analysis of input - and output data will be performed using IBM/SPSS ® ver. 22 using appropriate statistical methods.

3.8 Risks, inconvenience and patient exposure to adverse events

A) Since participating patients are all scheduled for PVI the added risk relate to the elements added by the study protocol. Directly related to the procedure are: 1) Added ablation (LAPW) compared to PVI alone. This increases the amount of ablation with estimated 30%, increases the total procedure time with an estimated 10% and increases the total fluoroscopy dose area product (DAP) with an estimated 5-10%. 2) A mandatory second procedure after 3 months. This procedure will be minor in most patients.

B) Risk to the oesophagus. Although inflammatory reactions and even devastating fistulae after ablation at or near LAPW are mentioned in the literature (including recently). We have never in our setting (15 years) experienced any clinical manifestations of damage to the oesophagus when performing ablation procedures - including PVI + BOX lesion sets. On the other hand this has not been studied systematically with oesophagoscopy. Thus the detection of subclinical superficial in-

flammation of the oesophageal wall in this study is a possibility and is in fact *the pivotal safety parameter under study*. The oesophagus temperature is monitored closely with a temperature probe during the procedures and the ablation process is tailored to avoid damage.

C) X-ray exposure. It is estimated that adding BOX to PVI in the first procedure will add extra 0,5 mSv. and the obligatory follow up procedure after 3 months an extra 3-4 mSv. This might increase the lifetime risk of developing mortal cancer from around 45% to 45,01 % (one in 10000 pts.).

D) Implantation of loop recorder (ILR). Is a minor surgical maneuver with subcutaneous insertion of a chip measuring 2 mm * 3 mm * 40 mm performed while the patient is still under full anesthesia at the end of the ablation procedure. The overall rate of adverse events related to that device is an estimated 1% - 2% including pain and local infection.

E) Oesophagoscopy the day after the procedure. This is a common invasive routine procedure performed by a highly skilled gastroenterologist. International literature estimates the overall complication rate to around 0,1%. However up to 2 % of patients may experience soreness of throat or annoying belching post procedure. The procedure is done under light sedation and overall this added oesophagoscopy is estimated to delay discharge for two hours.

F) Adverse effects from amiodarone treatment. Amiodarone is a highly effective and much used antiarrhythmic drug with many potential adverse effects. Most important are effects on the thyroid hormone synthesis due to the many iodine residues in the molecule. Patients with former adverse reactions/ experiences with the drug will be excluded from participation and patient who do not tolerate the drug in the 3 week run-in period pre procedure will also be excluded and treated by other means. The remaining patients who do not experience adverse effects during run-in will continue the drug for 3 weeks post procedure to stabilize sinus rhythm for one to two months.

3.9 Research ethical considerations.

This study is a part of the continuing effort to increase accessibility, efficiency and safety of catheter ablation in persistent atrial fibrillation (PeAF) which is notoriously difficult to treat both pharmacologically and invasively. Patients more often than not need more than one procedure to get rid of the disease. This study aims to establish the feasibility and safety of adding a second ablation element to the primary procedure in order to avoid the need for secondary procedures. Further we will evaluate by long term follow-up the efficacy. Patients scheduled for ablation of PeAF will be asked to give informed consent and participate. Participating patients will be exposed to several inconveniences and adverse effects (given in **paragraph 3.8**).

On the other hand participants will be followed very closely for symptoms or signs of recurrent arrhythmia and gain access to immediate qualified treatment. Further there is a possibility that the participating patients (PVI + BOX) will fare better and need fewer secondary procedures compared to non-participants (PVI only). Atrial fibrillation is a large and increasing disease entity worldwide. Therefore every well conducted collection of data on new methods/ approaches may importantly impact the treatment of these patients worldwide

It is the perception among the participating scientists that we, by conducting this study, can safely and in a routined manner obtain important data and still maintain *both* a favorable benefit/risk ratio for the participants *and* a favorable benefit/cost ratio to the surrounding society.

