Association of platelet activation markers with recurrence of atrial fibrillation after pulmonary vein isolation

Christian Pfluecke, Lina Plichta, Daniel Tarnowski, Mathias Forkmann, Stefan Ulbrich, Silvio Quick, Felix M. Heidrich, Stephan Wiedemann, Marian Christoph, David M. Poitz, Carsten Wunderlich, Ruth H. Strasser, & Karim Ibrahim

Abstract

Atrial fibrillation (AF) is known to cause platelet activation. AF and its degree of thrombogenesis could be associated with monocyte-platelet aggregates (MPAs). We investigated on whether the content of MPAs or other platelet activation markers is associated with the recurrence of AF after pulmonary vein isolation (PVI). A total of 73 patients with symptomatic AF underwent PVI. After 6 months, all patients were evaluated for episodes of AF recurrence. At the same time, flow-cytometric quantification analyses were performed to determine the content of MPAs. Further platelet activation parameters were detected by using either flow cytometric bead arrays or quantitative immunological determination. Patients with recurrent AF (n = 20) compared to individuals without AF relapse (n = 53) were associated with an increased content of MPAs (43 ± 3% vs. 33 ± 2%, p = 0.004), as well as an increased CD41 expression on monocytes (191 ± 20 vs. 113 ± 6, p = 0.001). The level of the soluble platelet activation markers such as D-dimer, sCD40L, and sP-selectin did not differ between these groups. The content of MPAs correlated weakly with the level of sCD40L (r = 0.26, p = 0.03), but not with sP-selectin and D-dimer, whereas sP-selectin and sCD40L correlated with each other (r = 0.38, p = 0.001). Only the cellular marker of platelet activation, the content of MPAs, was increased in patients with recurrent AF after PVI. In contrast, soluble markers remained unaltered. These data indicate a distinct mechanism and level of platelet activation in AF. The clinical relevance of MPAs in identifying AF recurrence or in guiding the therapy with anticoagulants remains to be elucidated.

Keywords

monocyte-platelet-aggregates, platelet activation, atrial fibrillation, stroke, pulmonary vein isolation, thrombogenicity

Introduction

Atrial fibrillation (AF) is well known for being one of the major causes of ischemic stroke. The mechanisms of developing emboli are multifarious. In addition to blood stasis in the left atrium (LA) [1], inflammatory processes [2], endothelial dysfunction [3,4], hemostatic influence [5], impaired fibrinolysis [6], and platelet activation [7] were also identified as contributors to the increased thromboembolic risk in patients with AF. A therapy with anticoagulants is able to reduce the risk of stroke but also entails an elevated risk of bleeding. Pulmonary vein isolation (PVI) is a well-established treatment option in patients with symptomatic AF. However, the need to continue therapy with anticoagulants after PVI is still a matter of debate, even in patients without obvious recurrence of AF after catheter ablation [8]. Therefore, reliable biomarkers that could predict AF recurrence or thromboembolic events of patients after PVI would be helpful. Several markers of coagulation, such as fibrinogen and D-dimer [9] or markers of platelet activation [10,11], for example soluble P-selectin (sP-selectin) [12] and soluble CD40 ligand (sCD40L) [13], could be associated with AF [10;11;11]. Though, controversy does exist, as some studies could not confirm these associations [14,15]. The sCD40L was detected immediately by provoking AF [16] and was further recognized as a potential link between inflammatory processes [17], atherothrombotic events [18], and platelet activation [19]. Both CD40L and P-selectin were initially expressed rapidly on platelets and secreted in a soluble form after activation. Therefore, these markers seem to be detectable only for a short period of time in the peripheral blood after activation [20]. Another interesting marker of platelet activation is the level of monocyte-platelet aggregates (MPAs) in the peripheral blood, which has been shown to correlate with other platelet activation markers [21]. Furthermore, in contrast to soluble markers, MPAs as cellular markers demonstrated advantages in sensitivity [22] and stability [23] in the peripheral blood after activation. MPAs and sCD40L could be associated with worse outcome after stroke [24]. In a previous study, we were able to show that the prevalence of both persistent and paroxysmal AF correlated well with an increased content of MPAs in patients with aortic stenosis [25]. Furthermore, we demonstrated that the level of MPAs correlated with the prevalence of left atrial thrombus and the degree of reduced flow in the left atrial appendage [26] in patients with AF. In this study we investigated whether the content of MPAs or other platelet activation markers is associated with the recurrence of AF after PVI, which may in turn help to identify the recurrence of obscure AF in the period after PVI without current AF.
Methods

