CLINICAL RESEARCH

Experience of atrial fibrillation ablation in a new cardiac centre using three-dimensional mapping and multielectrode duty-cycled radiofrequency ablation

L’expérience de l’ablation de la fibrillation auriculaire dans un nouveau centre cardiaque en utilisant la cartographie en trois dimensions et l’ablation par radiofréquence multiélectrodes décapolaire circulaire

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KEYWORDS
Atrial fibrillation;
Pulmonary vein isolation;
Three-dimensional mapping;
Multielectrode ablation

Summary
Background. — Catheter ablation is widely used to treat symptomatic atrial fibrillation (AF) refractory to drug therapy; and can be facilitated by a number of different techniques.
Aims. — To evaluate our performance as a new centre for AF ablation and to evaluate the efficacy of different AF ablation techniques.
Methods. — We employed three techniques in AF ablations; the three-dimensional (3D) mapping approaches (CARTO® or Ensite NavX®) or multielectrode catheter duty-cycled radiofrequency ablation (pulmonary vein ablation catheter [PVAC®]). The immediate restoration of sinus rhythm was considered as acute success; while success at 6 months was determined by the maintenance of sinus rhythm on Holter monitoring.
Results. — Between March 2008 and March 2010, 109 patients underwent AF ablations (mean age: 58 years; 72% male). Six-month success rates did not differ significantly between CARTO and NavX (40% vs 38%; P = 0.81), but the PVAC group achieved greater success than the two 3D-mapping groups combined (68% vs 39%; P = 0.004). Paroxysmal AF patients demonstrated greater

Abbreviations: 3D, Three-dimensional; AF, Atrial fibrillation; AFA, Atrial fibrillation ablation; ECG, Electrocardiogram; LA, Left atrium; LVEF, Left ventricular ejection fraction; PV, Pulmonary veins; PVAC, Pulmonary vein ablation catheter; PVI, Pulmonary vein isolation; RF, Radiofrequency; WACA, Wide area circumferential ablation.

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6-month success than persistent AF patients ($P = 0.005$); and although the ratio of paroxysmal to persistent AF patients was slightly higher among the PVAC group, logistic regression confirmed PVAC and paroxysmal AF as predictors of success. Single-procedure success at 6 months was 48%. Including redo-ations, some performed beyond the study period, our overall success rate at 6 months was 65%. Four patients experienced complications, but there were no deaths.

Conclusions. — Despite being a new centre with relative inexperience, we achieved success rates comparable to those of established tertiary centres. PVAC performed significantly better than the two 3D-mapping approaches.

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MOTS CLÉS
La fibrillation auriculaire ; L’isolation des veines pulmonaires ; La cartographie en trois dimensions ; L’ablation multiélectrodes

Résumé Afin d’évaluer notre performance comme un nouveau centre pour la fibrillation auriculaire (FA) dans trois techniques d’ablation, les approches cartographie en trois dimensions (3D) utilisant CARTO® ou NavX® et l’ablation par radiofréquence multiélectrodes décapolaire circulaire à l’aide du cathéter d’ablation des veines pulmonaires (PVAC®).

Méthodes. — Au cours des 24 premiers mois de mars 2008, nous avons réalisé 109 cas d’ablation de la FA. La restauration immédiate du rythme sinusal par ablation a été considéré comme un succès instantané. La réussite à six mois a été déterminée par le maintien du rythme sinusal au monitoring du Holter.

Résultats. — L’âge moyen de notre cohorte était de 58 ans. Le taux de réussite à six mois ne différait pas significativement entre les CARTO® et NavX® (40 % vs 38 %, $p = 0.81$) mais le groupe PVAC a connu plus de succès que les deux combinés groupes en 3D cartographie (68 % vs 39 %, $p = 0.004$). Les patients FA paroxystique (FAP) ont démontré une réussite plus grande de patients atteints de FA tenace à six mois ($p = 0.005$). La proportion des patients paroxystique et de FA tenace n’était pas significativement différente entre les groupes de cartographie 3D et PVAC. La régression logistique a confirmé FAP et PVAC comme prédicteurs de succès. Le succès de une seule procédure à six mois était de 48 %. Y compris les ablations refait au-delà de la période d’étude, notre taux de réussite global à six mois était de 65 %. Nous avons eu quatre complications, mais aucun décès liés à l’arythmie.

