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ORIGINAL RESEARCH PAPER

Myocardial Damage, Inflammation, Coagulation, and Platelet Activity During Catheter Ablation Using Radiofrequency and Pulsed-Field Energy

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ABSTRACT

BACKGROUND Pulsed-field ablation (PFA) represents a new, nonthermal ablation energy for the ablation of atrial fibrillation (AF). Ablation energies producing thermal injury are associated with an inflammatory response, platelet activation, and coagulation activation.

OBJECTIVES This study aimed to compare the systemic response in patients undergoing pulmonary vein isolation (PVI) using pulsed-field and radiofrequency (RF) energy.

METHODS Patients with AF indicated for PVI were enrolled and randomly assigned to undergo PVI using RF (CARTO Smart Touch, Biosense Webster) or pulsed-field (Farapulse, Boston-Scientific) energy. Markers of myocardial damage (troponin I), inflammation (interleukin-6), coagulation (D-dimers, fibrin monomers, von Willebrand antigen and factor activity), and platelet activation (P-selectin, activated GpIIb/IIIa antigen) were measured before the procedure (T1), after trans-septal puncture (T2), after completing the ablation in the left atrium (T3), and 1 day after the procedure (T4).

RESULTS A total of 65 patients were enrolled in the pulsed-field ablation (n = 33) and RF ablation (n = 32) groups. Both groups were similar in baseline characteristics (age 60.5 ± 12.7 years vs 64.0 ± 10.7 years; paroxysmal AF: 60.6% vs 62.5% patients). Procedural and left atrial dwelling times were substantially shorter in the PFA group (55:09 ± 11:57 min vs 151:19 ± 41:25 min; $P < 0.001$; 36:00 ± 8:05 min vs 115:58 ± 36:49 min; $P < 0.001$). Peak troponin release was substantially higher in the PFA group (10,102 ng/L [IQR: 8,272-14,207 ng/L] vs 1,006 ng/L [IQR: 603-1,433ng/L]). Both procedures were associated with similar extents (>50%) of platelet and coagulation activation. The proinflammatory response 24 h after the procedure was slightly but nonsignificantly higher in the RF group.

CONCLUSIONS Despite 10 times more myocardial damage, pulsed-field ablation was associated with a similar degree of platelet/coagulation activation, and slightly lower inflammatory response. (The Effect of Pulsed-Field and Radiofrequency Ablation on Platelet, Coagulation and Inflammation; NCT05603637) (J Am Coll Cardiol EP 2023;■:■–■) © 2023 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

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ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

CS = coronary sinus

CTI = cavotricuspid isthmus

DOAC = direct oral coagulants

ICE = intracardiac echocardiography

IL = interleukin

LA = left atrium

LIPV = left inferior pulmonary vein

PAC = procaspase-activating compound

PF = pulsed-field

PFA = pulsed-field ablation

PV = pulmonary vein

PVI = pulmonary vein isolation

RF = radiofrequency

RFA = radiofrequency ablation

RSPV = right superior pulmonary vein

vW = von Willebrand

vWF = von Willebrand factor

Pulmonary vein isolation (PVI) is the most effective treatment modality for atrial fibrillation (AF).[1] The rationale for PVI is to electrically isolate the pulmonary veins (PVs) using different energy sources. The ultimate mechanism for most energy sources, ie, radiofrequency (RF), cryo or laser energy, is similar, ie, it leads to thermal myocardial injury and coagulation necrosis. Therefore, all these energy sources are associated with a proinflammatory response, as well as the activation of platelets and the coagulation cascade.[2]

Pulsed-field (PF) energy is a new energy source for treating AF. It uses high-energy, ultra-short electrical pulses (of microsecond or nanosecond duration) to selectively and irreversibly increase the permeability (electroporation) of cardiomyocyte membranes, which leads to nonthermal cell death. In contrast to cryo- or radiofrequency ablation (RFA), lesions are made using nonthermal destruction of myocardial tissue. In vitro experiments show that the induction of myocardial cell death differs in pulsed-field ablation (PFA); in contrast to thermal energies, cell death is related to the induction of apoptosis in cardiomyocytes. Very importantly, coagulation necrosis induced with thermal energy is always associated with a proinflammatory response and activation of platelets and the coagulation cascade.[3]

The first clinically tested pentaspline catheters for PFA were approved by regulatory authorities for clinical praxis in 2021. Since then, the number of PFA procedures has grown exponentially. Despite the excellent safety profile reported in clinical studies,[4,5] many factors associated with PFA remain unknown. For instance, lower platelet and coagulation activation was expected due to the absence of coagulation necrosis. However, for example, the rate of silent strokes and small cerebral lesions caused by periprocedural microembolization was not lower in PFAs compared with RFAs. The exact myocardial and systemic response to PFA has yet to be described. The study aimed to compare markers of cell damage, platelet activation, and coagulation activation in patients undergoing PVI using PF and RF energy.

METHODS

TRIAL DESIGN. Ours was a prospective, randomized, single-center study to evaluate and compare the systemic effect of PFA vs RFA for AF. The study was approved by the Ethics Committee of the University Hospital Kralovske Vinohrady and was conducted in accordance with the Declaration of Helsinki. Each participant signed informed content before enrollment. The study was registered on clinicaltrials.gov (NCT05603637).

STUDY PARTICIPANTS. Patients with symptomatic paroxysmal or nonparoxysmal AF indicated for a first ablation were recruited and randomized to ablation using PFA or RFA. Several comorbidities, such as chronic heart failure or chronic obstructive pulmonary disease, are associated with higher proinflammatory activity or greater activation of platelets or coagulation systems. Therefore, such comorbidities were exclusion criteria for enrollment. Inclusion criteria were the presence of symptomatic paroxysmal or nonparoxysmal AF, age >18 years, and signed informed content. The exclusion criteria were heart failure with reduced ejection fraction (irrespective of NYHA functional class status), heart failure with preserved ejection fraction worse than NYHA functional class II, treated chronic obstructive pulmonary disease, history of left atrial ablation, presence of malignant, any hematologic, or systemic inflammatory disease, and treatment with antiplatelet agents (aspirin or adenosine-diphosphate antagonists). Because directl oral anticoagulants (DOAC) distinctively affect coagulation parameters compared with warfarin,[6] patients prescribed warfarin were also excluded. The randomization was done using a web-based electronic system and was stratified by AF type (paroxysmal vs nonparoxysmal AF), left atrial size, and age. Patients prescribed DOACs before the procedure were given their last dose of DOAC in the evening before (apixaban, dabigatran) or in the morning (rivaroxaban) on the day before the procedure.

