Effectiveness and safety of a single freeze strategy of cryoballoon ablation of atrial fibrillation – a systematic review and meta-analysis

Short title: Single freeze AF cryoablation meta-analysis

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Abstract

Aim

To conduct a systematic review and meta-analysis to evaluate the effectiveness and safety of cryoballoon ablation (CB) of atrial fibrillation (AF) performed using a single freeze strategy in comparison to an empiric double ('bonus') freeze strategy.

Methods

We systematically searched MEDLINE, EMBASE and CENTRAL databases from inception to 12 July, 2020, for prospective and retrospective studies of patients undergoing CB for paroxysmal or persistent AF comparing a single vs. bonus freeze strategy. The main outcome was atrial arrhythmia-free survival and eligible studies required at least 12 months follow-up; the primary safety outcome was a composite of all complications. Study quality was assessed using the Cochrane risk of bias tool and the Newcastle-Ottawa Scale as appropriate.

Results

Thirteen studies (three randomized controlled trials and 10 observational studies) comprising 3,163 patients were eligible for inclusion (64% males, 71.5% paroxysmal AF, mean CHA_2DS_2-VASc score 1.3±0.9). The pooled effectiveness of a single freeze strategy was similar to the double freeze strategy – OR 1.09; 95%CI: 0.9-1.32, I^2=0%. Single freeze procedures were associated with a significantly lower adverse event rate (RR 0.72; 95%CI: 0.53-0.98; I^2=0%) and shorter average procedure time (20 minutes, 95%CI 15-26 min; P<0.001), whereas a trend for lower risk of persistent phrenic nerve palsy was observed (RR 0.61; 95%CI: 0.37-1.01; I^2=0%). The quality of included studies was moderate/good, with no evidence of significant publication bias.

Conclusion

A single freeze strategy for CB of AF is as effective as an empiric double ('bonus') freeze strategy while appearing safer and quicker (PROSPERO registration number CRD42020158696).

Keywords: atrial fibrillation, ablation, cryoballoon, single freeze, effectiveness, safety, systematic review, meta-analysis

What's new?

- Main randomized controlled trials assessing cryoballoon ablation of atrial fibrillation (AF) employed an empiric double ('bonus') freeze strategy but a growing body of evidence suggests a single freeze cryoballoon ablation to be comparable in terms of clinical effectiveness and safety.
- This systematic review identified thirteen studies comparing single freeze to double ('bonus') freeze cryoballoon strategy, including three randomized controlled trials.
- During a ≥ 12 month follow-up, the single freeze cryoballoon strategy was as effective as the double freeze strategy, while appearing safer and quicker.

Introduction

Catheter ablation is the most effective rhythm control strategy for atrial fibrillation (AF) patients, with the potential to improve prognosis in selected populations.[1-3] Electrical isolation of the pulmonary veins (PVI) is considered a crucial endpoint of AF ablation and can be achieved by a variety of ablation techniques, with radiofrequency ablation (RFA) and cryoballoon ablation being the most widely utilized. [1, 4] A large body of evidence indicates clinical equivalence of both ablation techniques in terms of arrhythmia recurrence, albeit with significant differences in procedure duration or safety.[1, 4-6] In two pivotal trials which compared cryoballoon ablation to RFA, all pulmonary veins were subject to at least two cryoballoon applications (double or 'bonus' freeze strategy) with a fixed time of freezing.[7, 8] The cryoballoon ablation technique has evolved with time, leading to a reduction in both the number and duration of applications.[9-11] Recent studies report that even a single 180s application with or without time-to-isolation (TTI) guidance (single freeze strategy) may be sufficient for a favorable long term effect.[10, 12] Based on the published data, it is unclear how this evolution may influence the effectiveness and safety of cryoballoon ablation, especially considering that two recent multicenter trials investigating cryoballoon ablation effectiveness as a first-line treatment of AF used a 'bonus' freeze strategy.[13-15]

Therefore, we conducted a systematic review and meta-analysis of randomized and observational studies to evaluate the effectiveness and safety of a single freeze cryoballoon ablation of AF in comparison to the double ('bonus') freeze strategy.

Methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines.[16] The protocol is registered in the international prospective register of systematic reviews (CRD42020158696).

Search strategy and selection criteria

MEDLINE (Medical Literature Analysis and Retrieval System Online) via PubMED, EMBASE (Excerpta Medical Database) and CENTRAL (Cochrane Central Register of Controlled Trials) databases were searched from inception to the 25th of July 2019 using the following search string (cryo* AND ablation AND "atrial fibrillation"). Due to delay in completion of the review, the MEDLINE search was updated on the 12th of July 2020. Reference lists of eligible studies were searched for additional sources of information. Full text publications of conference abstracts or registered clinical trials were sought. We also contacted key opinion leaders in the field of CB.

We included all prospective and retrospective studies with a control group which assessed the first-time cryoballoon ablation performed in adult patients with either paroxysmal or persistent AF using second or newest generation cryoballoons. Generations 2 to 4 are currently commercially available and do not differ significantly in terms of clinical performance. Only studies published in English were included.

The intervention assessed was cryoballoon ablation of AF. The comparison of interest was intended single freeze cryoballoon procedure ('single freeze group') vs. intended two cryoballoon applications or an empirical 'bonus' application after demonstrating pulmonary vein isolation ('double/bonus freeze group') regardless of the method used for assessment of pulmonary vein isolation (spiral catheter, time-to-isolation, etc.) or length of application. An additional freeze was allowed in the 'single freeze group' when it was felt that isolation was either not achieved or was achieved very late in the freeze, causing concerns for lesion durability.