3.10 Patient recruitment and informed consent

Consecutive patients referred with PeAF and scheduled for ablation will be contacted by mail informing the patients about the study and that they will be approached by telephone. There they will be and asked if they are willing to let the study personnel conduct a screening of their eligibility to participate in the study. Then, patients who are willing to receive further information will receive the written information material by mail and summoned for a meeting with the study group at least two days later. These patients will be listed in the study´s screening log.

The patients will be strongly advised to bring a relative or other assessor for the meeting with a study nurse and one of the investigators, however if most convenient to the patient, the information and consent can be given without the participation of an assessor. In a calm secluded environment in an office in the research dept. of our clinic the aims, perspectives, risks and conductance of the study will be discussed and a brief physical examination carried out. IF the patient is willing to participate AND fulfill the in- and exclusion criteria THEN oral and written consent will be obtained at the same meeting.

If the patient - in spite of access to the patient information at least 2 days before the meeting has not decided for or against participation he or she will be granted another day´s decision time at home. If more convenient to the patient receipt and filing of the signed consent form can be postponed until the patient appears for the first study day.

3.11 Proposed substudies (not an integral part of the main protocol)

3.11.1 ECG study

Since WACA-PVI + LAPWI may affect pericardial nerves (containing vagal and sympathetic afferents and efferents there may be detectable effects on heart rate, heart rate variability, SVES, VES and intervals (PQ, QRS, QT).

3.11.2 ECHO study

LA contractility, LA size, LA ejection fraction, systolic and diastolic parameters.

3.11.3 Viability of posterior wall

PET?

3.11.4 Types of recurrence

(AT, Afl, AF)

3.11.5 Gap localization at 3 month per protocol re-evaluation

As a function of lesion characteristics such as FTI/ Ablation index, power, topographic localization, esophagus temperatures

3.11.6 Biochemical markers

Troponin/ CKMB profile post ablation and correlation to recurrence and LA size/ contractility/

3.12 Budget *)

A) Aggregate cost of submission and approval in the regional Ethics Committee		Dkr.	0
B) Estimated time consumption for study nurse/ EP technician:			
1) SCREENING an estimated 4:1 eligible ~ 100 screened	50 h		
2) Run-in + control amiodarone ; 23 pt x 2 hours	46 h		
3) F-U for one year: 1h/visit: 23 x 4 x 1	92 h		
4) Telemedicine F-U /analysis ILR for one year: 5h/patient x 23 pts	115 h .		
Aggregate cost of technical staff: 353 h x 250kr/h		Dkr.	88250
C) Esophagoscopy 2000 D.kr /pt x 23		Dkr.	69000
D) Estimated Cost of ILR **)		DKr.	0
E) Unforeseen 5 %		Dkr.	3145
Budget estimate (aggregate costs)		**Danish Kroner (DKr.)**	**160395**

*) This estimate only includes what is considered extra costs due to the study protocol *per se.*
Not included is the standard setup for AF ablation with an electroanatomic mapping system, cool tip force sensing ablation catheter, circular mapping catheter, esophagus temperature probe, coronary sinus pace/sense catheter etc. for the *index procedure*. This and the extra revenue for suppliers due to the F-U procedures (e.g. Circular mapping catheters, Temperature probes etc.) will be covered by the hospital/research institution responsible for variable production costs.
**) Devices in stock from related studies.

3.13 Economy and sponsorship

Commercial suppliers of technology pivotal to the conductance and perceived to be stakeholders regarding the results of this study have been approached to seek **economic support** for coverage of all or parts of the budget. "Suppliers" include but are not restricted to manufacturers of ablation catheters, implantable loop recorders and esophageal temperature monitoring devices. There **is no commercial sponsorship and no sponsor-investigator relationship agreement** to this study.

The budget will be funded by **unrestricted grants** donated by Biosense Webster (Denmark) and CIRCA electronics (Sweden) and the research foundation belonging to the arrhythmia research group at Gentofte Hospital. There will be no direct or indirect payments of compensation or costs to the participating patients.

