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## SPECIALTY SECTION

This article was submitted to Cardiac Rhythmology, a section of the journal Frontiers in Cardiovascular Medicine

RECEIVED 30 September 2022

ACCEPTED 15 February 2023

PUBLISHED 06 March 2023

## CITATION

Shi L-B, Wollner K, Chu S-Y, Wang Y-C, Solheim E, Schuster P and Chen J (2023) Thawing plateau time indicating the duration of phase transition from ice to water is the strongest predictor for long-term durable pulmonary vein isolation after cryoballoon ablation for atrial fibrillation—Data from the index and repeat procedures. *Front. Cardiovasc. Med.* 10:1058485. doi: 10.3389/fcvm.2023.1058485

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# Thawing plateau time indicating the duration of phase transition from ice to water is the strongest predictor for long-term durable pulmonary vein isolation after cryoballoon ablation for atrial fibrillation—Data from the index and repeat procedures

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**Introduction:** This study aimed to clarify the relationship between the durability of pulmonary vein (PV) isolation and the time of phase transition from ice to water indicated by thawing plateau time in a cryoballoon ablation for atrial fibrillation (AF).

**Methods and results:** In this retrospective study, 241 PVs from 71 patients who underwent a repeat AF ablation 526 (IQR: 412, 675) days after a cryoballoon ablation were analyzed. Reconnection was observed in 101 (41.9%) PVs of 53 patients (74.6%). Thawing plateau time ( $Time_{TP}$ ) was defined as the time from 0°C to 10°C inside the balloon in the thawing period. Durable PV isolation was associated with significantly longer  $Time_{TP}$  compared with PV reconnection (26.0 vs. 11.0 s,  $P < 0.001$ ). The proportion of durable PV isolations increased with  $Time_{TP}$  in a dose-proportional manner. The cut point for PV reconnection was  $Time_{TP} < 15$  s with a positive predictive value of 82.1% (sensitivity = 63.4%, specificity = 90.0%) while for durable PV isolation the cut point was  $Time_{TP} > 25$  s with a positive predictive value of 84.6% (sensitivity = 55.0%, specificity = 86.1%). In the analysis of multivariable logistic regression, location of PV reconnection ( $P < 0.01$ ),  $Time_{TP}$  ( $P < 0.05$ ) and thawing plateau integral ( $P < 0.01$ ) were shown as independent predictors for durable PV isolation.

**Conclusion:**  $Time_{TP}$  is an independent predictor for the durability of PV isolation, and it presents in a dose-proportional manner.  $Time_{TP} < 15$  s predicts long-term reconnection while  $Time_{TP} > 25$  s predicts durable PV isolation.

## KEYWORDS

atrial fibrillation, pulmonary vein isolation, cryoballoon, ablation, thawing plateau time

## Introduction

Atrial fibrillation (AF) is one of the most common arrhythmias and electrical isolation of the pulmonary veins (PV) remains the cornerstone of ablation therapy for AF. Recent studies have demonstrated similar clinical outcomes of PV isolation in AF patients achieved by

cryoballoon (CB) ablation compared to radiofrequency ablation (1). However, PV electrical reconnection and collateral tissue injuries are still challenges for this technique (2–6). An earlier study has already suggested that cryoablation effect is not only related to freezing temperature, but also other physical factors (7). During CB ablation, both freezing and thawing processes are essential for acute lesion formation. Procedure-related factors determining PV reconnection have also been investigated (8–13). Previous analyses (9, 11) have shown that thawing time, but not freezing time or temperature, impacts PV reconnection, which occurs mostly during long-term follow-up. However, it is unknown whether the entire or only part of the thawing stage plays an important role. During the thawing stage before the CB is deflated, the CB is still adhering to the myocardia of the PV-atrial junction and the blood flow outside the CB is unchanged. Therefore, the temperature in the CB is mostly affected by the temperature of iced tissue around the CB. At the ice melting point the temperature-time curve briefly plateaus, corresponding to the phase transition to water. The duration of the thawing plateau is closely related to the quantity of ice crystals in the myocardia. Thus, we hypothesize that thawing plateau time may reflect the extent of tissue injuries resulting from the freezing/thawing process. We aimed to investigate whether the time of phase transition, as measured by thawing plateau time, might indicate the lesion size and serve as a predictor for durable PV isolation after CB ablation.