ABLATION PROCEDURES. All procedures were performed under analgosedation with sufentanil, midazolam, propofol, and ketamine; sedation was mild in RFA patients and deep in PFA patients. Femoral

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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venous access was achieved using ultrasound guidance. In patients randomized to RFA, 2 sheaths (F6 and F11) were inserted in the left femoral vein, 1 for a 10-F phased-array intracardiac echocardiography (ICE) probe (AcuNav, Siemens) and the other for a decapolar catheter, which was inserted into the coronary sinus (CS) (Dynamic XT Catheter, Boston Scientific,). In patients randomized to PFA, the left femoral vein was used for the decapolar CS catheter only in patients for whom a cavotricuspid or mitral isthmus ablation was planned; otherwise, the left femoral vein was left untouched. In all patients, 2 sheaths were inserted in the right femoral vein: 1 11-F sheath for the ICE and an 8-F for the trans septal puncture in the PFA group, and 2 8-F sheaths, both for a trans septal puncture, in the RFA group.

Trans septal punctures were performed using a nonsteerable trans septal sheath (SL1, Abbott) under ICE guidance. In PFA patients, the SL1 sheath was replaced by a 13-F deflectable trans septal sheath (Faradrive, Boston Scientific) using the over-the-wire technique. Peri-procedural anticoagulation was managed using heparin at a dose of 5,000 IU before the trans septal puncture in both groups; another bolus of 5,000-10,000 IU was given immediately after the trans septal puncture. The activated clotting time was assessed every 10 min with a target value of 300 s; when this target was achieved, further activated clotting time checks were done every 20 min in the RFA group.

Patients in the PFA group underwent ablation using a pentaspline catheter (Farawave, Boston Scientific, Inc) with a PFA generator (Farastar, Boston Scientific, Inc). Ablations were performed using a biphasic bipolar waveform in the following order: 4 applications with the ablation catheter in the "basket" configuration and 4 applications with the ablation catheter in the "flower" configuration for each PV ostium. For the right superior PV, 2 additional applications in the flower configuration were used on the anterior aspect of the right superior PV. All PVs were checked for entrance (and exit, in sinus rhythm) block; if the block was not present, additional PF applications were made. In patients with nonparoxysmal fibrillation, ablation of the posterior wall and mitral isthmus was conducted at the discretion of the treating physician. Regarding the posterior wall, 2 applications were delivered at each overlapping posterior wall location to connect the right superior with the left superior PV and the right inferior with the left inferior pulmonary vein (LIPV). In nonparoxysmal patients, the mitral isthmus was ablated between the LIPV and the mitral annulus using 4-8 PF applications.

In patients randomized in the RFA group, all procedures were done using the CARTO 3 mapping system (Biosense-Webster). A circular mapping catheter (Lasso, Biosense-Webster) was inserted in all PVs for verification of entrance and exit blocks; in nonparoxysmal patients, left atrium (LA) mapping was done using an Octarey Mapping Catheter (Biosense-Webster). A 3.5-mm irrigated-tip CARTO catheter (ThermoCool SmartTouch, Biosense-Webster) was also used for mapping and ablation. Ablations were done using an ablation index with a target value of 400-450 on the anterior and superior aspects of the PVs and 350-400 and the posterior and inferior aspects, with RF ablation power of 30-35 W on the anterior/superior, and 25-30 W on the posterior/inferior parts of PVs. The surface areas of the isolated left- and right-sided veins were quantified. The CARTO system enables the calculation of the surface area from manually selected points. Because no voltage maps were done after ablations, the isolated areas were depicted through the middle of the ablation points. In nonparoxysmal patients, additional ablations were added at the discretion of the surgeon. These additional ablations involved the ablation of fractionated signals within the scar areas in the LA, the ablation of complex fractionated signals, and linear ablations.

BLOOD SAMPLING. All blood samples were taken while patients were in a fasting state. Four blood samples were drawn: 1) at the beginning of the procedure from the femoral vein before any intravenous anticoagulation was given; 2) from the LA immediately after the transseptal puncture (the first transseptal puncture in the RFA group); 3) from the LA at the end of the left-atrial ablations; and 4) in the morning on the day after the ablation (the antecubital vein). In all 4 samples, the first 5 mL of blood was discarded. The samples from antecubital veins were drawn without tourniquets. Samples for flow cytometry, troponin I, interleukin (IL)-6, and coagulation markers were analyzed immediately or within 3 h of collection.

BIOMARKER ANALYSIS. Markers of myocardial necrosis. Troponin I was quantified using a commercial Atellica IM High-Sensitivity Troponin I (TnIH) TR chemiluminescence test in a Siemens Atellica Solution Analyzer (Siemens Healthineers). The institutional physiological reference range for females was set as 0-34 ng/L and for males 0-53 ng/L, with a cutoff value of 2.5 ng/L.

Markers of platelet activation. Samples of citrated whole blood were used to determine the expression of platelet surface markers CD41a/CD61 (gpIIb/

IIIa complex), procaspase-activating compound (PAC)-1 (extracellular activation-induced conformational epitope on gpIIb/IIIa complex), CD62P (P-selectin), and CD42b (GPIbα) based on flow cytometry. Flow cytometry analysis was performed within 3 h of blood collection without adding ex vivo platelet agonists. Five microliters of citrated whole blood was diluted 1:9 in Tris-buffered saline (10 mmol/L TRIS, 0.15 mol/L sodium chloride) and then stained for 30 min with the following monoclonal antibodies: fluorescein isothiocyanate–conjugated anti-PAC1 (clone SP2), BV510-conjugated anti-CD41a (clone VI-PL2), BV510-conjugated anti-CD61 (clone HIP8), phycoerythrin-conjugated anti-CD62P (clone AK-4), and activated allophycocyanin-conjugated anti-CD42b (clone HIP1). All antibodies were purchased from Beckton Dickinson Biosciences. After incubation, samples were fixed using 400 μL of 1% paraformaldehyde solution. Platelets were acquired using a Navios EX (Beckman Coulter). Forward scatter and side scatter were set at a logarithmic gain, and platelets were identified based on the size and expression of CD41a and CD61. In each sample, platelets were further identified using the platelet-specific CD42b antibody. Expression of CD62P and PAC-1 was then evaluated on CD41a/CD61$^+$ CD42b$^+$ platelets.

Markers of coagulation. D-dimers were determined using a commercial INNOVANCE D-Dimer immunoturbidimetric assay (Siemens Healthineers) in a Sysmex CS-5100 (Sysmex Corporation) automated blood coagulation analyzer. The institutional physiological reference range was set to 0-500 ng/L, with an institutional cut-off set to 190 ng/L. Fibrin monomers were determined using a commercial STA-Liatest FM immunoturbidimetric test (Stago) in a Stago STA Compact Hemostasis System (Stago), with a reference range set to 0-5 mg/l and with an institutional cut-off set to 2.5 mg/L. Von Willebrand (vW) antigen and von Willebrand factor (vWF) activity were measured using commercial immunoturbidimetric assays (ie, von Willebrand Antigen-hemo-RGT and INNOVANCE VWF Ac assay) in a Sysmex CS-5100 (Sysmex Corporation) automated blood coagulation analyzer. Both assays were purchased from Siemens Healthineers. Results were reported as percentages, with the institutional reference range for vWF activity being 50%-150% and vW antigen being 50%-150%.