Study population

Seventy-three patients with a history of paroxysmal or permanent AF underwent elective PVI. Prior to PVI, all patients received a thorough anamnesis, physical examination, laboratory tests, and transthoracic echocardiography, combined with transesophageal echocardiography, in order to exclude the presence of atrial thrombi and to determine the left atrial appendage peak emptying flow velocity (LAAEV). After PVI, patients without preexisting pacemaker were equipped with an event recorder. A total of 19 out of 73 patients did not have continuous ECG-monitoring and were instead checked by a seven-day ECG (Holter monitoring) 3 months and 6 months after PVI to determine whether SR or AF occurred. Six months after the procedure, the patients underwent anamnestic and physical examination, as well as laboratory tests again. In addition, flow-cytometric quantification analyses of the peripheral blood were performed on these patients on the day of follow-up. Patients exhibiting acute coronary syndrome, infections, or inflammatory disorders were excluded. The study was performed in accordance with the Helsinki Declaration and approved by the Institutional Ethic Committee of the Technische Universität Dresden (EK406122012). All participants submitted their written informed consent.

Laboratory methods

Peripheral venous blood samples were collected through non-traumatic puncture from all 73 study participants with minimal stasis in tubes containing sodium citrate (Sarstedt) as anticoagulants and analyzed by flow cytometry within 30 min of collection. Shortly thereafter, 50 µl blood was labeled with CD45-FITC, CD14-APC, and CD41-PE (all antibodies were purchased from Becton Dickinson [BD], Oxford, United Kingdom) for 10 min. Flow cytometric measurements were performed by using a BD FACSCalibur flow cytometer. Monocytes were identified by gating strategies based on CD45, CD14 expression, and side scatter to select monocytes. The content of MPAs was determined by co-expression of CD41 and CD14 on monocytes. The quantification of MPAs was expressed as mean fluorescence intensity (MFI) and as relative count. Relative counts of MPAs represent the percentage of monocytes with coexpression of the platelet marker CD41 on all monocytes. All parameters, both intra- and inter-reproductibility had acceptable coefficients of variation (CVs). The CV of the percentage of MPAs was 3%. Soluble human P-selectin (sP-selectin) and soluble CD40 Ligand (sCD40L) were assessed by using the cytometric bead arrays (CBA) [27], according to the manufacturer’s instructions. Therefore, blood samples were collected in sodium citrate tubes and plasma prepared by centrifugation at 2000×g for 10 min at 4°C. The plasma aliquots were stored at −80°C until assayed. The lower limit of detection in our laboratory for sP-selectin and sCD40L was 80 pg/ml. Levels of D-dimer were determined by quantitative immunological determination of fibrin degradation products (Tina-quant D-dimer, Roche/Hitachi cobas c systems.1.2), according to the manufacturer’s instructions (lower limit of detection: 0.10 µg FEU/ml).