## Methods

### Study population

We performed a retrospective analysis in consecutive patients with AF who underwent a repeat ablation procedure in our institute between August 2013 and April 2021. The first-time CB ablation (index procedure) for symptomatic patients with paroxysmal or persistent AF was conducted between April 2012 and July 2020. Patients without complete index procedure data were excluded from the analysis. This study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of Western Norway.

### Index procedure and follow-up

All patients had taken oral anticoagulation for at least 4 weeks and transoesophageal echocardiography was performed before the procedure. The patients underwent the cryoablation procedure under conscious sedation. Heparin was administered immediately after transseptal access to the left atrium. Activated clotting time was kept between 250 and 350 s throughout the procedure. A multiple-electrode diagnostic mapping catheter (Liverwire™, Abbott) was positioned in the coronary sinus and later replaced in the superior caval vein for stimulation of the phrenic nerve.

A steerable 12-Fr sheath (Flexcath®, Medtronic) was placed in the left atrium and angiography of the PVs was performed. A

second-generation 28-mm-diameter cryoballoon catheter (Arctic Front Advance®, Medtronic) was introduced into the left atrium through the sheath with a circular mapping catheter (Achieve™, Medtronic) inserted in the lumen. The circular mapping catheter was advanced more distally, if necessary, to support the deployed CB stabilizing at the PV ostium. The PV occlusion after balloon inflation was confirmed by venography. Phrenic nerve stimulation at the high output (up to 20 mA), an inspection of diaphragmatic contraction, and monitoring of the diaphragmatic motor action potential were performed to prevent damage during cryoablation in the right PVs. Freezing duration varied from 180 to 300 s for every single application, with or without an extra application after the PV was isolated at the operator's discretion. Electrical PV isolation was demonstrated by the elimination of all PV ostial potentials recorded by the circular catheter, and the entrance and exit conduction block were proven by stimulation maneuvers.

Plasma level of troponin T (TnT) was determined before and 20–24 h after the index procedure, using an electrochemiluminescence immunoassay on a Modular E system (Roche Diagnostics, Mannheim, Germany). The analytical detection limit was 1 ng/L.

Patients without complications were discharged from the hospital within 1 day of the procedure. Oral anticoagulation was continued for at least 3 months. Patients were followed up in an out-patient clinic with routine ECG, 24-h or 7-day ambulatory electrocardiographic monitoring. Detection of any atrial tachyarrhythmia lasting for longer than 30 s was regarded as a clinical recurrence. Antiarrhythmic drugs were recommended to be discontinued after 3 months if no recurrence of atrial tachyarrhythmia was documented.

### Repeat ablation procedure

Symptomatic patients with AF recurrence were referred to a repeat ablation procedure. All patients were treated with an irrigated radiofrequency ablation catheter with the aid of a 3-dimensional mapping system (EnSite NavX, Abbott, or Carto 3, Biosense Webster, Inc). Procedural details were published (14) earlier. All PVs were checked and reconnection was verified by the recording of the PV potentials. Re-isolation was performed in those reconnected PVs until electrical isolation was achieved.

### Data collection and analysis

All procedural data were recorded during the index procedure and analyzed after the repeat ablation procedure. If more than one freezing was applied in a single PV, the application resulting in eventual PV isolation was included for investigation. All temperature values refer to the inner balloon temperature measured by the thermocouple inside the CB. For further analysis, the freezing stage was divided into two contiguous periods: initial freezing (IF, from start of freezing to temperature decreases to  $-40^{\circ}\text{C}$ ) and effective freezing (EF, from temperature

