



HAL
open science

Platelet function and microparticle levels in atrial fibrillation: Changes during the acute episode

Line Pourtau, Jean Marc Sellal, Romaric Lacroix, Philippe Poncelet, Olivier Bernus, Gisèle Clofent-Sanchez, Mélèze Hocini, Michel Haïssaguerre, Françoise Dignat-George, Frédéric Sacher, et al.

► **To cite this version:**

Line Pourtau, Jean Marc Sellal, Romaric Lacroix, Philippe Poncelet, Olivier Bernus, et al.. Platelet function and microparticle levels in atrial fibrillation: Changes during the acute episode. *International Journal of Cardiology*, 2017, 243, pp.216-222. 10.1016/j.ijcard.2017.03.068 . hal-01770208

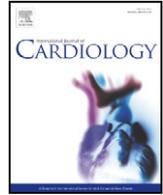
HAL Id: hal-01770208

<https://amu.hal.science/hal-01770208>

Submitted on 18 Apr 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Platelet function and microparticle levels in atrial fibrillation: Changes during the acute episode



Line Pourtau^{a,b,c,*}, Jean Marc Sellal^{a,d,e}, Romaric Lacroix^{f,g}, Philippe Poncelet^h, Olivier Bernus^{a,b,c}, Gisèle Clofent-Sanchezⁱ, Méléze Hocini^{a,b,c,d}, Michel Haïssaguerre^{a,b,c,d}, Françoise Dignat-George^{f,g}, Frédéric Sacher^{a,b,c,d,1}, Paquita Nurden^{a,1}

^a IHU Liryc, Electrophysiology and Heart Modeling Institute, fondation Bordeaux Université, 33600, Pessac, France

^b Univ. Bordeaux, Centre de recherche Cardio-Thoracique de Bordeaux, U1045, 33000 Bordeaux, France

^c INSERM, Centre de recherche Cardio-Thoracique de Bordeaux, U1045, 33000 Bordeaux, France

^d Bordeaux University Hospital (CHU), Electrophysiology and Ablation Unit, 33600 Pessac, France

^e Centre Hospitalier Régional Universitaire (CHRU) de Nancy, département de cardiologie, 54500 Vandœuvre-lès-Nancy, France

^f VRCM, UMR-S1076, Aix -Marseille Université, INSERM, UFR de Pharmacie, 13385 Marseille, France

^g Department of Haematology and Vascular Biology, CHU Conception, AP-HM, 13385 Marseille, France

^h Research & Technology Department, BioCytex, 13010 Marseille, France

ⁱ Univ Bordeaux, CNRS, Centre de Résonance Magnétique des Systèmes Biologiques, U5536, 33076 Bordeaux, France

ARTICLE INFO

Article history:

Received 24 November 2016

Received in revised form 8 March 2017

Accepted 10 March 2017

Keywords:

Atrial fibrillation

Platelets

Thrombin receptor

Tissue factor

Microparticle procoagulant activity

Microparticles fibrinolytic activity

ABSTRACT

Background: Thrombotic risk constitutes a major complication of atrial fibrillation (AF). Platelets and microparticles (MPs) are important for hemostasis and thrombosis, however their participation during AF is not well known. The aim of this study was to characterize platelet function and MPs procoagulant and fibrinolytic activity in AF patients and to determine the effects of an acute-AF episode.

Methods: Blood was collected from paroxysmal (21) and persistent (16) AF patients referred for AF catheter ablation. Ten patients in sinus rhythm for 10 days were induced in AF allowing comparisons of left atrium samples before and after induction. Platelet aggregation with ADP, TRAP, collagen, and ristocetin was studied. Platelet surface expression of PAR-1, α IIb β 3, GPIb and P-selectin were evaluated by flow cytometry, and MPs-associated procoagulant and fibrinolytic activity levels were determined by functional assays.

Results: A specific reduction in platelet aggregation to TRAP, activating the thrombin receptor PAR-1, was found in all AF patients. No differences in platelet receptor expression were found. Yet, after acute-induced AF, the platelet response was improved. Furthermore, a significant decrease of left atrium tissue factor-dependent procoagulant activity of MPs was observed.

Conclusion: Acute episodes of AF results in a decrease in MPs-associated tissue factor activity, possibly corresponding to consumption, which in turn favors coagulation and the local production of thrombin. A decreased platelet basal aggregation to TRAP may result from PAR1 desensitization, whereas the improved response after an induced episode of AF suggests activation of coagulation and PAR1 re-sensitization.

© 2017 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Abbreviations: ADP, adenosine diphosphate; AF, atrial fibrillation; DOAC, direct oral anti-coagulant; MPs, microparticles; NSAIDs, nonsteroidal anti-inflammatory drugs; TF, tissue factor; TRAP, thrombin receptor activating peptide; VKA, vitamin-K antagonist; VWF, von Willebrand factor.

* Corresponding author at: L'institut de Rythmologie et Modélisation cardiaque (LIRYC), Hôpital Xavier Arnoz, Avenue du Haut Lévêque, 33600 Pessac, France.

E-mail addresses: line.pourtau@ihu-liryc.fr (L. Pourtau), jeanmarc.sellal@free.fr (J.M. Sellal), romaric.lacroix@univ-amu.fr (R. Lacroix), philippe.poncelet@biocytex.fr (P. Poncelet), olivier.bernus@ihu-liryc.fr (O. Bernus), gisele.clofent-sanchez@rmsb.u-bordeaux2.fr (G. Clofent-Sanchez), meleze.hocini@chu-bordeaux.fr (M. Hocini), michel.haissaguerre@chu-bordeaux.fr (M. Haïssaguerre), francoise.dignat-george@univ-amu.fr (F. Dignat-George), frederic.sacher@chu-bordeaux.fr (F. Sacher), paquita.nurden@gmail.com (P. Nurden).

¹ These authors contributed equally.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, occurring in 1–2% of the world's population with risk increasing with age [1]. Radiofrequency ablation emerged in the late 1990's, and is now an important approach in treating AF decreasing the burden of the arrhythmia [2,3]. One of the main complications of paroxysmal or permanent forms of AF is increased risk of a stroke; with 20% of stroke patients being related to AF [1,4]. Vitamin-K antagonist (VKA), and more recently direct oral anticoagulants (DOAC) are a cornerstone of anti-thrombotic treatments for patients with AF. Nevertheless, even with these drugs, stroke may still occur in patients presenting AF.

