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# Non-ischaemic titrated cardiac injury caused by radiofrequency catheter ablation of atrial fibrillation mobilizes CD34-positive mononuclear cells by non-stromal cell-derived factor-1α mechanism

**Sook Kyoung Kim[1], Hui-Nam Pak[2]*, Jae Hyung Park[2], Jong Il Choi[1], Myung-Hyun Nam[3], Yunjung Jo[3], and Young-Hoon Kim[1]**

[1]Korea University Cardiovascular Center, Seoul, Republic of Korea; [2]Division of Cardiology, Yonsei University Hospital System, 250 Seongsan-no, Seodaemun-gu, Seoul 120-752, Republic of Korea; and [3]Department of Laboratory Medicine, Korea University, Seoul, Republic of Korea

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**Aims** It has been known that myocardial ischaemia mobilizes CD34+ bone marrow-derived cells by the stromal cell-derived factor (SDF)-1α pathway. We hypothesized that non-ischaemic titrated cardiac injury caused by radiofrequency catheter ablation (RFCA) of atrial fibrillation (AF) recruits CD34+ cells by an alternative mechanism.

**Methods and results** Fifty-six patients (39 males, 53.0 ± 13.5 years old) who underwent electrophysiology study (EPS; $n = 10$) or RFCA of AF ($n = 46$) were included. Peripheral blood CD34+ cell count and multiple serologic markers were evaluated before, immediately after, at 24 h, and 10 days after the procedure. The results are as follows: (i) the per cent increase in CD34+ cells (%ΔCD34+) was significant after RFCA compared with after EPS ($P < 0.01$), and correlated with RF duration and troponin I, respectively. (ii) In contrast, SDF-1α decreased after RFCA and had no correlation with %ΔCD34+ cells while matrix metalloproteinase (MMP)-9 ($P < 0.0001$) and GROβ ($P < 0.001$) increased after RFCA and had correlations with 24 h %ΔCD34+ cells.

**Conclusion** Non-ischaemic titrated cardiac injury caused by AF ablation mobilizes CD34+ cells to the peripheral blood through a non-SDF-1α pathway associated with MMP-9 and GROβ.

**Keywords** CD34+ mononuclear cell • Atrial fibrillation • Catheter ablation • Cardiac injury

## Introduction

It has been reported that endothelial progenitor cells can be isolated from circulating mononuclear cells[1–3] and bone marrow.[4] These cells express a number of endothelial cell surface markers and exhibit endothelial properties.[1,3] Circulating endothelial progenitor cells increase after vascular injury,[5,6] acute myocardial infarction,[5] and percutaneous coronary intervention[7] by stromal cell-derived factor (SDF)-1α.[8–12] Although there are significant differences in critical mass and tissue characteristics between ventricles and atria, Goette *et al.*[13] reported an increase in haematopoietic progenitor cells immediately after electrical cardioversion of atrial fibrillation (AF) related to SDF-1α. GROβ$_{\Delta 4}$ also mobilizes peripheral blood mononuclear cells by a mechanism less dependent on the SDF-1α/CXCR4 axis,[14] but is related to angiopoietin-1 and MMP-9.[15–17] Most studies of CD34+ cell mobilization have been related to myocardial ischaemia or endothelial function. Mobilization of circulating CD34+ mononuclear cells by non-ischaemic titrated cardiac injury has not been previously reported. The radiofrequency catheter ablation (RFCA) technique is an effective rhythm control strategy for AF.[18] Therefore, we hypothesized that non-ischaemic titrated cardiac injury

* Corresponding author. Tel: +82 2 2228 8459, Fax: +82 2 393 2041, Email: hnpak@yuhs.ac

caused by AF ablation can recruit CD34+ cells. The purpose of this study was to investigate the mechanism of the peripheral blood CD34+ cell mobilization after AF ablation and to determine its effects on the outcome of the ablation procedure.

# Methods

## Patient selection

Fifty-six patients (39 males, 53.0 ± 13.5 years old) who underwent electrophysiology study (EPS) ($n = 10$) or RFCA for AF [$n = 46$; paroxysmal AF (PAF):persistent AF (PeAF) = 26:20] were included in this study. In 10 patients who underwent EPS, we used basic programmed stimulation but did not perform RFCA. In 46 patients with AF, a mean of 1.8 ± 1.4 antiarrhythmic drugs were proven to be ineffective. The mean left atrial (LA) size measured by echocardiography was 42.2 ± 7.2 mm and did not show structural heart disease, except for left ventricular hypertrophy. Three-dimensional (3D) computed tomography (CT) scans were performed to visually define the anatomy of the pulmonary veins (PVs). The presence of LA thrombus was excluded by transoesophageal echocardiography. All antiarrhythmic drugs were discontinued for a period corresponding to at least five half-lives. Amiodarone was discontinued for at least 4 weeks. Anticoagulation therapy was continued until the day of the procedure.

