

# Effect of Atrial Fibrillation on Atrial Thrombogenesis in Humans: Impact of Rate and Rhythm

Han S. Lim, MBBS, PhD,\* Scott R. Willoughby, PhD,\* Carlee Schultz, BHLTHSc(Hons),\* Cheryl Gan, BSc,\* Muayad Alasady, MBChB,\* Dennis H. Lau, MBBS, PhD,\* Darryl P. Leong, MBBS, MPH, PhD,\* Anthony G. Brooks, PhD,\* Glenn D. Young, MBBS,\* Peter M. Kistler, MBBS, PhD,† Jonathan M. Kalman, MBBS, PhD,‡ Matthew I. Worthley, MBBS, PhD,\* Prashanthan Sanders, MBBS, PhD\*

*Adelaide and Melbourne, Australia*

- Objectives** We sought to assess the effect of atrial fibrillation (AF) on atrial thrombogenesis in humans by determining the impact of rate and rhythm.
- Background** Although AF is known to increase the risk of thromboembolic stroke from the left atrium (LA), the exact mechanisms remain poorly understood.
- Methods** We studied 55 patients with AF who underwent catheter ablation while in sinus rhythm; 20 patients were induced into AF, 20 patients were atrial paced at 150 beats/min, and 15 were control patients. Blood samples were taken from the LA, right atrium, and femoral vein at baseline and at 15 min in all 3 groups. Platelet activation (P-selectin) was measured by flow cytometry. Thrombin generation (thrombin-antithrombin [TAT] complex), endothelial dysfunction (asymmetric dimethylarginine [ADMA]), and platelet-derived inflammation (soluble CD40 ligand [sCD40L]) were measured using enzyme-linked immunosorbent assay.
- Results** Platelet activation increased significantly in both the AF ( $p < 0.001$ ) and pacing ( $p < 0.05$ ) groups, but decreased in control patients ( $p < 0.001$ ). Thrombin generation increased specifically in the LA compared with the periphery in both the AF ( $p < 0.01$ ) and pacing ( $p < 0.01$ ) groups, but decreased in control patients ( $p < 0.001$ ). With AF, ADMA ( $p < 0.01$ ) and sCD40L ( $p < 0.001$ ) levels increased significantly at all sites, but were unchanged with pacing (ADMA,  $p = 0.5$ ; sCD40L,  $p = 0.8$ ) or in control patients (ADMA,  $p = 0.6$ ; sCD40L,  $p = 0.9$ ).
- Conclusions** Rapid atrial rates and AF in humans both result in increased platelet activation and thrombin generation. Prothrombotic activation occurs to a greater extent in the human LA compared with systemic circulation. AF additionally induces endothelial dysfunction and inflammation. These findings suggest that although rapid atrial rates increase the thrombogenic risk, AF may further potentiate this risk. (J Am Coll Cardiol 2013;61:852-60)  
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Atrial fibrillation (AF) confers a 5-fold increased risk of stroke in the absence of valvular heart disease (1). Although epidemiological studies have linked various clinical and echocardiographic risk factors to stroke, the exact mecha-

nism of increased risk of stroke in AF remains poorly understood. Although the heightened risk of stroke after

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From the \*Centre for Heart Rhythm Disorders (CHRD), University of Adelaide and Royal Adelaide Hospital, Adelaide, Australia; †Baker IDI Heart and Diabetes Institute, Melbourne, Australia; and ‡Department of Cardiology, Royal Melbourne Hospital and the Department of Medicine, University of Melbourne, Melbourne, Australia. This study was supported by a Grant-in-Aid from the National Heart Foundation of Australia. Drs. Lim and Alasady are supported by Postgraduate Research Scholarships from the National Health and Medical Research Council of Australia (NHMRC) and Earl Bakken Electrophysiology Scholarships from the University of Adelaide. Dr. Willoughby is supported by a Career Development Fellowship from the NHMRC. Drs. Lau and Leong are supported by Postdoctoral Fellowships from the NHMRC. Drs. Brooks, Leong, and Sanders are supported by the National Heart Foundation of Australia. Dr. Kalman has received research

support from Medtronic Inc. and St. Jude Medical; has received a travel grant from St. Jude Medical; and has received Speakers' honoraria from Biotronik and Biosense Webster. Dr. Sanders has served on the advisory board of Bard Electrophysiology, Biosense-Webster, Medtronic, St. Jude Medical, Sanofi-Aventis, and Merck; received lecture fees from Biosense-Webster, St. Jude Medical, and Merck; and received research funding from Biosense-Webster, Boston Scientific, Biotronik, Medtronic, St. Jude Medical, and Merck. Drs. Willoughby and Sanders contributed equally to this paper as senior authors. Presented in oral abstract form at the Annual Scientific Sessions of the American Heart Association Scientific Sessions 2010, Chicago, Illinois, and published in abstract form (Circulation 2010;122:A17597).

Manuscript received August 16, 2012; revised manuscript received November 18, 2012, accepted November 20, 2012.

cardioversion has been attributed to atrial mechanical dysfunction, it has been increasingly recognized that AF may in itself exhibit a prothrombotic state (2). There have been suggestions that atrial flutter, a more organized rhythm, may also be associated with an increased risk of stroke (3). However, the mechanisms by which rapid atrial rates and/or rhythm contribute to left atrial (LA) thrombogenesis have not been well studied.