Statistical analysis

The study was powered to have a 90% chance of detecting a difference in the level of MPAs between the two groups of AF recurrence and stable SR after PVI. This was based on our previous study examining patients with aortic stenosis [25], which showed that MPAs were elevated in dependence of AF in the following range: Group SR mean 43 ± 19 vs. Group AF mean 61 ± 24, p = 0.002. The calculated minimum number of participants in each group with α < 5% was n = 20. Statistical analysis was performed using SPSS v.18. The distribution of continuous data was examined using the Kolmogorov–Smirnov test. Data are given as mean ± standard error of mean (SEM), unless otherwise stated. To compare group 1 (patients with stable SR after PVI) with group 2 (patients with any recurrence of AF after PVI), the data with normal Gaussian distribution were analyzed using an unpaired Student’s t-test after being controlled for equality of variances with the Levene’s test. Data with a non-Gaussian distribution were analyzed using the Mann–Whitney U test. A p-value of <0.05 was considered to be statistically significant. For logistic regression analysis, we included predictors that were identified in the univariate test. Considering the relatively small sample size, variables achieving p < 0.15 on univariate testing were entered into this regression analysis. Additionally, LA-diameter and C-reactive protein (CRP) were included due to their known association with the recurrence of AF (LA-diameter) or with the content of MPAs (CRP). Receiver-operating characteristic (ROC) curve analyses were used for evaluating the optimal cutoff value, and the related sensitivity and specificity for predicting the recurrence of AF. Odds ratio and 95% confidence interval were calculated.

Results

Baseline characteristics

Relevant clinical and demographic characteristics are presented in Table I. No differences in age, gender, or comorbidities like diabetes, obesity, hypertension, or coronary artery disease existed between patients with a recurrence of AF and patients with stable SR 6 months after PVI. The CHA2DS2-VASc score in both groups amounted to an average of 2.3. Before PVI, 58 out of 73 patients were on anticoagulants, whereas all patients after PVI were prescribed anticoagulants according to guidelines. Six months after the performed PVI, 20 out of 73 patients showed at least one documented episode of AF within the period of follow-up. Signs of AF were defined as any episode of AF lasting at least 30 seconds after a blanking period of 3 months revealed by a loop recorder, pacemaker, or seven-day ECG. From the group of AF-recurrence, 7 patients exhibited AF on the day of follow-up examination when flow-cytometric quantification analyses were performed. The group of patients with any episode of AF in the 6 months of follow-up showed only a slight trend toward a larger LA diameter, a reduced LAA peak emptying flow velocity and a reduced left ventricular ejection fraction (LVEF) before PVI. Similarly, the level of NT-proBNP was slightly higher but not statistically significant in the group of patients with a documented episode of AF-recurrence after 6 months.

Association of MPAs with AF recurrence after PVI

The relative proportion of MPAs on monocytes (33 ± 2% vs. 43 ± 3%, p = 0.004) and the MFI of CD41 on monocytes (113 ± 6 ± 20 vs. 191 ± 20, p = 0.001) was significantly increased in patients with any recurrence of AF compared to the group of stable SR after PVI (Figure 1, Table II). In regard to the ROC analysis, the level of MPAs in our study adduces an AUC of 0.74 (0.61–0.87). A relative content of MPAs above 30% predicted recurrence of AF with a sensitivity of about 90% (corresponding specificity 67%). A relative count of MPAs remained independently associated with the recurrence of AF after the adjustment of age, LVEF, LAA peak emptying flow velocity, the diameter of the LA, and CRP as shown in a multivariate logistic regression analysis (Table III). Elevated MPAs above a content of 30% had an odds ratio of 10.6 (95% CI 2.1–52.3; p = 0.004) for recurrent AF after PVI. Patients with documented episodes of AF and exhibiting AF on the day of examination showed an even higher level of MPAs (51 ± 3% vs. 39 ± 4%, p < 0.05) as compared to patients with SR at this point in time (S1).
Systemic soluble markers of platelet activation

The levels of sP-selectin and sCD40L were determined with immune beads assays while the levels of D-dimer were detected with quantitative immunological determination of fibrin degradation products. In contrast to the content of MPAs, none of these three markers for platelet activation showed relevant differences between the patients with stable SR and those with any recurrence of AF (Table II). The content of MPAs correlated moderately with the level of sCD40L (r = 0.26, p = 0.03), but not with sP-selectin and D-dimer, whereas the levels of sP-selectin and

