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

Running title: Stazi et al.; Preconditioning and ablation-activated platelets

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Abstract

**Background**—Radiofrequency (RF) ablation of atrial fibrillation (AF) 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 RF ablation of AF.

**Methods and Results**—We randomized 19 patients (54.7±11 years, 17 males), undergoing RF catheter of paroxysmal AF, to receive remote IPC or sham intermittent forearm ischemia (controls) 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 (MPA) formation, platelet CD41 in the MPA gate and platelet CD41 and CD62 in the platelet gate, in absence and in presence of ADP stimulation. At baseline there were no differences between groups in platelet variables. RF ablation induced platelet activation in both groups, which persisted after 24 hours. However, compared controls, remote IPC patients showed a lower increase in all platelet variables, including MPA formation (p<0.0001), CD41 in the MPA gate (p=0.002) and CD41 (p<0.0001) and CD62 (p=0.002) in the platelet gate. Compared to controls, remote IPC was also associated with a significant lower ADP-induced increase of all platelet markers.

**Conclusions**—Our data show that remote IPC before RF catheter ablation for paroxysmal AF significantly reduces the increased platelet activation and reactivity associated with the procedure.

**Key words:** preconditioning, atrial fibrillation, ablation, platelet
**Introduction**

Thromboembolic events constitute a serious complication of atrial fibrillation (AF).

Radiofrequency (RF) catheter ablation has become a standard form of treatment for cure and prevention of AF. Thromboembolic events, however, can occur as a complication of the procedure\(^1\)-\(^5\).

Ablation can indeed favor intra-atrial thrombogenesis through activation of the coagulation cascade related to both catheter placement and RF induced tissue injury\(^4,6\).

Importantly, platelet activation consequent to atrial endocardial injury likely also plays a significant role in initiating the mechanisms eventually leading to thrombosis\(^7,8\). Accordingly, in a recent study, a high-dose aspirin regimen for 3 days prior to AF ablation, followed by warfarin started immediately after ablation, significantly reduced thromboembolic events\(^5\).

Remote ischemic preconditioning (IPC) is a phenomenon that consists in a reduction of 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\(^9,10\).

In a recent study, we demonstrated that upper arm intermittent ischemia reduces the exercise-induced increase of platelet reactivity in patients with coronary artery disease\(^11\). In addition, Pedersen et al. showed that remote IPC is able to abolish systemic platelet activation induced by myocardial ischemia-reperfusion injury\(^12\). 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\(^13\). Independently of the mechanism(s), remote IPC might reduce platelet activation in several other kinds of conditions.

In this study we aimed at investigating 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 to undergo radiofrequency AF ablation, and meeting the inclusion criteria, over a predefined period of 18 months.

From November 2011 to April 2013, we enrolled 19 consecutive patients with paroxysmal AF, referred to undergo RF 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 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 ethical review board.

Remote ischemic preconditioning
The method to induce remote IPC has been described in detail elsewhere (11). Shortly, 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 to the right arm and inflated to a pressure value 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.

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

All patients underwent trans-septal atrial puncture and AF ablation using an intra-cardiac echocardiography-guided technique (Cypress Acuson system, Siemens, Mountain View, CA, with a Soundstar probe, Biosense Webster)\textsuperscript{14,15}. Trans-septal atrial catheterization was performed by trans-septal assembly consisting of (i) 8F Preface sheath (Biosense Webster, Diamond Bar, CA, USA), (ii) dilator, and (iii) 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, Diamond Bar, CA, USA) or an EnSite NavX mapping system (St Jude Medical, Inc., St Paul, MN, USA) in 12 and 7 patients, respectively. Irrigated RF energy was delivered with a target temperature of 43°C and a power between 25 and 35W, using an irrigated ablator catheter (Navistar Thermo Cool, Biosense Webster). The electrophysiological endpoint was 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\textsuperscript{16}. No additional lines were made.

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

Peri-procedural anticoagulation during the intervention was performed in accordance with current guidelines\textsuperscript{17}. A single bolus of 50 IU/kg of heparin was administered after trans-septal puncture; thereafter, additional boluses of heparin were given to maintain the activated
coagulation time between 250-350 s, which was checked at 30-minute intervals throughout the procedure, unless differently indicated. 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 RF 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 platelet receptors, glycoprotein (GP) IIb/IIIa (CD41) and P-selectin (CD62), by flow cytometry.