Conclusions. — En dépit d’un nouveau centre et de l’inexpérience relative, nous avons atteint les taux de succès comparables à ceux des centres de soins tertiaires établis. PVAC fait significativement mieux que les deux approches de cartographie en 3D. Nous recommandons donc l’ouverture de plusieurs installations d’ablation de la FA et l’adoption de PVAC dans ces centres.

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Background
Catheter ablation of atrial fibrillation (AF) has been widely used in the treatment of symptomatic AF, as well as AF refractory to drug therapy or cardioversion [1]. AF ablation (AFA) involves the isolation of triggers within pulmonary veins (PVs) using radiofrequency (RF) energy [2]. To facilitate this, CARTO (Biosense Webster, Diamond Bar, CA, USA) uses magnetic fields to construct a three-dimensional (3D) geometry of the left atrium (LA), while Ensite NavX (St Jude Medical, St Paul, MN, USA) uses electrical fields to generate 3D maps. Pulmonary vein ablation catheter (PVAC) (Medtronic, Ablation Frontiers, Carlsbad, CA, USA) simplifies PV isolation procedures by using a multielectrode catheter capable of circular mapping and duty-cycled RF energy delivery without 3D guidance.

The aim of this retrospective study was to compare the efficacy of CARTO and NavX with that of PVAC, and to evaluate the performance of our new institution in a 6-month follow-up.

Methods

Patients
Our institution started performing AFAs in March 2008, undertaken by two operators. Patients included those with paroxysmal AF (self-terminating AF lasting < 7 days) or persistent AF (lasting for > 7 days but < 1 year) [3].

Transoesophageal echocardiogram was routinely performed prior to ablation procedures to rule out atrial thrombi. Warfarin was discontinued 5 days prior to the procedures and was replaced with low-molecular-weight heparin. This study formed part of a service evaluation of our new institution.

Ablation procedures
Patients underwent ablation using CARTO, NavX, or PVAC. Techniques were selected on a random basis in our cohort. The ablation procedures were performed under conscious
sedation using diazepam and pethidine or general anaesthesia. Surface and intracardiac electrocardiograms (ECGs) were continuously monitored and recorded. In all three techniques, a steerable multipolar electrode catheter was positioned in the coronary sinus for pacing and recording.

LA access was gained through a transseptal puncture with Brockenbrough (St. Jude Medical) or Endrys (Cook Medical, Bloomington, IN, USA) needle, or through a patent foramen ovale when present. An SL1 (St. Jude Medical), Mullins (Cook Medical), or Channel (Bard, Covington, GA, USA) sheath was then introduced into the LA and infused with 0.9% saline. In the 3D-mapping approaches, a multipolar mapping catheter (Orbiter [Bard], Optima [St. Jude], or Lasso [Biosense Webster]) was passed through this sheath. An initial bolus of 10,000 units of heparin was given, followed by 2500—5000 units of additional boluses to maintain an activated clotting time of 300—400 seconds. Activated clotting times were determined every 30 minutes.

CARTO- and NavX-guided ablations were done with 30 W of RF energy delivered through an ablation catheter (Celsius or Navistar Thermocool [Biosense Webster] or Cool Path Duo [St. Jude Medical]) advanced into the LA through the initial puncture whenever possible. Otherwise, a second transseptal puncture was performed. The catheter tip was maintained <48°C with irrigated saline. Pulmonary vein isolation (PVI) was the primary goal in all cases. Wide area circumferential ablation (WACA) was performed around the PV antrum, proximal to PV ostia, in all patients. No ablations were done within or distal to PV ostia. Successful PVI was confirmed by circular mapping catheters positioned at the PV ostia. Linear ablations in the forms of roof, floor, and coronary sinus lines were done in addition to PVI in cases of persistent AF [4,5]. Burns were performed in the coronary sinus and LA surface of the coronary sinus until definite change in coronary sinus activation suggested electrical isolation. Other linear burns were assessed by juxtaposed atrial pacing and presence of homogenous line of split potentials.