Table I. Clinical and demographic characteristics of the study participants with history of symptomatic AF undergoing pulmonary vein isolation (PVI).

| Parameter                  | Patients without recurrence of AF after PVI (n = 53) | Patients with episodes of AF after PVI (n = 20) | p-Value |
|----------------------------|-----------------------------------------------------|------------------------------------------------|---------|
| Age, years                 | 62.4 ± 11.1                                         | 66.6 ± 8.1                                      | 0.14    |
| Sex (men), n (%)           | 30 (57)                                             | 10 (50)                                         | 0.62    |
| CHA2DS2-VASc.-Score        | 2.25 ± 1.34                                         | 2.30 ± 1.60                                     | 0.89    |
| HAS-BLED-Score             | 1.3 ± 0.9                                           | 1.6 ± 0.9                                       | 0.33    |
| Hypertension, n (%)        | 42 (79)                                             | 15 (75)                                         | 0.70    |
| Diabetes mellitus, n (%)   | 5 (9)                                               | 2 (10)                                          | 0.94    |
| CAD, n (%)                 | 10 (19)                                             | 3 (15)                                          | 0.71    |
| BMI (kg/m²)                | 28.2 ± 4.8                                          | 28.2 ± 4.0                                      | 0.98    |
| Smoking, n (%)             | 7 (13)                                              | 1 (5)                                           | 0.32    |
| Pacemaker, n (%)           | 3 (6)                                               | 2 (10)                                          | 0.52    |
| LVEF (%)                   | 57.7 ± 8.3                                          | 53.6 ± 9.7                                      | 0.07    |
| degree of MR               | 1.1 ± 0.6                                           | 1.4 ± 0.6                                       | 0.09    |
| LA diameter, mm            | 43.3 ± 5.2                                          | 43.9 ± 6.1                                      | 0.69    |
| LAAEV, cm/s                | 41.4 ± 19                                            | 35.6 ± 8.3                                      | 0.10    |
| Creatinine, µmol/l         | 90 ± 22                                             | 86 ± 17                                         | 0.34    |
| hsCRP, mg/l                | 3.1 ± 4.9                                           | 2.6 ± 1.7                                       | 0.64    |
| NT-proBNP, ng/l            | 284 ± 314                                           | 439 ± 431                                       | 0.20    |
| Troponin T, ng/l           | 9.8 ± 11.3                                          | 10.7 ± 6.5                                      | 0.83    |
| Medication                 |                                                     |                                                 |         |
| Oral anticoagulants        | 53 (100)                                            | 20 (100)                                        | 1.00    |
| Statins, n (%)             | 21 (40)                                             | 8 (41)                                          | 0.95    |
| Diuretics, n (%)           | 13 (25)                                             | 7 (35)                                          | 0.41    |
| ACEI/ARB, n (%)            | 32 (60)                                             | 13 (65)                                         | 0.71    |
| MRA, n (%)                 | 3 (5)                                               | 1 (5)                                           | 0.93    |
| Betablocker, n (%)          | 47 (89)                                             | 20 (100)                                        | 0.12    |
| Antiarrhythmics, n (%)     | 16 (30)                                             | 6 (31)                                          | 0.90    |

Table II. Parameters of platelet activation in patients with stable SR (n = 53) in comparison to patients with a documented episode of AF recurrence (n = 20) within 6 months after pulmonary vein isolation.

| Parameters               | Patients without recurrence of AF after PVI (n = 53) | Patients with episodes of AF after PVI (n = 20) | p-Value |
|--------------------------|-----------------------------------------------------|-------------------------------------------------|---------|
| D-dimer, mg FEU/l        | 0.13 ± 0.02                                          | 0.10 ± 0.14                                     | 0.41    |
| sP-selectin, pg/ml       | 7441 ± 393                                           | 6977 ± 551                                      | 0.53    |
| sCD40L, pg/ml            | 36.4 ± 3.2                                           | 36.3 ± 3.2                                      | 0.78    |
| MPA, %                   | 33 ± 2                                               | 43 ± 3                                          | 0.004** |
| CD41 on monocytes (MFI)  | 113 ± 6                                              | 191 ± 20                                        | 0.001** |