reaches  $-40^{\circ}\text{C}$  to end of freezing). Duration of initial freezing ( $\text{Time}_{\text{IF}}$ ), duration of effective freezing ( $\text{Time}_{\text{EF}}$ ), and nadir temperature were recorded. If  $-40^{\circ}\text{C}$  was never achieved,  $\text{Time}_{\text{IF}}$  was defined as the time from the start of freezing to the point of the nadir temperature. Freezing-temperature-time integrals under  $0^{\circ}\text{C}$  ( $\text{FTTI0}$ ) and  $-40^{\circ}\text{C}$  ( $\text{FTTI40}$ ) were defined as the areas bounded by the temperature curve and  $0^{\circ}\text{C}$  and  $-40^{\circ}\text{C}$ , respectively. The thawing stage was divided into three contiguous periods: initial thawing (IT, from the end of freezing to temperature reaches  $0^{\circ}\text{C}$ ), thawing plateau (TP, from  $0^{\circ}\text{C}$  to  $10^{\circ}\text{C}$ ) and late warming (LW, from  $10^{\circ}\text{C}$  to  $20^{\circ}\text{C}$  when CB deflated). Duration of the corresponding stages ( $\text{Time}_{\text{IT}}$ ,  $\text{Time}_{\text{TP}}$ , and  $\text{Time}_{\text{LW}}$ ) were recorded. Initial thawing integral, thawing plateau integral and late warming integral were defined as the areas bounded by the temperature curve and  $0^{\circ}\text{C}$ ,  $10^{\circ}\text{C}$  and  $20^{\circ}\text{C}$  for IT, TP and LW, respectively. Exemplified temperature-time curves during the cryoablation and the specified measurements are shown in **Figure 1**. The accumulated values of these parameters (referred to below) are the corresponding sums for all PVs.

### Statistical analysis

Continuous variables were presented as mean  $\pm$  standard deviation if normally distributed, or as median and interquartile ranges (IQR) if not normally distributed, after the Shapiro-Wilk test. To compare means of continuous data, a 2-sample *t*-test and Mann-Whitney *U* test were employed for parametric and nonparametric analysis. Categorical values were presented as percentages and analyzed using the  $\chi^2$  test or Fisher's exact test as appropriate. Logistic regression analysis was performed to evaluate the effects of the procedural parameters on the presence of PV reconnection. Variables with a *P*-value over 0.1 in the univariate analysis were removed from the model for further

analysis. The correlation was tested among continuous variables, using Pearson's correlation coefficient for normally distributed data. Otherwise, Spearman's correlation was applied as appropriate. Statistical analysis was performed with SPSS version 26 (IBM, USA). A *P*-value  $<0.05$  was considered statistically significant.

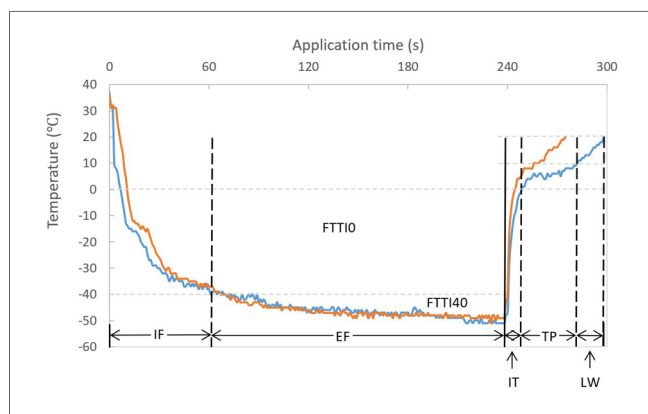
## Results

### Characteristics of the study population, ablation procedures and the pulmonary veins

A total of 71 patients with recurrent AF were enrolled in this study. The baseline characteristics of patients and information of index procedures are shown in **Table 1**. The common left pulmonary vein (LPV) was observed in 12 patients. Data were missed or applications were interrupted by technical problems in 6 PVs and cryoablation failed in index procedure in 4 PVs. A total of 21 PVs were excluded in which more than 3 applications were applied, to avoid the accumulative point-by-point freezing effect. Finally, 241 PVs were analyzed. Reconnection was observed in 101 (41.9%) PVs of 53 patients (74.6%). No extra application was performed in 168 PVs (69.7%). Complete occlusion was achieved in 173 PVs (71.8%), and suboptimal occlusion with minor leakage in 68 (28.2%). Successful isolation was achieved after the first freezing in 184 PVs (76.3%).