In each patient, blood samples of 15 ml were collected from the right femoral vein, via a 6F venous sheath: (i) at baseline, before remote IPC/sham intermittent ischemia; (ii) immediately after remote IPC/sham intermittent ischemia, before ablation; (iii) immediately after the end of ablation procedure; (iv) 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 discarding the first 5 ml 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. Appropriate fluorochrome-conjugated isotype-matched mAb, obtained from the different manufactures, were
used as control for background staining.

**Monocyte-platelet aggregates**

Blood (100 µl) was labeled within 10 minutes of collection with a saturating concentration of PerCP-conjugated CD14 (LPS protein receptor) and FITC-conjugated Glycoprotein IIb/IIIa (GP IIb, CD41) for 15 minutes at room temperature. Following incubation, erythrocytes were lysed with buffered ammonium chloride and analyzed by FACScan. MPAs were identified using the logical gating facility by combination of binding characteristics of anti-CD14 (monocyte marker) and of 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 phosphate-buffered saline (PBS) and labeled within 10 min of collection by incubation with specific antibodies. Blood aliquots (5 mL) were incubated for 15 min at room temperature with saturating concentrations of PE-conjugated CD41 and FITC-conjugated PAC-1 to study the basal and the active form of platelet expression of GP IIb/IIIa receptor (Becton–Dickinson, Milan, Italy), respectively. Following incubation, samples were diluted with 200 mL of PBS and immediately analysed by a flow cytometer Becton–Dickinson FACScan. An acquisition gate was first established on FSC/SSC signals. These 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 backgating 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 MFI.

**ADP stimulation**

Blood samples were incubated with ADP (10^{-7} M) for 15 min 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 experiments19.

Statistical analyses

Comparisons between groups of continuous variables were done by t-test. A generalized linear
model (GLM) for repeated measures was applied to compare the curves of platelet activation
markers throughout the RF 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-test, respectively, in order to obtain and provide evidence of where
significant changes did occur. Global differences were considered significant when \( p \leq 0.05 \). As
there were many individual between-group and within-group comparisons for markers of platelet
activation throughout RF ablation, for these multiple comparisons we considered as significant
only statistical differences with \( p \) value <0.01. Continuous data are reported as mean±standard
deviation. The SPSS version 12.02 statistical software (SPSS Inc. Florence, Italy) has been used
for statistical analyses.

Results

General findings

The main clinical characteristics of the 2 groups are summarized in Table 1, whereas the main
data relative to the characteristics of paroxysmal AF episodes and RF 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 to the main
findings concerning RF ablation procedure, which was always performed without any clinically
relevant complication in all patients.

**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 in the left panels of Figures 1-4. There were no significant differences between the 2 groups in spontaneous MPA formation, CD41 expression in the MPA and platelet gate, and in CD62 expression in the platelet gate, both in basal conditions and after the preconditioning/sham procedure.

A significant increase in platelet markers was observed in both groups during the RF ablation (p<0.01 for all variables in both groups), which persisted at 24 hours after the procedure. GLM for repeated measures showed a significant difference in the curve changes between the two groups (p<0.0001 for MPAs, MPA-related CD41 and platelet CD41; p=0.037 for CD62; Table 3), due to lower platelet increase in remote IPC patients, compared to controls, both at end ablation and 24 hours later (Figures 1-2).

**Platelet reactivity**

Platelet markers measured following ADP stimulation are summarized in Table 4 and their trends are shown in the right panels of Figures 1-4. As expected, ADP always increased the expression of platelet markers in either group, as compared to the respective basal time point. 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 RF ablation, which persisted at 24 hours after the procedure (p<0.01 for all variables in both groups).

GLM for repeated measures showed a significant difference in the curve changes between the two groups (p<0.0001 for MPAs, MPA-related CD41 and platelet CD41; p=0.007 for CD62;
Table 4), due to a lower increase in platelet markers in remote IPC patients, compared to controls, both at end ablation and 24 hours later, but also after the remote IPC/sham procedure (Figures 3-4).