Table III. Multivariate logistic regression analyses of predictors for recurrence of atrial fibrillation in patients 6 months after pulmonary vein isolation.

| Parameter               | Odds ratio | 95% CI     | p-Value |
|-------------------------|------------|------------|---------|
| Age                     | 1.02       | 0.95 – 1.09| 0.61    |
| LVEF                    | 0.96       | 0.90 – 1.03| 0.26    |
| LAAEV, cm/s             | 0.97       | 0.92 – 1.02| 0.22    |
| LA diameter             | 0.95       | 0.84 – 1.07| 0.36    |
| CRP                     | 1.04       | 0.80 – 1.35| 0.77    |
| Elevated MPA count      | 10.6       | 2.1 – 52.3 | 0.004** |

ACEI/ARB, angiotensin converting enzyme inhibitor/angiotensin receptor blocker; CAD, coronary artery disease; CHA2DS2-VASc.-Score, congestive heart failure, hypertension, age, diabetes, stroke, vascular disease, sex category female; HAS-BLED, hypertension, abnormal liver/renal function, stroke, bleeding history or predisposition to bleeding; hsCRP, high sensitive C-reactive protein; LA, left atrium; LAAEV, LAA peak emptying flow velocity; LVEF, left ventricular ejection fraction; MR, mitral regurgitation; MRA, mineralocorticoid receptor antagonist; NT-proBNP, NT-pro-B-type natriuretic peptide. Data are presented as mean ± SD.

Figure 1. Comparison of monocyte-platelet aggregates (MPAs) in regard to stable sinus rhythm (SR) and recurrent episodes of atrial fibrillation (AF) after pulmonary vein isolation (PVI); MPAs, in % as CD14⁺/CD41⁺ double positive monocytes in the group of patients with stable SR (n = 53) and the group with episodes of recurrent AF (n = 20) within the follow-up period of 6 months after PVI. **p < 0.001.

MPA, monocyte-platelet aggregate; sCD40L, soluble CD40 ligand; sP-selectin, soluble P-selectin. Results are presented as mean ± SEM. **p < 0.01.
sCD40L correlated with each other ($r = 0.38$, $p = 0.001$), but not with the occurrence of AF. In comparison to the small group of patients with persistent AF after PVI, the group with ongoing AF showed likewise no relevant differences in the levels of sP-selectin, sCD40L, and D-dimer. However, the content of MPAs (35 ± 2 vs. 51 ± 3, $p = 0.002$) and the MFI of CD41 on monocytes (128 ± 6 vs. 240 ± 29, $p < 0.001$) were also elevated in patients with ongoing AF in comparison to patients with SR on the day of examination (S2).

**Discussion**

The present study demonstrates that recurrence of AF is associated with a cellular marker of platelet activation as the level of MPAs is significantly elevated in patients with documented episodes of AF in the follow-up after performed PVI. In contrast to the level of MPAs, the additionally examined soluble parameters D-dimer, sCD40L, and sP-selectin were unaltered and, therefore, appear not to be suitable for detecting recurrence of obscure AF.

