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## Effect of Remote Ischemic Preconditioning on Platelet Activation and Reactivity Induced by Ablation for Atrial Fibrillation

Alessandra Stazi, MD; Giancarla Scalone, MD; Marianna Laurito, MD; Maria Milo, MD; Gemma Pelargonio, MD; Maria Lucia Narducci, MD; Rossella Parrinello, MD; Stefano Figliozzi, MD; Gianluigi Bencardino, MD; Francesco Perna, MD; Gaetano A. Lanza, MD; Filippo Crea, MD

**Background**—Radiofrequency ablation of atrial fibrillation has been associated with some risk of thromboembolic events. Previous studies showed that preventive short episodes of forearm ischemia (remote ischemic preconditioning [IPC]) reduce exercise-induced platelet reactivity. In this study, we assessed whether remote IPC has any effect on platelet activation induced by radiofrequency ablation of atrial fibrillation.

**Methods and Results**—We randomized 19 patients (age, 54.7±11 years; 17 male) undergoing radiofrequency catheter ablation of paroxysmal atrial fibrillation to receive remote IPC or sham intermittent forearm ischemia (control subjects) before the procedure. Blood venous samples were collected before and after remote IPC/sham ischemia, at the end of the ablation procedure, and 24 hours later. Platelet activation and reactivity were assessed by flow cytometry by measuring monocyte-platelet aggregate formation, platelet CD41 in the monocyte-platelet aggregate gate, and platelet CD41 and CD62 in the platelet gate in the absence and presence of ADP stimulation. At baseline, there were no differences between groups in platelet variables. Radiofrequency ablation induced platelet activation in both groups, which persisted after 24 hours. However, compared with control subjects, remote IPC patients showed a lower increase in all platelet variables, including monocyte-platelet aggregate formation ( $P<0.0001$ ), CD41 in the monocyte-platelet aggregate gate ( $P=0.002$ ), and CD41 ( $P<0.0001$ ) and CD62 ( $P=0.002$ ) in the platelet gate. Compared with control subjects, remote IPC was also associated with a significantly lower ADP-induced increase in all platelet markers.

**Conclusions**—Our data show that remote IPC before radiofrequency catheter ablation for paroxysmal atrial fibrillation significantly reduces the increased platelet activation and reactivity associated with the procedure. (*Circulation*. 2014;129:11-17.)

**Key Words:** atrial fibrillation ■ blood platelets ■ catheter ablation ■ ischemic preconditioning ■ platelet activation.

Thromboembolic events constitute a serious complication of atrial fibrillation (AF). Radiofrequency catheter ablation has become standard treatment for the cure and prevention of AF. Thromboembolic events, however, can occur as a complication of the procedure.<sup>1-5</sup>

### Editorial see p 5 Clinical Perspective on p 17

Ablation can indeed favor intra-atrial thrombogenesis through activation of the coagulation cascade related to both catheter placement—and radiofrequency-induced tissue injury.<sup>4,6</sup> Importantly, platelet activation consequent to atrial endocardial injury likely also plays a significant role in initiating the mechanisms eventually leading to thrombosis.<sup>7,8</sup> Accordingly, in a recent study, a high-dose aspirin regimen for 3 days before AF ablation, followed by warfarin started immediately after ablation, significantly reduced thromboembolic events.<sup>5</sup>

Remote ischemic preconditioning (IPC) is a phenomenon that consists of a reduction in myocardial damage caused by prolonged myocardial ischemia when the latter is preceded by the application of intermittent episodes of ischemia to organs or tissues other than the heart, usually arms or legs.<sup>9,10</sup>

In a recent study, we demonstrated that upper-arm intermittent ischemia reduces the exercise-induced increase in platelet reactivity in patients with coronary artery disease.<sup>11</sup> In addition, Pedersen et al<sup>12</sup> showed that remote IPC is able to abolish systemic platelet activation induced by myocardial ischemia/reperfusion injury. The mechanisms responsible for the favorable effect of remote IPC on platelet reactivity remain to be elucidated, but a possible role for the peripheral release of adenosine can be hypothesized.<sup>13</sup> Independently of the mechanism(s), remote IPC might reduce platelet activation in several other kinds of conditions.

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The aim of this study was to investigate whether remote IPC has any effects on platelet activation induced by radiofrequency catheter ablation of AF.

## Methods

This study was planned to enroll consecutive patients referred to our center for radiofrequency AF ablation who met the inclusion criteria over a predefined period of 18 months.

From November 2011 to April 2013, we enrolled 19 consecutive patients with paroxysmal AF who were referred to undergo radiofrequency catheter ablation. Patients were randomized to receive remote IPC (n=10) or a sham intermittent ischemia (n=9) immediately before the procedure. Patients with any evidence of significant cardiac or systemic disease, including any acute or chronic inflammatory or allergic disease, were excluded. A detailed clinical history was collected from all patients, including an assessment of cardiovascular risk factors and characteristics of AF episodes. Written informed consent for participation in the study was obtained from all patients. The study was approved by our institutional ethics review board.

### Remote IPC

The method to induce remote IPC has been described in detail elsewhere.<sup>11</sup> Briefly, remote IPC was induced by the application of 3 short episodes (5 minutes) of forearm ischemia by cuff sphygmomanometer inflation separated by 5 minutes of reperfusion. The cuff of the sphygmomanometer was placed in the standard position on the right arm and inflated to a pressure 50 mmHg higher than the systolic blood pressure of the patient. In the control group of sham remote IPC, the cuff was inflated for 3 times at 10 mmHg for 5 minutes with 5-minute intervals.