Several studies have found baseline regional differences in platelet activation and hypercoagulability in the LA compared with systemic circulation in patients with valvular and nonvalvular AF (4,5), suggesting local contributing factors. Animal studies have demonstrated increased platelet activation and endothelial dysfunction with acute AF (6,7). However, the acute effect of AF on thrombogenesis in the human LA has never been studied before.

We hypothesized that acute-onset AF results in increased prothrombotic risk (by platelet activation, thrombin generation, endothelial dysfunction, and inflammation) within the human atria. Furthermore, we aimed to distinguish whether this effect was rate or rhythm related.

## Methods

**Study population.** The study comprised 55 patients with a history of AF and 15 patients with left-sided accessory pathways as a reference group who underwent catheter ablation. Consecutive patients with paroxysmal or persistent AF who were in sinus rhythm (SR)  $\geq 48$  h before the procedure (by continuous monitoring) were included. Exclusion criteria were history of valvular heart disease, left ventricular dysfunction, previous myocardial infarction, unstable angina, surgical or ablation procedure within the preceding 3 months, congenital heart disease, chronic inflammatory condition, chronic infection, chronic renal failure, chronic liver disease, and patients on antiplatelet agents.

All patients underwent baseline transthoracic echocardiography and transesophageal echocardiography within 2 days of the procedure (8). All antiarrhythmic medications were ceased 5 half-lives before the procedure. All patients underwent anticoagulation with warfarin to maintain their international normalized ratio between 2 and 3 for  $\geq 6$  weeks before the procedure. Warfarin was stopped 7 days before the procedure and substituted with enoxaparin at a dose of 1 mg/kg twice a day until  $\geq 12$  h before the procedure.

All patients provided written informed consent to the study protocol, which was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, Adelaide, Australia.

**Electrophysiology study and ablation.** Electrophysiological study and ablation were performed with sedation using midazolam and fentanyl. The technique used for mapping and ablation of AF in our laboratory was previously described (9). In brief, the following catheters were utilized for the procedure: 1) 10 pole catheter (Daig Electrophysiology,

Minnetonka, Minnesota) positioned within the coronary sinus; 2) 10-pole circumferential catheter (Lasso; Biosense-Webster, Diamond Bar, California) to map the pulmonary veins; and 3) a 3.5-mm-tip externally irrigated ablation catheter (Thermocool, Biosense-Webster) for ablation. All patients underwent circumferential ablation of the pulmonary veins with the endpoint of electrical isolation. Additional substrate modification using either linear ablation (roofline and/or mitral isthmus) and/or ablation of complex fractionated atrial electrograms was undertaken in patients with long episodes of AF ( $> 48$  h), evidence of structural heart disease, or with a large LA (largest dimension  $> 57$  mm). **Study protocol.** For the clinical procedure, a conventional single transeptal puncture was performed using an SLO sheath (St. Jude Medical, St. Paul, Minnesota) and a BRK-1 needle (Daig Corporation). The ablation catheter was advanced through the same puncture into the LA. Following transeptal puncture, and 5 min after intravenous administration of unfractionated heparin (bolus of 100 IU/kg), blood samples were simultaneously collected from the peripheral femoral venous (FV) sheath (systemic sample), right atrial (RA) sheath, and LA sheath. Samples from the RA and LA were collected with care using a slow withdrawal technique, with the sheath positioned in the midchamber. Patients were then randomized either into the AF group, pacing group, or to serve as control patients.

Of the 55 patients with a history of AF who presented in SR, AF was induced by burst atrial pacing in 20 patients, commencing at a cycle length of 250 ms and ramping down to loss of 1:1 capture. This process was repeated up to 3 times from 3 sites, as required. Another 20 patients underwent atrial pacing at 150 beats/min. The rate of atrial pacing was limited to prevent induction of AF. To control for the effects of the transeptal puncture and procedure duration, 15 patients served as a control group, who neither underwent AF induction nor pacing. After 15 min of AF, atrial pacing, or in control patients, blood sampling was repeated from the LA, RA, and FV. No ablation was performed before the completion of the study protocol.

In addition to the control group of AF patients, to evaluate the effect of transeptal puncture on prothrombotic markers, 15 non-AF patients with left-sided accessory pathways who underwent an electrophysiological study and transeptal puncture during this period were also recruited as a reference group. Blood samples were obtained from the LA, RA, and FV following transeptal puncture at baseline. With the same exclusion criteria, consecutive patients with

### Abbreviations and Acronyms

<b>ADMA</b> = asymmetric dimethylarginine
<b>AF</b> = atrial fibrillation
<b>CV</b> = coefficient of variation
<b>FV</b> = femoral vein
<b>LA</b> = left atrium
<b>RA</b> = right atrium
<b>sCD40L</b> = soluble CD40 ligand
<b>SR</b> = sinus rhythm
<b>TAT</b> = thrombin-antithrombin-complex

left-sided accessory pathways and no history of AF were evaluated.