Thromboembolic risk is linked to different pathophysiological mechanisms, including hemodynamic dysfunction and modifications of hemostatic parameters. Hemodynamic problems are most often due to modifications of blood flow in the left atrium (with the loss of atrial contraction and atrio-ventricular synchronization), atrial tissue remodeling and inflammation processes [5]. Among hemostatic parameters, platelets are important players. They are small, anucleate blood cells that upon endothelial damage form a hemostatic plug, helping to prevent blood loss at sites of vascular injury. They adhere to sites of vascular injury through interactions between glycoprotein (GP) receptors with von Willebrand factor (VWF) and collagen, present in the exposed subendothelial matrix. Platelets then aggregate and secrete biologically active substances, proteins and newly synthesized active metabolites [6]. They form a procoagulant surface enhancing the coagulation cascade ending in the formation and stabilization of the hemostatic plug favoring thrombin generation and fibrin formation. Secreted proteins are capable of influencing processes such as angiogenesis, inflammation and the immune response. During activation or apoptotic processes, large numbers of small vesicles called microvesicles or microparticles (MPs) may form and be shed from the surface of the platelets, leukocytes and vascular cells [7]. MPs are important in thrombosis, inflammation and vascular reactivity. They behave as biological drones playing a key role in the fine-tuning of vascular homeostasis. Beyond their well-described procoagulant properties, accumulating data has shown that specific endothelial cell or leukocyte-derived MPs bind plasminogen and vectorize plasminogen activators, leading to an efficient plasmin generation and matrix metalloproteinases activation [8]. Studies with AF patients did not show increased plasmin activity, but an abnormal fibrinolytic balance [9]. If AF is associated with an increased thrombotic tendency the role of platelets and other changes needs to be established.

We have analyzed in non-valvular AF, the platelet aggregation response, expression of selected platelet membrane GPs, and the levels of MPs-linked procoagulant and fibrinolytic activities. These parameters were measured in paroxysmal and persistent AF patients from peripheral blood samples and directly from blood in the left atrium during the ablation procedure. As atrial fibrillation had to be induced in some patients to map atrial fragmented signals, this procedure allowed us to examine if the newly identified biomarkers were directly linked to an acute episode of fibrillation.

2. Methods

2.1. Patients

Paroxysmal and persistent AF patients referred for primary AF catheter ablation were included in this study and baseline characteristics are described in Table 1. Paroxysmal AF patients ($n = 21$) were defined as having at least 2 self-converting episodes lasting <7 days. Persistent AF patients ($n = 16$) were defined as AF lasting longer than 7 days [3]. Healthy control subjects ($n = 11$) were from the hospital and research center staff. Exclusion criteria were active smokers, antiplatelet treatment, history of acute cardiac events in the preceding 3 months, valvular heart diseases, chronic inflammatory diseases, chronic renal and hepatic disease, and uncontrolled hypertension. In our 37 patient cohort, 24 patients were administered with 100 mg of ketoprofen (nonsteroidal anti-inflammatory drugs (NSAIDs)) before the beginning of the procedure and heparin was given after the trans-septal puncture.

This study was in accordance with the declaration of Helsinki and was approved by the local institutional review committee (2016-A00603-48). Informed consent was signed by the patients and controls.

2.2. Blood collection

Blood samples from AF patients were taken during AF ablation procedures, using a trans-septal approach [3]. Citrated and EDTA blood samples were collected from the femoral vein, and the left atrium immediately after trans-septal puncture and heparin administration. Among the 37 patients included in the study, a subgroup of patients in sinus rhythm ($n = 10$) throughout the 10 days prior to ablation were subjected to induced-AF by pacing at the coronary sinus catheter (paroxysmal AF $n = 5$, persistent AF $n = 5$). After 20 min of continuous AF, additional blood samples were collected from the left atrium (before radiofrequency energy delivery). Peripheral blood samples were collected from healthy volunteers via the cubital vein.

Table 1
Baseline characteristics of AF patients.

	Paroxysmal AF ($n = 21$)	Persistent AF ($n = 16$)	<i>p</i> value
Age	60 ± 2.6	59 ± 2.0	<i>p</i> = 0.63
Men/women	14:7	14:2	<i>p</i> = 0.25
BMI	26.3 ± 1.0	26.6 ± 1.0	<i>p</i> = 0.84
CHA2DS2-VASc Score	1.3 ± 0.2	0.9 ± 0.3	<i>p</i> = 0.44
Duration of AF (years)	6 ± 1.2	5.8 ± 1.1	<i>p</i> = 0.90
Comorbidities			
Hypertension (%)	28.6	18.8	<i>p</i> = 0.70
Diabetes mellitus (%)	4.8	0	<i>p</i> = 1.00
Stroke/transient ischemic attack (%)	9.5	0	<i>p</i> = 0.50
Treatment			
Class I antiarrhythmics (%)	57.1	25	<i>p</i> = 0.05
Class II antiarrhythmics (%)	14.3	43.8	<i>p</i> = 0.07
Class III antiarrhythmics (%)	33.3	81.3	** <i>p</i> = 0.01**
VKA (%)	47.6	25.0	<i>p</i> = 0.19
DOAC (%)	42.9	75.0	<i>p</i> = 0.09
Echocardiographic parameters			
LA surface (cm ²)	19.6 ± 0.9	24.5 ± 2.9	<i>p</i> = 0.12
LVEF (%)	62.1 ± 1.4	58.3 ± 4.5	<i>p</i> = 0.92

Value are mean ± SEM or %. ***p* ≤ 0.01. AF = atrial fibrillation; BMI = body mass index; CHA2DS2-VASc Score = congestive heart failure, hypertension, age ≥ 75 years, diabetes, stroke, vascular disease, age 65 to 75 years, sex category (female); LA = left atrium; LVEF = left ventricular ejection fraction, DOAC = direct oral anti-coagulant; VKA = vitamin K antagonist.

2.3. Platelet function

Blood cell counts were recorded. Platelet-rich plasma (PRP) and platelet-poor plasma were prepared following the SSC/ISTH recommendations. PRP platelet count lower than $150 \times 10^9 \text{ l}^{-1}$ and higher than $600 \times 10^9 \text{ l}^{-1}$ were excluded [10]. Platelet aggregation was assessed using an APACT4004 aggregometer (Elitech, Puteaux, France) by light transmission over 10 min after adding adenosine diphosphate (ADP) 10 μM, ristocetin 1.5 mg/ml (Elitech, Puteaux, France), Horm collagen 2 μg/ml (Stago, Taverny, France) or thrombin receptor activating peptide (TRAP-6mer) 10 μM (Bachem, Bubendorf, Switzerland). Percentage of maximal intensity aggregation and maximal disaggregation after addition of agonists were determined from the recordings [9].