Markers of inflammation. IL-6 was analyzed using commercial Atellica IM IL-6 chemiluminescence tests in a Siemens Atellica Solution Analyzer (Siemens Healthineers). The institutional physiological reference range was 0-4.4 ng/L, with the cut-off value set to 2.7 ng/L.

STATISTICAL ANALYSIS AND POWER CALCULATION. Standard descriptive statistics were used for the analysis. Binary or categorical parameters of patients were characterized by absolute and relative frequencies, whereas continuous parameters were described as mean ± SD. Because most markers did not follow a normal probability distribution, the median (IQR) were used to describe those parameters. The Mann-Whitney test was used to assess the statistical significance of the differences between groups (PFA vs RFA) for continuous parameters and the Fisher exact test for categorical parameters. In related samples, Friedman 2-way analysis of variance by ranks with a Bonferroni correction for post hoc testing was used for evaluating the progression of individual markers. However, when only baseline (T1) and discharge (T4) marker levels were assessed (specifically for troponin I hs and IL-6), the related samples Wilcoxon signed-rank test was used. Univariate and multivariate stepwise linear regression was performed to predict the maximum biomarker value obtained during the procedure with selected clinical and procedural characteristics used as predictors. A log transformation of dependent variable was applied where appropriate.

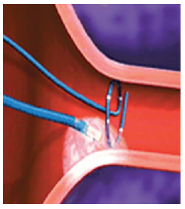
The level of statistical significance used in all analyses was $P = 0.05$. Analyses were performed in SPSS 28.0.1.1 (IBM Corporation).

Based on a previous observations of troponin levels between PFA and RFA,[7] we assumed at least a 50% relative increase in troponin levels after PFA. So far, no studies have compared platelet activation or inflammatory response during RFA and PFA procedures. Therefore, based only on the known physical principles and the expected biological response to PFA and RFA, which cause entirely different kinds of cell death and could lead to differing degrees of inflammatory response, we expect 50% less activation of proinflammatory markers and markers of platelet and coagulation activation in PFA patients. Because the concentrations of troponin, markers of platelet activation, and inflammation after RFA in published studies were reported as mean ± SD, power calculation was done using *t* test. Using a 2-tailed value of 0.05 and a power of 80% resulted in a sample size of 25 patients per group for troponin concentrations, and 30 patients per group for markers of platelet activation and inflammation. No power calculation was done for markers of coagulation.

RESULTS

PATIENTS AND PROCEDURES. Sixty-five patients were enrolled: 33 in the PFA group and 32 in the RFA group (**Central Illustration**). The baseline

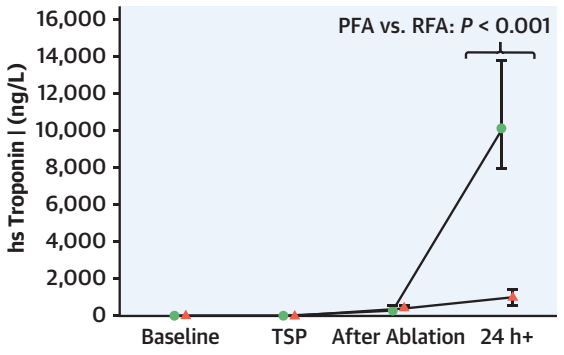
CENTRAL ILLUSTRATION Summary of Results

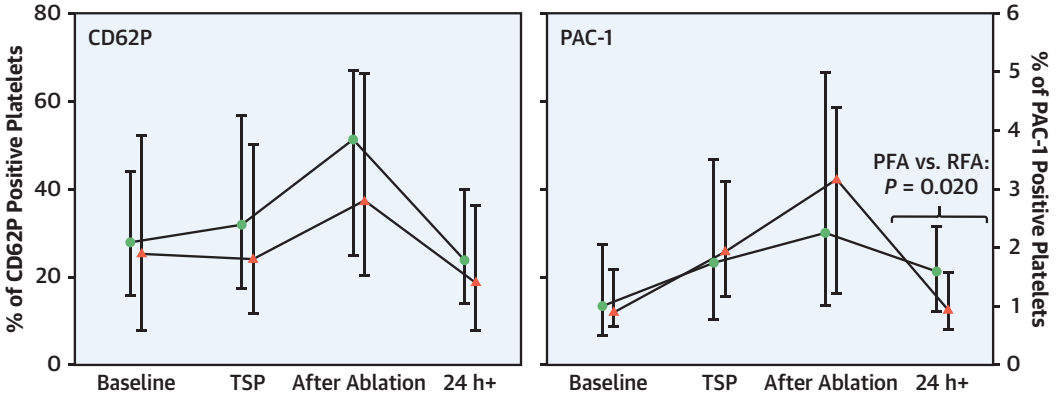


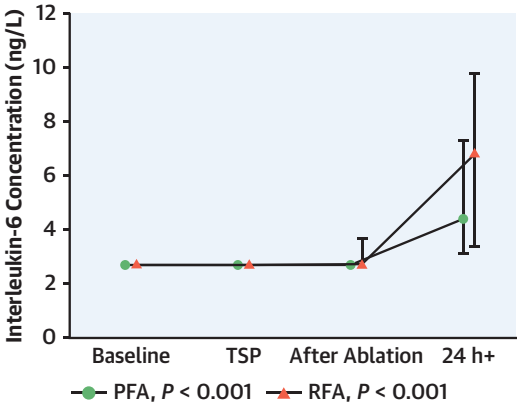
65 patients with atrial fibrillation
(age 62.2 ± 11.8 yrs, CHA_2DS_2VASc 2.2 ± 1.8)

RANDOMIZED

- Blood samples drawn before the procedure, after transeptal puncture, after the completion of ablation, 24h after the procedure
- Assessment of markers of myocardial damage, platelet activity (CD62P, PAC-1), coagulation (von Willebrand activity, D-dimers, fibrin monomers) and inflammation (IL-6)







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Figures display the concentration of troponin, platelet activity determined as the percentage of CD62P and PAC-1 positive platelets, and the concentration of IL-6 during PVI performed by PF and RF energy. PFA = green circles; RFA = red triangles. IL = interleukin; PAC- = procaspase-activating compound; PF = pulsed-field; PFA = pulsed-field ablation; PVI = pulmonary vein isolation; RF = radiofrequency; RFA = radiofrequency ablation.