The association of MPAs with the recurrence of AF after PVI supports the notion of an interdependency between AF and platelet activation. This aligns with reports on the increase of platelet activation and tissue factor expression after induction of AF [28], the coincidence of postoperative AF with high platelet reactivity [29], or similar reports describing the association of AF with platelet activation [9,16]. Even a reduction in platelet activation has been demonstrated after cardioversion of AF [30] or after successful PVI [31]. In patients with severe aortic stenosis, MPAs, as established markers for platelet activation, have been reported to correlate not only with ongoing AF, but also with paroxysmal AF, even while patients are in SR [25]. Our seen association of MPAs with the recurrence of AF after PVI confirmed the association of MPAs with persistent and paroxysmal AF and extended these findings on the cohort of patients who had undergone a PVI for treatment of AF. Additionally, our previous study showed that the extent of thrombogenicity correlated with the level of MPAs in patients with AF [26]. However, our results confirm previous associations of AF with hypercoagulability or platelet activation only partially. In contrast to the level of MPAs, the known markers for assessing the degree of hypercoagulability [32], D-dimer and further markers for ongoing platelet activation, sP-selectin and sCD40L are not elevated in the group with recurrence of AF. One possible explanation might be the differences in time dependency for these markers. In particular, the investigated parameters sP-selectin and sCD40L [20,33] are known to have a limited detectability in peripheral blood due only to transient activation. In contrast, the level of MPAs seems to persist over a longer period of time after activation, which has been shown in patients with acute heart failure [23]. In patients with episodes of recurrent AF after PVI, the burden of AF is often reduced markedly. Therefore, MPAs seem to have advantages for detecting previous episodes of AF. Interestingly, when the subgroups of patients with ongoing AF and paroxysmal episodes of AF were compared, the level of MPAs was the only different parameter remaining. However, these results must be viewed with caution due to the small number of patients with ongoing AF during the mentioned time dependency, platelet specificity, and inflammatory processes on influencing the degree of different kinds of individual platelet reactions [41]. MPAs are known to represent a link between platelet activation, inflammation [42], and hemostasis [43]. Also inflammatory circumstances [44] or predisposition to reduced blood flow, like acute heart failure, alone, can lead to a raise of MPAs [23,37]. However, elevated MPAs, within our study group, remained an independent predictor for the recurrence of AF after adjustment for age, LVEF, LA, and CRP. The level of MPAs adduces a respectable AUC of about 0.74 for predicting the recurrence of AF. MPAs above 30% showed a high sensitivity of 90%, an acceptable corresponding specificity of 67% and a negative predictive value of about 95% for predicting AF after the performed PVI. Given that even short episodes of AF can lead to platelet activation and thromboembolic events, the decision to stop the therapy with anticoagulants after PVI should take the individual thromboembolic risk into account as well. The only modest discriminative capability of the CHA2DS2-VASc Score with a reported AUC of about 0.65 [45] for the prediction of stroke might not be sufficient for the individual patient. Together with the aforementioned qualities of MPAs to predict thrombogenicity in patients with AF [26] and the hereby found association with the recurrence of AF, MPAs may prove to be a suitable marker for identifying patients who no longer benefit from a therapy with anticoagulants after PVI or who rather should continue taking anticoagulants. However, the possible clinical relevance of MPAs as a marker for successful PVI or as a decision-making aid for the therapy with anticoagulants needs to be proven in further studies, powered high enough for clinical outcome.

From a mechanistic view, the salient finding of the present study is that MPAs as cellular markers are elevated even in patients with SR but with recurrent episodes of AF, in contrast to soluble markers of platelet activation, such as sP-selectin. Whether this observation indicates that platelet activation at the cellular level, i.e. MPAs, is mediated by distinct mechanisms or by kinetic aspects, such as the rapid degradation of soluble markers, cannot be answered at this point of time. It may be speculated that platelet activation indicated by MPAs may have distinct targets, potentially more localized in the heart itself and at the cellular level, such as endothelial cells or cardiomyocytes. Within the formation of MPAs, platelets form linkages between leukocytes, particularly mediated by platelet P-selectin binding to leukocyte PSGL-1, but also via leukocyte CD11b/CD18 binding to platelet GP1b or platelet bound fibrinogen [46]. This way, endothelium-bound platelets can bind leukocytes and can therefore promote leukocyte transendothelial migration. Although the physiological role is not fully understood, MPAs are able to influence inflammatory processes, such as the upregulation of leukocyte proinflammatory functions [47], and are known to be enhanced by inflammatory circumstances, like sepsis [48] by peripheral arterial disease [49] or by acute coronary syndrome [50]. Furthermore, monocyte–platelet interactions are reported to be able to induce a proinflammatory phenotype in circulating monocytes [51] which could contribute to processes of remodeling in the left atrium of patients with AF [52] as well. Therefore, new treatment options for reducing the level of MPAs seem to be promising. However, whether MPAs contribute to the recurrence of AF and therefore be more than bystanders, could not be answered in our study and remains to be elucidated.
Study limitations