## Electrophysiological mapping

The study protocol was approved by the Institutional Review Board of Anam Hospital, Korea University, and adhered to the declaration of Helsinki. All patients provided written informed consent. Intracardiac electrograms were recorded using a Prucka CardioLab™ electrophysiology system (General Electric Medical Systems, Inc., Milwaukee, WI, USA). For the EPS group ($n = 10$), we used three mapping catheters and performed basic EPS by programmed stimulation. Atrial and ventricular stimulation protocols were conducted without RF energy delivery. For the AF RFCA group ($n = 46$), we used five mapping catheters and a 7 Fr deflectable 3.5 mm tip open irrigation tip ablation catheter (Celsius, Johnson & Johnson Inc., Diamond Bar, CA, USA) and catheter ablation procedures were performed using a 3D electroanatomical mapping system (NavX; St Jude Medical Inc., Minneapolis, MN, USA) in all patients. If the initial rhythm was AF lasting >5 min, AF was terminated by internal cardioversion (2, 5, 7, to 10 J serially, depending on the result of cardioversion, biphasic shocks with R-wave synchronization, anodal decapolar catheter in the high right atrium to cathodal duodecapolar catheter inside of the coronary sinus. Lifepak12, Physiocontrol Ltd). Immediately after cardioversion, the earliest atrial premature beat initiating AF was mapped as we described previously.[19] Before catheter ablation, we generated a 3D LA electroanatomical map and voltage map by contact bipolar electrograms from 70–100 points in the LA endocardium during high right atrial pacing (cycle length, 500 ms). The voltage map was colour coded. Atrial fibrillation ablation was performed at the level of the LA antrum without touching the PV directly.

## Radiofrequency catheter ablation

Radiofrequency applications (30 W, 47°C, irrigation flow rate 20–30 mL/min, 40 s of RF delivery at each ablation point, Stockert generator, Biosense Webster Inc., Diamond Bar, CA, USA) were performed with a 7 Fr deflectable 3.5 mm tip open irrigation tip catheter (Celsius, Johnson & Johnson Inc.). Electrical isolation of all four PVs and circumferential antral ablation were performed in all patients. Cavotricuspid isthmus block and linear ablation of the left lateral isthmus, LA roof, and septum were carried out in patients with PeAF, depending on AF inducibility in those with PAF. The total procedure time, fluoroscopic time, duration of RF energy delivery, and non-ablation procedure time (total procedure time − ablation time) were counted in all patients. After the procedure, patients were followed without antiarrhythmic medications and asked to visit the outpatient clinic at 1, 3, 6, 9, and 12 months, and every 6 months thereafter. Electrocardiography (ECG) was performed at every visit or anytime the patient reported palpitations. Holter ECG (24 or 48 h) and/or event recorder was evaluated at 3, 6, and 12 months after RFCA.

## Protocol for quantification of CD34+ mononuclear cells

Peripheral blood samples were taken before, immediately after, at 24 h, and 10 days after the procedure to quantify CD34+ haematopoietic progenitor cells and biochemical analyses. Peripheral blood circulating CD34+ mononuclear cell count was measured by a fluorescence-activated cell sorter (FACS) within 4 h of sampling. Flow cytometry was done using a monoclonal antibody to CD34 (Becton–Dickinson, Heidelberg, Germany). The Stem-Kit Reagents (Beckman–Coulter, USA) utilized a calibrated number of fluorescent beads with direct quantification of CD34+ cell concentration. Samples were gated to the CD45+ population from which CD34+ cells were identified. The cut-off point for CD34 positivity was determined from isotype-matched antibodies.

## Biochemical analyses

We measured serum levels of SDF-1α, granulocyte colony-stimulating factor (G-CSF), GRO-β, MMP-9, and angiopoietin-1 using ELISA kits (R&D Systems, Minneapolis, MN, USA) to evaluate the mechanism of haematopoietic progenitor cell mobilization after non-ischaemic cardiac injury. Serum concentration of troponin I at 24 h after RFCA was also quantified by the Vitros ECiQ (OCD, Johnson & Johnson, Holmers Farm Way, UK) method to estimate the degree of cardiac injury.

## Analyses of left atrial electroanatomic voltage map

To quantify the degree of atrial substrate remodelling, we analysed the colour-coded LA electroanatomical voltage maps as reported previously.[20,21] Both anterior–posterior and posterior–anterior views of each map were captured and converted to image files. All of the colour voltage images were divided into four quadrants, and the percentages of each colour were measured and calculated. Pulmonary veins were not included in the analysis. The percentage of colour-coded areas in each quadrant (eight quadrants per patient) of voltage maps was analysed by custom software (Image Pro) and referenced to colour scale bars. The NavX system detected peak-to-peak voltage differences in each contact bipolar electrogram and changed them to colour codes. Low-voltage areas <0.2 mV were coded grey and high-voltage areas ≥1.0 mV were purple. The mean LA voltage was calculated by summation of per cent area of each colour multiplied by representative voltage, and then divided by the total LA area.