2.4. Platelet membrane receptor expression

Expression of αIIbβ3 and GPIb was evaluated by flow cytometry with a FacsCanto I (BD biosciences, Franklin Lakes, New Jersey, USA) as previously described [11]. For PAR-1 and P-selectin detection, mouse anti-thrombin receptor antibody PE conjugates (clones WEDE15 and SPAN12) (Beckman-Coulter, Brea, California, USA) and mouse anti-CD62P-PE (BD, biosciences, Franklin Lakes, New Jersey, USA) were mixed with 10^6 platelets. Negative controls consisted of the addition of isotype mouse IgG1-PE antibodies (Beckman Coulter, Brea, California, USA and BD Biosciences, Franklin Lakes, New Jersey, USA). Levels of αIIbβ3, GPIb and PAR-1 were determined as mean fluorescence intensity (MFI) and the level of P-selectin expression was determined as a percentage of positive cells compared to the negative control set at 1%.

2.5. Evaluation of microparticle functions

Platelet free plasma samples were prepared following the SSC/ISTH recommendations [12]. TF-dependent procoagulant activity of MPs was determined using a fluorogenic assay of factor Xa generated by purified MPs as described in Agouti et al. [13]. The fibrinolytic activity of MPs was determined using a chromogenic test of plasmin generated by purified MPs as modified by BioCyte (separation of plasma MPs by immuno-magnetic separation) [14].

2.6. Statistical analysis

Categorical variables were compared using Fisher's exact or Pearson's chi-square test as deemed appropriate. As normality or equal variance test failed on several continuous variables and the small size of the study, statistical analysis was performed by non-parametric tests. To compare the control, paroxysmal and persistent AF groups for platelet function, membrane receptor expression and MPs, the Kruskal-Wallis test was performed. A Mann-Whitney test was used to determine the effect of NSAID administration and cardiac rhythm during blood sampling on maximal aggregation variables. The effect of an acute AF episode and site sampling on platelet function, membrane receptor expression and MPs was analyzed by Wilcoxon test. Statistical significance was established at $p < 0.05$. All data was analyzed using GraphPad Prism (version 6.07).

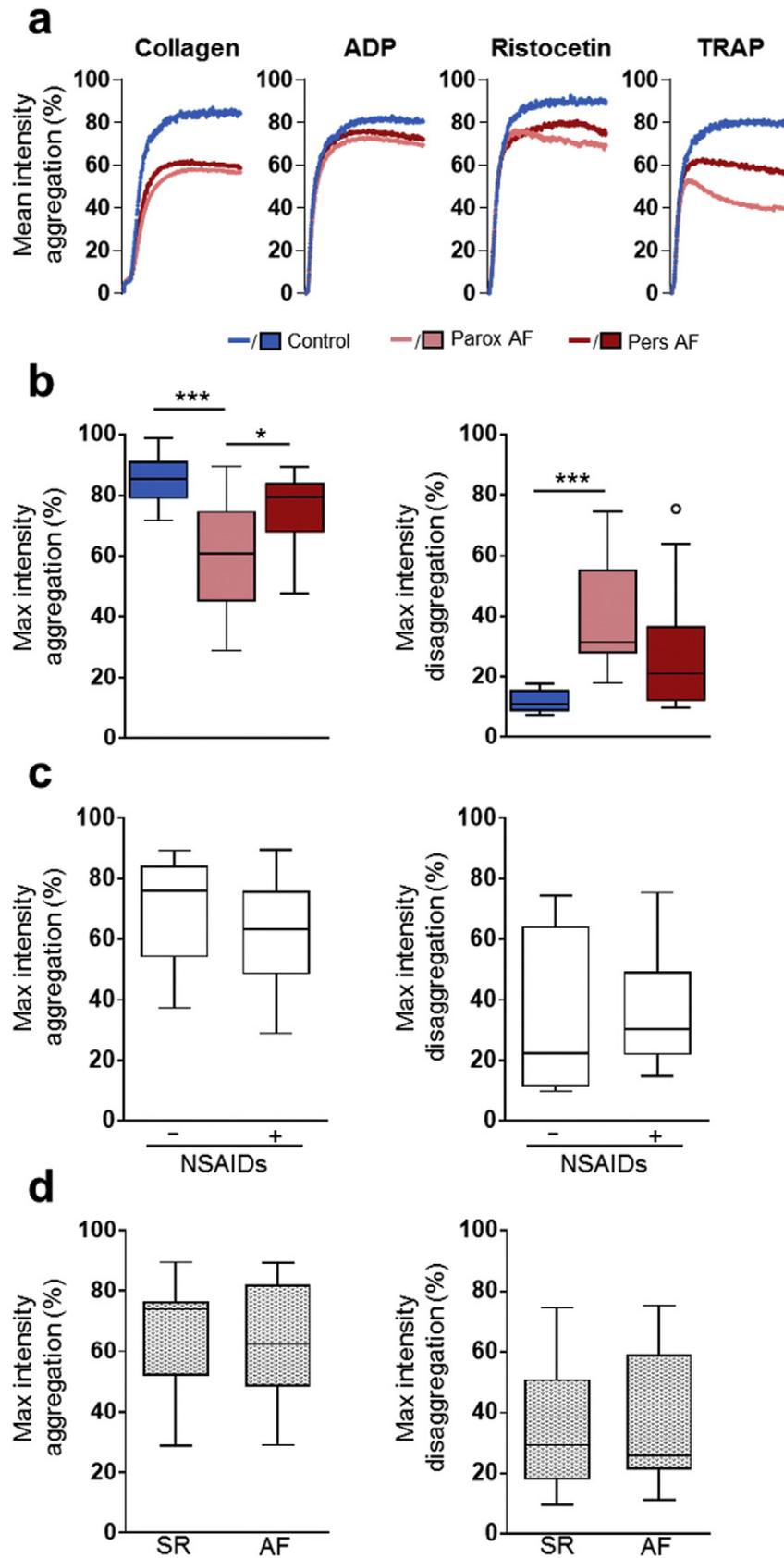


Fig. 1. Platelet aggregation response. Platelet aggregation profiles in response to collagen (2 $\mu\text{g/ml}$), ADP (10 μM), ristocetin (1.5 mg/ml) and TRAP (10 μM) for control, paroxysmal (Parox AF) and persistent (Pers AF) AF patients (a). Composite data representing the mean percent aggregation intensity of platelets from peripheral blood for controls ($n = 11$) and from samples taken from the left atrium for paroxysmal ($n = 21$) and persistent ($n = 16$) AF patients. Comparison of the maximal aggregation intensity and the residual intensity after disaggregation in response to TRAP, between control, paroxysmal and persistent AF patients (b), between patients receiving anti-inflammatory drugs or not (c), between blood samples taken in sinus rhythm and in fibrillation (d). Data are represented as box and whiskers. Box plots display first and third quartiles and horizontal lines display median. Whiskers are represented with Tukey's method. Symbols represent outliers. * $p \leq 0.05$ *** $p \leq 0.001$. AF = atrial fibrillation, NSAIDs = nonsteroidal anti-inflammatory drugs, SR = sinus rhythm.