In PVAC ablations, after access into LA was gained, the PVAC was positioned at the antrum of each PV to record local electrical activity. RF energy was applied with a target temperature of 60°C and 4:1 ratio between bipolar and unipolar energy. Multiple applications of RF were delivered to achieve antral isolation. Then, the PVAC was used to map all PV ostia. If PVs appeared to be incompletely isolated, additional RF applications were delivered using the PVAC until PVs were completely disconnected, based on PVAC signals and differential pacing manoeuvres. DC cardioversion was performed if sinus rhythm was not restored following the ablations.

Post-ablation and follow-up

After ablation, patients remained in hospital for ≥24 hours for telemetry monitoring. Patients were anti-coagulated with warfarin for ≥3 months. Low-molecular-weight heparin was administered until a therapeutic international normalized ratio >2 was achieved. A transthoracic echocardiogram was performed the day after the procedure in all cases. A blanking period of 3 months was employed in this study as per international consensus [1]. All patients were followed up in the outpatient clinic at 3, 6, and 12 months. A 12-lead ECG was recorded at each visit; and 24—48 hours of Holter monitoring was performed at each follow-up for all patients.

The successful restoration of sinus rhythm after ablations was considered as acute success. Success on follow-up was determined by the absence of symptoms and a lack of sustained AF of >30 seconds on Holter monitoring. Data were collected from TOMCAT (Philips, Netherlands) and local electronic patient records.

Statistical analyses

Continuous data are presented as mean±SD, and comparisons were performed with Student’s t test. Categorical variables are expressed as frequencies and percentages, and comparisons were performed with χ² analyses or Fisher’s exact test. Multivariable analyses were performed with logistic regression. All P values are two-sided and P < 0.05 indicates statistical significance. Statistical analyses were performed using SPSS (SPSS Inc, Chicago, IL, USA).

Results

Patient characteristics

In the 24 months from March 2008, we performed 117 AFAs. Two cases using cryoballoon (Arctic Front, Cryocath, Quebec, Canada) were excluded from this study. Six patients who had incomplete procedures due to vascular access difficulties such as femoral stenoses were also excluded. Therefore, only 109 patients (mean age 57.8±10 years; 72% male) who received AFAs during the study period were analysed. All patients were symptomatic and refractory to drug treatment prior to ablation. Echocardiographic recordings demonstrated a mean LA size of 41.6±7.3 mm (range 25—58 mm) and left ventricular ejection fraction (LVEF) of 57.1±7.8% (range 26—70%).

We performed 47 ablations with CARTO (43%), 24 with NavX (22%), and 38 with PVAC (35%). There were no significant differences in baseline characteristics between the groups (Table 1). Our cohort was composed of 73 patients with paroxysmal AF (67%) and 36 with persistent AF (33%). The ratio of paroxysmal to persistent AF patients was higher in the PVAC group than in the 3D-mapping groups, but this was not significant (P = 0.052; Table 1).

The mean total procedure time was 221±67 minutes (median 218 minutes; range 60—450 minutes) and the mean fluoroscopy time was 62±29 minutes (median 54 minutes; range 18—165 minutes). PVAC ablations were completed significantly faster than 3D-guided ablations (168 vs 252 minutes; P < 0.0001; Table 1), taking and average of 159 minutes for paroxysmal AF and 206 minutes for persistent AF. As a result of a shorter procedure time, PVAC also demonstrated a shorter duration of fluoroscopy, with an average screening time of 39 minutes, compared to >70 minutes for the 3D systems (P < 0.0001; Table 1).

Predictors of success

We achieved a 96% acute success rate with CARTO, 100% with NavX, and 97% with PVAC (P = 0.42; Table 2). Six-month
success rates were similar in the CARTO and NavX groups (40% vs 38%, \( P = 0.81 \)), therefore, we merged the CARTO and NavX groups together for comparison against PVAC. At 6 months, the PVAC group performed significantly better than the 3D-mapping groups (68% vs 39%; \( P = 0.004; \) Table 2).