### Radiofrequency Catheter Ablation

On the day before the procedure, standard transthoracic echocardiography and a computerized tomography scan of the heart were performed to assess left atrial parameters and to define anatomy of pulmonary veins, respectively.

All patients underwent transseptal atrial puncture and AF ablation with an intracardiac echocardiography-guided technique (Cypress Acuson System, Siemens, Mountain View, CA, with a Soundstar probe, Biosense Webster, Diamond Bar, CA).<sup>14,15</sup> Transseptal atrial catheterization was performed by transseptal assembly consisting of an 8F Preface sheath (Biosense Webster), a dilator, and a Brockenbrough needle. We also used dye injection to confirm the correct positioning of the needle in the left atrium as soon as the most distal part of the dilator had passed the atrial septum at the fossa ovalis.

Pulmonary vein antrum isolation was performed under the guidance of a Carto mapping system (Biosense Webster) or an EnSite NavX mapping system (St. Jude Medical, Inc, St. Paul, MN) in 12 and 7 patients, respectively. Irrigated radiofrequency energy was delivered with a target temperature of 43°C and a power between 25 and 35 W with an irrigated ablator catheter (Navistar Thermo Cool, Biosense Webster). The electrophysiological end point was the absence or dissociation of all pulmonary vein potentials as documented by circular mapping catheter (Lasso, Biosense Webster) within the ipsilateral superior and inferior pulmonary veins and along their antrum.<sup>16</sup> No additional lines were made.

All patients were in stable sinus rhythm at the time of radiofrequency ablation. Peripheral oxygen saturation, heart rate, and blood pressure were monitored continuously throughout the ablation procedure.

Periprocedural anticoagulation during the intervention was performed in accordance with current guidelines.<sup>17</sup> A single bolus of 50 IU/kg heparin was administered after transseptal puncture; thereafter, additional boluses of heparin were given to maintain the activated coagulation time between 250 and 350 seconds, which was checked at 30-minute intervals throughout the procedure, unless indicated

otherwise. Protamine administration was never required to correct for heparin excess dose.

An intravenous bolus of midazolam (1 mg) was administered in case of chest pain during the procedure. The dose could be repeated up to a maximal total dose of 5 mg. No additional analgesic drugs were required for pain control.

The success of radiofrequency ablation was verified again in all pulmonary veins 20 minutes after the last pulmonary vein isolation. No adenosine or isoproterenol infusion tests were used to assess inducibility of the arrhythmia both before and after ablation. No clinically relevant hemodynamic perturbations requiring specific medical interventions occurred during the procedures.

### Platelet Reactivity

Platelet activation and reactivity were assessed by measuring monocyte-platelet aggregates (MPAs) and the expression of the platelet receptors glycoprotein IIb/IIIa (CD41) and P-selectin (CD62) by flow cytometry.

In each patient, 15-mL blood samples were collected from the right femoral vein via a 6F venous sheath (1) at baseline, before remote IPC/sham intermittent ischemia; (2) immediately after remote IPC/sham intermittent ischemia, before ablation; (3) immediately after the end of ablation procedure; and (4) 24 hours after the procedure.

Special care was taken to avoid platelet activation during sampling procedures. Blood was drawn directly into plastic tubes containing 3.8% buffered sodium citrate after the first 5 mL was discarded to minimize the formation of platelet aggregates. Blood was kept at room temperature and immune labeled within 10 minutes of collection for analyses by flow cytometry.<sup>18</sup> Appropriate fluorochrome-conjugated isotype-matched monoclonal antibodies, obtained from the different manufacturers, were used as control for background staining.

### Monocyte-Platelet Aggregates

Blood (100  $\mu$ L) was labeled within 10 minutes of collection with a saturating concentration of PerCP-conjugated CD14 (lipopolysaccharide protein receptor) and FITC-conjugated glycoprotein IIb/IIIa (glycoprotein IIb, CD41) for 15 minutes at room temperature. After incubation, erythrocytes were lysed with buffered ammonium chloride and analyzed by FACScan. MPAs were identified by use of the logical gating facility with a combination of binding characteristics of anti-CD14 (monocyte marker) and anti-CD41 (platelet marker) antibodies. A minimum of 3000 monocytes were counted for each test. MPAs were expressed as percentage of monocyte-binding platelets.

### Platelet Surface Receptors

Blood was diluted 1:10 in PBS and labeled within 10 minutes of collection by incubation with specific antibodies. Blood aliquots (5 mL) were incubated for 15 minutes at room temperature with saturating concentrations of PE-conjugated CD41 and FITC-conjugated PAC-1 to study the basal and the active forms of platelet expression of glycoprotein IIb/IIIa receptor (Becton-Dickinson, Milan, Italy), respectively. After incubation, samples were diluted with 200 mL PBS and immediately analyzed by the Becton-Dickinson FACScan flow cytometer. An acquisition gate was first established on FSC/SSC signals. They were collected in a logarithmic mode to improve discrimination between viable platelets and unwanted events (erythrocytes, white blood cells, debris, and aggregates). The purity of the gate was always confirmed by back-gating on CD41 staining. A low flow rate was used to minimize coincident events. A minimum of 10 000 platelets were counted for each test. Fluorescence data were evaluated as mean fluorescence intensity.