## Data analyses

We analysed the per cent change in CD34+ (%ΔCD34+) cell count and serum levels of protein biological markers according to time sequence before and after EPS or RFCA. Left atrial size, the mean LA voltage, total duration of RF energy delivery, non-ablation procedure time, and incidence of recurrence of AF after ablation were compared with %ΔCD34+ cells and biological markers. Data are

expressed as mean ± standard deviation (SD). Linear correlation, Student's *t*-test, and Fisher's exact test were used for analyses of comparisons. A *P*-value of $<0.05$ was considered statistically significant.

# Results

## Non-ischaemic titrated cardiac injury by radiofrequency catheter ablation mobilizes peripheral blood CD34+ mononuclear cells

The %ΔCD34+ cells were significantly elevated immediately (60.2 ± 87.3%, $P < 0.001$) and 24 h after AF ablation (59.4 ± 112.6%, $P < 0.001$), and remained increased for 10 days after RFCA (58.7 ± 130.8%, $P < 0.01$). They were also significantly elevated after RFCA for AF compared with after EPS (*Figure 1*). We compared %ΔCD34+ cells instead of absolute cell count because FACS was performed within 4 h of blood sampling and reset the control value in each blood sample. The total duration of RF energy delivery, the specific marker for cardiac injury (troponin I), and %ΔCD34+ cells were correlated with one other with statistical significance (*Figure 2A–C*). However, non-ablation procedure time (total procedure time − total duration of RF ablation) and fluoroscopic time were not associated with %ΔCD34+ cells ($P$ = NS; *Figure 2D* and *E*). We delivered 2.85 ± 2.46 times of low-energy internal cardioversion shocks (total shock, 2.85 ± 20.32 J) during the procedure, but they did not have a correlation with post-ablation %ΔCD34+ cells ($P$ = NS). Post-ablation absolute CD34+ cell count (1.99 ± 1.09 vs. 3.59 ± 3.40/μL, $P$ = NS) and %ΔCD34+ cells (49.2 ± 86.1 vs. 57.1 ± 103.1%, $P$ = NS) were not significantly different with ($n = 35$) or without low-energy electrical cardioversion ($n = 11$).

## CD34+ cell mobilization after non-ischaemic titrated cardiac injury by non-stromal cell-derived factor-1α mechanism

To estimate the mechanism of CD34+ mononuclear cell mobilization, we quantified the serial change of SDF-1α, G-CSF, angiopoietin-1, MMP-9, and GROβ. Stromal cell-derived factor-1α, which plays a role in the mobilization of CD34+ cells after ischaemic cardiovascular injury, was not increased after RFCA, but rather decreased at 24 h after RFCA (2.09 ± 0.69–1.60 ± 0.43 ng/mL, $P < 0.01$; *Figure 2E*). Neither did G-CSF after RFCA (7.88 ± 13.64–9.50 ± 16.02 μg/mL, $P$ = NS). In contrast, both MMP-9 (39.97 ± 26.34–119.65 ± 71.42 ng/mL, $P < 0.01$) and GROβ (0.29 ± 0.22–0.82 ± 0.60 ng/mL, $P < 0.01$) were significantly increased immediately after RFCA for AF (*Figure 2F* and *G*). We compared the correlations between immediate post-ablation plasma levels of each serologic marker to those related to progenitor cell mobilization and %ΔCD34+ cells at 24 h after RFCA. Although SDF-1α did not have any correlation with %ΔCD34+ cells (*Figure 3A*), GROβ ($R = 0.61$, $P < 0.001$) and MMP-9 ($R = 0.44$, $P < 0.01$) had significant correlations with the degree of CD34+ mononuclear cell mobilization (*Figure 3B* and *C*). GROβ and MMP-9 may play a role in mononuclear cell mobilization and homing as a non-SDF-1α alternative mechanism, and