Two investigators (MF and MK) independently screened and selected potentially eligible studies based on title and abstract. Final eligibility was decided after evaluation of full-text publications. All disagreements were resolved via discussion or through the involvement of a third referee (DG).

Data extraction and quality assessment

Data extraction was done independently by two investigators (MF and MK) and all disagreements were resolved via discussion, or through the involvement of a third referee (DG). A standardized form was used to extract the following information from each study: 1) study design and methodology, 2) details of the ablation procedure (single or double application, time-to-isolation), 3) information on the assessment of the main clinical outcome (surface ECG, Holter monitoring), including length of follow-up, 4) baseline characteristics of participants (age, CHA_2DS_2-VASc score, paroxysmal and persistent AF, left atrial diameter), 5) measures of effect and safety as stated in the protocol of the current meta-analysis. In case of missing data on main outcomes, authors of the original publications were contacted via email.

The risk of bias in randomized controlled trials (RCTs) was assessed using the Cochrane risk of bias tool [17], while the Newcastle-Ottawa Scale (NOS) was used to assess the quality of non-randomized studies. Critical assessments on the risk of bias (high, low, unclear) were done separately for each domain. The risk of bias assessment was done independently by two investigators (MF and MK) and all disagreements were resolved via discussion, or through the involvement of a third referee (DG). A trial was considered of high quality if no domains scored as high risk, or low quality if three or more domains scored as high risk. High-quality non-randomized studies were defined as those with a Newcastle-Ottawa score of ≥7.

Outcome measures

The main clinical outcome was atrial arrhythmia-free survival assessed at least 12 months from the date of the ablation, defined as the lack of any atrial arrhythmia (AF or other) lasting more than 30s as assessed by repeated ECG and/or Holter monitoring. The main safety outcome was any adverse effect (AEs) of the ablation. Secondary outcome measures included: persistent phrenic nerve palsy (PNP), defined as palsy lasting longer than until the end of the index ablation, transient phrenic nerve palsy, defined as palsy which resolved before the end of the index ablation, tamponade, atrio-esophageal fistula formation, procedure duration and fluoroscopy time.

Data synthesis and analysis

Data were synthesized if reported in at least two included studies. Continuous variables were presented as mean with standard deviation (SD). If means and SD were not reported, these were estimated from sample size, medians and quartiles.[18, 19]

We used random effect models to calculate pooled: (i) odds ratios for freedom from any atrial arrhythmia in a ≥12 month follow-up, (ii) relative risk of ablation-associated persistent phrenic nerve palsy, (iii) relative risk of ablation-associated transient phrenic nerve palsy, (iv) relative risk of any ablation-associated adverse event, (v) difference in procedure duration time and (vi) difference in fluoroscopy time, all with 95% confidence intervals (95%CI).

Analyses were conducted separately for randomized and non-randomized studies and subgroups were compared using the Z statistics. Heterogeneity between study populations was calculated using I^2 statistics, where values of less than 25%, 50% to less than 75%, and more than 75% were regarded as evidence of low, moderate, and high levels of heterogeneity, respectively.[20] Funnel plots were used for evaluating the presence of publication bias.

Analyses were performed using Statistica 13.3 (TIBCO Software Inc., California).

Results

Selection and description of studies

The PRISMA flow diagram in Figure 1 summarizes the study selection process. Primary searches identified 3,930 citations and, after removal of duplicates, 2,666 abstracts were independently screened by two investigators. Taking also into account additional searches (MEDLINE update, reference lists), a total of 16 potentially relevant studies were selected for full-text examination. Three studies were excluded based on the wrong comparator (no single freeze group in all cases [13, 29, 30]) and, finally, thirteen studies were included in the data synthesis: three RCT's [11, 12, 21] and 10 non-RCT's [9, 10, 12, 22-28].

Characteristics of included studies

The characteristics of included studies comprising methodology, demographic data, cryoballoon ablation details and follow-up strategies are provided in Tables 1 and 2. Five studies [11, 21, 22, 25, 31] used a single freeze strategy based on the TTI principle, whereas investigators of two studies [10, 26] changed the ablation technique from empirical to TTI-guided applications over the course of their studies and six others used empirical 180/240s applications[9, 12, 23, 24, 27, 28]. In all cases, pulmonary vein isolation was confirmed before the bonus freeze application. The diaphragmatic compound motor action potential (CMAP) assessment, an additional precaution measure for preventing PNP, was used in five studies [12, 22, 24-26]. The definition of primary efficacy and safety endpoints were homogenous among included studies. Apart from two studies [21, 31], effectiveness was defined as freedom from any atrial arrhythmia lasting > 30s recorded after a three-month blanking period. Effectiveness was assessed during clinical visits and through repeated elective electrocardiograms (ECG) or Holter ECG monitoring of varying duration. In all studies, persistent PNP was defined as PNP lasting longer than the index procedure.

Risk of bias

The overall quality of the included studies was good/moderate (Supplementary Table 1 and 2). All three RCTs lacked blinding of participants and/or personnel to the intervention, but otherwise had no other major source of bias. All observational studies had a control group and derived data directly from medical records. The NOS score varied between 8 and 9 which was consistently above the established cut-off for high-quality observational studies; in one case [26] there was not sufficient data to conduct a proper classification.