### ADP Stimulation

Blood samples were incubated with ADP ( $10^{-7}$  mol/L) for 15 minutes at room temperature and labeled as previously described for the assessment of MPA and platelet receptors. The concentrations of ADP chosen for the study were the lowest that were found to activate platelets in healthy subjects in preliminary experiments.<sup>19</sup>

## Statistical Analyses

Comparisons between groups of continuous variables were done by *t* tests. A generalized linear model for repeated measures was applied to compare the curves of platelet activation markers throughout the radiofrequency procedure between the 2 groups. In case of global significant differences, we proceeded with post hoc multiple comparisons between and within groups using unpaired and paired *t* tests, respectively, to obtain and provide evidence of where significant changes occurred. Global differences were considered significant at values of  $P \leq 0.05$ . Because there were many individual between-group and within-group comparisons for markers of platelet activation throughout radiofrequency ablation, for these multiple comparisons, we considered significant only statistical differences with a value of  $P < 0.01$ . Continuous data are reported as mean  $\pm$  SD. SPSS version 12.02 statistical software (SPSS Inc, Florence, Italy) was used for statistical analyses.

## Results

### General Findings

The main clinical characteristics of the 2 groups are summarized in Table 1, and the main data relative to the characteristics of paroxysmal AF episodes and radiofrequency ablation procedure are summarized in Table 2. The 2 groups were well balanced with regard to the main clinical and laboratory characteristics, drug therapy, and characteristics of AF episodes, as well as the main findings concerning the radiofrequency ablation procedure, which was performed without any clinically relevant complication in all patients.

**Table 1. Main Basal Clinical Findings of the Patients**

	Remote IPC (n=10)	Control Subjects (n=9)
Clinical data		
Age, y	58.1 $\pm$ 8	50.1 $\pm$ 13
Male/female, n	9/1	8/0
Smoking, n	4	5
Hypertension, n	7	5
Hypercholesterolemia, n	2	3
Family history of CAD, n	3	4
Laboratory data		
Platelet count, $10^9/\text{mm}^3$	229 $\pm$ 45	199 $\pm$ 65
Prothrombin activity, %	59.8 $\pm$ 26	67.9 $\pm$ 29
aPTT, s	38.1 $\pm$ 6.8	37.2 $\pm$ 5.2
Fibrinogen, mg/dL	314 $\pm$ 66	281 $\pm$ 52
INR	1.47 $\pm$ 0.6	1.42 $\pm$ 0.6
Preablation medications, n		
$\beta$ -Blockers	10	7
Diuretics	1	1
Ca <sup>2+</sup> channel blockers	1	2
ACE inhibitors	2	2
ARBs	1	0
Antiarrhythmic drugs	8	5
Aspirin	3	3
Anticoagulants	4	7

ACE indicates angiotensin-converting enzyme; aPTT, activated partial thromboplastin time; ARB, angiotensin receptor blocker; CAD, coronary artery disease; INR, international normalized ratio; and IPC, ischemic preconditioning.

**Table 2. Main Findings of AF and Radiofrequency Ablation Procedure in the 2 Patient Groups**

	Remote IPC (n=10)	Control Subjects (n=9)
AF duration, y	4.6 $\pm$ 4.3	6.3 $\pm$ 3.5
Time from the last AF episode, d	35.0 $\pm$ 20.3	44.7 $\pm$ 31.3
Duration of the last AF episode, min	483 $\pm$ 392	567 $\pm$ 467
Duration of RF ablation, min	324 $\pm$ 34	330 $\pm$ 60
Procedural activated coagulation time, c		
Average	327.6 $\pm$ 13.8	331.7 $\pm$ 10.3
Minimal	299.8 $\pm$ 17.5	308.9 $\pm$ 22.5
Maximum	345.3 $\pm$ 18.1	351.7 $\pm$ 16.4
At 24 hours after ablation		
aPTT, s	37.0 $\pm$ 7.1	36.1 $\pm$ 6.8
Fibrinogen, mg/dL	402.4 $\pm$ 82.4	386.1 $\pm$ 102.3

AF indicates atrial fibrillation; aPTT, activated partial thromboplastin time; IPC, ischemic preconditioning; and RF, radiofrequency.

### Platelet Activation

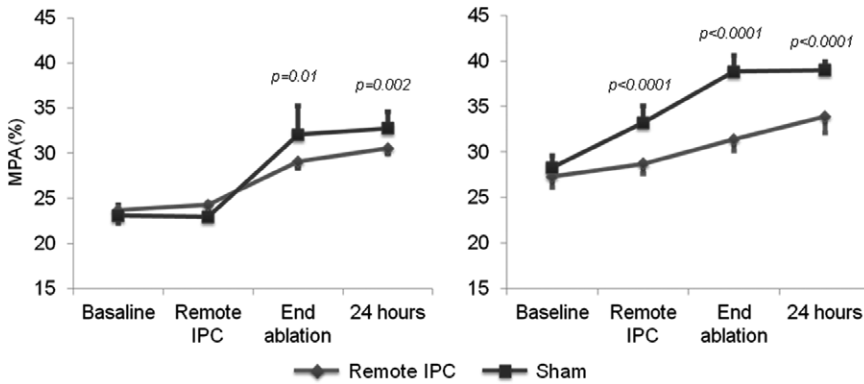
The results of platelet markers in the absence of agonist stimulation are summarized in Table 3, and their trends in relation to AF ablation are shown on the left sides of Figures 1 through 4. There were no significant differences between the 2 groups in spontaneous MPA formation, CD41 expression in the MPA and platelet gates, and CD62 expression in the platelet gate both in basal conditions and after the preconditioning/sham procedure.