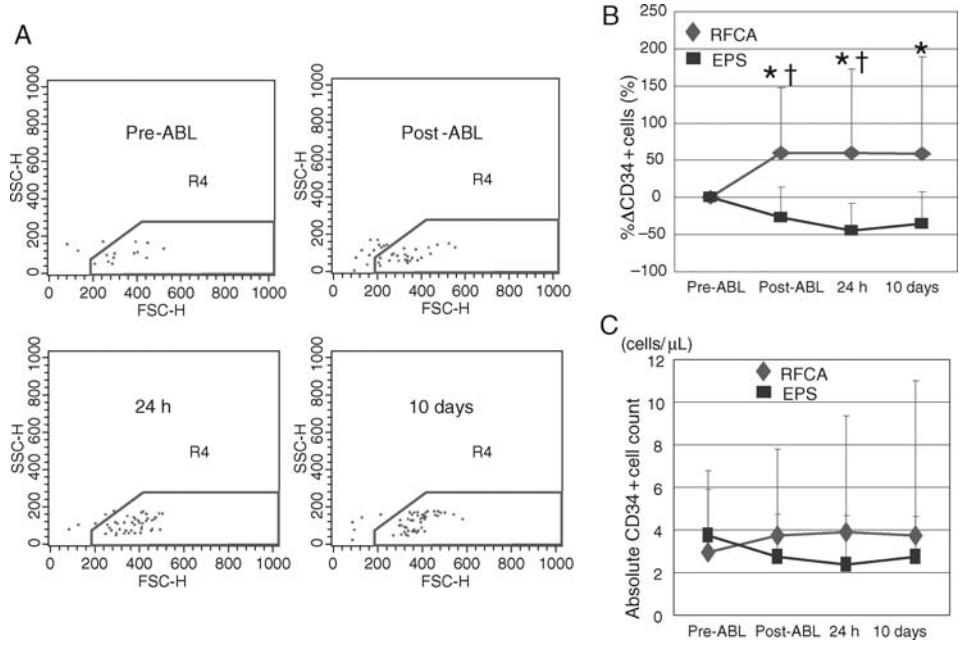


**Figure 1** (*A*) Flow cytometry detection of endothelial progenitor cells double-labelled with CD45 and CD34 detected in gate R4 by FACS. (*B*) The %ΔCD34+ cells were significantly increased after RFCA immediately, at 24 h, and 10 days in patients with AF, and immediate and 24 h post-ablation %ΔCD34+ cells were higher than after EPS. (*C*) Absolute CD34+ cell count showed a similar pattern of change to %ΔCD34+ cells without statistical significance. $^{*}P < 0.01$ vs. pre-ABL; $^{\#}P < 0.01$ vs. EPS.