**Blood analysis. WHOLE BLOOD FLOW CYTOMETRY.** Blood was collected utilizing a slow withdrawal technique, with the first 10 mL discarded, and immediately transferred into citrated tubes. Flow cytometry was performed within 24 h. The surface expression of the platelet activation receptor, CD62P (P-selectin) was determined by flow cytometry using the CD62P monoclonal antibody. All monoclonal antibodies were obtained from BD Biosciences (San Jose, California). Citrated whole blood was diluted 1:9 in tris-buffered saline (10-mM tris, 0.15-M sodium chloride) before 5- $\mu$ l antibody per 500- $\mu$ l tris-buffered blood was added (5). After incubation, the sample was fixed by adding 400  $\mu$ l of CellFix solution (BD Biosciences). The presence of platelet expressing ligands was determined using flow cytometry (FACSCanto, Becton Dickinson, Oxford, United Kingdom). Forward (size-dependent) scatter and 90° sideways (density-dependent) scatter were set at logarithmic gain, and platelets were identified on the basis of size using a platelet immunoglobulin bead suspension. For each sample, platelets were further identified using the platelet-specific CD42b antibody. The control ligand (mouse IgG2a-monoclonal antibody fluorescein isothiocyanate isotype control) was used to detect a nonspecific association and to define the threshold for activation-dependent binding.

Data acquisition and analysis was performed with BD FACSDiva Software Version 4.1.2 (Becton Dickinson). The threshold for nonspecific binding (the percentage defined with the immunoglobulin-G-fluorescein isothiocyanate conjugate) was set at 1%. The percentage of platelets expressing CD62P (P-selectin) monoclonal antibody was defined as the fraction exhibiting specific binding. The intra-assay coefficient of variation (CV), interassay CV, and lower limit of detection for platelet P-selectin were 5.8%, 6.1%, and 1.1% positive, respectively.

**ENZYME-LINKED IMMUNOSORBENT ASSAY.** The obtained blood samples were centrifuged at 2,500 g for 15 min at 4°C and stored at -80°C for batch analysis utilizing enzyme-linked immunosorbent assay. Thrombin generation was assessed by measuring thrombin-antithrombin (TAT) complex (Siemens Healthcare Diagnostics, Marburg, Germany). Endothelial dysfunction was assessed by measuring asymmetric dimethylarginine (ADMA) (Immunodiagnostik, Bensheim, Germany). Platelet-derived inflammation was assessed by measuring soluble CD40 ligand (sCD40L) (R&D Systems, Minneapolis, Minnesota), as per company instructions. The intra-assay CV, interassay CV, and lower limit of detection for each marker were: TAT, intra-assay CV 4% to 6%, interassay CV 6% to 9%, lower limit of detection 2.0  $\mu$ g/L; ADMA, intra-assay CV 5.7 %, interassay CV 5.3 %, lower limit of detection 0.05  $\mu$ mol/L; and sCD40L, intra-assay CV 4.5% to 5.4 %, interassay CV 6.0%

to 6.4%, lower limit of detection 4.2 pg/ml. All assays were performed in duplicate or triplicate.

**Statistical analysis.** All data are expressed as mean  $\pm$  SD or number (percentages) for continuous and categorical variables, respectively, unless otherwise stated. Continuous variables were compared using 1-way analysis of variance. Categorical variables were compared using Fisher's exact or Pearson's chi-square tests as appropriate. Data were tested for normality and log-transformed as appropriate. A constant of 1 was added to ADMA values before log-transformation. To compare changes in the outcome measures over time between the sites within each group, a linear mixed effects model was fitted to the data. In the model, time, sampling site, and the interaction between time and site were fitted as fixed effects, whereas individual patients were fitted as a random effect. This model takes the repeated measurements over time into account. Where the interaction was not significant, this was removed from the model so that the main effects of time and site could be interpreted. To compare between treatment groups, data were pooled across sites. A linear mixed effects model was fitted to the data. In the model, treatment group and time and the interaction between treatment group and time were fitted as fixed effects. Differences between sampling sites in the reference group were analyzed using repeated measures 1-way analysis of variance. Statistical significance was established at  $p < 0.05$ . All data were analyzed using PASW Statistics 18 (version 18.0.0, SPSS, Chicago, Illinois).

## Results

**Patient characteristics.** There were no significant differences among the AF, pacing, and control groups with regard to age, gender, comorbidities, medications, and echocardiographic parameters (Table 1). There was no difference in mean ventricular rate between the AF and pacing groups.

**Platelet activation.** Platelet P-selectin increased significantly with both AF induction ( $p < 0.001$ ) and pacing ( $p = 0.02$ ), taking into consideration all sites, as shown in Figures 1A and 1B. However, platelet P-selectin levels decreased in control patients over time ( $p < 0.001$ ) (Fig. 1C). There was a significant difference between the sites measured ( $p = 0.04$ ) in the pacing group.

**Thrombin generation.** Thrombin generation (TAT) increased significantly in the LA and RA compared with peripheral samples in both the AF group ( $p < 0.001$ , site and time interaction) and the pacing group ( $p = 0.002$ ), as shown in Figures 2A and 2B. Similar to the peripheral levels in the AF and pacing groups, TAT levels in the control group decreased with time ( $p < 0.001$ ), with no differences in sites ( $p = 0.6$ ) (Fig. 2C).

**Endothelial dysfunction.** ADMA levels increased significantly over time with the onset of AF ( $p = 0.008$ ) (Fig. 3A). There was no change in ADMA levels with atrial pacing ( $p = 0.5$ ) (Fig. 3B) or in control patients ( $p = 0.6$ ) (Fig.