TABLE 1 Baseline Clinical Characteristics of Patients

	PFA Group (n = 33)	RFA Group (n = 32)	*P* Value
Age (y)	60.5 ± 12.7	64.0 ± 10.7	0.23
Female	12 (36.4)	7 (21.9)	0.27
Hypertension	22 (66.7)	22 (68.8)	0.99
Diabetes mellitus	6 (18.2)	10 (31.3)	0.26
Body mass index, kg/m^2	28.9 ± 4.9	30.5 ± 5.2	0.20
Coronary artery disease	4 (12.1)	2 (6.3)	0.67
CHA_2DS_2VASc score	2.39 ± 1.68	2.28 ± 1.69	0.79
Stroke	3 (9.1)	1 (3.1)	0.61
AF type			
Paroxysmal	20 (60.6)	20 (62.5)	0.99
Persistent	10 (30.3)	9 (28.1)	
Long-lasting persistent	3 (9.1)	3 (9.4)	
Electrical cardioversion	18 (54.6)	17 (53.1)	0.99
Echocardiography			
LVEF (%)	58.7 ± 4.8	58.2 ± 4.9	0.75
LA size (mm)	41.7 ± 5.8	43.3 ± 5.1	0.25
Medication			
No. of antihypertensive drugs	1.97 ± 1.31	1.75 ± 1.32	0.41
Current AAD use	15 (45.5)	17 (53.1)	0.62
Current anticoagulation	32 (97.0)	30 (93.8)	0.61
Rivaroxaban	20 (60.6)	21 (65.6)	0.79
Apixaban	11 (33.3)	8 (25.0)	
Dabigatran	1 (3.0)	1 (3.1)	

Values are mean ± SD or n (%).

AAD = antiarrhythmic drug; AF = atrial fibrillation; LA = left atrium; LVEF = left ventricular ejection fraction; PFA = pulsed-field ablation; RFA = radiofrequency ablation.

TABLE 2 Procedural Characteristics

	PFA Group (n = 33)	RFA Group (n = 32)	*P* Value
Procedure duration (min)	55:09 ± 11:57	151:19 ± 41:25	<0.001
Procedure duration, PVI-only patients (min)	52:06 ± 11:19	136:23 ± 27:54	<0.001
Fluoroscopy duration (min)	9.4 ± 3.3	6.8 ± 2.4	<0.001
LA dwelling time (min)	36:00 ± 8:05	115:58 ± 36:49	<0.001
Total heparin dose (IU)	15,697 ± 2,023	16,565 ± 4,185	0.50
Maximum ACT (s)	357 ± 37	338 ± 24	0.02
Midazolam dose (mg)[a]	2.4 ± 1.1	1.8 ± 1.4[a]	0.01
Sufentanil dose (mg)	9.7 ± 2.8	16.3 ± 6.4	<0.001
Propofol dose (mg)[b]	277.4 ± 144.4	102.2 ± 81.1[b]	<0.001
Electrical cardioversion	8 (24)	9 (28)	0.78
SR at discharge	33 (100)	32 (100)	1.00
Ablation			
Total no. of LA PFA applications)/ total LA ablation time (min)	43.1 ± 11.8	31.7 ± 10.1	NA
No. of PFA applications on PVs (no)/PV ablation time (min)	34.6 ± 2.6	28.4 ± 8.4	NA
No. of patients with left atrial ablation outside PVs	12 (36.4)	11 (34.4)	1.00
No. of patients with CTI ablation	4 (12.1)	3 (9.4)	1.00
No. of patients with PVI only (without additional LA ablations or CTI ablation)	17 (51.5)	19 (59.4)	0.62

Values are mean ± SD or n (%). [a]Midazolam was used in 32 (97%) PFA and 18 (56.2%) RFA patients. [b]Propofol was used in 33 (100%) PFA and 18 (56.2%) RFA patients. [c]1 patient in the RFA group underwent both additional LA ablation and CTI ablation.

ACT = activated clotting time; CTI = cavotricuspid isthmus; NA = not available; PV = pulmonary vein; PVI = pulmonary vein isolation; SR = sinus rhythm; other abbreviations as in Table 1.

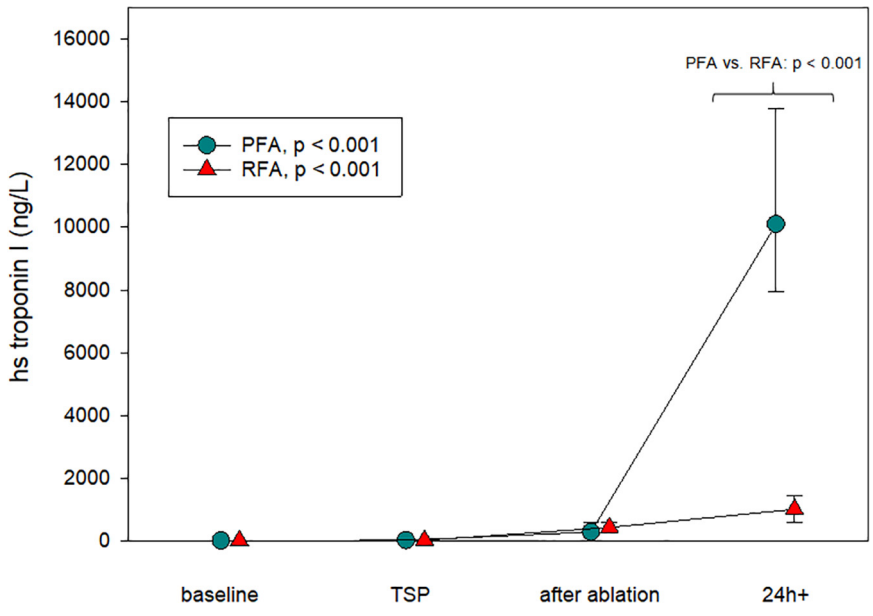
characteristics are shown in Table 1; both groups were similar in important baseline clinical characteristics. Twenty (60.6%) patients had paroxysmal AF and 13 (39.4%) had nonparoxysmal AF in the PFA group; in the RFA group, the proportion was 20 (62.5%) and 12 (37.5%) patients, respectively (Table 1). LA area was also similar between groups (23.0 ± 5.4 cm^2 PFA group vs 23.9 ± 5.5 cm^2 RFA group; $P = 0.54$).

Important procedural characteristics are shown in Table 2. As expected, the procedure was substantially shorter in the PFA group (55:09 ± 11.57 min vs 151:19 ± 41.25 min; $P < 0.001$), as was LA dwelling time (36:00 ± 8:05 min vs 115:58 ± 36:49 min; $P < 0.001$). Procedural durations were also shorter in PVI-only PFA patients compared with PVI-only RFA patients (52:06 ± 11.19 min vs 136:23 ± 27:54 min; $P < 0.001$). Electrical cardioversion was done in 8 (24.3%) patients in the PFA group, and in 9 (28.1%) patients in the RFA group. Blood samples (the "after ablation" samples) were drawn before electrical cardioversion in all 8 PFA patients. In the RFA group, blood samples were drawn before electrical cardioversion in 6 patients, and after cardioversion in 3 patients.