3. Results

3.1. AF pathology is associated with platelet aggregation abnormalities

For all patients and control subjects, platelet counts (control: 257 ± 19 G/l, paroxysmal: 219 ± 12 G/l, persistent: 237 ± 17 G/l) and mean platelet volumes (control: $7.8 \pm 0.3 \mu\text{m}^3$, paroxysmal: $7.9 \pm 0.2 \mu\text{m}^3$, persistent: $7.9 \pm 0.2 \mu\text{m}^3$) were within normal ranges and there was no significant differences between groups.

Platelet aggregation in patients with paroxysmal or persistent AF showed a lower maximal intensity compared to controls for all agonists, including collagen, ADP, ristocetin and TRAP (Fig. 1a). It was with TRAP that the difference between platelet responses from paroxysmal AF compared to persistent AF was highly significant. No significant difference of platelet aggregation in response to collagen, ADP, ristocetin and TRAP, between peripheral and left atrium blood samples was

observed, suggesting that platelet function in AF patients was globally affected (Supplementary Fig. A.1).

Statistical comparative analysis between control, paroxysmal and persistent forms revealed that maximal percentage aggregation induced by TRAP was significantly lower in paroxysmal AF compared to control ($p < 0.001$) and persistent AF ($p = 0.02$) (Fig. 1b). Furthermore, the lower maximal intensity of platelet aggregation was associated with a high reversibility of aggregation in paroxysmal AF patients compared to control ($p < 0.001$) (Fig. 1b). The platelet aggregation response to TRAP agonist in patients was statistically unrelated to NSAID administration (Fig. 1c) and to cardiac rhythm at the time of blood sampling (Fig. 1d).

3.2. Acute-AF induced increased platelet aggregation

Subsequently, to evaluate whether a short burst of AF influences platelet functions, we analyzed the immediate effect of 20 min of

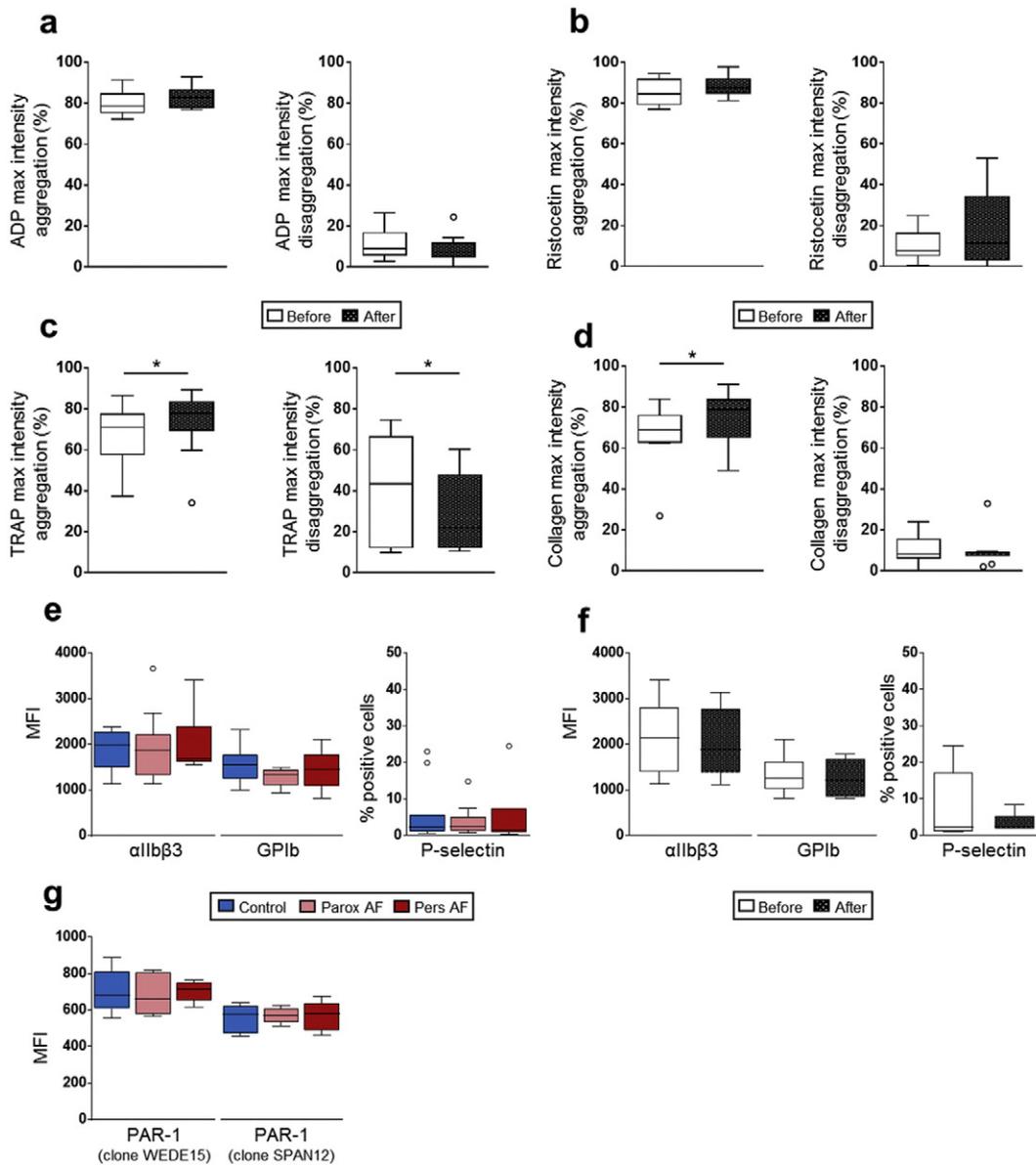


Fig. 2. Left atrium platelet aggregation response after acute-AF episode and expression of major platelet membrane receptors including PAR-1. Data are represented as box and whiskers as in the legend to Fig. 1. Data of maximal intensity aggregation and percent disaggregation in response to ADP (a), ristocetin (b), TRAP (c) and collagen (d) in left atrium blood samples from patients in sinus rhythm during a period of 10 days and following AF induction. Comparison of αIIbβ3, GPIb and P-selectin expression between controls, paroxysmal (Parox AF) and persistent (Pers AF) AF patients (e), between left atrium blood samples from patients in sinus rhythm during 10 days and following AF induction (f). Comparison of PAR-1 epitopes expression between controls, paroxysmal and persistent AF patients (g).