**Table 1** Baseline Characteristics of Patients in the AF, Pacing, and Control Groups

	AF Group (n = 20)	Pacing Group (n = 20)	Control Group (n = 15)	p Value
Age (yrs)	53.5 ± 8.6	54.9 ± 14.5	56.0 ± 11.2	0.5
Male gender	10 (50.0)	15 (75.0)	7 (46.7)	0.1
BMI	31.5 ± 8.0	28.1 ± 4.2	28.4 ± 5.2	0.3
<b>Comorbidities</b>				
Congestive heart failure	0 (0)	0 (0)	0 (0)	NS
NYHA functional class				1.0
I	13 (65.0)	12 (60.0)	9 (60.0)	
II	7 (35.0)	8 (40.0)	6 (40.0)	
III and IV	0 (0)	0 (0)	0 (0)	
Hypertension	8 (40.0)	7 (35.0)	7 (46.7)	0.5
Diabetes mellitus	2 (10.0)	1 (5.0)	0 (0)	0.6
Stroke/transient ischemic attack	0 (0)	1 (5.0)	1 (6.7)	0.7
Mean CHA <sub>2</sub> DS <sub>2</sub> -VASc score	1.3 ± 1.1	1.2 ± 0.9	1.3 ± 1.1	0.9
CHA <sub>2</sub> DS <sub>2</sub> -VASc score				1.0
0	5 (25.0)	5 (25.0)	3 (20.0)	
1	8 (40.0)	8 (40.0)	7 (46.7)	
≥2	7 (35.0)	7 (35.0)	5 (33.3)	
Mean HAS-BLED score	0.6 ± 0.6	0.9 ± 0.7	0.8 ± 0.8	0.4
HAS-BLED score				0.3
0–2	20 (100.0)	20 (100.0)	14 (93.3)	
≥3	0 (0)	0 (0)	1 (6.7)	
<b>Usual medications</b>				
Flecainide	8 (40.0)	7 (35.0)	8 (53.3)	0.7
Sotalol	3 (15.0)	6 (30.0)	2 (13.3)	0.6
Beta-blockers	5 (25.0)	4 (20.0)	7 (46.7)	0.1
Calcium-channel blockers	4 (20.0)	3 (15.0)	3 (20.0)	0.7
Warfarin	15 (75.0)	15 (75.0)	11 (73.3)	0.7
Baseline heart rate (beats/min)	64.1 ± 10.0	66.3 ± 13.6	68.2 ± 10.8	0.7
Atrial rate after 15 min (beats/min)	293.1 ± 58.0	150.0 ± 0	66.0 ± 10.1	<0.01*
Ventricular rate after 15 min (beats/min)	108.4 ± 23.0	114.6 ± 31.2	66.0 ± 10.1	<0.01†
Heparin bolus dose (IU)	8,438 ± 1,632	9,077 ± 1,256	8,667 ± 1,155	0.5
ACT after 15 min (s)	237.4 ± 27.4	237.0 ± 26.5	238.7 ± 30.0	1.0
<b>Echocardiographic parameters</b>				
LA diameter	39.2 ± 5.0	41.6 ± 7.3	43.1 ± 7.0	0.3
LA size	22.6 ± 3.5	25.0 ± 5.2	24.4 ± 5.5	0.3
RA size	20.3 ± 3.0	20.9 ± 4.5	20.6 ± 4.9	0.9
LVEF	61.6 ± 6.7	63.1 ± 9.3	62.5 ± 6.9	0.9
LASEC grade	0 ± 0	0.1 ± 0.3	0.1 ± 0.4	0.4
LAAEV (cm/s)	77.9 ± 28.8	73.9 ± 32.2	68.6 ± 20.9	0.8

Values are mean ± SD or n (%). \*AF versus pacing group, p < 0.01; AF versus control group, p < 0.01; pacing versus control group, p < 0.01. †AF versus pacing group, p = 1.0; AF versus control group, p < 0.01; pacing versus control group, p < 0.01.

ACT = activated clotting time; AF = atrial fibrillation; BMI = body mass index; CHA<sub>2</sub>DS<sub>2</sub>-VASc = congestive heart failure, hypertension, age ≥75 years (doubled), diabetes, stroke (doubled), vascular disease, age 65 to 74 years, sex category (female); HAS-BLED = hypertension, abnormal renal/liver function (1 point each), stroke, bleeding tendency or predisposition, labile international normalized ratio, elderly (e.g., age >65 years), drugs/alcohol concomitantly (1 point each); LA = left atrium; LAAEV = left atrial appendage emptying velocity; LASEC = left atrial spontaneous echocardiographic contrast; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

3C). There was no significant difference in ADMA levels between sites in the AF group (p = 0.2).