3.14 Publication policy

The study group consists of René Worck MD, Arne Johannessen MD, Jim Hansen MD, Michael Vinther MD and Ebbe Langholtz MD.

René Worck is the author of the protocol and will be the PI and first author of publications derived from this study.

The results from this study will be published in international peer reviewed periodicals independently of the results being positive (e.g. clearly showing that the procedure under study can be done safely, efficiently and durably in all the enrolled patients), negative (e.g. difficult, time consuming and not durable to obtain the sought additional electrical blockade in the left atrium) or *inconclusive* (e.g. the power of the data turns out to be insufficient to draw firm conclusions).

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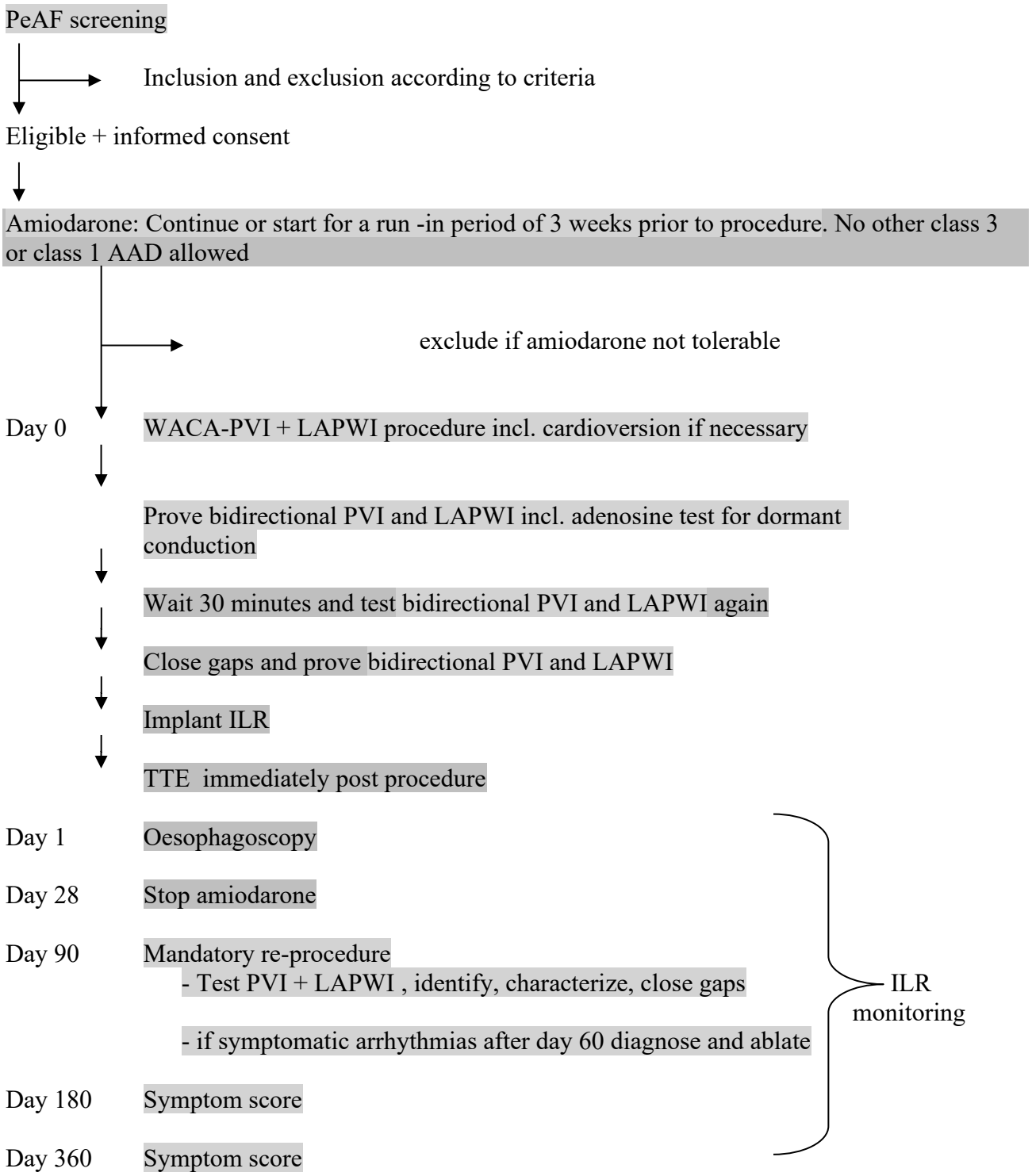
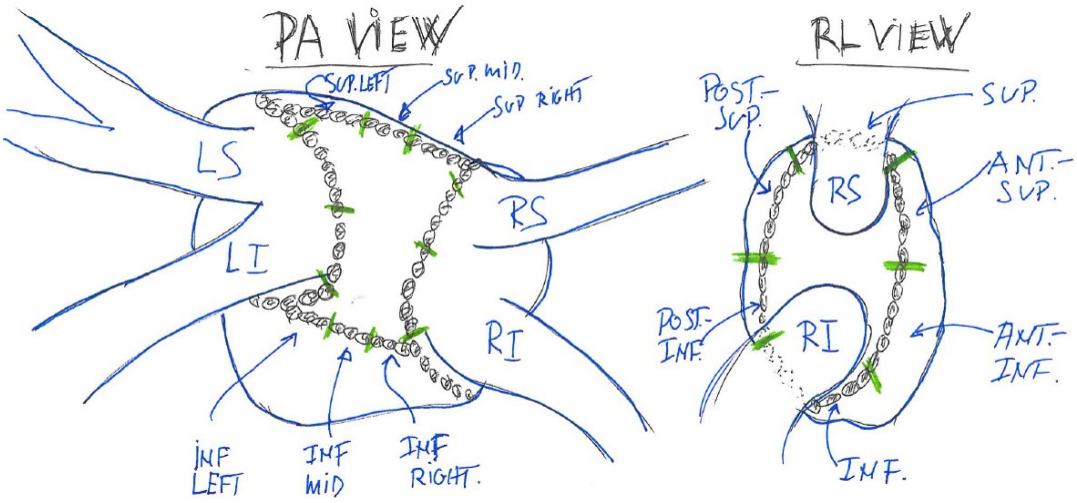
Appendix A. PeAF-BOX - feasibility. Flow-sheet