Discussion

Two main results emerge from our data: 1) RF ablation for paroxysmal AF is associated with a significant increase in platelet activation and reactivity, which persists up to 24 hours after the procedure; 2) 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.

RF catheter ablation has become a reference therapy for AF and its success varies from 16 to 84%, based on clinical features of patients, method of ablation, characteristics of the arrhythmia (e.g., paroxysmal vs. permanent AF) and atrial substrate.

A potential benefit of successful AF ablation is the abolition of thromboembolic events related to the persistence and/or recurrence of the arrhythmia. RF ablation of AF, however, is by itself associated with an increased risk of thromboembolic events in the early period following the procedure, mostly in the first 2 weeks. This undesired complication has been reported in up to 7% of patients, even in spite of the use of appropriate anticoagulation. Moreover, silent cerebral embolic events have been detected in up to 35% of patients.

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. Several factors may lead to increased platelet activation in this context, including the RF-induced injury of subendocardial left atrium wall, which also results in the activation of the...
coagulation cascade and inflammatory reaction\textsuperscript{4,6,8}. Antiplatelet agents can be administered to reduce this untied effect, but their efficacy in this setting remains unknown\textsuperscript{5,8}.

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\textsuperscript{27,28}.

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

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\textsuperscript{9,10}. In the clinical setting, remote IPC has been shown to limit myocardial suffering and damage following transient ischemic episodes\textsuperscript{13} or persistent thrombotic coronary occlusion\textsuperscript{33}.

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 of platelet reactivity.

Hata et al., showed that IPC was able to reduce platelet-mediated thrombosis in a model of damaged and stenotic coronary arteries in dogs\textsuperscript{34}. In a similar model Linden et al. 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\textsuperscript{35}. Finally,
Posa et al., in a model of acute myocardial infarction in pigs, found that IPC resulted in a lower mean platelet volume, suggesting attenuation of platelet activation\textsuperscript{36}.

The experimental evidence that myocardial IPC can reduce platelet activation was recently confirmed in clinical studies\textsuperscript{13,37}. Importantly, while no experimental studies 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\textsuperscript{11,12}.

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 RF ablation of AF and that its anti-platelet effect persists up to 24 hours after the procedure.

The mechanisms responsible for the anti-platelet effect of remote IPC during RF 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\textsuperscript{34,38}, might play some role. A modulation of the complex dynamic interaction between endothelial cells and platelets, however, might also be involved\textsuperscript{9,12}.

Independently of the mechanisms, a major consequence of our findings is that remote IPC might be applied to reduce platelet activation and reactivity during RF 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.

Some limitations of our study should be acknowledged. First, we enrolled only a small number of patients, which resulted in some little, although not significant, differences between groups (table 1) and in the absence of some kinds of patients with a potential increased risk of
platelet activation, as those with diabetes or with heart disease; thus, our findings require
confirmation in larger populations, also including 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\textsuperscript{34,38}, appropriate
studies are needed to define the mechanism(s) underlying the phenomenon.

Conflict of Interest Disclosures: None.

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Table 1. Main basal clinical findings of patients.

<table>
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<tr>
<th></th>
<th>Remote IPC (n=10)</th>
<th>Controls (n=9)</th>
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<tbody>
<tr>
<td><strong>Clinical data</strong></td>
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<tr>
<td>Age (years)</td>
<td>58.1±8</td>
<td>50.1±13</td>
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<tr>
<td>Male/female</td>
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<tr>
<td>Smoking</td>
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<td>Hypertension</td>
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<td>Hypercholesterolemia</td>
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<td>3</td>
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<tr>
<td>Family history of CAD</td>
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<td>4</td>
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<tr>
<td><strong>Laboratory data</strong></td>
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<tr>
<td>Platelet count (10³/mm³)</td>
<td>229±45</td>
<td>199±65</td>
</tr>
<tr>
<td>Prothrombin activity (%)</td>
<td>59.8±26</td>
<td>67.9±29</td>
</tr>
<tr>
<td>aPTT (sec)</td>
<td>38.1±6.8</td>
<td>37.2±5.2</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>314±66</td>
<td>281±52</td>
</tr>
<tr>
<td>INR</td>
<td>1.47±0.6</td>
<td>1.42±0.6</td>
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<tr>
<td><strong>Pre-ablation medications</strong></td>
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<tr>
<td>ß-blockers</td>
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<td>7</td>
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<tr>
<td>Diuretics</td>
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<tr>
<td>Ca²⁺ channel blockers</td>
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<td>ACE-inhibitors</td>
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<tr>
<td>ARBs</td>
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<td>Antiarrhythmic drugs</td>
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<td>Aspirin</td>
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<td>3</td>
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<tr>
<td>Anticoagulants</td>
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</table>

ACE=Angiotensin-converting enzyme; aPTT=activated partial thromboplastin time; ARBs=angiotensin receptor blockers; INR=International Normalized Ratio.