continuous AF on the ex vivo platelet aggregation response for patients in sinus rhythm over at least 10 days. Platelet responses to ADP (Fig. 2a) and ristocetin (Fig. 2b) were not modified in left atrium blood samples taken in sinus rhythm and after 20 min of continuous AF. However, induction of acute-AF increased the maximal intensity of platelet aggregation induced by TRAP ($p = 0.02$) (Fig. 2c). This increase of platelet aggregation was associated with a reduced reversibility of aggregation ($p = 0.05$) (Fig. 2c). An acute-AF episode also increased maximal platelet aggregation induced by collagen ($p = 0.03$) without any effect on the reversibility (Fig. 2d).

These results indicated that an acute-AF episode was sufficient to modify platelet function, in particular, the response to the thrombin analog. Thus AF influences platelet functionality differently in the long and short term.

3.3. Platelet α IIb β 3, GPIb and expression of P-selectin remain normal

Flow cytometric analysis of the expression of α IIb β 3 and GPIb, the major platelet receptors involved in platelet aggregation and adhesion showed normal results in left atrium blood samples (Fig. 2e). P-selectin is a measure of platelet activation and granule secretion. The percentage of platelets expressing P-selectin in the left atrium from paroxysmal and persistent AF patients was not significantly different to that of the controls (Fig. 2e). Platelet α IIb β 3, GPIb and P-selectin receptor expression was not significantly different when comparing peripheral blood to that taken from the left atrium (Supplementary Fig. A.2). Finally, an acute-AF episode induced in patients in sinus rhythm over 10 days did not generate any modification to platelet α IIb β 3, GPIb and P-selectin expression levels (Fig. 2f).

3.4. Thrombin receptor PAR-1 expression and activation

Because the platelet aggregation response to TRAP was abnormal, we analyzed the expression of the thrombin receptor PAR-1 by flow cytometry using two different commercial antibodies. The peptide TRAP mimics a ligand domain of PAR-1 that interacts with the receptor resulting in activation. A neopeptide becomes exposed after cleavage of the receptor by thrombin. The WEDE15 antibody reacts with both cleaved and uncleaved receptors whereas the SPAN12 antibody reacts exclusively with the uncleaved form. As shown in Fig. 2g, neither WEDE15 nor SPAN12 binding was modified in AF compared to healthy volunteers, suggesting that (i) the receptor has not previously been cleaved by thrombin and (ii) that the abnormal response to TRAP was not associated with a significant modification of the receptor expression levels.

3.5. Fibrinolytic and TF-dependent procoagulant activities of MPs in AF patients

Analysis of MPs by functional assays revealed different behavior between fibrinolytic and TF-dependent procoagulant MPs-associated activities. Levels of fibrinolytic activity were consistent between patients and controls, although a small reduction was found in paroxysmal AF patients compared to controls ($p = 0.01$) (Fig. 3a). Levels of procoagulant activity (of MPs) were lower in both paroxysmal and persistent AF patients compared to controls ($p < 0.01$) (Fig. 3b). Similar studies were performed before and after acute induction of AF. Levels of fibrinolytic activity in the left atrium showed no difference between AF patients in sinus rhythm versus 20 min after the onset of AF (Fig. 3c), whereas TF-dependent

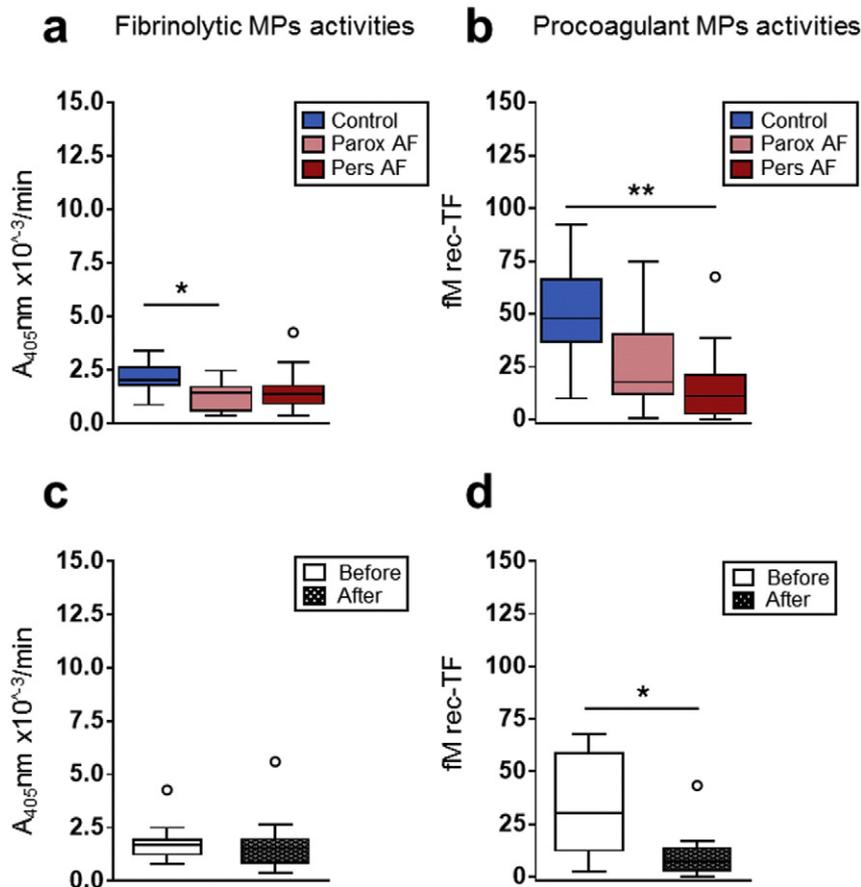


Fig. 3. Assessment of MPs-linked fibrinolytic and TF-dependent procoagulant activities. Data are represented as in the legend to Fig. 1. Results for peripheral blood samples are presented for controls and results for samples taken from the left atrium are presented for patients. Compared levels of fibrinolytic (a) and TF-dependent procoagulant (b) functions between controls, paroxysmal (Parox AF) and persistent (Pers AF) AF. Compared levels of fibrinolytic (c) and TF-dependent procoagulant (d) functions of MPs between left atrium blood samples from patients in sinus rhythm during 10 days and following AF induction. * $p \leq 0.05$, ** $p \leq 0.01$.