Data synthesis

The 13 included studies comprised 3,163 patients, predominantly males (64%) with paroxysmal AF (71.5%) and low risk of stroke (Table 3). The pooled data revealed that the cryoballoon ablation single freeze strategy was as effective as the double freeze strategy in preventing recurrent atrial arrhythmia (OR 1.09, 95%CI (0.90-1.32), p=0.386, I^2=0%, Figure 2A) while associating with a significantly lower risk of overall adverse events (RR 0.72, 95%CI (0.53-0.98), p=0.037, I^2=0%, Figure 2B) and borderline lower risk of persistent PNP (RR 0.61, 95%CI (0.37-1.01), p=0.056, I^2=0%, Figure 3A). These results were similar when calculated for RCTs and non-RCTs separately (Figure 2A and B). Furthermore, single freeze procedures had a significantly shorter duration (90±27 min. versus 121±36 min., p<0.001, Figure 3B). There was no difference between both strategies in terms of fluoroscopy times or risk of transient PNP (Supplementary Figure 1 and 2). Funnel plots did not reveal any significant publication bias for the main efficacy and safety endpoints (Supplementary Figure 3).

Discussion

The main findings of this systematic review are: 1) a single freeze cryoballoon ablation is as effective as a bonus/double-freeze strategy, while 2) associating with a lower risk of adverse events (with a strong trend for lower risk of persistent phrenic nerve palsy) and 3) a shorter procedure duration. These findings question the need for routine bonus freeze and suggest that a single freeze strategy should be the preferred technique for cryoballoon ablation of AF.

CB ablation is widely considered as equivalent to RF ablation of AF in terms of efficacy. [1, 7, 8] Recent publications indicate that AF ablation using the cryoballoon might become the first-line treatment of choice for patients with paroxysmal AF as it is more effective than antiarrhythmic drug therapy. [14, 15] However, in these multicenter RCTs, the cryoballoon ablation was routinely conducted using a double freeze strategy.

A growing body of evidence suggests the possibility of reducing the number of freezes to shorten the procedure and lower the risk of persistent PNP, a relatively common complication of cryoballoon ablation, without compromising the long-term effectiveness. [11, 12, 21] This systematic

review and meta-analysis summarizes the current knowledge on the effectiveness and safety of the cryoballoon ablation single freeze strategy. Included studies had good overall quality and described similar cryoballoon ablation techniques, had mandatory confirmation of PVI, and used relatively homogeneous definitions of effectiveness, safety outcomes and follow-up methods, which adds credence to the results. We highlight that patients included in these studies were relatively healthy, with a low average CHA_2DS_2-VASc score and predominantly with paroxysmal AF, and therefore it is unclear whether our results can be extrapolated to cohorts of persistent AF patients.

In terms of effectiveness, none of the individual studies reported a significant difference between strategies, yet the pooled analysis as well as the sub-group analysis based on study design confirmed that a single freeze strategy is as effective as using a routine bonus freeze (Figure 2 and 3). Simultaneously, the single-freeze strategy was associated with a lower risk of adverse events, which was seen in both RCTs and non-RCTs and was largely driven by a borderline significant reduction in persistent PNPs (Figure 2B and 3A). Given that this lower risk of adverse events was seen consistently across studies, with no significant heterogeneity between studies or evidence of significant publication bias, it reinforces the idea that adding a routine second freeze may be deleterious and should not be generally pursued. The single freeze procedures were also on average 20 minutes shorter, which may be useful to improve lab efficiency, allowing more cryoballoon ablation cases per week (and thus reducing waiting list times), without compromising patient safety and treatment efficacy.

This systematic review does not, however, provide any indications on the optimal technique for a single cryoballoon application. Included studies differed in terms of utilization of TTI guidance or length of cryoapplications - 180/240s in general (Table 1). However, due to occasionally short TTI, the total application times may have been even lower than 180s in selected cases [22, 25, 31]. It must be emphasized that studies using TTI-guided cryoablation had a formal protocol for quality control of the lesion formation which anticipated prolongation of the application based on prespecified TTI thresholds. [10, 11, 21, 22, 25, 26, 31] Still, there was virtually no heterogeneity between studies in terms of

effectiveness and safety regardless of the technique used during the single freeze strategy (Figure 2). While the influence of the length of cryoapplication on the long-term effectiveness of AF ablation was clearly beyond the scope of this review, it should be bore in mind that there is evidence suggesting a significant association between longer freeze times and better rate of durable pulmonary vein isolation. [32]

Although the results of this systematic review and meta-analysis support the use of a single freeze strategy, our findings must be interpreted in the broader setting and should also take into account the data obtained at the time of re-do ablation in patients with recurrent AF.[32-36] Published data suggest that gaps identified during re-do AF procedures after initial CB ablations tend to accumulate in the vicinity of the left atrial appendage/left superior pulmonary vein ridge, the superior aspect of right superior and inferior aspect of right inferior pulmonary veins. While we could argue that additional 'bonus' freezes in those areas may improve durability of CB-derived PVI, the present meta-analysis suggests that additional applications in the vicinity of the right pulmonary veins should be discouraged and operators should instead take time to properly position the cryoballoon so it achieves optimal occlusion at the time of the first lesion. As far as the left pulmonary veins are concerned, the potential for fistula formation associated with very low temperatures and repeat freezes, especially in the inferior vein, has been previously reported[37]. Our study does not support the need for routine bonus freeze in this area.

Limitations

This study has the typical limitations of systematic reviews and meta-analyses. Most of the included studies were observational and, although these were generally of good quality, they cannot replace large scale randomized controlled trials. However, our results were consistent across RCTs and non-RCTs, which adds robustness to our findings. In addition, where non-randomized studies are concerned, a learning curve effect cannot entirely be ruled out; some studies noted that a single-freeze strategy was adopted later than a double-freeze strategy. However, this notion is not

supported by outcomes of included randomized trials which were all conducted by experienced investigators and generally reported similar effectiveness and safety to observational studies.

Conclusion

A single freeze strategy for cryoballoon ablation of atrial fibrillation is as effective as an empiric double ('bonus') freeze strategy while appearing safer and quicker (PROSPERO registration number CRD42020158696).