The present study has two main limitations. First, the size of the study group was limited, especially the number of patients with documented recurrence of AF. Although the results of power calculation indicate that the number of patients was sufficient to detect differences between the two groups, the present study has to be considered as hypothesis-generating and needs to be confirmed in further studies with larger sample sizes, powered high enough for clinical outcome.

Second, the parameters for each patient were acquired at a single time point. Therefore, we cannot rule out variations of markers over time. This question warrants further investigation. Although the majority of patients had continuous rhythm monitoring, in patients who were assessed by seven-day ECG, silent episodes of AF may have been missed.

Conclusion

Among the examined markers of platelet activation, only the content of MPAs was increased in dependence on detected recurrent episodes of AF within 6 months after PVI. Whether the content of MPAs could achieve clinical relevance as a marker for successful PVI or as a decision-making aid to stop therapy with anticoagulants remains to be proven in further studies.

Acknowledgments

Peggy Barthel is highly acknowledged for expert technical assistance.

Declaration of interest

All authors state that they have no interests that could be perceived as posing a conflict or bias. None of the authors have conflicts of interest to disclose.

Funding

C.P. was supported by the Deutsche Herzstiftung.

References

1. Heppell RM, Berkin KE, McLenachan JM, Davies JA. Haemostatic and haemodynamic abnormalities associated with left atrial thrombosis in non-rheumatic atrial fibrillation. Heart 1997;77:407–411.
2. Hu YF, Chen YJ, Lin YJ, Chen SA. Inflammation and the pathogenesis of atrial fibrillation. Nat Rev Cardiol 2015;12(4):230–243.
3. Cai H, Li Z, Goette A, Mera F, Honeycutt C, Feterik K, Wilcox JN, Dudley SC, Jr., Harrison DG, Langberg JJ. Downregulation of endocardial nitric oxide synthase expression and nitric oxide production in atrial fibrillation: Potential mechanisms for atrial thrombosis and stroke. Circulation 2002;106:2854–2858.
4. Nakamura Y, Nakamura K, Fukushima-Kusano K, Ohta K, Matsuura H, Hamuro T, Yutani C, Ohe T. Tissue factor expression in atrial endothelium associated with nonvalvular atrial fibrillation: Possible involvement in intracardiac thrombogenesis. Thromb Res 2003;111:137–142.
5. Alonso A, Tang W, Agarwal SK, Soliman EZ, Chamberlain AM, Folsom AR. Hemostatic markers are associated with the risk and prognosis of atrial fibrillation: The ARIC study. Int J Cardiol 2012;155:217–222.
6. Drabik L, Wolkow P, Undas A. 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 2015;136:408–414.
7. Lip GY, Patel JV, Hughes E, Hart RG. High-sensitivity C-reactive protein and soluble CD40 ligand as indices of inflammation and platelet activation in 880 patients with nonvalvular atrial fibrillation: Relationship to stroke risk factors, stroke risk stratification schema, and prognosis. Stroke 2007;38:1229–1237.
8. Karasoy D, Gislaason GH, Hansen J, Johannessen A, Kober L, Hvidtfield M, Ozcan C, Torp-Pedersen C, Hansen ML. Oral anticoagulation therapy after radiofrequency ablation of atrial fibrillation and the risk of thromboembolism and serious bleeding: Long-term follow-up in nationwide cohort of Denmark. Eur Heart J 2015;36:307–144.