procoagulant activity levels decreased significantly ($p = 0.02$) after 20 min of acute-AF (Fig. 3d). The mechanism below these observations remains to be clarified, a first hypothesis being that TF-dependent procoagulant MPs are preferentially used locally following tissue modification induced by AF.

4. Discussion

We have investigated platelet function as well as the procoagulant and fibrinolytic activity of MPs in patients with persistent and paroxysmal non-valvular AF with, for some cases, an analysis of the direct effect of an acute-AF episode. Initial results showed a significant reduction in the platelet aggregation response to TRAP, a synthetic peptide activating the thrombin receptor PAR-1. Present in both groups of patients, this decrease was greater for paroxysmal than for persistent AF, not only locally in atrial blood but also in the peripheral circulation. Interestingly, an acute episode of induced-AF improved the platelet response to TRAP, indicating that (i) short AF episodes are able to directly influence platelet aggregation and (ii) the decreased response of platelets to TRAP observed for paroxysmal and permanent AF can be partially reversed. The results showing an abnormal platelet thrombin receptor response, strongly suggest prior contact of platelets with thrombin.

Abnormal formation of thrombin during AF has been previously reported, with the presence of increased markers of thrombin generation in the circulation such as thrombin-antithrombin complexes and D-dimer [15,16] but without increased fibrinolytic activity [17,18]. Thrombin not only has a crucial role for fibrin generation, but it is also a potent platelet activator. When thrombin is formed, platelet PAR-1 and PAR-4 receptors can be cleaved, first activating platelets, leading to a secondary platelet refractory state through receptor desensitization [19]. To explain the decreased platelet response to TRAP for AF patients, one hypothesis is that some of the PAR-1 receptors were cleaved or missing from the surface. Quantification of PAR-1 performed using cleavage-sensitive and insensitive antibodies showed no significant decrease in platelet surface expression. However, it cannot be excluded that many of the affected platelets are rapidly removed from the circulation. A recent investigation of platelet PAR-1 receptors on AF patients receiving dabigatran, a direct thrombin inhibitor that blocks thrombin catalytic activity, showed an increased expression of PAR-1 after the patients had received this treatment [20]. This finding would be in agreement with a direct action of thrombin on platelets of AF patients.

PAR-1 activation can also result from interactions with proteases other than thrombin [21]. Plasmin cleaves PAR-1, but for AF patients increased circulating levels of plasmin have not been described [9] and the stable amount of fibrinolytic activity in MPs after induced-AF in our study is not in favor of this hypothesis. Metalloproteases (MMP)-1 and -2 are present in platelets, it was recently demonstrated that MMP-2 stimulates PAR-1 through Gq and G12/13 signaling pathways though it is insufficient to cause platelet aggregation and only predisposes platelets to a full response with a Gi activating signaling [22]. We cannot exclude a contribution of MMPs for our patients, but trace amounts of thrombin have already been reported in the circulation in AF [23]. Interaction of proteases with PAR-1 initiates receptor cleavage, signaling and desensitization. Desensitization can be brought about by internalization of PAR-1 receptors, a process often seen in vascular cells, but limited in platelets as shown by electron microscopy and immunogold labeling of platelet sections of thrombin-stimulated platelets [24]. Alternatively, desensitization can be mediated through an arrestin-dependent pathway, again reported in nucleated cells but for platelets only reported for PAR-4 [25,26]. A third way of modulating PAR-1 activation has been shown for platelets incubated with TRAP peptide and consists of the desensitization of PAR-1 signaling, including a decrease in Ca^{2+} mobilization, restricted protein kinase C substrate phosphorylation and reduced secretion but not associated with a modification of PAR-1

expression on platelet surface [27]. Further tests are required to see if the platelets of patients such as those examined in our study exhibit this profile.

We provide evidence that, after a short-time of induced-AF, platelets became more reactive and decreased PAR-1-dependent aggregation was partially corrected. In vitro experiments showed that PAR-1 desensitized platelets can respond to amounts of a second thrombin receptor (PAR-4) agonist, suggesting that thrombin can abrogate rapidly the inhibitory state and that this is sufficient to re-establish aggregation. Importantly, this also signifies that a partial refractory state does not protect platelets from a second wave of thrombin. These results are highly suggestive of: (i) a desensitization process greater in paroxysmal AF than in the permanent form and (ii) an equilibrium between platelet activation and desensitization; whereby activation is elevated relative to desensitization in permanent AF (Supplementary Fig. A.3). Typically, AF evolves from a paroxysmal to a persistent form. For paroxysmal AF, restoration to sinus rhythm can in most cases be achieved by “anatomical” catheter ablation limited to the pulmonary veins. Whereas for the persistent form, zones of electrical remodeling in the atrium are more diffuse leading to changes in ion channels, signaling pathways, calcium handling, and structural changes in the atrial tissue associated with oxidative stress, inflammation and atrial fibrosis [28]. These observed modifications during the evolution of AF may explain the increased platelet response to TRAP observed in permanent AF.

Elevated P-selectin expression was observed for two AF patients but statistically significant differences were not found between the grouped AF patients and controls. Small increases in expression of this marker, commonly used to evaluate platelet activation, have been reported in AF but the results are still controversial [29]. Willoughby et al. found a mild increase in platelet activation for blood taken either from the right or left atrium of patients with AF [30]. However, Choudhury et al. found low levels of activation in the presence of patient comorbidities (e.g. diabetes) [31]. Another possibility to explain the absence or low levels of P-selectin-expressing platelets is rapid removal from the circulation [32]. Platelet P-selectin was shown to be increased after inducing AF [33] but as measurements were obtained following fixation with methanol-based solution (CellFix®, BD biosciences), which gives access to the internal pool of this receptor, these observations cannot be compared to our results.