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Table 1: Summary of methodology and cryoablation details of included studies.

First author, year, reference	Study design (number of centers)	Sample size		Description of the procedure				Demographic data					
		Study group	Control group	Study group	Control group	TTI	CMAP	Patients' mean age		Male sex (%)		Paroxysmal AF (%)	
								Study group	Control group	Study group	Control group	Study group	Control group
Heeger 2016	Prospective cohort study (1)	60	60	Single 240s application.	1 bonus 240s application after PVI.	0	1	61±11	62±11	63	60	83	75
Tebbenjohanns 2016	Prospective cohort study with a historical control group (2)	53	139	Single 240s application, adenosine challenge.	Two 240s applications.	0	0	66±10	61±11	51	54	72	63
Chun 2017	Randomized controlled trial (1)	50	50	TTI <75s or within 25s after a pull-down, application	Two 240s applications.	1	0	66 ± 10	63 ± 12	60	58	100	100

				240s; TTI > 75s or not recorded, application 240s and 240s bonus.									
Ekizler 2017	Prospective cohort study (1)	56	80	Single application 240s.	1 bonus application after PVI.	0	0	58 (48-67)	62 (49-68)	57	55	100	100
Aryana 2017	Prospective cohort study (5)	355	400	TTI ≤60s, one application TTI + 120s; TTI 60-90s, one application TTI+120s and bonus 120s; TTI > 90s, application aborted; no TT-PVI, 180s	2-3 applications lasting 2-4 min.	1	1	64±11	63±11	69	74	72	74

				application plus 120s bonus.									
Ströker 2018	Case-control study with propensity scoring (2)	256	256	Single 180/240 min application; bonus application if: temperature > −40 °C within 1 min, no PVI or early spontaneous PV reconnection	1-2 bonus application(s) (240s/180s) after PVI.	0	0	59 ± 12	60 ± 11	62	67	82	79
Pott 2018	Case-control study (1)	100	100	TTI <30s, application 120s; TTI 30-60s, single application 180s; TTI >60s, 180s application +	Two 240s applications.	1	1	65 ± 10.9	65.3 ± 11.3	56	57	65	69

				180s bonus; no TTI recording, single 180s application.									
Rottner 2018*	Prospective cohort study (2)	352	211	Single 240s application; later single application TTI + 120s and if no TTI recording, single 180s application.	Two 240s applications.	1	1	63.3 ± 10.9	63.3 ± 10.9	64	64	58	58
Mortsell 2018	Randomized controlled trial (1)	69	70	Single 240s application; either TTI or temp. ≤40 °C within 120s.	Two 240s applications.	1	0	61.9 ± 9.08	68.3 ± 10.0	70	77	49.3	40.0
Yoshiga 2019	Prospective cohort study (1)	67	33	Single application ≥180 min.	1 bonus 120s application after PVI.	0	0	65.1±10.0	67.5±8.3	58	70	100	100

Cordes 2019	Prospective cohort study (1)	35	35	TTI + 120s; if TTI not recorded, single 180s application with target temp. < 40 °C; if TTI >90s, application aborted.	Two 180s applications.	1	0	58 (IQR 16)	60 (IQR 20)	71	80	60	57
Miyamoto 2019	Randomized controlled trial (3)	55	55	Single 180s application	1 bonus 180s application after PVI.	0	1	63.1±11.8	64.0±11.0	65.5	63.6	100	100
Koektuerk 2019	Case-control study (1)	77	92	Single 240s application	Two 240s applications.	0	0	61±10	64± 10	63.6	85.9	100	100

* Rottner 2018 reported aggregated demographic data for all patient groups.

AF, atrial fibrillation; AT, atrial tachycardia; CMAP, compound motor action potentials; PVI, pulmonary vein isolation, TTI, time to isolation

Table 2. Summary of the follow-up strategies among included studies.

First author, year, reference	**Assessment of the primary end point**	**Standard AF ablation endpoint***	**Mean follow-up time**			AADs management	Information on redo procedures
			Study group	Control group	All patients		
Heeger 2016	ECG and 24h Holter at 3, 6, 12 months and in 6-months intervals thereafter; regular telephonic interviews, additional outpatient visits in symptomatic patients.	1	848 ± 101 days	849 ± 74 days		AADs continued for 3 months.	A total of 26/34 (76 %) patients suffering from atrial arrhythmia recurrences underwent a second ablation.
Tebbenjohanns 2016	Clinical visit and 24h Holter at 3, 6, 12, and 18 months; external event recording for 4 weeks in symptomatic patients.	1			458±107 days	Not reported.	Not reported.
Chun 2017	Visits at 3, 6, 12 months; ECG and 72-hour Holter ECG; additional telephone interviews and event recording in symptomatic patients.	1	372 (351-455) days	378(361-483) days		AADs discontinued after the procedure. Resumption of AADs in case of AF relapse during blanking period.	Not reported.
Ekizler 2017	ECG and 24h Holter at 1, 3, 6 nad 12 month, biannually thereafter.	1	12 ± 3 months after	13 ± 3 months after blanking		Discontinued at the end of the blanking period.	A redo procedure was performed in 5 patients in Study group and in 6

			blanking period	period			patients in Control group.
Aryana 2017	ECG during each follow-up visit; 2- to 4-week ambulatory electrocardiographic monitoring at 6 weeks, 3 and 6 months.	1	15±2 month	16±3 month		Antiarrhythmic therapy was discontinued within 6 weeks of ablation.	35 patients (9.9%) in Study group and 63 patients (15.7%) in Control group underwent a repeat catheter ablation during the study period.
Ströker 2018	ECG, Holter at 1, 3, 6 month and every 6 month after or in case of symptoms.	1			18 ± 10 months	AAD's were discontinued 3 months post ablation if no recurrence.	A redo procedure was performed in 44 (66%) patients in the Control group and 37 (50%) patients in the Study group.
Pott 2018	Clinical assessment, echocardiography, ECG, and 7-day-Holter-monitoring at 1, 3, and 6 months and thereafter every 6 months.	1			436 ± 184 days	AADs withheld either after the procedure or after blanking period.	Not reported.
Rottner 2018	No information provided; paper focused on procedure safety.	NA	NA	NA	NA	AADs recommended to be continued for 3 months.	NA
Mortsell 2018	Clinical visit at 3, 6, and 12months; a 7 day	1 (one month	12 months	12 months		Antiarrhythmic drugs were	Re-ablations