A local hematoma in a patient with RFA prolonged their hospitalization by 1 day but did not require blood products, surgical revision, or other procedures. Neither group had other complications (eg, cardiac tamponade, phrenic nerve palsy, stroke, or femoral access complications). Procedures were done under analgosedation, which was substantially deeper in the PFA group (Table 2). Although the dose of midazolam was higher in the RFA group, the doses of sufentanil and propofol were higher in the PFA group (Table 2). Propofol was used in 18 (56.2%) patients in the RFA group compared with 33 (100%) patients in the PFA group; the average dose of propofol in the patients with PFA was more than twice as high (Table 2). Additionally, 16 (48.5%) patients in the PFA group (but none in the RFA group) received ketamine (34.1 ± 9.2 mg/procedure). The surface areas of the isolated veins were quantified in the RFA group. The isolated areas were 5.67 ± 1.02 cm^2 for left-sided veins, 2.89 ± 0.51 cm^2 for right superior pulmonary vein (RSPV), and 2.32 ± 0.47 cm^2 for LIPV (left-sided PVs were isolated by a single circular ablation, right-sided veins were ablated separately). Because 3D mapping was not used in the PFA group, the ablated areas in the PFA group could not be quantified.

MARKERS OF MYOCARDIAL DAMAGE. Compared with baseline concentrations, there was a significant increase in high-sensitivity troponin I in both groups 24 hours after the procedure ($P < 0.001$). However,

FIGURE 1 The Time Course of High-Sensitivity Troponin I Concentrations During PFA and RFA



Figures display medians with IQRs (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. RFA = radiofrequency ablation; PFA = pulsed-field ablation.

the increase was significantly higher in the PFA (Figure 1) compared with the RFA group. The maximum troponin I concentrations were obtained 24 hours postprocedure in patients with PFA (median: 10,102; IQR: 8,272-14,207) and were almost 10 times higher than those in patients with RFA (median: 1,006; IQR: 603-1,433) with $P < 0.001$.

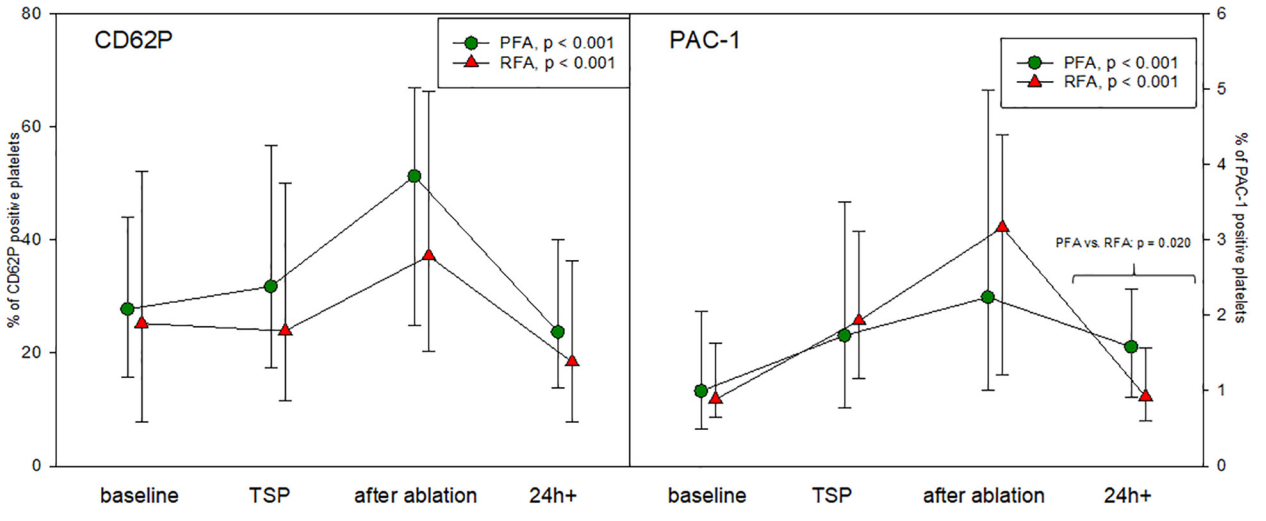
MARKERS OF PLATELET ACTIVATION. The time course of CD62P (P-selectin) during the procedure was very similar in both groups. There were similar significant increases by ~50% in CD62P after trans-septal punctures and at the end of the ablation; a return to preprocedural values occurred the following day in both groups ($P < 0.001$ for each group) (Figure 2). Similarly, a significant increase in PAC-1 was observed during the procedure in both groups ($P < 0.001$ for each group) (Figure 2). The time course of PAC-1 (ie, an increase during the procedure and a subsequent decrease to preprocedure values 1 day after the procedure) was very similar between groups. The only exception was the 24-hour value, which was higher in the PFA group than in the RFA group (Figure 2).

MARKERS OF COAGULATION ACTIVITY. There were no significant changes in the coagulation markers (ie, D-dimers, fibrin monomers, vW antigen, or vWF activity) between groups (Figure 3). There were no differences in D-dimer concentrations during the procedure in either group ($P = 0.46$ in the PFA or 0.43 in the RFA, respectively). The time course of fibrin monomers changed significantly during the procedure in both groups (Figure 3), with a slight decrease after trans-septal punctures, but without differences between groups. The time course of vW antigen and vWF activity changed significantly in both groups (Figure 3), with gradual increases in both vW antigen and vWF activity during the procedure, again without differences between groups.

MARKER OF INFLAMMATION. The concentrations of IL-6 increased significantly 24 hours postprocedure in both groups ($P < 0.001$) (Figure 4). The increase was slightly higher in the RFA group, although the difference did not reach statistical significance ($P = 0.07$).

SUBGROUP ANALYSIS AND LINEAR REGRESSION. In the PFA group, 17 patients underwent pulmonary vein isolation only (PVI-only), and 16 patients underwent left atrial ablations or cavotricuspid isthmus (CTI) ablations in addition to PVI (PVI-plus). The time-course of concentrations for all measured biomarkers was compared between PVI-only and PVI-plus subgroups of patients with PFA. The time course of D-dimers was not significant in either subgroup (but it was also not significant in the main analysis). The time course of all other analyzed

FIGURE 2 The Time Course of Markers of Platelet Activation CD62P and PAC-1 During PVI Using PF and RF Energy



Figures display the medians with IQRs and express the percentage of antigen-positive platelets (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. The difference between groups at time point 24 h+ is shown. PAC = procaspase-activating compound; PF = pulsed-field; PVI = pulmonary vein isolation; RF = radiofrequency; other abbreviations as in Figure 1.

biomarkers differed significantly during ablation in each subgroup, except for fibrin monomers ($P = 0.18$) and PAC-1 ($P = 0.058$) in the PVI-only PFA group. Importantly, no differences between PVI-only vs PVI-plus patients were found; additionally, the troponin I concentration did not reach statistical significance between PVI-only and PVI-plus PFA subgroups.

A similar analysis was performed for patients with RFA. In the RFA group, 19 patients only underwent PVI, and 13 patients had additional left atrial or CTI ablations. The time course of D-dimers was not significant in either subgroup (but was also not significant in the main analysis). The time course of all other analyzed biomarkers differed significantly in each subgroup, with exceptions of vW antigen ($P = 0.21$) and vW activity ($P = 0.70$) in PVI-only patients and fibrin monomers ($P = 0.15$) and CD6P ($P = 0.082$) in PVI-plus RFA patients. As with PFA patients, no differences between PVI-only vs PVI-plus RFA patients were found.