**Table 3. Platelet Cytometry Variables in the 2 Patient Groups in the Absence of Agonist Stimulation**

	Remote IPC	Control Subjects	P Value*
MPA, %			
Basal	23.67 $\pm$ 1.50	23.07 $\pm$ 1.22	
After remote IPC	24.27 $\pm$ 1.42	22.97 $\pm$ 1.54	
End of procedure	29.09 $\pm$ 0.79	32.08 $\pm$ 3.13	<0.0001
24 h after procedure	30.53 $\pm$ 0.65	32.76 $\pm$ 1.81	
CD41 in MPA gate, MFI			
Basal	21.98 $\pm$ 1.81	21.97 $\pm$ 1.45	
After remote IPC	23.09 $\pm$ 1.33	22.01 $\pm$ 1.59	
End of procedure	27.51 $\pm$ 1.17	31.04 $\pm$ 0.89	<0.0001
24 h after procedure	29.85 $\pm$ 0.63	31.76 $\pm$ 0.72	
CD41 in platelet gate, MFI			
Basal	22.87 $\pm$ 1.46	22.46 $\pm$ 1.16	
After remote IPC	23.34 $\pm$ 1.08	24.32 $\pm$ 1.51	
End of procedure	26.70 $\pm$ 1.12	33.61 $\pm$ 1.42	<0.0001
24 h after procedure	29.33 $\pm$ 0.89	34.41 $\pm$ 1.19	
CD62 in platelet gate, MFI			
Basal	9.12 $\pm$ 1.67	10.18 $\pm$ 1.29	
After remote IPC	9.59 $\pm$ 2.11	10.94 $\pm$ 1.05	
End of procedure	9.80 $\pm$ 1.94	12.08 $\pm$ 1.09	0.037
24 h after procedure	10.16 $\pm$ 1.93	12.51 $\pm$ 1.00	

IPC indicates ischemic preconditioning; MFI, mean fluorescence intensity; and MPA, monocyte-platelet aggregates.

\*P values for differences between groups in group-variable interaction.



**Figure 1.** Monocyte-platelet aggregates (MPA) in the absence (**left**) and presence (**right**) of ADP in the 2 groups. *P* values refer to between-group comparisons at each time point. IPC indicates ischemic preconditioning.

A significant increase in platelet markers was observed in both groups during the radiofrequency ablation ( $P < 0.01$  for all variables in both groups), which persisted at 24 hours after the procedure. A generalized linear model for repeated measures showed a significant difference in the curve changes between the 2 groups ( $P < 0.0001$  for MPAs, MPA-related CD41, and platelet CD41;  $P = 0.037$  for CD62; Table 3) as a result of a smaller platelet increase in remote IPC patients compared with control subjects both at end ablation and 24 hours later (Figures 1 and 2).

**Platelet Reactivity**

Platelet markers measured after ADP stimulation are summarized in Table 4, and their trends are shown on the right sides of Figures 1 through 4. As expected, ADP always increased the expression of platelet markers in either group compared with the respective basal time markers. In basal conditions, no significant differences were found after ADP stimulation between the 2 groups in all platelet cytometry variables. A significant increase in platelet markers was observed in both groups during the radiofrequency ablation, which persisted at 24 hours after the procedure ( $P < 0.01$  for all variables in both groups).

A generalized linear model for repeated measures showed a significant difference in the curve changes between the 2 groups ( $P < 0.0001$  for MPAs, MPA-related CD41, and platelet CD41;  $P = 0.007$  for CD62; Table 4) resulting from a smaller increase in platelet markers in remote IPC patients compared with control subjects both at end ablation and 24 hours later but also after the remote IPC/sham procedure (Figures 3 and 4).

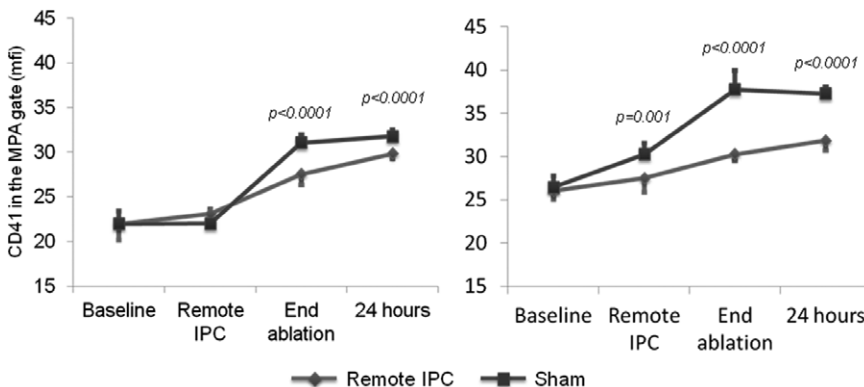
**Discussion**

Two main results emerge from our data: radiofrequency ablation for paroxysmal AF is associated with a significant increase in platelet activation and reactivity, which persists up to 24 hours after the procedure; and remote IPC is able to reduce the increased platelet activation and reactivity related to the ablation procedure. Of note, remote IPC consistently reduced all markers of platelet activation assessed in the study.