Table 2. Main findings of atrial fibrillation and radiofrequency ablation procedure in the 2 groups of patients.

<table>
<thead>
<tr>
<th></th>
<th>Remote IPC (n=10)</th>
<th>Controls (n=9)</th>
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<tbody>
<tr>
<td>AF duration (years)</td>
<td>4.6±4.3</td>
<td>6.3±3.5</td>
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<tr>
<td>Time from the last AF episode (days)</td>
<td>35.0±20.3</td>
<td>44.7±31.3</td>
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<tr>
<td>Duration of the last AF episode (min)</td>
<td>483±392</td>
<td>567±467</td>
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<tr>
<td>Duration of RF ablation (min)</td>
<td>324±34</td>
<td>330±60</td>
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<tr>
<td><strong>Procedural activated coagulation time</strong></td>
<td></td>
<td></td>
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<tr>
<td>Average (s)</td>
<td>327.6±13.8</td>
<td>331.7±10.3</td>
</tr>
<tr>
<td>Minimal (s)</td>
<td>299.8±17.5</td>
<td>308.9±22.5</td>
</tr>
<tr>
<td>Maximum (s)</td>
<td>345.3±18.1</td>
<td>351.7±16.4</td>
</tr>
<tr>
<td><strong>24 hours after ablation</strong></td>
<td></td>
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<tr>
<td>aPTT (s)</td>
<td>37.0±7.1</td>
<td>36.1±6.8</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>402.4±82.4</td>
<td>386.1±102.3</td>
</tr>
</tbody>
</table>

AF=atrial fibrillation; aPTT=activated partial thromboplastin time.
Table 3. Platelet cytometry variables in the two groups in the absence of agonist stimulation

<table>
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<tr>
<th></th>
<th>Remote IPC</th>
<th>Controls</th>
<th>p*</th>
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<tbody>
<tr>
<td><strong>MPA (%)</strong></td>
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<tr>
<td>Basal</td>
<td>23.67±1.50</td>
<td>23.07±1.22</td>
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<tr>
<td>After RIPC</td>
<td>24.27±1.42</td>
<td>22.97±1.54</td>
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<tr>
<td>End of procedure</td>
<td>29.09±0.79</td>
<td>32.08±3.13</td>
<td>&lt;0.0001</td>
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<tr>
<td>24-h after procedure</td>
<td>30.53±0.65</td>
<td>32.76±1.81</td>
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</tr>
<tr>
<td><strong>CD41 in MPA gate (mfi)</strong></td>
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<td></td>
</tr>
<tr>
<td>Basal</td>
<td>21.98±1.81</td>
<td>21.97±1.45</td>
<td></td>
</tr>
<tr>
<td>After RIPC</td>
<td>23.09±1.33</td>
<td>22.01±1.59</td>
<td></td>
</tr>
<tr>
<td>End of procedure</td>
<td>27.51±1.17</td>
<td>31.04±0.89</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>24-h after procedure</td>
<td>29.85±0.63</td>
<td>31.76±0.72</td>
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<tr>
<td><strong>CD41 in platelet gate (mfi)</strong></td>
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<tr>
<td>Basal</td>
<td>22.87±1.46</td>
<td>22.46±1.16</td>
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<tr>
<td>After RIPC</td>
<td>23.34±1.08</td>
<td>24.32±1.51</td>
<td></td>
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<tr>
<td>End of procedure</td>
<td>26.70±1.12</td>
<td>33.61±1.42</td>
<td>&lt;0.0001</td>
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<td>24-h after procedure</td>
<td>29.33±0.89</td>
<td>34.41±1.19</td>
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<tr>
<td><strong>CD62 in platelet gate (mfi)</strong></td>
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<tr>
<td>Basal</td>
<td>9.12±1.67</td>
<td>10.18±1.29</td>
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<tr>
<td>After RIPC</td>
<td>9.59±2.11</td>
<td>10.94±1.05</td>
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</tr>
<tr>
<td>End of procedure</td>
<td>9.80±1.94</td>
<td>12.08±1.09</td>
<td>0.037</td>
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<tr>
<td>24-h after procedure</td>
<td>10.16±1.93</td>
<td>12.51±1.00</td>
<td></td>
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mfi=mean fluorescence intensity; MPA=monocyte-platelet aggregates.
*p values for differences between groups in group-variable interaction.
Table 4. Platelet cytometry variables in the two groups after ADP stimulation