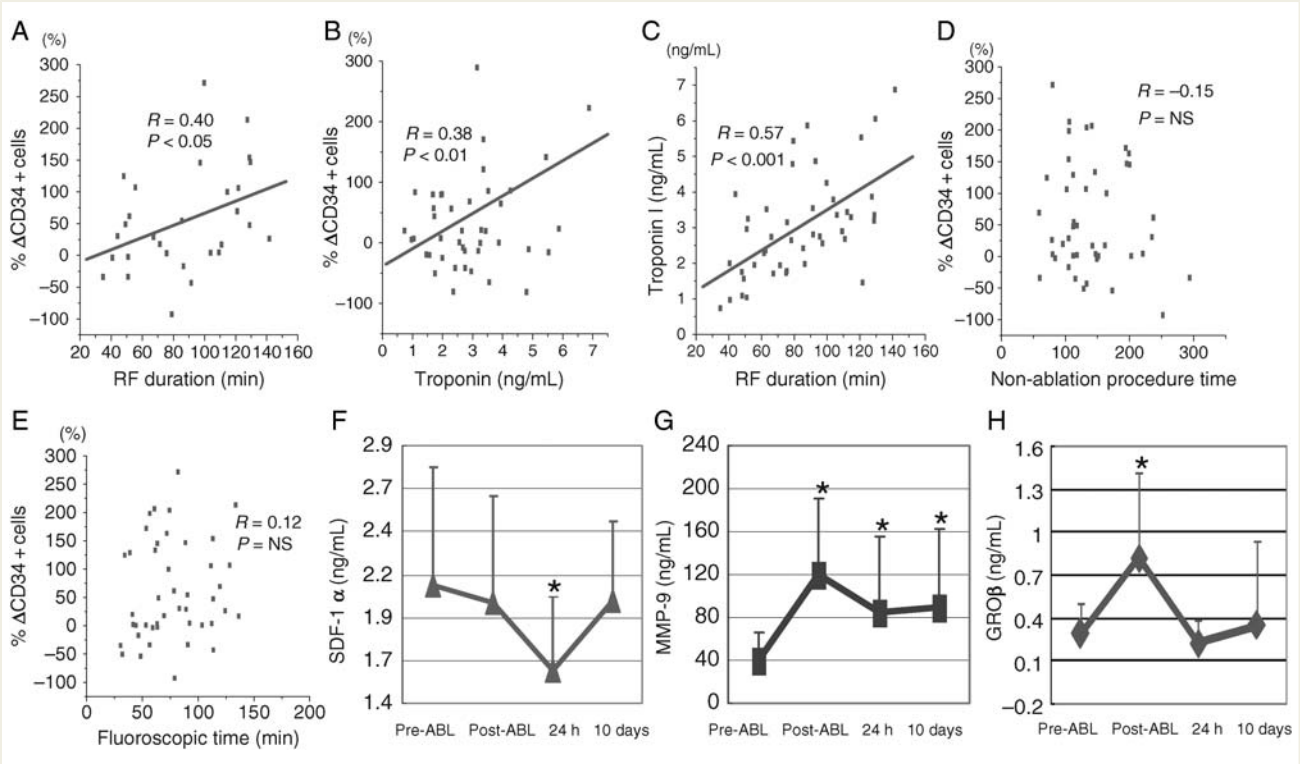


**Figure 2** The %ΔCD34+ cells correlated with non-ischaemic titrated cardiac injury after RFCA. (*A*) Correlation between %ΔCD34+ cells and the total duration of RF energy delivery. (*B*) Correlation between %ΔCD34+ cells and the cardiac-specific injury marker troponin I. (*C*) Correlation between RF duration and level of troponin I. (*D* and *E*) Non-ablation procedure time (total procedure time − RF ablation time) or fluoroscopic time did not have any correlation with %ΔCD34+ cells. (*F*) The plasma level of SDF-1α did not increase after RFCA for AF, but rather decreased 24 h after RFCA. (*G* and *H*) Both plasma levels of MMP-9 and GROβ increased immediately after RFCA for AF. *$P < 0.01$.

plasma levels of GROβ and MMP-9 had a good correlation ($R = 0.62$, $P < 0.01$; *Figure 3D*).

## Atrial fibrillation burden and mobilization of CD34+ cells

In terms of AF burden, we compared patients with PAF and PeAF (*Table 1*). Patients with PeAF were older, had a larger LA size, and received a longer duration of RFCA compared with those with PAF. Pre-ablation absolute CD34+ cell count was not different between patients with PAF and PeAF. In contrast, post-ablation absolute CD34+ cell count was significantly higher in patients with PeAF ($P < 0.05$, *Figure 4A*) who underwent a longer duration of RF delivery (91.43 ± 26.64 vs. 68.34 ± 26.36 min, $P < 0.01$) than in those with PAF. Because low LA voltage reflects long-lasting AF with structural remodelling, we compared LA voltage and %ΔCD34+ cells in 16 patients. Post-ablation %ΔCD34+ cell count was significantly higher in patients with a mean LA voltage <2.3 mV (147.7 ± 85.9%) than in those with a mean LA voltage ≥2.3 mV (43.7 ± 78.2%, $P < 0.01$, *Figure 4C*). Both LA voltage ($R = -0.50$, $P < 0.05$) and its heterogeneity (SD of LA voltages in eight different segments of the LA; $R = -0.65$, $P < 0.01$) exhibited a negative correlation with post-ablation %ΔCD34+ cells (*Figure 4D* and *E*).

# Discussion

In this study, we documented the effects of non-ischaemic titrated cardiac injury on the mobilization of bone marrow-derived CD34+ mononuclear cells in peripheral blood after RFCA of AF. We also suggest that the non-SDF-1α mechanism is associated with MMP-9 and GROβ in the mobilization of CD34+ cells after RFCA. CD34+ cell mobilization was correlated with the duration of RF delivery, amount of cardiac injury reflected by troponin I levels, and more significant in patients with a mean LA voltage ≥2.3 mV than in those with a mean LA voltage <2.3 mV.

## Circulating CD34+ mononuclear cells and cardiovascular disease

Endothelial progenitor cells derived from bone marrow circulate in peripheral blood and are involved in neoangiogenesis after tissue ischaemia.[1,22,23] These cells express a number of endothelial cell surface markers, such as CD34+, and exhibit endothelial properties.[1,3] Circulating endothelial progenitor cells are capable of proliferating and differentiating into endothelial cells and contribute to vascular regeneration.[24] In addition, they have been shown to differentiate into cardiomyocytes and smooth muscle cells following myocardial infarction.[25] It has been reported that endothelial progenitor cells are mobilized after acute myocardial infarction,[5]