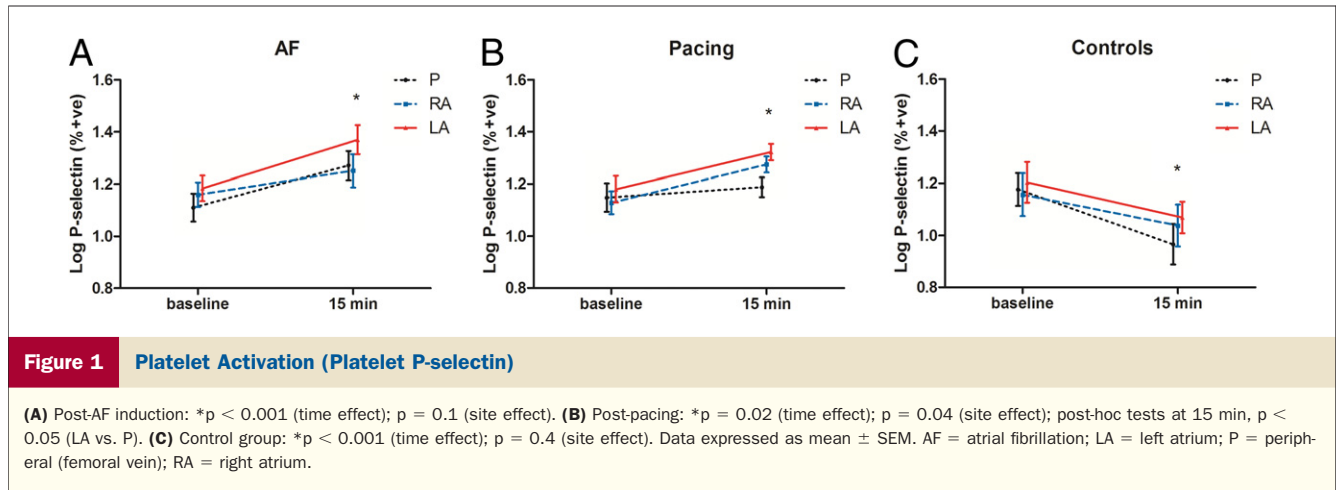
**Platelet-derived inflammation.** Soluble CD40L levels increased significantly over time with acute AF (p < 0.001), but were unchanged with atrial pacing (p = 0.8) and in control patients (p = 0.9), as shown in Figures 4A to 4C. There was no significant difference between the sites measured in the AF group (p = 0.7).

**AF versus pacing.** We compared intracardiac levels (LA and RA) of thrombin generation and platelet activation among the AF, pacing, and control groups over time, given

the difference between intracardiac and peripheral levels. For ADMA and sCD40L levels, comparison was made among the groups, taking into account all sites, given that no significant difference in sites were found.

Intracardiac platelet activation was significantly elevated in the AF and pacing groups compared with the control group (Fig. 5A). However, the difference between AF and pacing was not significant (p = 0.9). Intracardiac thrombin generation was significantly elevated in the AF and pacing groups compared with the control group (p < 0.01), but no significant difference was found between the AF and pacing





groups ( $p = 0.4$ ) (Fig. 5B). In contrast, endothelial dysfunction was significant in the AF group compared with the pacing group ( $p < 0.05$ ) and the control group ( $p < 0.05$ ) (Fig. 5C). In addition, inflammatory mediator sCD40L was raised in the AF group compared with the pacing group ( $p = 0.01$ ) and the control group ( $p < 0.01$ ) (Fig. 5D).

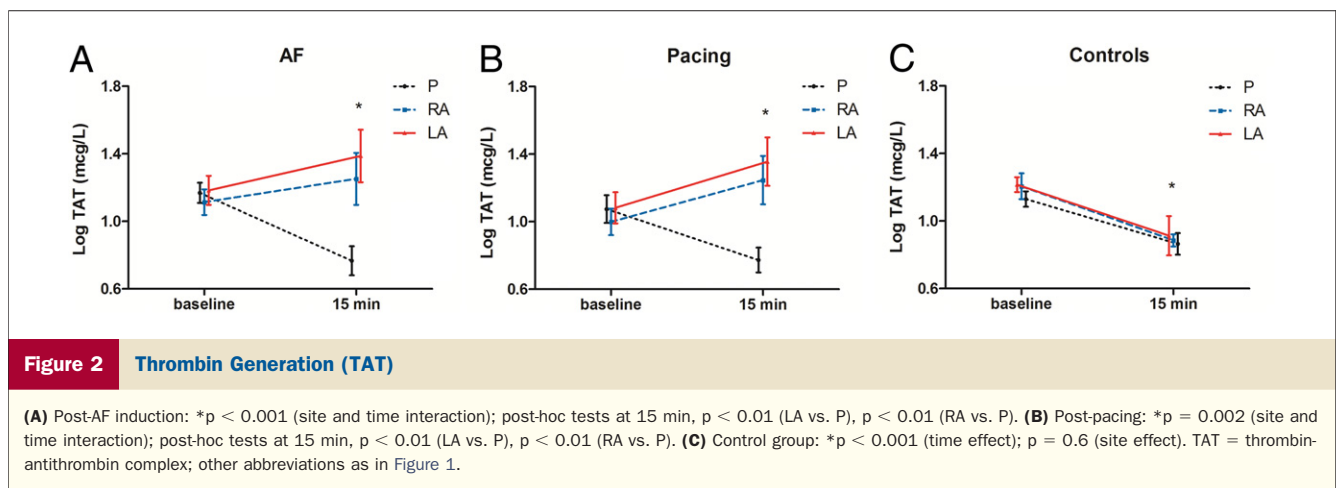
**Effect of transeptal puncture on prothrombotic markers.** To eliminate transeptal puncture as a possible cause of the increased atrial prothrombotic markers observed in AF patients, a cohort of patients who underwent ablation of a left-sided accessory pathway was studied (8 men, 7 women; mean age  $38 \pm 7$  years). Blood samples were obtained from the LA, RA, and FV following transeptal puncture. There were no significant differences in the levels of platelet P-selectin (in mean  $\pm$  SEM) (log P-selectin  $1.22 \pm 0.07$  in FV vs.  $1.09 \pm 0.08$  in RA vs.  $1.18 \pm 0.07$  in LA,  $p = 0.2$ ), TAT (log TAT  $1.29 \pm 0.08$  in FV vs.  $1.23 \pm 0.06$  in RA vs.  $1.22 \pm 0.06$  in LA,  $p = 0.2$ ), ADMA (log ADMA  $0.13 \pm 0.01$  in FV vs.  $0.13 \pm 0.01$  in RA vs.  $0.13 \pm 0.01$  in LA,  $p = 0.5$ ), and sCD40L (log sCD40L  $2.20 \pm 0.05$  in FV vs.  $2.20 \pm 0.05$  in RA vs.  $2.19 \pm 0.05$  in LA,  $p = 1.0$ )