<table>
<thead>
<tr>
<th></th>
<th>Remote IPC</th>
<th>Controls</th>
<th>p*</th>
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<td><strong>MPA (%)</strong></td>
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<tr>
<td>Basal</td>
<td>27.30±1.24</td>
<td>28.30±1.27</td>
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<tr>
<td>After RIPC</td>
<td>28.72±1.11</td>
<td>33.24±1.79</td>
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<tr>
<td>End of procedure</td>
<td>31.41±1.29</td>
<td>38.86±1.77</td>
<td>&lt;0.0001</td>
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<tr>
<td>24-h after procedure</td>
<td>33.88±1.78</td>
<td>39.01±0.92</td>
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<tr>
<td><strong>CD41 in MPA gate (mfi)</strong></td>
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<td></td>
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<tr>
<td>Basal</td>
<td>26.03±1.00</td>
<td>26.43±1.34</td>
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<tr>
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<td>27.49±1.62</td>
<td>30.25±1.29</td>
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<tr>
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<td>30.26±0.72</td>
<td>37.77±2.21</td>
<td>&lt;0.0001</td>
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<tr>
<td>24-h after procedure</td>
<td>31.84±1.14</td>
<td>37.27±0.81</td>
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<td><strong>CD41 in platelet gate (mfi)</strong></td>
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<tr>
<td>Basal</td>
<td>26.24±1.18</td>
<td>27.05±1.07</td>
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<tr>
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<td>26.14±1.29</td>
<td>32.67±1.58</td>
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<tr>
<td>End of procedure</td>
<td>27.62±1.38</td>
<td>37.01±2.14</td>
<td>&lt;0.0001</td>
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<tr>
<td>24-h after procedure</td>
<td>32.33±1.52</td>
<td>38.31±1.22</td>
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<tr>
<td><strong>CD62 in platelet gate (mfi)</strong></td>
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<tr>
<td>Basal</td>
<td>11.21±2.03</td>
<td>12.81±2.10</td>
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<tr>
<td>After RIPC</td>
<td>11.16±2.16</td>
<td>13.70±2.13</td>
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<tr>
<td>End of procedure</td>
<td>11.91±2.56</td>
<td>14.20±1.68</td>
<td>0.007</td>
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<tr>
<td>24-h after procedure</td>
<td>11.34±2.28</td>
<td>14.29±2.03</td>
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</tr>
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</table>

mfi=mean fluorescence intensity, MPA=monocyte-platelet aggregates.
*p values for differences between groups in group-variable interaction.

Figure Legends:

Figure 1. Monocyte-platelet aggregates in the absence (left) and in presence of adenosine diphosphate (ADP) (right) in the 2 groups. p values refer to between-group comparisons at each time point.

Figure 2. CD41 expression in the monocyte-platelet aggregate (MPA) gate in the absence (left) and in presence of adenosine diphosphate (ADP) (right) in the 2 groups. p values refer to between-group comparisons at each time point.
Figure 3. CD41 expression in the platelet gate in the absence (left) and in presence of adenosine diphosphate (ADP) (right) in the 2 groups. p values refer to between-group comparisons at each time point.

Figure 4. CD62 expression in the platelet gate in the absence (left) and in presence of adenosine diphosphate (ADP) (right) in the 2 groups. p values refer to between-group comparisons at each time point.
Figure 3
Effect of Remote Ischemic Preconditioning on Platelet Activation and Reactivity Induced by Ablation for Atrial Fibrillation

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

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