Radiofrequency catheter ablation has become a reference therapy for AF, and its success varies from 16% to 84% on the basis of clinical features of the patients, ablation method used, characteristics of the arrhythmia (eg, paroxysmal versus permanent AF), and atrial substrate.<sup>1,20</sup>

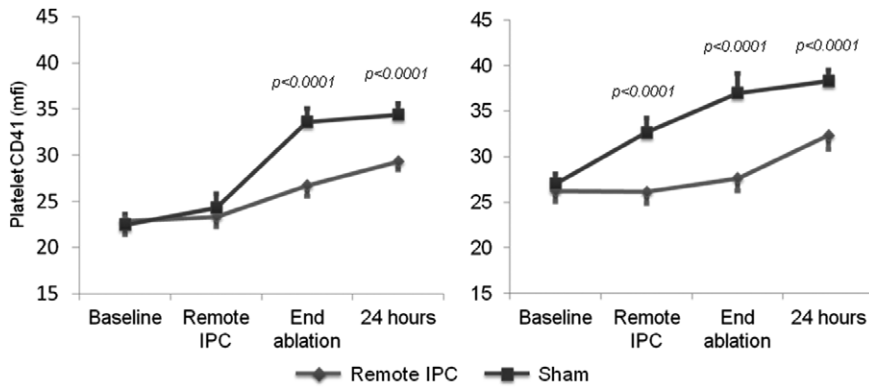
A potential benefit of successful AF ablation is the abolition of thromboembolic events related to the persistence or recurrence of the arrhythmia. Radiofrequency ablation of AF, however, is by itself associated with an increased risk of thromboembolic events in the early period after the procedure, mostly in the first 2 weeks.<sup>1-5</sup> This undesired complication has been reported in up to 7% of patients, despite the use of appropriate anticoagulation.<sup>20-23</sup> Moreover, silent cerebral embolic events have been detected in up to 35% of patients.<sup>24-26</sup>

Platelets play a significant role in this transient increased risk of thromboembolism. Increased platelet activation and reactivity have indeed been reported to occur during AF ablation.<sup>6,8</sup> Several factors may lead to increased platelet activation in this context, including the radiofrequency-induced injury of the subendocardial left atrial wall, which also results in the activation of the coagulation cascade and inflammatory reaction.<sup>4,6,8</sup> Antiplatelet agents can be



**Figure 2.** CD41 expression in the monocyte-platelet aggregate (MPA) gate in the absence (**left**) and presence (**right**) of ADP in the 2 groups. *P* values refer to between-group comparisons at each time point. IPC indicates ischemic preconditioning.





**Figure 3.** CD41 expression in the platelet gate in the absence (left) and presence (right) of ADP in the 2 groups. P values refer to between-group comparisons at each time point. IPC indicates ischemic preconditioning.

administered to reduce this undesired effect, but their efficacy in this setting remains unknown.<sup>5,8</sup>

IPC is a phenomenon consisting of the capacity of brief recurrent bursts (3–5 minutes) of myocardial ischemia to induce protection of myocardial cells against the damage caused by a subsequent prolonged episode of severe ischemia.<sup>27,28</sup>

In the clinical setting, IPC is believed to contribute to the smaller myocardial infarct extension of acute myocardial infarction patients with compared with those without a history of preinfarction angina,<sup>29</sup> as well as in patients with progressively milder degrees of myocardial suffering detectable during repeated ischemic episodes induced by balloon coronary occlusion,<sup>30</sup> coronary artery spasm,<sup>31</sup> or exercise stress test.<sup>32</sup>

Subsequent studies showed that IPC can also be induced by the application of short episodes of ischemia in peripheral tissues, typically in the forearm, a phenomenon defined as remote IPC.<sup>9,10</sup> In the clinical setting, remote IPC has been shown to limit myocardial suffering and damage after transient ischemic episodes<sup>13</sup> or persistent thrombotic coronary occlusion.<sup>33</sup>

Although the clinical benefits of IPC are largely related to some favorable changes in myocardial cells that make them more resistant to ischemic injury, a few studies suggested that IPC can also induce other beneficial pathophysiological effects, including a reduction in platelet reactivity.

Hata et al<sup>34</sup> showed that IPC was able to reduce platelet-mediated thrombosis in a model of damaged and stenotic coronary arteries in dogs. In a similar model, Linden et al<sup>35</sup> showed that the lower recurrence of thrombosis induced by IPC was associated with reduced platelet-fibrinogen binding,

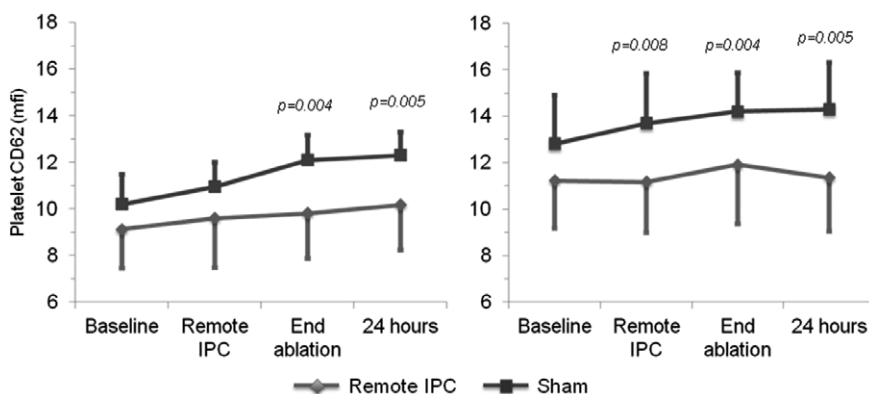
neutrophil-platelet aggregates, and platelet P-selectin expression. Finally, Posa et al,<sup>36</sup> in a model of acute myocardial infarction in pigs, found that IPC resulted in a lower mean platelet volume, suggesting attenuation of platelet activation.