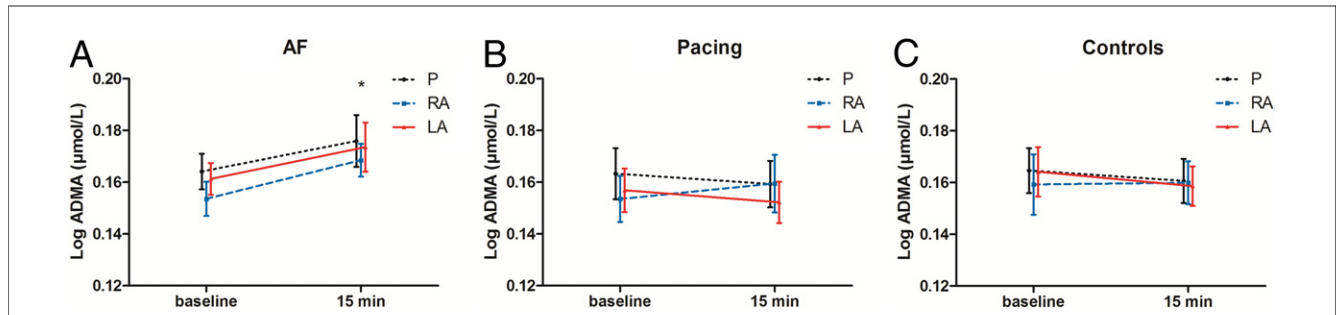
before or after transeptal puncture or between sampling sites in these patients.

### Discussion

This study provides new information on the relative contribution of atrial rate and rhythm to thrombogenesis due to atrial arrhythmias. By performing sampling from the LA, RA, and the peripheral circulation, it demonstrates the following: 1) rapid atrial rates are associated with increased platelet activation and thrombin generation; 2) AF, although also demonstrating changes in platelet activation and thrombin generation, additionally leads to endothelial dysfunction and activation of the inflammatory cascade; and 3) interestingly, these factors occurred to a much greater extent in the human LA compared with peripheral circulation.

**Left atrial platelet activation with AF and pacing.** Patients with AF are recognized to exhibit a prothrombotic state and abnormal platelet activation. This has been documented peripherally in various subsets of patients with AF (10–12). Platelets play an essential role in thrombogenesis by interacting with the endothelium, inflammatory cells,





**Figure 3** ADMA Levels

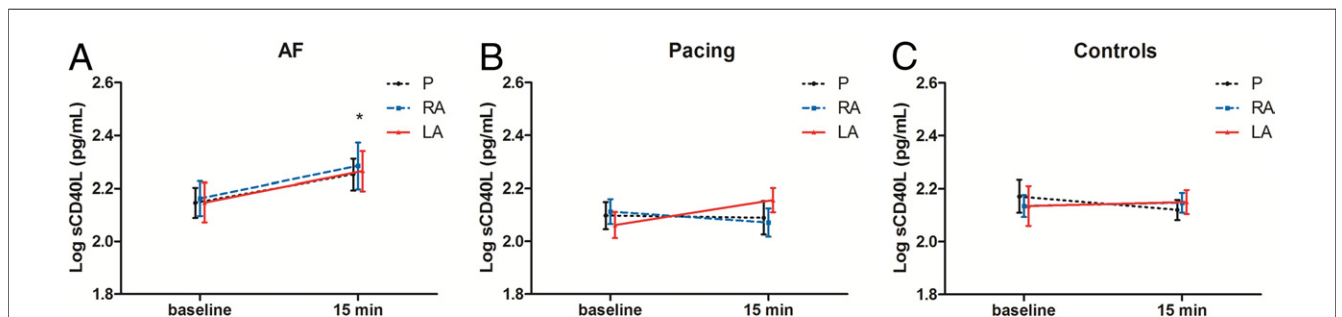
(A) Post-AF induction: \* $p = 0.008$  (time effect);  $p = 0.2$  (site effect). (B) Post-pacing:  $p = 0.5$  (time effect);  $p = 0.3$  (site effect). (C) Control group:  $p = 0.6$  (time effect);  $p = 0.9$  (site effect). ADMA = asymmetric dimethylarginine; other abbreviations as in Figure 1.

and proteins from the coagulation cascade (5,13). Platelet expression of P-selectin is commonly used as a marker of platelet activation (5). Platelet P-selectin expression has been associated with spontaneous echo contrast, presence of LA thrombus or embolic events, and silent cerebral infarction in patients with AF (6,14).

Although several studies have shown increased platelet activation in patients with AF compared with control patients (11,15,16), other studies have shown that the difference could be attributed to patient comorbidities (17). These inconsistent results could partly be explained by sampling from a heterogeneous population of AF patients, sampling from peripheral versus central sites, and by measuring at different times during AF rather than at a predefined time (5,15). In this study, the effect of AF was compared with each patient's individual baseline state, and sampling was performed in the LA at a predefined time. We showed that AF per se and rapid atrial rates resulted in elevated platelet activation.