Appendix B. PeAF-BOX – feasibility - Procedure details

A) Topographical registration in the left atrium:
The operator will ablate the circles and lines in a point-by-point fashion. This allows for the registration of each point according to the guide given in the sketch below.
For each ablation point the topography, contact force, time, impedance drop, power and the concomitant temperature in the esophagus will be registered
Annotation guide for LA ablations



B) Esophagus temperature. We aim to keep measured esophagus (ESO) temperatures below a maximum of 41.5° C while still attempting to obtain durable lesions on the LAPW. To achieve that goal as a main rule:
1) Ablate at the LAPW with max energy output 20-25 watts (maximum)
2) If ESO temp reaches 39.5° C stop ablating and check if the temperature continues to rise to or above 41.5°
3) If the temperature stays below 40° decrease the energy output and/or decrease the contact force (> 10 grams) and continue ablating keeping the ESO temperature ≤ 40° for a maximum of 15 seconds at the same point if deemed necessary.
4) If the ESO temp rises above 41.5 ° and there are still signals at that point then wait until complete cool down. Then ablate again at the same point using less power and less contact force.
5) If abatement of signals at that point is not achievable using the above algorithm then attempt if possible to ablate close to the point.
6) If impossible to obtain block using this algorithm then the patient will be classified as "LAPWI (or PVI) not feasible."