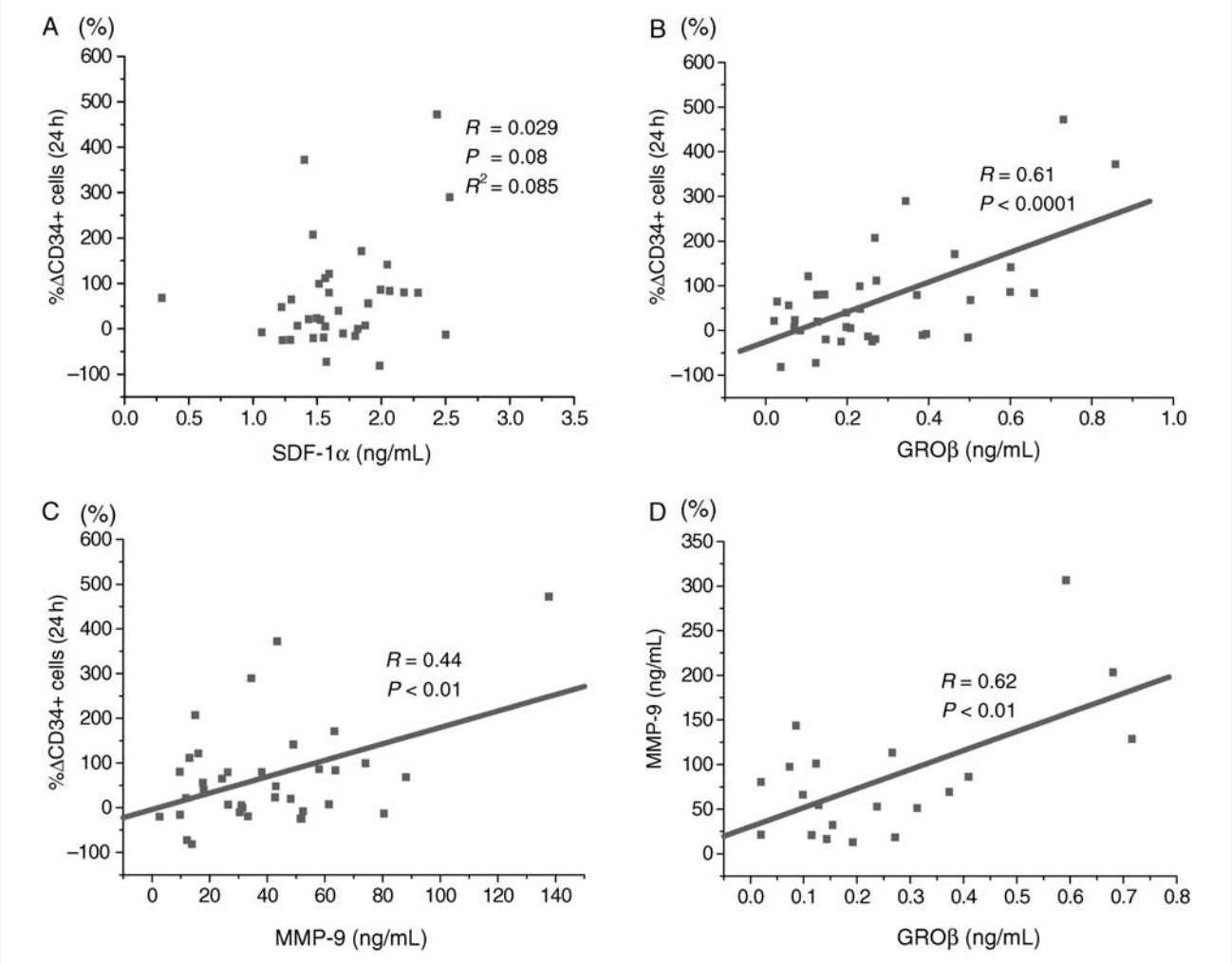


**Figure 3** (*A*) Immediate post-ablation SDF-1α did not have a correlation with %ΔCD34+ cells at 24 h post-ablation. (*B* and *C*) Immediate post-ablation plasma levels of GROβ and MMP-9 had weak correlations with 24 h post-RFCA %ΔCD34+ cells. (*D*) Post-ablation plasma levels of GROβ had correlations with those of MMP-9.

percutaneous coronary intervention,[7] and microvascular angina.[26] The level of circulating CD34+ endothelial progenitor cells has been reported to be able to predict the occurrence of cardiovascular events and death in patients with coronary artery disease.[27] Theses findings suggest that CD34+ cells play an important role in repair mechanisms after cardiac injury.[28] However, most clinical studies have been focused on myocardial ischaemia or vascular injury. To the best of our knowledge, this study is the first to prove non-ischaemic titrated cardiac injury-induced mobilization of CD34+ progenitor cells in peripheral blood, even after excluding the effects of AF burden, internal electrical cardioversion, or the non-ablation procedure itself.