	Holter at 6 and 12 months.	blanking period)				withdrawn after 3months in asymptomatic patients free from clinical AF recurrences.	were performed in 7 of 70 (10%) patients in the Study group and in 8 of 70 (11.4%) patients in the Control group.
Yoshiga 2019	ECG and Holter at 1, 3, 6, and 12 months; event monitoring in symptomatic patients.	1	12 months	12 months		In all the patients the anti-arrhythmic drugs were discontinued after the procedure.	Not reported.
Cordes 219	Standardized telephone interview at 3 and 6 months.	**	6 months	6 months		Not reported.	Not reported.
Miyamoto 2019	ECG at 1 month, and then every 1 to 3 months; 24h Holter at 3 and 12 month; event recorder in symptomatic patients.	1	12 months	12 months		Discontinuation of antiarrhythmic drugs was recommended after the ablation.	Not reported.
Koektuerk 2019	Outpatient visit at 1, 3, 6, 12 months or earlier if symptomatic; 7-day Holter recording at 3 and 6 months and 24-h Holter later; telephone interview at the end of the follow-up.	1	16.4±7.5	19.0±8.6		AADs continued for at least 3 months after ablation.	7 patients in the Study group and 14 in the Control group underwent repeated ablation.

*Defined as AF/AT episode lasting more than 30s recorded after a 3 month blanking period.

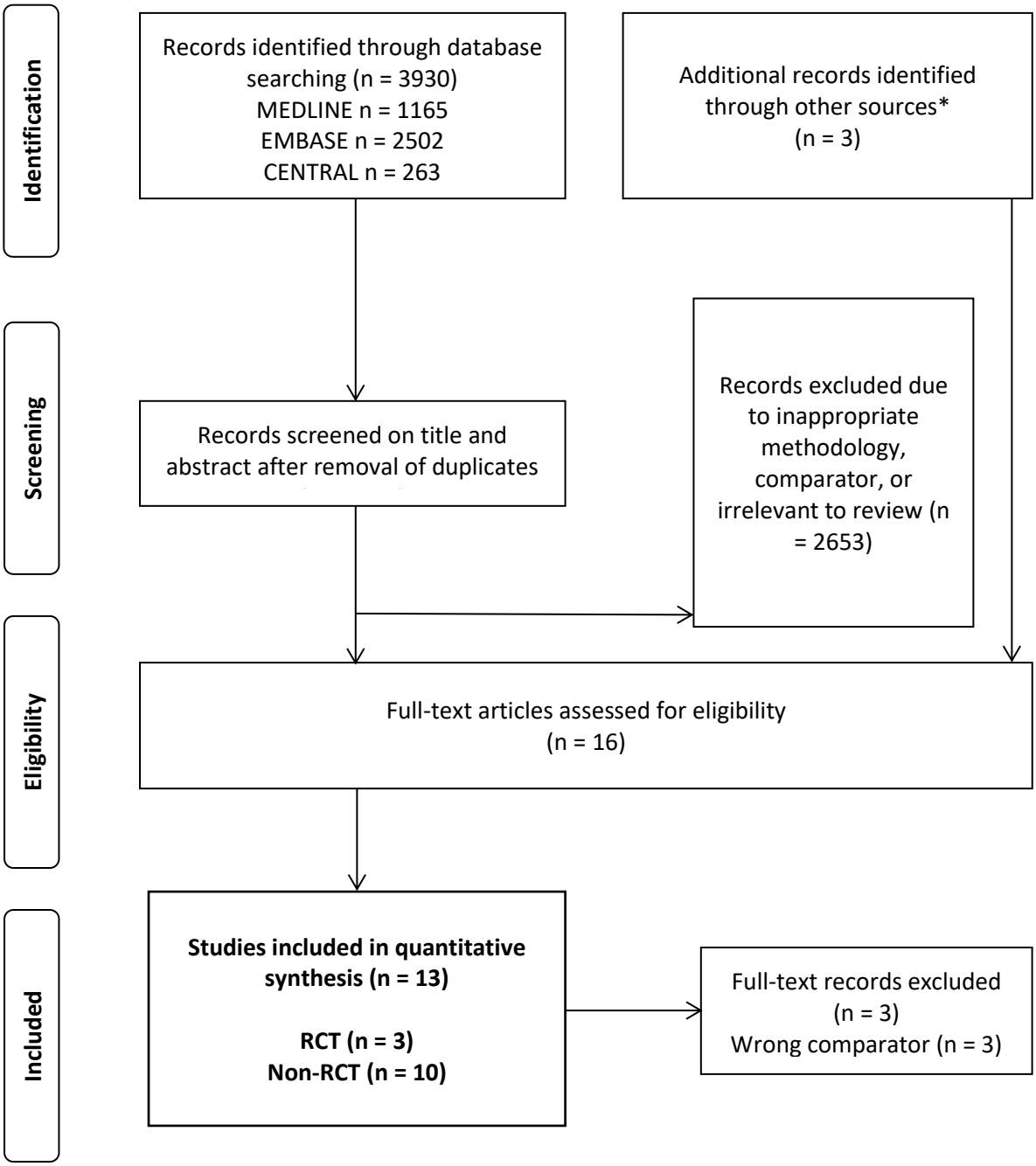
** Only 6 month follow-up results available.