In our study, levels of MPs-associated, TF dependent activity were measured in the left atrium of AF patients, using a highly sensitive functional assay that has the capacity of detecting TF activity even in normal healthy control samples. The most important result that we report are lower levels of TF-dependent activity in the left atrium observed after an acute-induced AF episode in comparison with measurements performed under the same conditions before inducing AF. This may suggest that (i) TF-dependent MPs may be captured by the surrounding tissues and/or incorporated into micro-clots or (ii) that higher levels of inhibitors such as TF pathway inhibitor may be bound to MPs. In previous studies, levels of PS-expressing MPs were found to be increased in AF patients [34]. But these studies were performed under basal conditions and did not evaluate the effect of an induced acute-AF episode. Jesel et al. have previously shown that levels of TF-dependent MPs measured in both upper cardiac chambers were slightly lower in the left atrium, here again suggesting a local consumption [35]. We show that MPs-linked fibrinolytic activity was not modified in patients with AF, but it would be important to examine these fibrinolytic MPs in the long-term especially as they have been shown to play a role in fibrosis formation by TGF- β 1 activation [36].

The observed reduced TF-dependent procoagulant activity of an increased platelet aggregation after an induced-AF episode, highlighted the pro-thrombotic effects of arrhythmia, also affecting platelets and their participation in this process. All of these results indicate that in addition to plasma coagulation involvement in the AF pathology the cellular participation in the evolution of this disorder is important.

5. Limitations

A limitation to this study is the absence of options to study blood from the atrium of normal donors as controls. Also there is not enough information about the pathophysiology of other abnormal cardiac arrhythmia, such as Bouveret syndrome, to consider these patients as potential controls. Future studies will focus on platelet signalization through the PAR-1 receptor to better explain the platelet desensitization to TRAP. We will also determine the cellular origin of the MPs. Finally the mechanisms bringing about the disappearance of TF-dependent procoagulant MPs in the left atrium remain to be elucidated, their attachment to the endocardium or other cells/tissues is an intriguing option to test.

6. Conclusion

Patients with AF showed significant changes in the platelet response to PAR-1 activation in favor of a desensitization, partially corrected during an acute induced episode of AF, suggesting a continuous rebalancing of platelet function. Equally, the MPs expressing tissue factor found in the atrium immediately after an acute AF episode can trigger coagulation and may represent a new therapeutic target.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ijcard.2017.03.068>.

Funding sources

This study received financial support from the French government as part of the «investments of the future» program managed by the national research agency (ANR) [grant number ANR-10-IAHU-04] and the French Federation of Cardiology (FFC).

Disclosures

Philippe Poncelet is a full-time employee of BioCytex, a Stago group company. There are no financial disclosures or relationships with the industries in connection with this article or for the other authors.

Acknowledgments

The authors would like to acknowledge Marie-José Jacobin-Valat from CNRS 5536 (Bordeaux) for flow cytometry technical assistance, Eliane Berrou and Marijke Bryckaert from INSERM 1176 (Kremlin-Bicêtre) for technical advice, Emma Abell and Fanny Vaillant from IHU Liryc (Bordeaux) for help in preparing the manuscript.