### Relationship between the procedural parameters and the durability of PV isolation

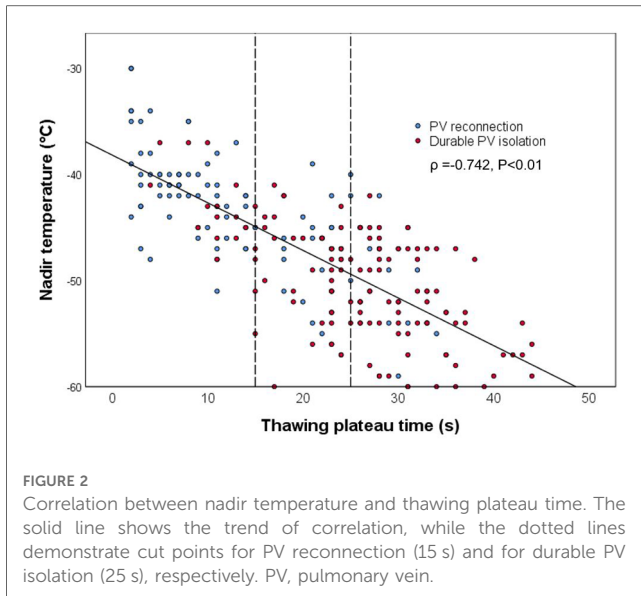
The mean  $\text{Time}_{\text{TP}}$  in this cohort was 23.0 (11.0, 28.0) s, and it correlated with the nadir temperature ( $\rho = -0.742$ ;  $P < 0.01$ ; **Figure 2**). The possible procedural and biophysical parameters associated with PV reconnection during long-term follow-up are listed and compared between groups in **Table 2**. Besides longer  $\text{Time}_{\text{TP}}$  ( $P < 0.01$ ), those cryoablations leading to durable PV



**FIGURE 1** Temperature-time curve during a cryoballoon ablation and descriptions of stages. Similar temperature and time interval during freezing but a short (orange) and long (blue) TP time during thawing are demonstrated. EF, effective freezing; FTTI0, freezing-temperature-time integral under  $0^{\circ}\text{C}$ ; FTTI40, freezing-temperature-time integral under  $-40^{\circ}\text{C}$ ; IF, initial freezing; IT, initial thawing; LW, late warming; TP, thawing plateau. Partition and annotation are based on the blue curve (definition see "Methods").

**TABLE 1** Baseline characteristics of the patients and index cryoablation procedures.

Characteristic ( <i>n</i> = 71)	Value
Age (year)	61.8 $\pm$ 10.5
Male, <i>n</i> (%)	43 (60.6)
Body mass index ( $\text{kg}/\text{m}^2$ )	27.7 $\pm$ 4.8
Hypertension, <i>n</i> (%)	32 (45.1)
Diabetes mellitus, <i>n</i> (%)	2 (2.8)
Coronary heart disease, <i>n</i> (%)	7 (9.9)
Paroxysmal atrial fibrillation, <i>n</i> (%)	42 (59.2)
<b>Information on index procedure</b>	
Accumulated time of freezing (minute)	25.0 (17.0, 32.0)
Fluoroscopy time (minute)	14.0 (10.0, 20.0)
Procedure time (minute)	90.0 (70.0, 120.0)
Duration from index to repeat procedure (day)	526 (412, 675)



isolation showed lower nadir temperature ( $P < 0.01$ ), shorter  $Time_{IF}$  and longer  $Time_{EF}$  ( $P < 0.01$ ), higher  $FTTI0$  and  $FTTI40$  ( $P < 0.01$ ), longer total thawing time and  $Time_{IT}$  ( $P < 0.01$ ), and higher initial thawing integral and thawing plateau integral ( $P < 0.01$ ). Univariate analysis in **Table 2**). In the analysis of

multivariable logistic regression, location of PV reconnection (most notably the left superior PV,  $P < 0.01$ ),  $Time_{TP}$  ( $P < 0.05$ ) and thawing plateau integral ( $P < 0.01$ ) were shown as independent predictors for durable PV isolation (**Table 2**).

The durability of PV isolation increased with  $Time_{TP}$  in a dose-proportional manner (**Figure 3**). The cut point for PV reconnection was  $Time_{TP} < 15$  s with a positive predictive value of 82.1% (sensitivity = 63.4%, specificity = 90.0%), while for durable PV isolation the cut point was  $Time_{TP} > 25$  s with a positive predictive value of 84.6% (sensitivity = 55.0%, specificity = 86.1%). For those applications with a  $Time_{TP}$  between 15 and 25 s, nadir temperature lower than  $-45^{\circ}C$  was employed as a supplementary predictor for durable PV isolation with a positive predictive value of 78.4% (sensitivity = 59.2%, specificity = 68.0%).