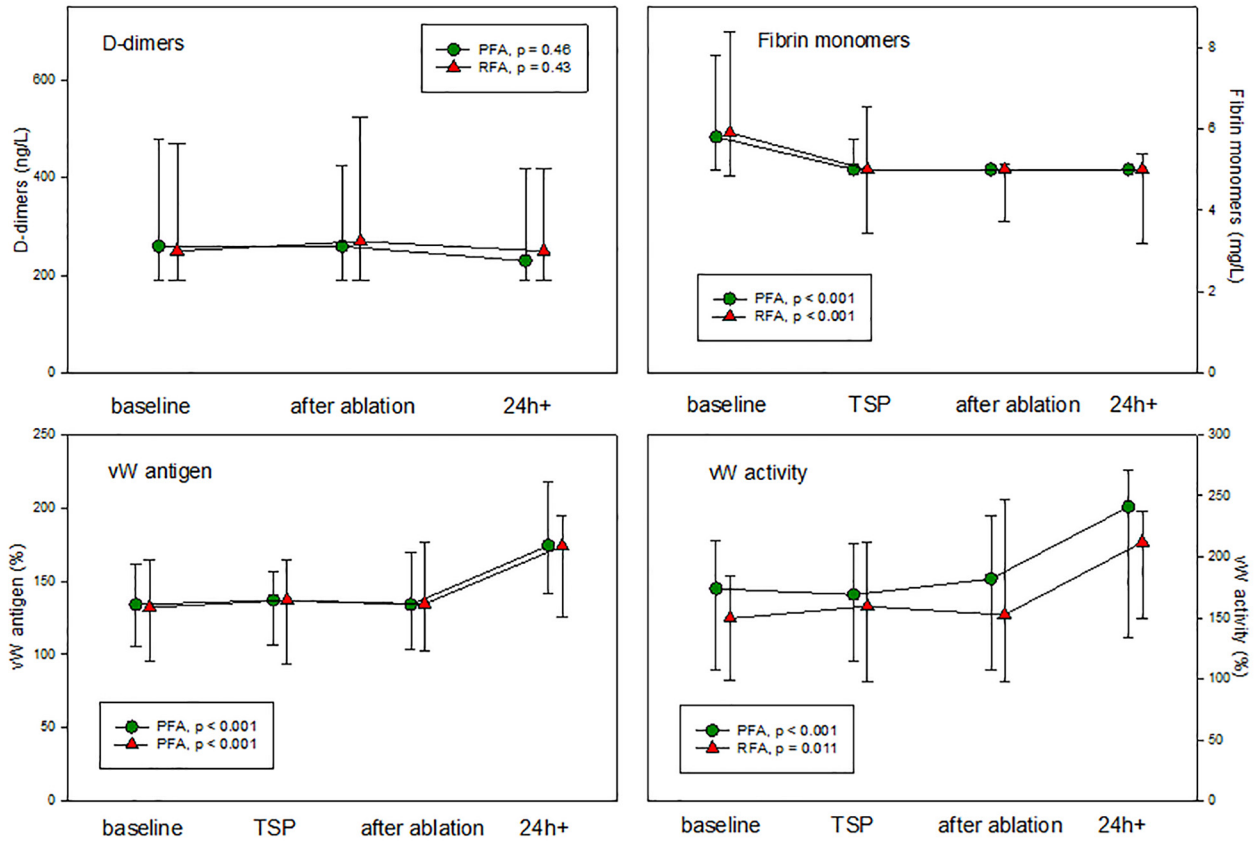
Finally, the concentrations of all measured biomarkers were compared between PFA (n = 17) and RFA (n = 19) patients who underwent the PVI-only procedure. Graphic presentations of biomarker concentrations are shown in Supplemental Figures 1 to 4. Similarly, as in the main analysis, the concentrations of hs-troponin I obtained 24 hours + after the procedure differed significantly between PFA and RFA PVI-only patients ($P < 0.001$). Furthermore, platelet expression of PAC-1 24 h+ after the procedure was slightly higher in the PFA patients compared with RFA patients ($P = 0.02$). No other biomarker differences were found between PFA and RFA PVI-only patients.

Univariate and multivariate linear regression was performed to predict the maximum biomarker value obtained during the procedure (the values immediately after ablations for CD62P and PAC-1 and for the remaining biomarkers at 24 hours+). A log transformation of dependent variable was applied where appropriate (eg, troponin concentration). Selected clinical (ie, age, sex, hypertension, coronary artery disease, and AF type) and procedural (ie, type of procedure, cardioversion) characteristics were used as predictors. Allocation to the PFA group was the only independent statistically significant predictor associated with high 24 hours + troponin levels in the multivariate linear regression model (exp(β) = 11.1; 95% CI: 7.9-15.6).

DISCUSSION

In this study, PVI performed using PF energy was associated with a substantially higher degree of myocardial damage compared with PVI performed using RF energy. Despite an almost 10 times higher degree of myocardial injury, it was not accompanied by a greater extent of platelet activation or coagulation. Moreover, the extent of inflammatory activation is slightly higher in RFA compared with PFA.

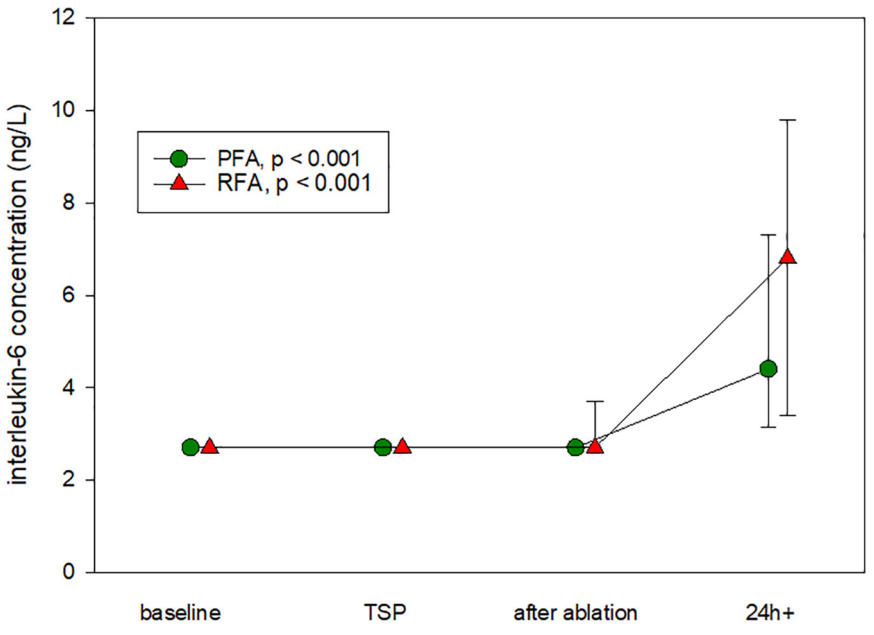
FIGURE 3 The Time Course of Markers of Coagulation During PFA and RFA



Figures display medians with IQRs (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. Abbreviations as in Figure 1.