References

- J. Heeringa, D.A.M. van der Kuip, A. Hofman, J.A. Kors, G. van Herpen, B.H.C. Stricker, et al., Prevalence, incidence and lifetime risk of atrial fibrillation: the Rotterdam study, *Eur. Heart J.* 27 (2006) 949–953.
- M. Haïssaguerre, P. Jaïs, D.C. Shah, A. Takahashi, M. Hocini, G. Quiniou, et al., Spontaneous initiation of atrial fibrillation by ectopic beats originating in the pulmonary veins, *N. Engl. J. Med.* 339 (1998) 659–666.
- H. Calkins, K.H. Kuck, R. Cappato, J. Brugada, A.J. Camm, S.-A. Chen, et al., HRS/EHRA/ECAS expert consensus statement on catheter and surgical ablation of atrial fibrillation: recommendations for patient selection, procedural techniques, patient management and follow-up, definitions, endpoints, and research trial design, *Europace* 14 (2012) 528–606.
- G.V. Naccarelli, H. Varker, J. Lin, K.L. Schulman, Increasing prevalence of atrial fibrillation and flutter in the United States, *Am. J. Cardiol.* 104 (2009) 1534–1539.
- Y.-H. Kim, S.-Y. Roh, The mechanism of and preventive therapy for stroke in patients with atrial fibrillation, *J. Stroke* 18 (2016) 129–137.
- A.T. Nurden, P. Nurden, M. Sanchez, I. Andia, E. Anitua, Platelets and wound healing, *Front. Biosci.* 13 (2008) 3532–3548.
- O. Morel, F. Toti, B. Hugel, B. Bakouboula, L. Camoin-Jau, F. Dignat-George, et al., Procoagulant microparticles: disrupting the vascular homeostasis equation? *Arterioscler. Thromb. Vasc. Biol.* 26 (2006) 2594–2604.
- R. Lacroix, C. Dubois, A.S. Leroyer, F. Sabatier, F. Dignat-George, Revisited role of microparticles in arterial and venous thrombosis, *J. Thromb. Haemost.* 11 (Suppl. 1) (2013) 24–35.
- L. Drabik, P. Wolkow, A. Undas, Denser plasma clot formation and impaired fibrinolysis in paroxysmal and persistent atrial fibrillation while on sinus rhythm: association with thrombin generation, endothelial injury and platelet activation, *Thromb. Res.* 136 (2015) 408–414.
- M. Cattaneo, C. Cerletti, P. Harrison, C.P.M. Hayward, D. Kenny, D. Nugent, et al., Recommendations for the standardization of light transmission aggregometry: a consensus of the working party from the platelet physiology subcommittee of SSC/ISTH, *J. Thromb. Haemost.* (2013) <http://dx.doi.org/10.1111/jth.12231>.
- C. Bihour, C. Durrieu-Jais, L. Macchi, C. Poujol, P. Coste, P. Besse, et al., Expression of markers of platelet activation and the interpatient variation in response to abciximab, *Arterioscler. Thromb. Vasc. Biol.* 19 (1999) 212–219.
- R. Lacroix, C. Judicone, M. Mooberry, M. Bouckine, N.S. Key, F. Dignat-George, et al., Standardization of pre-analytical variables in plasma microparticle determination: results of the international society on thrombosis and Haemostasis SSC collaborative workshop, *J. Thromb. Haemost.* (2013) <http://dx.doi.org/10.1111/jth.12207>.
- I. Agouti, S. Cointe, S. Robert, C. Judicone, A. Loundou, F. Driss, et al., Platelet and not erythrocyte microparticles are procoagulant in transfused thalassaemia major patients, *Br. J. Haematol.* 171 (2015) 615–624.
- R. Lacroix, L. Plawinski, S. Robert, L. Doeuvre, F. Sabatier, S. Martinez de Lizarondo, et al., Leukocyte- and endothelial-derived microparticles: a circulating source for fibrinolysis, *Haematologica* 97 (2012) 1864–1872.
- J.G. Akar, W. Jeske, D.J. Wilber, Acute onset human atrial fibrillation is associated with local cardiac platelet activation and endothelial dysfunction, *J. Am. Coll. Cardiol.* 51 (2008) 1790–1793.
- H. Inoue, T. Nozawa, K. Okumura, L. Jong-Dae, A. Shimizu, K. Yano, Prothrombotic activity is increased in patients with nonvalvular atrial fibrillation and risk factors for embolism, *Chest* 126 (2004) 687–692.
- V. Roldan, F. Marin, P. Marco, J.G. Martinez, R. Calatayud, F. Sogorb, Hypofibrinolysis in atrial fibrillation, *Am. Heart J.* 136 (1998) 956–960.
- F. Marin, V. Roldan, G.Y. Lip, Fibrinolytic function and atrial fibrillation, *Thromb. Res.* 109 (2003) 233–240.
- S.R. Coughlin, Protease-activated receptors in hemostasis, thrombosis and vascular biology, *J. Thromb. Haemost.* 3 (2005) 1800–1814.
- A. Achilles, A. Mohring, L. Dannenberg, M. Grandoch, T. Hohlfeld, J.W. Fischer, et al., Dabigatran enhances platelet reactivity and platelet thrombin receptor expression in patients with atrial fibrillation, *J. Thromb. Haemost.* 15 (3) (2017) 473–476.
- F. Adam, M.-C. Guillin, M. Jandrot-Perrus, Glycoprotein Ib-mediated platelet activation. A signalling pathway triggered by thrombin, *Eur. J. Biochem.* 270 (2003) 2959–2970.
- M. Sebastiano, S. Momi, E. Falcinelli, L. Bury, M.F. Hoylaerts, P. Gresele, A novel mechanism regulating human platelet activation by MMP-2-mediated PAR1 biased signaling, *Blood* 129 (2017) 883–895.
- R.C. Becker, Biomarkers in atrial fibrillation: investigating biologic plausibility, cause, and effect, *J. Thromb. Thrombolysis* 19 (2005) 71–75.
- M. Molino, D.F. Bainton, J.A. Hoxie, S.R. Coughlin, L.F. Brass, Thrombin receptors on human platelets. Initial localization and subsequent redistribution during platelet activation, *J. Biol. Chem.* 272 (1997) 6011–6017.
- M. Schaff, N. Receveur, C. Bourdon, P. Ohlmann, F. Lanza, C. Gachet, et al., Beta-arrestin-1 participates in thrombosis and regulates integrin α IIb β 3 signalling without affecting P2Y receptors desensitisation and function, *Thromb. Haemost.* 107 (2012) 735–748.
- D. Li, L. D'Angelo, M. Chavez, D.S. Woulfe, Arrestin-2 differentially regulates PAR4 and ADP receptor signaling in platelets, *J. Biol. Chem.* 286 (2011) 3805–3814.
- K. Fälker, L. Haglund, P. Gunnarsson, M. Nylander, T.L. Lindahl, M. Grenegård, Protease-activated receptor 1 (PAR1) signalling desensitization is counteracted via PAR4 signalling in human platelets, *Biochem. J.* 436 (2011) 469–480.
- M. Haïssaguerre, A.J. Shah, H. Cochet, M. Hocini, R. Dubois, I. Efmov, et al., Intermittent drivers anchoring to structural heterogeneities as a major pathophysiological mechanism of human persistent atrial fibrillation, *J. Physiol.* 594 (2016) 2387–2398.
- N. Wu, X. Chen, T. Cai, L. Wu, Y. Xiang, M. Zhang, et al., Association of inflammatory and hemostatic markers with stroke and thromboembolic events in atrial fibrillation: a systematic review and meta-analysis, *Can. J. Cardiol.* 31 (2015) 278–286.
- S.R. Willoughby, R.L. Roberts-Thomson, H.S. Lim, C. Schultz, A. Prabhu, P. De Sciscio, et al., Atrial platelet reactivity in patients with atrial fibrillation, *Heart Rhythm* 7 (2010) 1178–1183.
- A. Choudhury, I. Chung, A.D. Blann, G.Y.H. Lip, Platelet surface CD62P and CD63, mean platelet volume, and soluble/platelet P-selectin as indexes of platelet function in atrial fibrillation: a comparison of “healthy control subjects” and “disease control subjects” in sinus rhythm, *J. Am. Coll. Cardiol.* 49 (2007) 1957–1964.
- A.T. Nurden, L. Macchi, C. Bihour, C. Durrieu, P. Besse, P. Nurden, Markers of platelet activation in coronary heart disease patients, *Eur. J. Clin. Investig.* 24 (Suppl. 1) (1994) 42–45.
- H.S. Lim, S.R. Willoughby, C. Schultz, C. Gan, M. Alasady, D.H. Lau, et al., Effect of atrial fibrillation on atrial thrombogenesis in humans: impact of rate and rhythm, *J. Am. Coll. Cardiol.* 61 (2013) 852–860.
- S. Ederhy, E. Di Angelantonio, Z. Mallat, B. Hugel, S. Janower, C. Meuleman, et al., Levels of circulating procoagulant microparticles in nonvalvular atrial fibrillation, *Am. J. Cardiol.* 100 (2007) 989–994.
- L. Jesel, T. Arentz, C. Herrera-Siklody, D. Trenk, F. Zobairi, M. Abbas, et al., Do atrial differences in endothelial damage, leukocyte and platelet activation, or tissue factor activity contribute to chamber-specific thrombogenic status in patients with atrial fibrillation? *J. Cardiovasc. Electrophysiol.* 25 (2014) 266–270.
- F. Blasi, P. Carmeliet, uPAR: a versatile signalling orchestrator, *Nat. Rev. Mol. Cell Biol.* 3 (2002) 932–943.