The average plasma TnT after the index procedure was  $835.3 \pm 244.1$  ng/L. The correlation between the accumulated  $Time_{TP}$  and TnT level ( $\rho = 0.624$ ,  $P < 0.01$ ) was stronger than the accumulated values of thawing plateau integral,  $Time_{IT}$  and nadir temperature (**Figure 4**).

## Discussion

In this retrospective study, we demonstrated that the durability of PV isolation increased with longer  $Time_{TP}$  during the thawing

**TABLE 2** Association between procedural parameters and durable PV isolation.

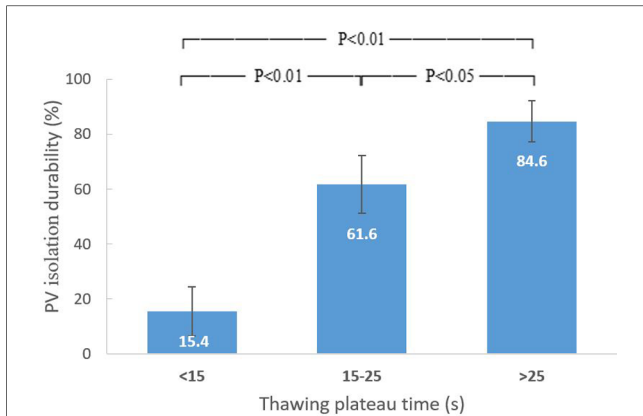
Factors	Durable PV isolation		Univariate <i>P</i> value	Multivariable	
	No ( <i>n</i> = 102)	Yes ( <i>n</i> = 140)		<i>P</i> value	OR (95% CI)
<b>Location of the reconnection, <i>n</i> (%)</b>			0.002	0.005	
Left common PV	5 (55.6%)	4 (44.4%)	0.906		
Left superior PV	14 (24.6%)	43 (75.4%)**	0.002	0.005	4.36 (1.57, 12.07)
Left inferior PV	17 (31.5%)	37 (68.5%)*	0.020		
Right superior PV	34 (54.0%)	29 (46.0%)	0.954		
Right inferior PV	31 (53.4%)	27 (44.6%)	1.000		
Time to isolation (s) ( <i>n</i> = 43 and 85)	50.0 (39.0, 60.0)	48.0 (33.5, 70.0)	†		
Total application number	1 (1, 2)	1 (1, 2)	0.939		
No extra application, <i>n</i> (%) ( <i>n</i> = 168)	71 (42.2%)	97 (57.8%)	0.676		
PV isolation at the first application, <i>n</i> (%) ( <i>n</i> = 184)	75 (40.8%)	109 (59.2%)	0.226		
<b>Freezing stage</b>					
Nadir temperature ( $^{\circ}C$ )	$-43.9 \pm 5.6$	$-50.2 \pm 5.4^{**}$	0.000		
Total freezing duration (s)	$240.1 \pm 35.0$	$228.8 \pm 37.6^{*}$	0.005		
$Time_{IF}$ (s)	78.0 (56.0, 125.0)	54.5 (42.0, 74.0)**	0.000		
$Time_{EF}$ (s)	158.0 (110.5, 184.0)	170.5 (141.0, 191.0)**	0.002		
$FTTI0$ ( $\times 10^3$ , $^{\circ}C \cdot s$ )	8.6 (8.0, 9.6)	9.2 (8.4, 10.3)**	0.002		
$FTTI40$ ( $\times 10^3$ , $^{\circ}C \cdot s$ )	0.3 (0, 0.9)	1.2 (0.6, 1.7)**	0.000		
<b>Thawing stage</b>					
Total thawing time (s)	32.0 (21.0, 45.5)	50.0 (41.0, 60.0)**	0.000		
$Time_{IT}$ (s)	5.0 (4.0, 7.5)	9.0 (7.3, 12.0)**	0.000	0.052	
$Time_{TP}$ (s)	11.0 (6.0, 22.5)	26.0 (22.0, 31.0)**	0.000	0.025	1.08 (1.01, 1.16)
$Time_{LW}$ (s)	13.0 (9.0, 18.5)	16.0 (12.0, 20.0)*	0.126		
Initial thawing integral ( $^{\circ}C \cdot s$ )	101.0 (79.5, 140.0)	171.0 (136.3, 214.5)**	0.000		
Thawing plateau integral ( $^{\circ}C \cdot s$ )	37.0 (17.0, 83.5)	127.5 (86.3, 168.5)**	0.000	0.003	1.02 (1.01, 1.03)
Late warming integral ( $^{\circ}C \cdot s$ )	59.0 (41.0, 85.0)	72.5 (58.0, 88.0)**	0.138		