The experimental evidence that myocardial IPC can reduce platelet activation was recently confirmed in clinical studies.<sup>13,37</sup> Importantly, although no experimental studies have assessed the effect of remote IPC on platelet function, recent data show that remote IPC is able to blunt platelet activation associated with myocardial ischemia.<sup>11,12</sup>

In the present study, we provide evidence that remote IPC, induced by intermittent forearm ischemia, is also able to significantly reduce platelet activation and reactivity during radiofrequency ablation of AF and that its antiplatelet effect persists up to 24 hours after the procedure.

The mechanisms responsible for the antiplatelet effect of remote IPC during radiofrequency catheter ablation of AF remain to be elucidated. According to previous observations, a peripheral release of adenosine, which exerts antiplatelet effects through A2 adenosine receptor stimulation,<sup>34,38</sup> might play a role. Modulation of the complex dynamic interaction between endothelial cells and platelets, however, might also be involved.<sup>9,12</sup>

Independently of the mechanisms, a major consequence of our findings is that remote IPC might be applied to reduce platelet activation and reactivity during radiofrequency ablation for AF, with possible favorable effects on the occurrence of thromboembolic events. This potential clinical effect, however, needs to be assessed in appropriately designed large clinical trials, even considering that the IPC-induced reduction of platelet activation in our study appeared relatively modest.



**Figure 4.** CD62 expression in the platelet gate in the absence (left) and presence (right) of ADP in the 2 groups. P values refer to between-group comparisons at each time point. IPC indicates ischemic preconditioning.

**Table 4. Platelet Cytometry Variables in the 2 Patient Groups After ADP Stimulation**

	Remote IPC	Control Subjects	P Value*
MPA, %			
Basal	27.30±1.24	28.30±1.27	
After remote IPC	28.72±1.11	33.24±1.79	
End of procedure	31.41±1.29	38.86±1.77	<0.0001
24 h after procedure	33.88±1.78	39.01±0.92	
CD41 in MPA gate, MFI			
Basal	26.03±1.00	26.43±1.34	
After remote IPC	27.49±1.62	30.25±1.29	
End of procedure	30.26±0.72	37.77±2.21	<0.0001
24 h after procedure	31.84±1.14	37.27±0.81	
CD41 in platelet gate, MFI			
Basal	26.24±1.18	27.05±1.07	
After remote IPC	26.14±1.29	32.67±1.58	
End of procedure	27.62±1.38	37.01±2.14	<0.0001
24 h after procedure	32.33±1.52	38.31±1.22	
CD62 in platelet gate, MFI			
Basal	11.21±2.03	12.81±2.10	
After remote IPC	11.16±2.16	13.70±2.13	
End of procedure	11.91±2.56	14.20±1.68	0.007
24 h after procedure	11.34±2.28	14.29±2.03	

IPC indicates ischemic preconditioning; MFI, mean fluorescence intensity; and MPA, monocyte-platelet aggregates.

\*P values for differences between groups in group-variable interaction.

Some limitations of our study should be acknowledged. First, we enrolled only a small number of patients, which resulted in some small, although not significant, differences between groups (Table 1) and the absence of some kinds of patients with a potential increased risk of platelet activation such as those with diabetes mellitus or heart disease; thus, our findings require confirmation in larger populations that include patients with higher risks of platelet activation. Second, we did not attempt to clarify the mechanism by which remote IPC reduced platelet activation; thus, although previous studies suggested a role for adenosine release,<sup>34,38</sup> appropriate studies are needed to define the mechanism(s) underlying this phenomenon.

## Disclosures

None.