## Mechanism of CD34+ mononuclear cell mobilization after non-ischaemic cardiac injury

The mechanism of endothelial progenitor cell mobilization after cardiac injury remains elusive. There have been several reports of potential biological messengers that induce mobilization or homing of peripheral blood- or bone marrow-derived endothelial progenitor cells. The chemokine SDF-1α has been shown to play a key role in CD34+ trafficking in the myocardial ischaemia model.[9,11,29] However, SDF-1α was not elevated nor related to CD34+ cell concentration in this study or other studies.[30] The mechanism for this result has yet to be clarified whether due to counter-balanced SDF-1α by alternative pathway, difference between ventricular tissue vs. atrial tissue, or ischaemic vs. non-ischaemic cardiac injury. In contrast, MMP-9 and GROβ were elevated immediately after RFCA and had good correlations with post-ablation 24 h %ΔCD34+ cells and to each other. Angiopoietin-1 has been known to induce stoichiometric changes in pro-MMP-9 and tissue inhibitor of metalloproteinase-1, and activated MMP-9 enhances GROβ/CXCR2 ligands that promote mobilization of bone marrow-derived progenitor and endothelial cells.[14–17] Fukuda *et al.*[14] reported that $GRO\beta_{\Delta 4}$ mobilizes peripheral blood mononuclear cells that adhere to vascular cell adhesion molecule-1 and endothelial cells, and this

**Table 1 Comparisons of clinical, electrophysiological, and biological parameters in patients with PAF vs. PeAF**

| | PAF ($n = 26$) | PeAF ($n = 20$) | *P*-value |
|---|---|---|---|
| Sex (M:F) | 17:9 | 17:3 | NS |
| Age (years) | 51.27 ± 12.54 | 59.65 ± 8.60 | <0.01 |
| LA size (mm) | 39.23 ± 5.58 | 46.6 ± 8.02 | <0.001 |
| Ejection fraction (%) | 53.42 ± 12.25 | 55.29 ± 5.53 | NS |
| Mean LA voltage (mV) | 6.75 ± 5.00 | 6.39 ± 4.43 | NS |
| RF duration (min) | 73.86 ± 28.16 | 95.28 ± 25.02 | <0.01 |
| Conc. of CD34+ cells (per μL) | | | |
| Pre-ABL | 2.64 ± 3.19 | 3.19 ± 4.43 | NS |
| Post-ABL | 3.18 ± 3.04 | 4.07 ± 5.02 | <0.05 |
| 24 h | 3.35 ± 5.22 | 3.78 ± 6.01 | NS |
| 10 days | 2.74 ± 3.49 | 4.77 ± 10.15 | NS |
| %ΔCD34+ cells (%) | | | |
| Post-ABL | 47.06 ± 73.49 | 80.47 ± 99.22 | NS |
| 24 h | 55.96 ± 82.62 | 73.0 ± 151.05 | NS |
| 10 days | 51.06 ± 141.69 | 64.78 ± 123.06 | NS |