EF, effective freezing;  $FTTI0$ , freezing-temperature-time integral under  $0^{\circ}C$ ;  $FTTI40$ , freezing-temperature-time integral under  $-40^{\circ}C$ ; IF, initial freezing; IT, initial thawing; LW, late warming; PV, pulmonary vein; TP, thawing plateau.

\* $P < 0.05$ , compared with the group without durable PV isolation.

\*\* $P < 0.01$ , compared with the group without durable PV isolation.

†Not involved in regression analysis because of fewer observation cases.



**FIGURE 3**  
Relation between thawing plateau time and durability of PV isolation. The durability of PV isolation increased with thawing plateau time in a dose-proportional manner. PV, pulmonary vein.

stage in a dose-proportional manner. The accumulated  $Time_{TP}$  positively correlated with TnT level after cryoablation.

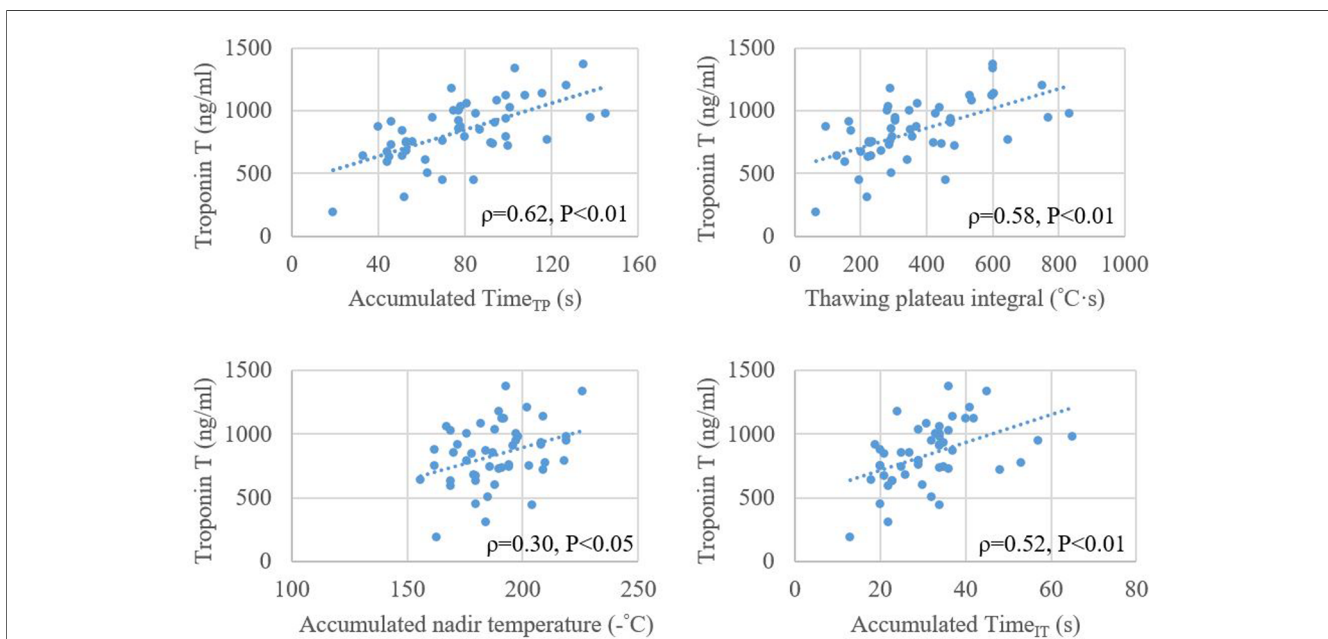
### The thawing plateau reflects the mass of the frozen tissue