## References

- Cappato R, Calkins H, Chen SA, Davies W, Iesaka Y, Kalman J, Kim YH, Klein G, Packer D, Skanes A. Worldwide survey on the methods, efficacy, and safety of catheter ablation for human atrial fibrillation. *Circulation*. 2005;111:1100–1105.
- Oral H, Chugh A, Ozaydin M, Good E, Fortino J, Sankaran S, Reich S, Iqbal P, Elmouchi D, Tschopp D, Wimmer A, Dey S, Crawford T, Pelosi F Jr, Jongnarangsin K, Bogun F, Morady F. Risk of thromboembolic events after percutaneous left atrial radiofrequency ablation of atrial fibrillation. *Circulation*. 2006;114:759–765.
- Zhou L, Keane D, Reed G, Ruskin J. Thromboembolic complications of cardiac radiofrequency catheter ablation: a review of the reported incidence, pathogenesis and current research directions. *J Cardiovasc Electrophysiol*. 1999;10:611–620.
- Ren JF, Marchlinski FE, Callans DJ. Left atrial thrombus associated with ablation for atrial fibrillation: identification with intracardiac echocardiography. *J Am Coll Cardiol*. 2004;43:1861–1867.
- Mortada ME, Chandrasekaran K, Nangia V, Dhala A, Blanck Z, Cooley R, Bhatia A, Gilbert C, Akhtar M, Sra J. Periprocedural anticoagulation for atrial fibrillation ablation. *J Cardiovasc Electrophysiol*. 2008;19:362–366.
- Dorbala S, Cohen AJ, Hutchinson LA, Menchavez-Tan E, Steinberg JS. Does radiofrequency ablation induce a prethrombotic state? Analysis of coagulation system activation and comparison to electrophysiologic study. *J Cardiovasc Electrophysiol*. 1998;9:1152–1160.
- Wang TL, Lin JL, Hwang JJ, Tseng CD, Lo HM, Lien WP, Tseng YZ. The evolution of platelet aggregability in patients undergoing catheter ablation for supraventricular tachycardia with radiofrequency energy: the role of antiplatelet therapy. *Pacing Clin Electrophysiol*. 1995;18:1980–1990.
- Manolis AS, Maounis T, Vassilikos V, Melita-Manolis H, Psarros L, Terzoglou G, Cokkinos DV. Pretreatment with antithrombotic agents during radiofrequency catheter ablation: a randomized comparison of aspirin versus ticlopidine. *J Cardiovasc Electrophysiol*. 1998;9:1144–1151.
- Kharbanda RK, Mortensen UM, White PA, Kristiansen SB, Schmidt MR, Hoschtitzky JA, Vogel M, Sorensen K, Redington AN, MacAllister R. Transient limb ischemia induces remote ischemic preconditioning in vivo. *Circulation*. 2002;106:2881–2883.
- Schmidt MR, Smerup M, Konstantinov IE, Shimizu M, Li J, Cheung M, White PA, Kristiansen SB, Sorensen K, Dzavik V, Redington AN, Kharbanda RK. Intermittent peripheral tissue ischemia during coronary ischemia reduces myocardial infarction through a KATP-dependent mechanism: first demonstration of remote ischemic preconditioning. *Am J Physiol Heart Circ Physiol*. 2007;292:H1883–H1890.
- Battipaglia I, Scalone G, Milo M, Di Franco A, Lanza GA, Crea F. Upper arm intermittent ischaemia reduces exercise-related increase of platelet reactivity in patients with obstructive coronary artery disease. *Heart*. 2011;97:1298–1303.
- Pedersen CM, Cruden NL, Schmidt MR, Lau C, Bøtker HE, Kharbanda RK, Newby DE. Remote ischemic preconditioning prevents systemic platelet activation associated with ischemia-reperfusion injury in humans. *J Thromb Haemost*. 2011;9:404–407.
- Scalone G, Coviello I, Barone L, Pisanello C, Sestito A, Lanza GA, Crea F. Brief low-workload myocardial ischaemia induces protection against exercise-related increase of platelet reactivity in patients with coronary artery disease. *Heart*. 2010;96:263–268.
- Khaykin Y, Marrouche NF, Saliba W, Schweikert R, Bash D, Chen MS, Williams-Andrews M, Saad E, Burkhardt DJ, Bhargava M, Joseph G, Rossillo A, Erciyes D, Martin D, Natale A. Pulmonary vein antrum isolation for treatment of atrial fibrillation in patients with valvular heart disease or prior open heart surgery. *Heart Rhythm*. 2004;1:33–39.
- Marrouche NF, Martin DO, Wazni O, Gillinov AM, Klein A, Bhargava M, Saad E, Bash D, Yamada H, Jaber W, Schweikert R, Tchou P, Abdul-Karim A, Saliba W, Natale A. Phased-array intracardiac echocardiography monitoring during pulmonary vein isolation in patients with atrial fibrillation: impact on outcome and complications. *Circulation*. 2003;107:2710–2716.
- Verma A, Marrouche NF, Natale A. Pulmonary vein antrum isolation: intracardiac echocardiography-guided technique. *J Cardiovasc Electrophysiol*. 2004;15:1335–1340.
- 2012 focused update of the ESC guidelines for the management of atrial fibrillation: an update of the 2010 ESC guidelines for the management of atrial fibrillation: developed with the special contribution of the European Heart Rhythm Association. *Eur Heart J*. 2012;33:2719–2747.
- Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. *Circulation*. 2001;104:1533–1537.
- Aurigemma C, Fattorossi A, Sestito A, Sgueglia GA, Farnetti S, Buzzonetti A, Infusino F, Landolfi R, Scambia G, Crea F, Lanza GA. Relationship between changes in platelet reactivity and changes in platelet receptor expression induced by physical exercise. *Thromb Res*. 2007;120:901–909.
- European Heart Rhythm Association (EHRA); European Cardiac Arrhythmia Society (ECAS); American College of Cardiology (ACC); American Heart Association (AHA); Society of Thoracic Surgeons (STS). HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of atrial fibrillation: recommendations for personnel, policy, procedures and follow-up: a report of the Heart Rhythm Society (HRS) Task Force on Catheter and Surgical Ablation of Atrial Fibrillation. *Heart Rhythm*. 2007;4:816–861.