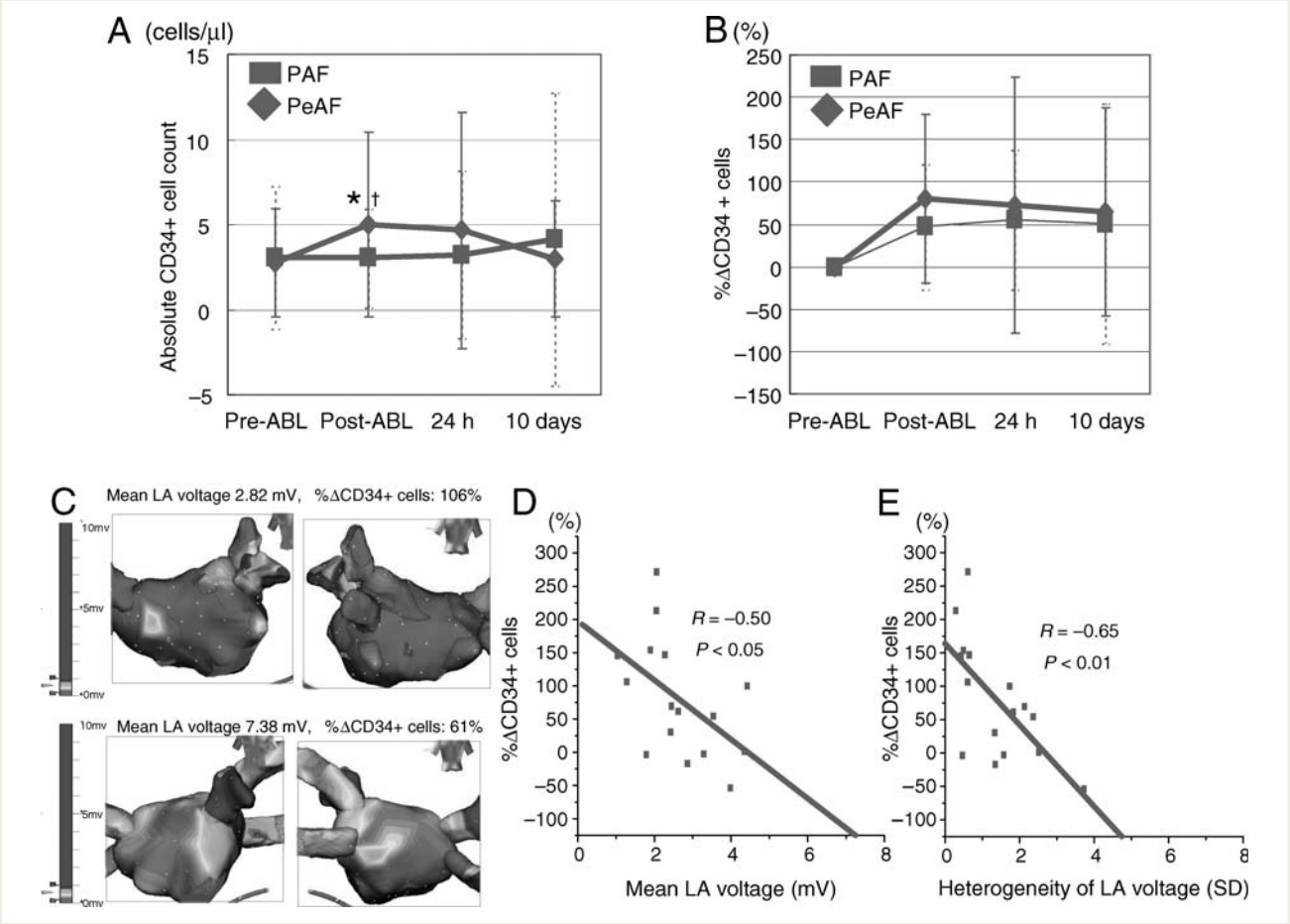


**Figure 4** (*A*) Comparison of PeAF and PAF over time and absolute CD34+ cell count. The increase in CD34+ mononuclear cells in peripheral blood was significant after RFCA for PeAF, but not for PAF. Immediate post-ablation CD34+ cell count was also higher in patients with PeAF than those with PAF. (*B*) Comparison of PeAF and PAF over time and %ΔCD34+ cells. (*C*) Examples of colour voltage maps with low and homogeneous LA voltage (upper panel) and high and heterogeneous LA voltage (lower panel). Left atrial voltage (*D*) and its spatial heterogeneity (*E*) had a negative correlation with the per cent change of CD34+ cells immediately after RFCA. $^{*}P < 0.05$ vs. PAF; $^{\dagger}P < 0.01$ vs. pre-ABL.

pathway is less dependent on the SDF-1α/CXCR4 axis. Therefore, non-ischaemic titrated cardiac injury mobilizes peripheral blood CD34+ mononuclear cells by non-SDF-1α mechanism in our clinical study model.

## CD34+ mononuclear cells and atrial fibrillation

Goette *et al.*[13] reported that PeAF appears to be associated with an increase in haematopoietic progenitor cells compared with PAF and controls. In contrast, low-energy internal electrical shocks did not affect CD34+ cell mobilization in our study. The reasons for the discrepancies were likely due to differences in clinical settings, lower energy used for electrical cardioversion, and RF-induced direct cardiac tissue injury. Recently, Stein *et al.*[30] reported that RFCA for PV isolation did not mobilize bone marrow-derived stem cells in patients with PAF. In their study, progenitor cell response to RFCA was minimal and delayed in patients with PAF as shown in *Figure 4A*. However, RFCA increased %ΔCD34+ cells in patients with PeAF significantly. The total duration of RF energy delivery was longer and the increase in troponin level was higher in our study. We also compared %ΔCD34+ cells instead of the absolute count of CD34+ cells because all cell counts were measured within 4 h of blood sampling and reset the control value in every FACS study.

## Study limitations

We tested our hypothesis on a relatively small number of selected patients who underwent RFCA. Because we performed RF energy delivery by continuous ablation, we could not count the exact number of RF lesions. Peripheral blood samples may not reflect the remote non-ischaemic cardiac injury of the atria, which is thinner and has a lower muscular mass than the ventricles. Although CD34+ cells include various types of non-specific bone marrow-derived cells, it reflects the cellular migration of endothelial progenitor cells as reported in previous studies.[5,7] Therefore, additional basic study analysing atrial tissue is warranted to prove the mechanism of CD34+ cell mobilization after non-ischaemic cardiac injury by RF energy delivery.

# Conclusions

This is the first study to document the effects of non-ischaemic titrated cardiac injury on the mobilization of CD34+ mononuclear cells in peripheral blood after RFCA of AF. CD34+ cell mobilization was correlated with the duration of RF delivery, amount of cardiac injury measured by troponin I level, and low and heterogeneous LA voltage. The potential mechanism was non-SDF-1α, non-G-CSF pathway, associated with MMP-9 and GROβ. Further study with a larger sample size will define the role of CD34+ mononuclear cell mobilization in the clinical recurrence of AF or long-term prognosis in patients who undergo RFCA.

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