All of our paroxysmal AF cases achieved acute success, compared to only 92% of persistent AF patients (\( P = 0.03; \) Table 2). Also, more patients with paroxysmal AF than persistent AF remained in sinus rhythm at 6 months (59% vs 31%; \( P = 0.005; \) Table 2). A subgroup analysis was performed to examine the influence of different AF types on the success of each ablation technique. Significantly, more paroxysmal AF patients in the PVAC group achieved 6-month success than those in the 3D-mapping group (73% vs 49%; \( P = 0.04 \)), but the difference was not significant among persistent AF patients in these groups (50% vs 25%; \( P = 0.21 \) (Table 2).

Age, LA size, LVEF, and gender did not influence success at 6 months; but success attributed to paroxysmal AF and PVAC was confirmed by logistic regression (Table 3).

### Table 1 Baseline clinical and procedural characteristics.

<table>
<thead>
<tr>
<th></th>
<th>CARTO (n = 47)</th>
<th>NavX (n = 24)</th>
<th>3D mapping (CARTO + NavX) (n = 71)</th>
<th>PVAC (n = 38)</th>
<th>( P ) (PVAC vs 3D mapping)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>56.2 ± 10.5</td>
<td>62.2 ± 7.7</td>
<td>58.2 ± 10.0</td>
<td>56.9 ± 10.2</td>
<td>0.51</td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>32 (68)</td>
<td>17 (71)</td>
<td>49 (69)</td>
<td>29 (76)</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>LA size (mm)</strong></td>
<td>40.5 ± 7.5</td>
<td>43.9 ± 6.3</td>
<td>41.5 ± 7.3</td>
<td>42.0 ± 7.5</td>
<td>0.79</td>
</tr>
<tr>
<td><strong>LVEF (%)</strong></td>
<td>58.9 ± 6.4</td>
<td>55.4 ± 6.3</td>
<td>57.9 ± 6.5</td>
<td>55.6 ± 9.8</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>AF type</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>28 (60)</td>
<td>15 (63)</td>
<td>43 (61)</td>
<td>30 (79)</td>
<td></td>
</tr>
<tr>
<td>Persistent</td>
<td>19 (40)</td>
<td>9 (38)</td>
<td>28 (39)</td>
<td>8 (21)</td>
<td></td>
</tr>
<tr>
<td><strong>Procedure time (min)</strong></td>
<td>246 ± 60</td>
<td>265 ± 60</td>
<td>252 ± 60</td>
<td>168 ± 41</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Fluoroscopy time (min)</strong></td>
<td>73 ± 27</td>
<td>79 ± 25</td>
<td>75 ± 26</td>
<td>39 ± 14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td>1 (2)</td>
<td>1 (4)</td>
<td>2 (3)</td>
<td>2 (5)</td>
<td>0.61</td>
</tr>
</tbody>
</table>

Data are mean ± standard deviation or number (%). 3D: three-dimensional; AF: atrial fibrillation; LA: left atrium; LVEF: left ventricular ejection fraction; PVAC: pulmonary vein ablation catheter.