Injury to cells during the freezing process is associated with extracellular and intracellular ice formation and the development of an osmotic gradient between the cells and interstitium. If lower temperatures are reached ( $-20^{\circ}C$  to  $-40^{\circ}C$ ), recrystallization may take place during thawing with further structural destruction (15). The freeze and thaw cycle is

important for lesion formation. However, previous studies have shown that biophysical parameters in the thawing stage, but not freezing duration or nadir temperature, may predict PV reconnection (9, 11). Frozen tissue around the CB forms an “ice cap” after the delivery of pressurized cryorefrigerant (nitrous oxide). During the thawing period, the flow of refrigerant has been interrupted and the balloon temperature measured by the thermocouple rises, warmed by the blood flow. When the temperature reaches  $0^{\circ}C$  the ice cap starts to melt. At this point the temperature-time curve exhibits a plateau corresponding to the ice-water phase transition. Energy that would otherwise raise the temperature of the ice-cap, instead provides the latent heat of fusion required to melt the ice. The greater the mass of the frozen tissue, the more energy is required and the longer the time of phase transition. The thawing plateau time therefore serves as a surrogate for the phase transition time. Furthermore, it represents the mass of the ice cap that forms during freezing, which is the critical factor for lesion formation.

### The predictive value of $time_{TP}$ for the durability of PV isolation

The thawing time has been suggested as a predictor for the durability of PV isolation. Ghosh et al. reported that a thawing time of  $\geq 25$  s between  $-30^{\circ}C$  and  $+15^{\circ}C$  may predict permanent PV isolation (9). Aryana et al. indicated a thawing time (defined as time to reach  $0^{\circ}C$ , the same as  $Time_{IT}$  in our study) of  $\geq 10$  s may predict PV isolation durability (11). Our findings further support their arguments. More importantly, we have demonstrated that the most decisive part in the thawing period is



**FIGURE 4**  
Relationship between plasma level of troponin T after cryoballoon ablation and accumulated values of  $time_{TP}$ , thawing plateau integral, nadir temperature, and  $time_{IT}$ .  $Time_{IT}$ , initial thawing time;  $Time_{TP}$ , thawing plateau time.



the thawing plateau and have shown that  $\text{Time}_{\text{TP}}$  is linked to the durability of PV isolation in a dose-proportional manner. We found that  $\text{Time}_{\text{TP}} < 15$  and  $> 25$  s can predict 82.1% of reconnection and 84.6% of durable PV isolation, respectively. Multivariable regression analysis showed  $\text{Time}_{\text{TP}}$  to be the strongest biophysical predictor among others that were significant in the univariate investigation. This might be explained by the following facts and observations. First,  $\text{Time}_{\text{TP}}$  represents the amount of frozen tissue created by the CB ablation, as abovementioned. Second, the balloon temperature at the thawing plateau is much closer to the tissue temperature than at other stages (16). Third, recrystallization frequently takes place in this stage and even prolongs the duration (10, 17). Finally, the rate of thawing has a significant effect on osmotic stress, and slow thawing, in general, is more likely to cause fluid shifts and increase osmotic trauma to the cells (10, 17). This hypothesis was further supported by the correlation between  $\text{Time}_{\text{TP}}$  and TnT. Although TnT is not a predictor for the clinical outcome of CB ablation for AF, it precisely quantifies the magnitude of myocardial injury (18). In the present study, accumulated  $\text{Time}_{\text{TP}}$  positively correlated with the elevation of TnT after the index procedure, which suggested  $\text{Time}_{\text{TP}}$  may serve as a parameter to estimate lesion size. From clinical perspective, the time between 0°C and 10°C is automatically recorded by the system, and it is easy to read out and can be potentially displayed after an application software is developed in the future.

Our results indicate that after a CB ablation with the elimination of the PV potentials, if the  $\text{Time}_{\text{TP}} < 15$  s, an extra application is probably necessary; if the  $\text{Time}_{\text{TP}} > 25$  s, no further application is needed. In the “grey zone” between 15 and 25 s, it is difficult to find a cutoff value. Combined with the nadir temperature not reaching  $-45^\circ\text{C}$ , additional applications should be considered.