MYOCARDIAL DAMAGE. As previously shown, the extent of myocardial injury in cryoablation and RFAs is similar. In this study, we found that the extent of myocardial injury was substantially greater in the PFA group. Kawamura et al[8] compared the isolated areas on voltage maps in patients after PVI performed using PFA and RFA and found that the isolated areas were similar between patients with PFA and patients with RFA. In our study, the isolated areas were measured only in RFA patients (3D mapping was not used in the PFA group), and the values of the isolated areas were similar to the values reported by Kawamura et al[8] (eg, 2.89 ± 0.51 cm^2 and 2.32 ± 0.47 cm^2 for RSPV and left superior pulmonary vein (LSPV) in our RFA patients, and 2.9 ± 1.1 cm^2 for RSPV and 2.5 ± 1.2 cm^2 for right inferior pulmonary vein (RIPV) in the report by Kawamura et al[8]). Because our PFA patients underwent ablation using a similar method as in Kawamura et al[8] and other reports on PFA ablation using the Farawave catheter (ie, 4 basket and 4 flower applications for each vein),[4,5] it is not probable that the areas in our patients with PFA would be significantly higher than in previous studies. In our opinion, the explanation for the difference in the greater extent of myocardial injury in the PFA group lies in the differences in the nature between PFAs and RFAs. In the RFA, the area around the PVs is isolated, but, in the PFA, the whole area is ablated. In RFA patients, only the circumference of the area around the PV is ablated, ie, a thin line (only a few millimeters wide) of cardiomyocytes around the vein is damaged. In contrast, PFA involves ablating the entire surface area surrounding the PVs. This means, that the number of damaged cardiomyocytes is significantly higher. Furthermore, as was shown in the preclinical and the first clinical studies, the degree of transmural injury is higher in ablations using PF energy, which, combined with a wide area of ablated cardiomyocytes, corresponds to the high degree of myocardial damage. In animal studies, markers of myocardial damage

FIGURE 4 The Time Course of Markers of IL-6 During PFA and RFA



Figures display medians with IQRs (PFA = green circles; RFA = red triangles). The results of progression in each group are shown in rectangles. IL = interleukin; other abbreviations as in Figure 1.

increased significantly after PFA, peaking 1-3 days after the procedure,[9] which corresponds to our results.

In a previous study, higher troponin release after RFA was associated with greater reversal of structural LA remodeling, and with a higher chance for sinus rhythm maintenance.[10] Whether this finding will also be confirmed in PFA ablation, which is associated with substantially higher troponin I concentrations, needs verification in further clinical studies. On the one hand, it could present a marker of high success rates in terms of durable PVI; on the other hand, it could represent more left atrial damage, which could produce a substrate for LA re-entry and could be associated with impaired left atrial function.

INFLAMMATORY RESPONSE AFTER PFA AND RFA. In an in vivo human study, Herrera Siklody et al[2] demonstrated that several proinflammatory markers increased significantly and similarly after ablation using cryo or RF energy. In a study by Yano et al,[11] cryoablation caused more myocardial injury than RFA; on the other hand, RFA was associated with a higher proinflammatory response. No study has yet compared the in vivo inflammatory response during RFA and PFA.

Previous in vitro studies using RF energy demonstrated that this kind of ablation energy triggers an inflammatory response. Histopathologic studies have established that RFA induces necrosis followed by infiltration of inflammatory cells leading to a fibrotic scar. In an animal study comparing PFA and RFA, PFA lesions were composed of organized, homogeneous fibrosis replacing the myocardium. In contrast, in RF lesions, fibrosis was disorganized and heterogeneous, and infiltration with mononuclear cells was present to a higher degree, which is consistent with a greater inflammatory response.[12] In another animal model of PFA, PFA caused selective atrial myocardial damage. The general architecture of the atrial wall was unaltered in histologic findings 7 days after the PFA procedure.[13] Also, 7 days after ablation, within regions where there was a loss of myocardial fibers, there were rare instances of slight thermal denaturation and mineralization with the presence of inflammatory cells as a consequence of occasional ongoing inflammation. Both these reports agree with our finding (ie, RFA was associated with a slightly higher degree of inflammation than PFA).

In nonparoxysmal AF patients, both troponins and proinflammatory markers were elevated, and a significant correlation between troponin and IL-6 was demonstrated.[14] A significant correlation between markers of myocardial necrosis and inflammation also exists in other cardiovascular disorders, such as acute coronary syndrome. In PFA, despite substantially

greater myocardial damage, there was no increase in the inflammatory response like that seen in myocardial injury caused by ischemia or thermal (RF) damage.

PLATELET ACTIVATION DURING PFA AND RFA. Previous studies have already shown that RFA induces platelet activation. In vitro studies have shown that RF lesions displayed higher thrombus formation than cryo-lesions,[15] and this finding was confirmed in clinical studies with human subjects. Hochholzer et al[16] reported significant platelet activation, indicated by platelet membrane CD62P expression, after ablation of the CTI using RF energy but not when using cryo-energy. Similarly, platelet activation after PVI using RF energy, but not after cryo-energy, was described by Bin Waleed et al.[17] Hererra Siklody et al[2] also described higher platelet activity during PVI in a randomized comparison between cryo-ablation and RFA; however, in this report, both kinds of ablation energies were associated with a similar extent of platelet activation during the ablation procedure.

In the past, myocardial necrosis has been reported to induce platelet activation in other clinical situations than catheter ablations (eg, due to ischemia). However, in our series of patients, despite a 10× increase in troponin after PFA compared with RFA, it was not associated with enhanced platelet activation.

Our data suggest that catheter ablations, in general, result in enhanced platelet activation. In studies comparing thermal energies (ie, cryo and RF energy), platelet activation was less dependent on the type of energy and more on the size of the lesion induced (indicated by peak troponin release). In contrast, in this study, the extent of platelet activation was similar using both RF and PF energy and was not related to the extent and size of the myocardial lesions, which confirms different biological responses to thermal and nonthermal ablation energy.

THE EFFECT OF RFA AND PFA ON COAGULATION MARKERS. The levels of D-dimers or vWF were higher in patients with AF than in controls without AF, and RFA was associated with a further increase in coagulation markers.[18] In our patients, neither PFA nor RFA significantly affected D-dimer concentrations.

Lee et al[19] described an elevation in coagulation markers after RFA. In 37 patients who underwent RFA, there was an increase in D-dimers after trans-septal punctures, with a further increase up to 24 h after the procedure. On the other hand, Lim et al[20] reported that, after RFA, D-dimers increased significantly but not earlier than 1 week after the ablation. Such a late increase would have been missed in our study because our last sample was taken 24 h after the procedure.

Kornej et al[21] described an elevation in the vWF after RFA, which peaked 24 h after the procedure. This agrees with our results (ie, both vW antigen concentrations and vWF activity were higher 24 h after the procedure compared with baseline values.