### Table 2 Comparison of CARTO with NavX and 3D mapping with PVAC.

<table>
<thead>
<tr>
<th></th>
<th>CARTO (n = 47)</th>
<th>NavX (n = 24)</th>
<th>( P )</th>
<th>3D mapping (n = 71)</th>
<th>PVAC (n = 38)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute success</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>28/28 (100)</td>
<td>15/15 (100)</td>
<td>0.99</td>
<td>43/43 (100)</td>
<td>30/30 (100)</td>
<td>0.99</td>
</tr>
<tr>
<td>Persistent</td>
<td>17/19 (89)</td>
<td>9/9 (100)</td>
<td>0.31</td>
<td>26/28 (93)</td>
<td>7/8 (88)</td>
<td>0.54</td>
</tr>
<tr>
<td>Combined</td>
<td>45/47 (96)</td>
<td>24/24 (100)</td>
<td>0.55</td>
<td>69/71 (97)</td>
<td>37/38 (97)</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>6-month success</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paroxysmal</td>
<td>16/28 (57)</td>
<td>5/15 (33)</td>
<td>0.14</td>
<td>21/43 (49)</td>
<td>22/30 (73)</td>
<td>0.04</td>
</tr>
<tr>
<td>Persistent</td>
<td>3/19 (16)</td>
<td>4/9 (44)</td>
<td>0.17</td>
<td>7/28 (25)</td>
<td>4/8 (50)</td>
<td>0.21</td>
</tr>
<tr>
<td>Combined</td>
<td>19/47 (40)</td>
<td>9/24 (38)</td>
<td>0.81</td>
<td>28/71 (39)</td>
<td>26/38 (68)</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are number/total (%). 3D: three-dimensional; PVAC: pulmonary vein ablation catheter.

### Single-procedure success

Single-procedure success at 6 months was 48% (Fig. 1). For the purpose of this analysis, redo ablations outside the study period were included. Only one patient received redo ablation for the second time, but continued to remain in sustained AF at 6 months. Single-procedure success combined with success following redo ablations yielded an overall success rate of 65% at 6 months (Fig. 1).

### Complications

Four patients (3.7%) had serious complications: one pericardial tamponade (CARTO); one pulmonary vein puncture (NavX); one pericardial effusion (PVAC), and one periprocedure stroke (PVAC). The difference in complication rates between the 3D-mapping groups and PVAC was not statistically significant (\( P = 0.61; \) Table 1). The patient who suffered from pulmonary vein puncture developed self-limiting haemoptysis during the procedure. Subsequent
investigation was consistent with trauma or perforation of pulmonary veins. The patient with periprocedure stroke developed unilateral weakness. All complications were managed conservatively, apart from pericardial tamponade, which was treated with pericardiocentesis. None of these complications resulted in death, and there were no arrhythmia-related deaths at 6-month follow-up.

No formal screening for PV stenosis was done. However, no patients suffered from symptoms consistent with PV stenosis in our follow-up.

Discussion

In this study, the best outcomes at 6 months were seen in paroxysmal AF patients (versus those with persistent AF) and with PVAC (versus 3D mapping) ablations. While the slightly higher ratio of paroxysmal AF patients in the PVAC group may have influenced the success in this group, we feel that the success of PVAC in this study is largely independent of the type of AF. The imbalance in AF types between the different groups has been accounted for in the multivariable analysis (Table 3). In a subgroup analysis of only paroxysmal AF patients, PVAC also performed significantly better than 3D mapping at 6 months. PVAC was a quicker procedure and the patients were exposed to the least radiation. As a new centre performing AFAs, we were able to achieve a satisfactory overall success rate of 65% at 6 months, taking redo ablations into consideration.

Randomized clinical trials have demonstrated 56–89% success with AFA at 1 year [6–10]. Almost all trials enrolled patients with a mean age <60 years, the majority of whom were men, similar to the baseline characteristics of patients in our study.

Single-procedure efficacy of catheter ablation has been reported to be 38–78% for paroxysmal AF and 22–45% for persistent AF [1]. Recurrences of AF after initial ablation usually require redo ablation in around 20–40% of patients [11]. In our study, 30% of patients received at least one redo ablation. Our success in multiple procedures lied within the published range of 54–80% [1].

At present, there is no consensus in selecting a technique for AFA. Sommer et al. (n = 129) did not find any significant differences in outcomes and fluoroscopy times between their CARTO and NavX groups [12]; and Liu et al. (n = 75) also demonstrated similar results at 7-month follow-up (p = 0.06) [13]. At present, there are no large randomized control trials comparing the outcomes of these two 3D approaches.

Our PVAC follow-up outcomes were comparable to those of recent non-randomized studies. Beukema et al. (n = 102) showed a 61% success with PVAC at 1 year [14]. In a 6-month follow-up on patients with paroxysmal AF, Boersma et al. (n = 98) demonstrated an 83% success with PVAC [15], while Wieczorek et al. (n = 73) showed a success rate of 86% [16].