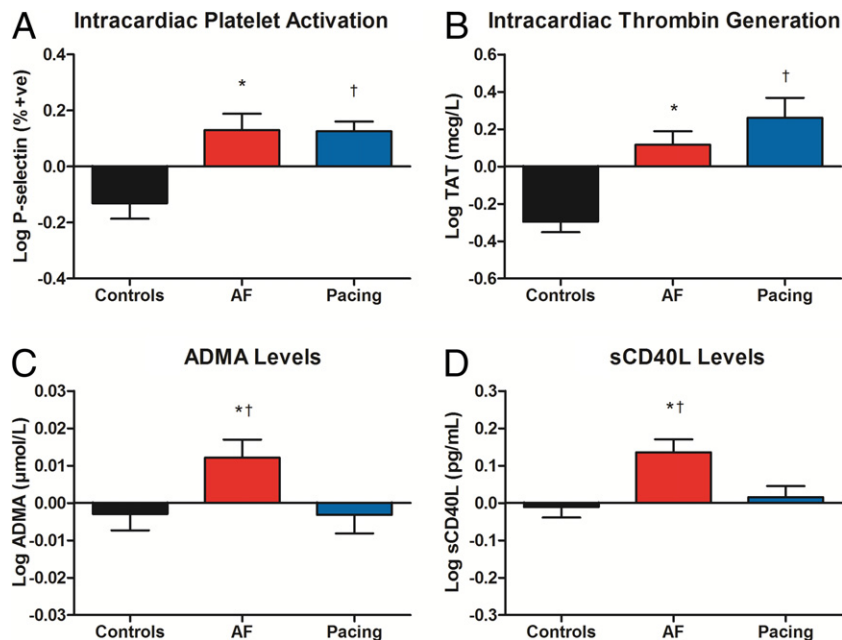
In addition, platelet expression of P-selectin has been suggested to be linked more with acute changes in platelet activation (18). Although studies measuring patients with AF at a baseline state have yielded varying results, studies examining patients with acute episodes of AF have consistently

shown the involvement of platelets (15,16,19). Our present findings are consistent with other studies in that platelet activation is enhanced in the setting of acute AF, after 3 to 12 h from peripheral sampling (16,19), and after 15 min from coronary sinus sampling (15). Platelet activation may thus play a role in the initiation of various prothrombotic pathways in the acute or paroxysmal setting. **Left atrial thrombin generation with AF and pacing.** Increased thrombin generation reflected by elevated TAT levels has been found in the LA in patients with mitral stenosis and AF (4). In chronic nonvalvular AF, increased peripheral levels of coagulation markers have been demonstrated (10,12). Abnormal expression of coagulation factors in the atrial endocardium has been demonstrated in an animal model of rapidly paced atria and in human patients presenting with AF and cardiogenic thromboembolism (20,21). Akar et al. (15) found increased thrombin generation and platelet activation with AF induction from samples taken from the coronary sinus. In this study, acutely elevated thrombin generation was observed specifically at the LA and RA with the onset of AF and rapid atrial rates, which was not seen in the peripheral circulation. This study demonstrates that with the onset of AF and rapid atrial rates, the LA is significantly more thrombogenic compared



**Figure 4** Soluble CD40L Levels

(A) Post-AF induction: \* $p < 0.001$  (time effect);  $p = 0.7$  (site effect). (B) Post-pacing:  $p = 0.8$  (time effect);  $p = 0.9$  (site effect). (C) Control group:  $p = 0.9$  (time effect);  $p = 1.0$  (site effect). Abbreviations as in Figure 1.



**Figure 5** Change in Prothrombotic Markers Between Groups

(A) Change in intracardiac platelet activation between groups:  $p < 0.001$  (group and time interaction); post-hoc tests: \* $p < 0.01$  (AF vs. control group), † $p < 0.01$  (pacing vs. control group),  $p = 0.9$  (AF vs. pacing). (B) Change in intracardiac thrombin generation between groups:  $p < 0.001$  (group and time interaction); post-hoc tests: \* $p < 0.01$  (AF vs. control group), † $p < 0.01$  (pacing vs. control group),  $p = 0.4$  (AF vs. pacing). (C) Change in ADMA levels between groups.  $p = 0.02$  (group and time interaction); post-hoc tests: \* $p < 0.05$  (AF vs. control group), † $p < 0.05$  (AF vs. pacing),  $p = 1.0$  (pacing vs. control group). (D) Change in soluble CD40L (sCD40L) levels between groups:  $p = 0.001$  (group and time interaction); post-hoc tests: \* $p < 0.01$  (AF vs. control group), † $p = 0.01$  (AF vs. pacing),  $p = 0.6$  (pacing vs. control group). NB: Descriptive plots shown. Statistical analyses performed using mixed effects models in which statistical significance was achieved. Abbreviations as in Figures 1 and 3.

with the peripheral circulation, and may explain the propensity for LA thrombus formation and cardioembolic stroke seen in these patients.

**Endothelial dysfunction with AF induction.** LA thrombus formation in AF has traditionally been attributed to atrial mechanical dysfunction (8,22,23). However, it is now accepted that multiple mechanisms contribute to LA thrombus formation. Abnormal endothelial function (endothelial dysfunction) has been shown in patients with AF and is a component of Virchow's triad for thrombogenesis (2,24,25). ADMA is an endogenous inhibitor of endothelial nitric oxide synthase and is known to result in endothelial dysfunction in experimental human studies (26). Nitric oxide has potent antithrombotic properties on the endothelium and inhibits platelet and monocyte adhesion (27). There is also evidence that ADMA mediates endothelial dysfunction through oxidative stress (26). Clinically, ADMA is associated with numerous cardiovascular conditions and is a predictor of mortality in cardiovascular patients (28,29).