Time-to-isolation (TTI) has been proven as a predictor for the durability of PV isolation, and several patient-tailored dosing strategies based on TTI showed similar clinical outcomes with the conventional 240-second procedure (19, 20). We did not analyze the relationship between TTI and the durability of PV isolation in this study. This was due to the unavailability of TTI in many cases, because the operators tended to advance the circular catheter further distal into the PVs to support a stable PV occlusion during freezing.

The CB temperature during freezing has also been studied. In an animal study, a rapid temperature drop to below  $-40^\circ\text{C}$  associated with maximal tissue damage (17). Fürnkranz et al. reported an association between nadir temperature at  $-51^\circ\text{C}$  and acute PV isolation (8). Unfortunately, the predictive value of the nadir temperature during freezing for the durability of PV isolation has not been confirmed by clinical trials. Our data were consistent with the study by Aryana et al. showing that the nadir temperature in the durable PV isolation group was lower than that in the reconnection group, but it was not an independent predictor for durable PV isolation. Cicone et al. reported the achievement of  $-40^\circ\text{C}$  within 60 s as an independent predictor for durable PV isolation (21). Later, a similar finding was

demonstrated in a propensity score-matched case-control study, comparing a dosing strategy based on achieving  $-40^\circ\text{C}$  within 60 s with a real-time signal guiding approach (22). In the present study, we evaluated the time below  $-40^\circ\text{C}$  and FTTI40 which combined the impacts of time and temperature. There was a difference between the reconnection group and the durable group, but neither was an independent predictor. A possible explanation for these findings might be that the balloon temperature does not accurately reflect the tissue temperature during freezing, due to the remote location of the thermocouple. Another could be the interference of delivery of pressurized cryorefrigerant. As shown in Figure 1, two different thawing patterns are demonstrated following the same freezing profile. The finding that the left superior PV was related to higher durability of PV isolation was probably due to simple anatomy, a more favorable fitting angle making it easier to achieve optimal CB ablation. In contrast, the left common and the right PVs were associated with worse outcomes.

## Limitations

First, to be accurate,  $\text{Time}_{\text{TP}}$  should be measured by the precise interval of the thawing plateau. However, the beginning and end of the plateau on the curve are difficult to define in practice and variation exists even for a single observer. We employed the thawing time between 0°C and 10°C to standardize and simplify the measurement. Since the plateau always lies in this narrow range, the difference is little and acceptable. Second,  $\text{Time}_{\text{TP}}$  is a parameter that is acquired after an application, it cannot be used for adjusting the duration during the application. Third, the impacts of TTI and freezing dosage/duration were not analyzed because of the unavailability of data or identical regimen. Fourth, variations of anatomical and pathophysiological features, including the size and angle of the PVs, and mitral regurgitation, may influence freezing and thawing to some extent. We could not conduct subgroup analysis of a single PV because of the limited sample size. Finally, as there was no major complication observed among study patients, we could not verify the predictive value of  $\text{Time}_{\text{TP}}$  for complications.

## Conclusion

$\text{Time}_{\text{TP}}$  is an independent predictor for the durability of PV isolation, and it presents in a dose-proportional manner.  $\text{Time}_{\text{TP}} < 15$  s predicts long-term reconnection while  $\text{Time}_{\text{TP}} > 25$  s predicts durable PV isolation. These findings may guide the regulation of further cryoballoon ablation regimens.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

## Ethics statement

The studies involving human participants were reviewed and approved by Regional Ethics Committee of Western Norway. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

## Author contributions

L-BS, KW, S-YC, Y-CW, ES, PS, JC have significantly contributed to this manuscript. L-BS, KW, JC are responsible for conception of the study. L-BS, KW, S-YC, JC are responsible for design and interpretation of data, as well as revising of the manuscript. L-BS, KW, S-YC, Y-CW, ES, PS, JC are responsible for data collection, data analysis and drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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## Funding

This study was partly supported by Helse Vest, Norway (L-BS), and the Research Council of Norway (S-YC).

## Conflict of interest

JC serves as a consultant for Biosense Webster, Johnson & Johnson, The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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