In a recent randomized study, Bulava et al. (n = 102) did not find any statistically significant differences between the efficacy of PVAC and CARTO at 6-month follow up [17]. AF recurrence was documented in 23 and 29% of patients in the PVAC and CARTO groups, respectively (P = 0.8). Similar to our study, Bulava et al. found significantly shorter procedural and fluoroscopic times in the PVAC group (P < 0.0001) [17]. CLARITY-AF, a larger multicentre randomized controlled trial comparing PVAC with CARTO, is currently in the enrolment phase [18].

Table 3. Univariate and multivariable analyses on predictors of success at 6 months.

<table>
<thead>
<tr>
<th>Comparison or OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (success vs failure)</td>
<td>57.2 vs 58.3</td>
</tr>
<tr>
<td>LA size (mm) (success vs failure)</td>
<td>40.9 vs 42.3</td>
</tr>
<tr>
<td>LVEF (%) (success vs failure)</td>
<td>59.5 vs 57.9</td>
</tr>
<tr>
<td>Male vs female</td>
<td>0.891 (0.391–2.03)</td>
</tr>
<tr>
<td>Paroxysmal vs persistent AF</td>
<td>3.26 (1.41–7.53)</td>
</tr>
<tr>
<td>PVAC vs 3D mapping</td>
<td>3.32 (1.46–7.58)</td>
</tr>
</tbody>
</table>

Multivariable analysis by logistic regression

<table>
<thead>
<tr>
<th>Comparison</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxysmal vs persistent AF</td>
<td>2.83 (1.18–6.78)</td>
<td>0.020</td>
</tr>
<tr>
<td>PVAC vs 3D mapping</td>
<td>2.91 (1.24–6.86)</td>
<td>0.014</td>
</tr>
</tbody>
</table>

3D: three-dimensional; AF: atrial fibrillation; CI: confidence interval; LA: left atrium; LVEF: left ventricular ejection fraction; OR: odds ratio; PVAC: pulmonary vein ablation catheter.

Figure 1. Six-month outcomes on initial and redo ablations.
Tuan et al. (n = 20) did not find any statistically significant differences in recurrence rates between PVC and NavX (5% vs 10%; P > 0.05) [19]. However, they found shorter procedure duration with PVC (P < 0.001), but no differences in fluoroscopy times. At present, there are no large trials comparing PVC with NavX.

Success in PVC-guided ablations could be explained by the better lesion integrity created using circumferential ablation catheters compared to a point-by-point ablation with 3D systems. Better lesion integrity could reduce the chances of a reconnection between trigger foci. Although 3D systems allow precise visualization of mapping and ablation catheters, the construction of these complex maps may require a steeper learning curve, therefore resulting in poorer success in new centres like ours.

Although our study was not a randomized trial, consecutive patients were enrolled. Six patients with incomplete procedures were excluded from this study to avoid any unintended influence they may have had on outcomes. Baseline data were similar in the three groups, so this is not expected to influence the results of the study. Despite it being a retrospective study, we are confident with the accuracy of our data due to the standards of documentation required in our electronic database.

Although the sample size of our study may be small, we consider it to be sufficiently large to represent the number of ablations performed at other new AFA centres. The number of cases performed at our institution increased substantially throughout and after the study period.

Data from 12-month follow-up, when available, will reflect a longer-term success of each technique more accurately. A longer follow-up with the inclusion of cost-benefit analysis would increase the strength of future studies.

Conclusions

Despite being a new centre with relatively inexperienced operators, we have achieved outcomes comparable to those of established tertiary centres. Although 3D visualization by CARTO and NavX allows precise localization of mapping and ablation catheters, the PVC group achieved better outcomes and lower procedural and fluoroscopy times. With a gentler learning curve, PVC could help new centres to succeed in AFA. We would recommend the opening of more AFA facilities, and the use of PVC in these centres. We would also recommend new centres to start with paroxysmal AF ablations as opposed to the more complex persistent AF ablations.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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