The present study found that induction of AF was associated with increased ADMA levels both peripherally and in the human atria. The finding that induction of AF upregulates ADMA is consistent with animal models, such as the porcine AF model by Goette et al. (28). In another

animal study, Cai et al. (7) demonstrated decreased atrial nitric oxide levels and endothelial nitric oxide synthase expression in a rapid atrial pacing model of AF, which was not seen in the control group of atrial pacing at 100 beats/min. Minamino et al. (6) found decreased nitric oxide levels with associated increased P-selectin expression on platelets in a canine model of AF. These findings suggest that endothelial dysfunction induced by AF, mediated by ADMA and the nitric oxide pathway, with resultant loss of its antithrombotic properties, plays an important contributory role to thrombogenesis in patients with AF.

**Inflammation with AF induction.** Inflammation is being increasingly recognized to play a significant role in the genesis and perpetuation of AF (24,30). C-reactive protein elevation is found in a stepwise fashion in patients with increasing AF burden (31). Studies also show that high-sensitivity C-reactive protein decreases after successful ablation for long-standing persistent AF, suggesting that AF itself may cause an inflammatory response (32).

Soluble CD40 ligand is increased in a number of cardiovascular settings (33). It is an important mediator in the pathogenesis of atherothrombotic disease and predicts mortality in patients with acute coronary syndromes (33,34). CD40 ligand on activated platelets plays a pivotal role in inflammatory responses by inducing endothelial secretion of

chemokines and expression of adhesion molecules, thereby promoting leukocyte recruitment (35). The CD40/CD40 ligand system has been proposed to provide an important link between inflammation and thrombosis (34). This study showed that the onset of AF increased sCD40L levels, which was not seen with rapid atrial rates alone. This study newly demonstrates that induction of AF in humans evidently results in an increase in inflammatory signals. This provides further insight into the link between inflammation and thrombogenesis in patients with AF.

**Clinical implications.** This study demonstrates that AF or abnormal rhythm per se confers additional prothrombotic effects in the LA beyond the patient's comorbidities. These findings point toward the benefit of maintaining SR and provide an explanation for the "on-treatment" analysis of the AFFIRM (Atrial Fibrillation Follow-up Investigation of Rhythm Management) study, which found the presence of SR associated with a lower risk of death (36). The present study also explains why increased AF burden and subclinical atrial tachyarrhythmias (from implantable devices) are associated with increased thromboembolic risk (37,38).

The finding that rapid atrial rates increase platelet and thrombotic markers in the LA provides mechanistic insight into thrombogenesis in atrial flutters, which may differ slightly from AF, but nevertheless confer increased risk of stroke (3,39,40). Furthermore, AF potentiates the thrombotic risk over that of rate alone by activating other mechanisms, such as endothelial dysfunction and the inflammatory cascade. This highlights the importance of other therapeutic modalities that improve endothelial function and mediate the inflammatory response, and the management of concomitant cardiovascular risk factors associated with AF (30).

**Study limitations.** With access to the human LA, the study would have been ethically impossible without the administration of heparin. Heparin is known to possibly affect TAT levels by initially enhancing binding before causing irreversible inhibition of thrombin's activity (41). In our study, TAT levels at all sites in the control group and at peripheral sites in the AF and pacing groups decreased with time after the administration of heparin, which was consistent with previous studies (15). However, despite decreased TAT levels in control patients and in peripheral samples with heparin, atrial levels significantly increased with AF and atrial pacing.

The half-life of TAT is 15 min (42) and that of ADMA is approximately 23 min (43), close to the study sampling time. However, previous studies in humans have documented acute changes in levels of platelet P-selectin at 15 min (15), TAT at 15 min (15), and ADMA when measured at 15-min intervals (44).

Underlying patient comorbidities could have contributed to the prothrombotic state. However, the study measured the effects of AF and pacing compared with each patient's baseline state. Hence, these results indicated that the effects

of AF and high atrial rates were in addition to a patient's underlying comorbidities.

Ventricular rate in both AF and pacing groups were faster than the control group and could have contributed to the increased effects. However, other studies have demonstrated that patients controlled for ventricular rate either in a paced setting or in paroxysmal supraventricular tachycardia did not show any significant difference in prothrombotic markers (11,15,19,28). The degree of rapid atrial pacing was limited to 150 beats/min to prevent inadvertent induction of AF. It is possible that the further findings observed in the AF group were due to its higher atrial rates, although this could not be explicated in the study.

## Conclusions

Rapid atrial rates and AF in humans both result in increased platelet activation and thrombin generation. Prothrombotic activation occurs to a greater extent in the human LA compared with systemic circulation. In addition, AF also induces endothelial dysfunction and inflammation. These findings suggest that although rapid atrial rates increase thrombotic risk, AF may further potentiate this risk.

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**Reprints and correspondence:** Dr. Prashanthan Sanders, Centre for Heart Rhythm Disorders (CHRD), Department of Cardiology, Royal Adelaide Hospital, Adelaide, SA 5000, Australia. E-mail: prash.sanders@adelaide.edu.au.

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**Key Words:** atrial fibrillation ■ atrium ■ stroke ■ thrombosis.