Importantly, in our patients, the time course of all coagulation parameters did not differ between patients with RF and patients with PF, and, from this point of view, PFA should not be associated with a higher risk of thrombus formation.

STUDY LIMITATIONS. The sheaths used for RFA and PFA differ in size and material, which could have influenced platelet or coagulation activation parameters. Intraprocedural blood samplings were performed from the LA, and the 24 h sampling was from a peripheral vein. The venipuncture site could have influenced platelet activation results; however, it would have influenced them similarly in both groups. The dose of drugs using for analgosedation differed between groups, and although it is known that anesthesia affects systemic coagulation, platelets, and inflammatory markers, it hardly explains the very similar results between groups. Patients with coronary artery disease, which can affect the levels of biomarkers, were not excluded. The patient sample was low and could be underpowered for secondary analyses, and no power calculation was done for markers of coagulation.

CONCLUSIONS

PFA is associated with significantly more myocardial necrosis. Despite greater myocardial damage, platelet activation during PFA was similar to RFA, whereas the inflammatory response was slightly greater after RFA.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: This study extends our knowledge of the effect of PFA on platelets, coagulation, and myocardial damage. It shows that myocardial damage after PFA is approximately 10 times higher compared with RFA. Despite significantly greater myocardial damage, platelet and coagulation activation is similar to RFA. Moreover, the inflammatory response is even slightly greater after RFA.

TRANSLATIONAL OUTLOOK: Similar extent of platelet and coagulation activation during PFA and RFA for AF implicates a pathophysiological background for similar antithrombotic regimens during RFAs and PFAs.

REFERENCES

1. Calkins H, Hindricks G, Cappato R, et al. 2017 HRS/EHRA/ECAS/APHRS/SOLAECE expert consensus statement on catheter and surgical ablation of atrial fibrillation: executive summary. *Heart Rhythm*. 2017;14:e445–e494.

2. Herrera Siklódy C, Arentz T, Minners J, et al. Cellular damage, platelet activation, and inflammatory response after pulmonary vein isolation: a randomized study comparing radiofrequency ablation with cryoablation. *Heart Rhythm*. 2012;9:189–196.

3. Jesel L, Arentz T, Herrera-Siklody C, et al. Do atrial differences in endothelial damage, leukocyte and platelet activation, or tissue factor activity contribute to chamber-specific thrombogenic status in patients with atrial fibrillation? *J Cardiovasc Electrophysiol*. 2014;25:266–270.

4. Reddy VY, Dukkipati SR, Neuzil P, et al. Pulsed field ablation of paroxysmal atrial fibrillation: 1-year outcomes of IMPULSE, PEFCAT, and PEFCAT II. *J Am Coll Cardiol EP*. 2021;7:614–627.

5. Ekanem E, Reddy VY, Schmidt B, et al. Multinational survey on the methods, efficacy, and safety on the post-approval clinical use of pulsed field ablation (MANIFEST-PF). *Europace*. 2022;24:1256–1266.

6. Voukalis C, Lip GYH, Shantsila E. Effects of antithrombotic drugs on the prothrombotic state in patients with atrial fibrillation: the West Birmingham atrial fibrillation project. *Thromb Res*. 2021;200:149–155.

7. Krisai P, Knecht S, Badertscher P, et al. Troponin release after pulmonary vein isolation using pulsed field ablation compared to radiofrequency and cryoballoon ablation. *Heart Rhythm*. 2022;19:1471–1472.

8. Kawamura I, Neuzil P, Shivamurthy P, et al. How does the level of pulmonary venous isolation compare between pulsed field ablation and thermal energy ablation (radiofrequency, cryo, or laser)? *Europace*. 2021;23:1757–1766.

9. Zhao Z, Chen Y, Wu B, et al. Study of necrotic apoptosis by pulsed electric field ablation in rabbit left ventricular myocardium. *Front Cardiovasc Med*. 2022;9:1012020.

10. Yoshida K, Yui Y, Kimata A, et al. Troponin elevation after radiofrequency catheter ablation of atrial fibrillation: relevance to AF substrate, procedural outcomes, and reverse structural remodeling. *Heart Rhythm*. 2014;11:1336–1342.

11. Yano M, Egami Y, Yanagawa K, et al. Comparison of myocardial injury and inflammation after pulmonary vein isolation for paroxysmal atrial fibrillation between radiofrequency catheter ablation and cryoballoon ablation. *J Cardiovasc Electrophysiol*. 2020;31:1315–1322.

12. Koruth J, Kuroki K, Iwasawa J, et al. Preclinical evaluation of pulsed field ablation: electrophysiological and histological assessment of thoracic vein isolation. *Circ Arrhythm Electrophysiol*. 2019;12:e007781.

13. Grimaldi M, Di Monaco A, Gomez T, et al. Time course of irreversible electroporation lesion development through short- and long-term follow-up in pulsed-field ablation-treated hearts. *Circ Arrhythm Electrophysiol*. 2022;15:e010661.

14. Horjen AW, Ulimoen SR, Norseth J, et al. High-sensitivity troponin I in persistent atrial fibrillation - relation to NT-proBNP and markers of inflammation and haemostasis. *Scand J Clin Lab Invest*. 2018;78:386–392.

15. Khairy P, Chauvet P, Lehmann J, et al. Lower incidence of thrombus formation with cryoenergy versus radiofrequency catheter ablation. *Circulation*. 2003;107:2045–2050.

16. Hochholzer W, Schlittenhardt D, Arentz T, et al. Platelet activation and myocardial necrosis in patients undergoing radiofrequency and cryoablation of isthmus-dependent atrial flutter. *Europace*. 2007;9:490–495.

17. Bin Waleed K, Yin X, Yang X, et al. Short and long-term changes in platelet and inflammatory biomarkers after cryoballoon and radiofrequency ablation. *Int J Cardiol*. 2019;285:128–132.

18. Tilly MJ, Geurts S, Pezzullo AM, et al. The association of coagulation and atrial fibrillation: a systematic review and meta-analysis. *Europace*. 2023;25:28–39.

19. Lee DS, Dorian P, Downar E, et al. Thrombogenicity of radiofrequency ablation procedures: what factors influence thrombin generation? *Europace*. 2001;3:195–200.

20. Lim HS, Schultz C, Dang J, et al. Time course of inflammation, myocardial injury, and prothrombotic response after radiofrequency catheter ablation for atrial fibrillation. *Circ Arrhythm Electrophysiol*. 2014;7:83–89.

21. Kornej J, Dinov B, Blann AD, et al. Effects of radiofrequency catheter ablation of atrial fibrillation on soluble P-selectin, von Willebrand factor and IL-6 in the peripheral and cardiac circulation. *PLoS One*. 2014;9:e111760.

KEY WORDS apoptosis, atrial fibrillation, coagulation, inflammation, platelet, pulsed-field ablation, radiofrequency ablation

APPENDIX For supplemental figures, please see the online version of this paper.