AADs, antiarrhythmic drugs; AF, atrial fibrillation; AT, atrial tachycardia.

Table 3. Baseline patient characteristics and procedural details.

	Overall			
	N	value		
Age (years)	3163	61.3±10.8		
Male sex	3163	2025 (64.0)		
Paroxysmal AF	3163	2261 (71.5)		
CHA2DS2-VASC score	2069	1.3±0.90		
BMI (kg/m^2)	2339	28.0±5.3		
LA (mm)	2818	42.7±6.9		
	Single freeze		**Repeated freeze**	
	N	value	N	value
Fluoroscopy time (min)	1700	16.6±7.5	1393	22.3±9.7
Procedure time (min)	1700	90.5±27.0	1393	122±35.2
Periprocedural death	1735	1 (0.00)	1428	0 (0.0)
Periprocedural stroke or TIA	1735	7 (0.40)	1428	3 (0.21)
Tamponade	1735	2 (0.12)	1428	4 (0.28)
Atrioesophageal fistula	1735	0 (0.00)	1428	0 (0.0)

Figure 1. PRISMA flow diagram for study selection process.

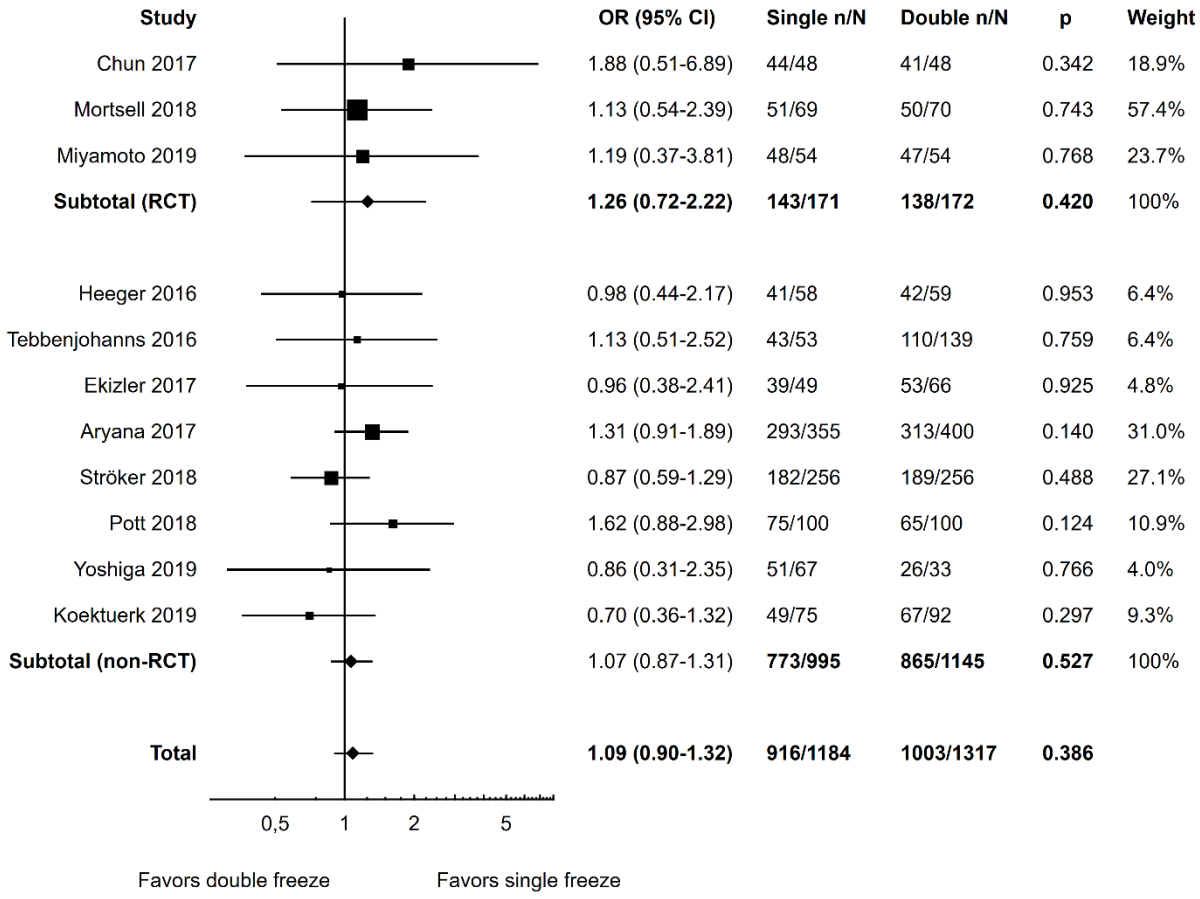


*Update of the MEDLINE search, manual search of reference lists of included studies, contact with key opinion leaders.

RCT, randomized controlled trial.

Figure 2. Forest plots of primary efficacy and safety outcomes.