21. Zhou L, Keane D, Reed G, Ruskin J. Thromboembolic complications of cardiac radiofrequency catheter ablation: a review of the reported incidence, pathogenesis and current research directions. *J Cardiovasc Electrophysiol*. 1999;10:611–620.
22. Cauchemez B, Extramiana F, Cauchemez S, Cosson S, Zouzou H, Meddane M, d'Almones LR, Lavergne T, Leenhardt A, Coumel P, Houdart E. High-flow perfusion of sheaths for prevention of thromboembolic complications during complex catheter ablation in the left atrium. *J Cardiovasc Electrophysiol*. 2004;15:276–283.
23. Kok LC, Mangrum JM, Haines DE, Mounsey JP. Cerebrovascular complication associated with pulmonary vein ablation. *J Cardiovasc Electrophysiol*. 2002;13:764–767.
24. Di Biase L, Burkhardt JD, Mohanty P, Sanchez J, Horton R, Gallinghouse GJ, Lakkireddy D, Verma A, Khaykin Y, Hongo R, Hao S, Beheiry S, Pelargonio G, Dello Russo A, Casella M, Santarelli P, Santangeli P, Wang P, Al-Ahmad A, Patel D, Themistoclakis S, Bonso A, Rossillo A, Corrado A, Raviele A, Cummings JE, Schweikert RA, Lewis WR, Natale A. Periprocedural stroke and management of major bleeding complications in patients undergoing catheter ablation of atrial fibrillation: the impact of periprocedural therapeutic international normalized ratio. *Circulation*. 2010;121:2550–2556.
25. Haeusler KG, Kirchhof P, Endres M. Left atrial catheter ablation and ischemic stroke. *Stroke*. 2012;43:265–270.
26. Gaita F, Leclercq JF, Schumacher B, Scaglione M, Toso E, Halimi F, Schade A, Froehner S, Ziegler V, Sergi D, Cesarani F, Blandino A. Incidence of silent cerebral thromboembolic lesions after atrial fibrillation ablation may change according to technology used: comparison of irrigated radiofrequency, multipolar nonirrigated catheter and cryoballoon. *J Cardiovasc Electrophysiol*. 2011;22:961–968.
27. Tomai F, Crea F, Chiariello L, Gioffrè PA. Ischemic preconditioning in humans: models, mediators, and clinical relevance. *Circulation*. 1999;100:559–563.
28. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74:1124–1136.
29. Ottani F, Galli M, Zerboni S, Galvani M. Prodromal angina limits infarct size in the setting of acute anterior myocardial infarction treated with primary percutaneous intervention. *J Am Coll Cardiol*. 2005;45:1545–1547.
30. Laskey WK. Brief repetitive balloon occlusions enhance reperfusion during percutaneous coronary intervention for acute myocardial infarction: a pilot study. *Catheter Cardiovasc Interv*. 2005;65:361–367.
31. Pasceri V, Lanza GA, Patti G, Pedrotti P, Crea F, Maseri A. Preconditioning by transient myocardial ischemia confers protection against ischemia-induced ventricular arrhythmias in variant angina. *Circulation*. 1996;94:1850–1856.
32. Tomai F, Perino M, Ghini AS, Crea F, Gasparone A, Versaci F, Chiariello L, Gioffrè PA. Exercise-induced myocardial ischemia triggers the early phase of preconditioning but not the late phase. *Am J Cardiol*. 1999;83:586–588, A7.
33. Bøtker HE, Kharbanda R, Schmidt MR, Böttcher M, Kalltoft AK, Terkelsen CJ, Munk K, Andersen NH, Hansen TM, Trautner S, Lassen JF, Christiansen EH, Krusell LR, Kristensen SD, Thuesen L, Nielsen SS, Rehling M, Sørensen HT, Redington AN, Nielsen TT. Remote ischaemic conditioning before hospital admission, as a complement to angioplasty, and effect on myocardial salvage in patients with acute myocardial infarction: a randomised trial. *Lancet*. 2010;375:727–734.
34. Hata K, Whittaker P, Kloner RA, Przyklenk K. Brief antecedent ischemia attenuates platelet-mediated thrombosis in damaged and stenotic canine coronary arteries: role of adenosine. *Circulation*. 1998;97:692–702.
35. Linden MD, Whittaker P, Frelinger AL 3rd, Barnard MR, Michelson AD, Przyklenk K. Preconditioning ischemia attenuates molecular indices of platelet activation-aggregation. *J Thromb Haemost*. 2006;4:2670–2677.
36. Posa A, Pavo N, Hemetsberger R, Csonka C, Csont T, Ferdinandy P, Petrás Z, Varga C, Pavo IJ, Laszlo F Jr, Huber K, Gyöngyösi M. Protective effect of ischaemic preconditioning on ischaemia/reperfusion-induced microvascular obstruction determined by on-line measurements of coronary pressure and blood flow in pigs. *Thromb Haemost*. 2010;103:450–460.
37. Scalone G, Aurigemma C, Tomai F, Corvo P, Battipaglia I, Lanza GA, Crea F. Effect of pre-infarction angina on platelet reactivity in acute myocardial infarction. *Int J Cardiol*. 2013;167:51–56.
38. Kitakaze M, Hori M, Sato H. Endogenous adenosine inhibits platelet aggregation during myocardial ischaemia in dogs. *Circulation Res*. 1991;69:1402–1408.

### CLINICAL PERSPECTIVE

Radiofrequency ablation is an established therapy for atrial fibrillation. Radiofrequency ablation, however, has been associated with some risk of thromboembolic events, which have been reported to occur in up to 7% of patients despite the use of appropriate antithrombotic therapy. Platelet activation occurs during the procedure and likely contributes to this increased risk. In this randomized, controlled study, we show that remote ischemic preconditioning, achieved before radiofrequency catheter ablation of paroxysmal atrial fibrillation by the application of 3 short episodes (5 minutes) of forearm ischemia by cuff sphygmomanometer inflation separated by 5 minutes of reperfusion, reduced platelet activation induced by the procedure compared with a sham control group. The most relevant potential clinical implication of our findings is that remote ischemic preconditioning might be applied to reduce platelet activation and reactivity during radiofrequency ablation, with possible favorable effects on the occurrence of thromboembolic events. This potential clinical effect, however, needs to be assessed in appropriately designed large clinical trials.