A. Odds ratios for freedom from any atrial arrhythmia in the 12 month follow-up.

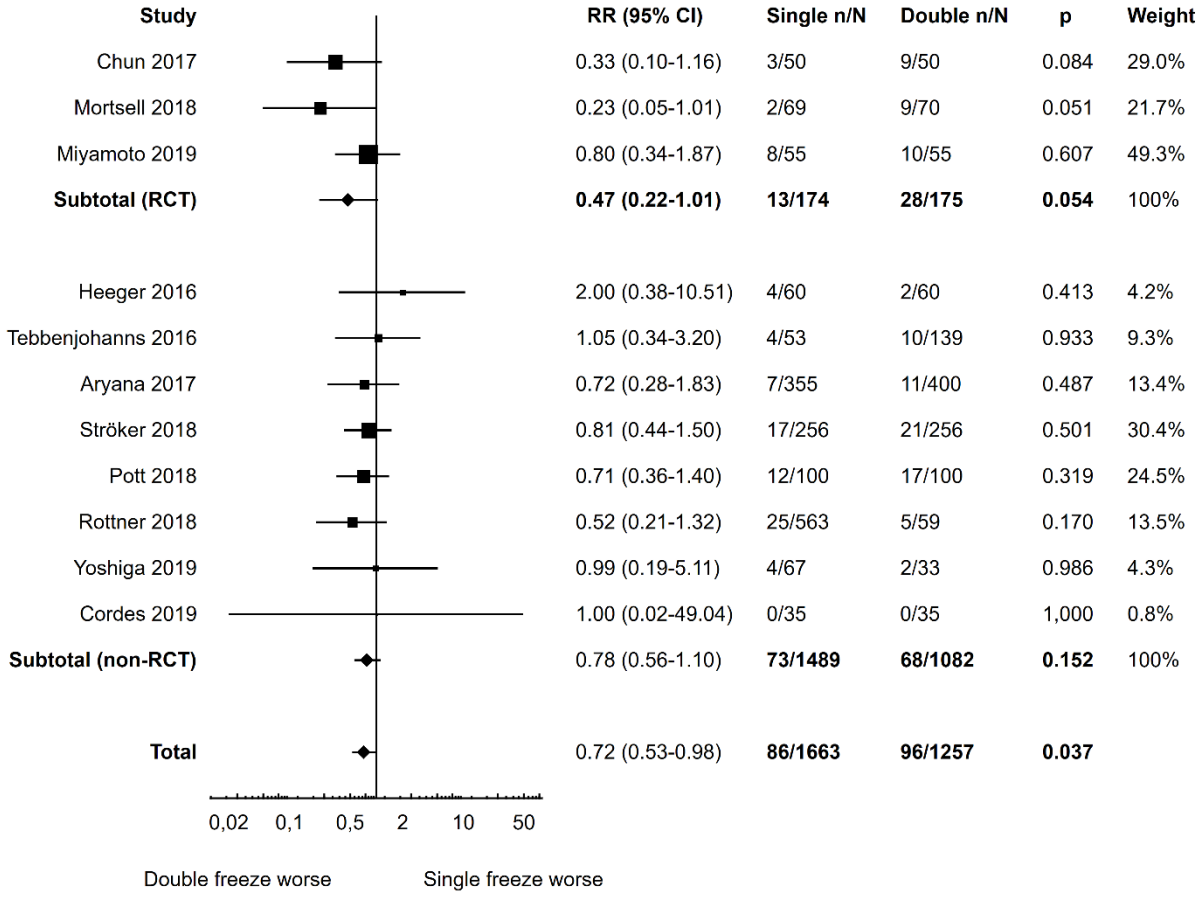


Test for subgroup differences: Z = 0.547 (p=0.585)

Overall heterogeneity: I^2 = 0.00%

95% CI, 95% confidence interval; OR, odds ratio; RCT, randomized clinical trial; Rep., repeated.

B. Relative risk of any adverse event associated in randomized and non-randomized studies.



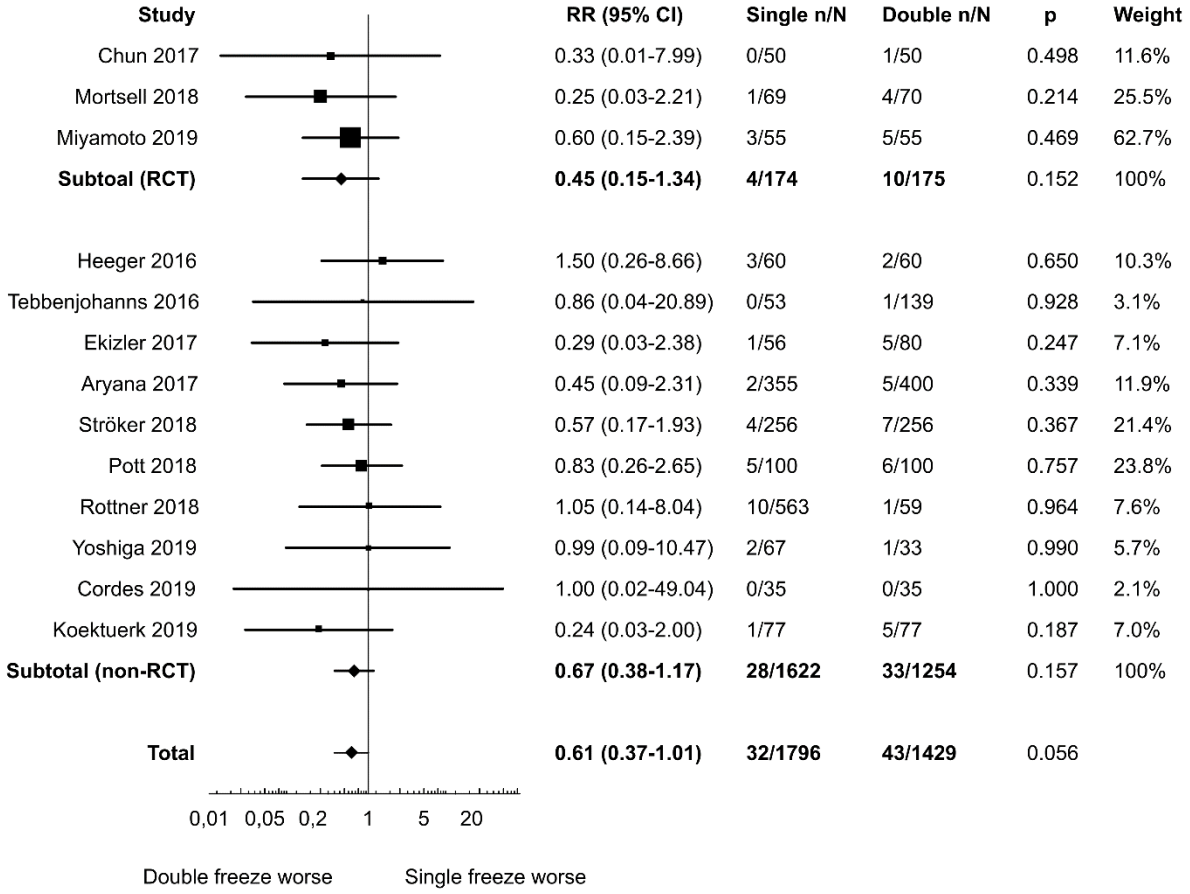
Test for subgroup differences: Z = -1.183 (p=0.238)

Overall heterogeneity: I^2 = 0.00%

95% CI, 95% confidence interval; RCT, randomized clinical trial; Rep., repeated; RR, relative risk.

Figure 3. Forest plots of most important secondary outcomes.

A. Relative risk of ablation-associated persistent phrenic nerve palsy in randomized and non-randomized studies.

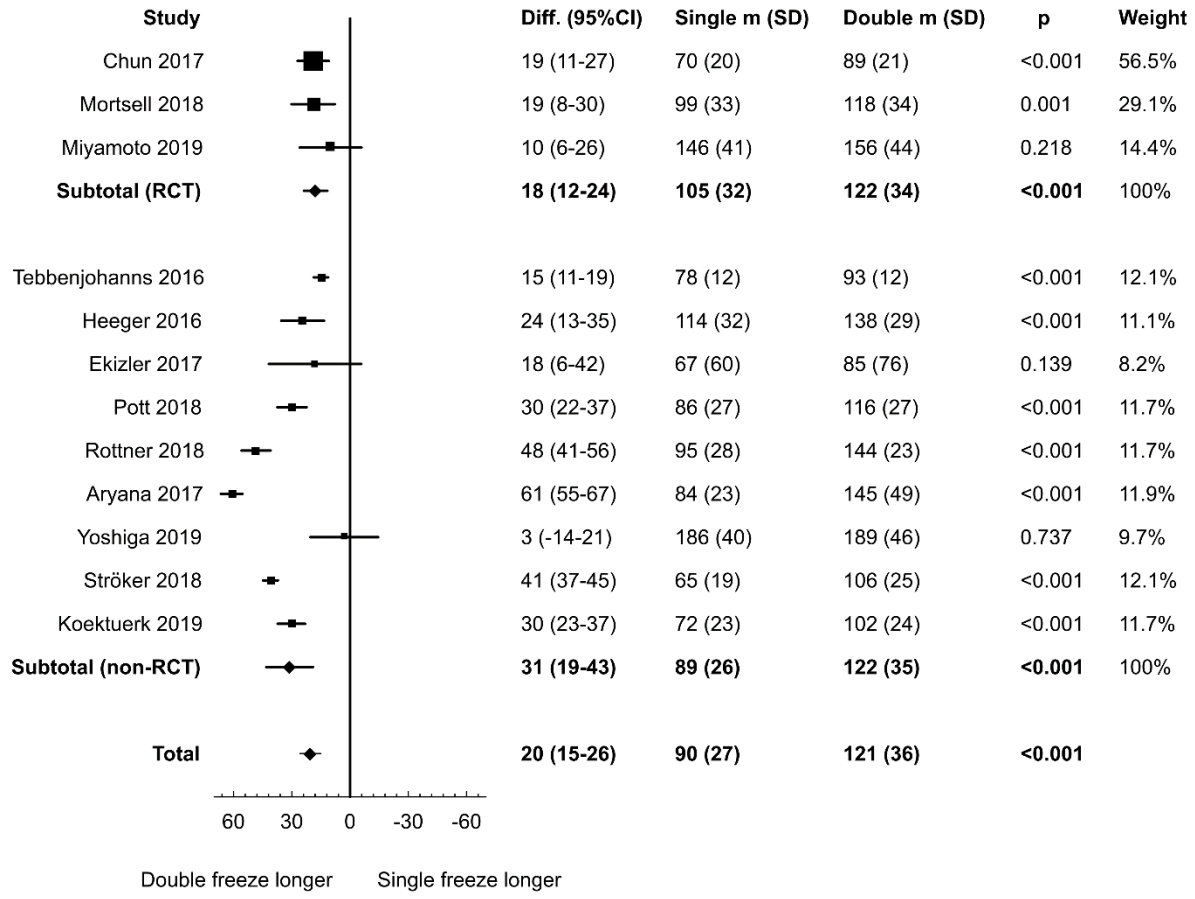


Test for subgroup differences: Z = -0.627 (p=0.531)

Overall heterogeneity: I^2 = 0.00%

95% CI, 95% confidence interval; RCT, randomized clinical trial; Rep., repeated; RR, relative risk.

B. Difference between double freeze and single freeze procedure duration in randomized and non-randomized studies (presented in minutes).



Test for subgroup differences: Z = 1.957 (p=0.050)

Overall heterogeneity: I^2 = 95.72%