9. Lip GY, Lip PL, Zarifis J, Watson RD, Bareford D, Lowe GD, Beevers DG. Fibrinogen, D-dimer and beta-thromboglobulin as markers of thrombogenesis and platelet activation in atrial fibrillation. Effects of introducing ultra-low-dose warfarin and aspirin. Circulation 1996;94:425–431.
10. Atalar E, Haznedaroglu IC, Acil T, Ozer N, Kilic H, Ovunc K, Aksoyek S, Nazli N, Kes S, Kabakci G, et al. Patients with paroxysmal atrial fibrillation but not paroxysmal supraventricular tachycardia display evidence of platelet activation during arrhythmia. Platelets 2003;14:407–411.
11. Erdem K, Ayhan S, Ozurtk S, Bugra O, Bozoglan O, Dursin H, Yazici M, Daglar B. Usefulness of the mean platelet volume for predicting new-onset atrial fibrillation after isolated coronary artery bypass grafting. Platelets 2014;25:23–26.
12. Choudhury A, Chung I, Blann AD, Lip GY. 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 2007;49:1957–1964.
13. Blann AD, Choudhury A, Freestone B, Patel J, Lip GY. Soluble CD40 ligand and atrial fibrillation: Relationship to platelet activation, and endothelial damage/disfunction. Int J Cardiol 2008;127:135–137.
14. Azzam H, Abousamra NK, Wafa AA, Hafez MM, El-Gilany AH. Upregulation of CD40/CD40L system in rheumatic mitral stenosis with or without atrial fibrillation. Platelets 2013;24:516–520.
15. Choudhury A, Chung I, Panja N, Patel J, Lip GY. Soluble CD40 ligand, platelet surface CD40 ligand, and total platelet CD40 ligand in atrial fibrillation: Relationship to soluble P-selectin, stroke risk factors, and risk factor intervention. Chest 2008;134:574–581.
16. Lim HS, Schultz C, Dang J, Alasades M, Lau DH, Brooks AG, Wong CX, Roberts-Thomson KC, Young GD, Worthley MI, et al. Time course of inflammation, myocardial injury, and prothrombotic response after radiofrequency catheter ablation for atrial fibrillation. Circ Arrhythm Electrophysiol 2014;7:83–89.
17. Elgueta R, Benson MJ, de Vries VC, Wasuiak A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev 2009;229:152–172.
18. Heeschchen C, Dimmelner S, Hamm CW, van den Brand MJ, Boersma E, Zeiher AM, Simoons ML. Soluble CD40 ligand in acute coronary syndromes. N Engl J Med. 2003;348:1104–1111.
19. Anand SX, Viles-Gonzalez JF, Badimon JJ, Casuvsoglu E, Marmur JD. Membrane-associated CD40L and sCD40L in atherothrombosis disease. Thromb Res 2003;90:377–384.
20. Aloui C, Prigent A, Sut C, Tariket S, Hamzeh-Cognasse H, Pozzetto B, Richard Y, Cognasse F, Laradi S, and Garraud O. The signaling role of CD40 ligand in platelet biology and in platelet component transduction. Int J Mol Sci 2014;15:22342–22364.
21. Burdess A, Michelsen AE, Brostad F, Fox KA, Newby DE, Nimmo AF. Platelet activation in patients with peripheral vascular disease: Reproducibility and comparability of platelet markers. Thromb Res 2012;129:50–55.
22. Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of in vivo platelet activation than platelet surface P-selectin: Studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation 2001;104:1533–1537.
23. Wirgley BJ, Shantsila E, Tapp LD, Lip GY. Increased formation of monocyte-platelet aggregates in ischemic heart failure. Circ Heart Fail 2015;8:127–135.
24. Lukasik M, Dworacki G, Kufel-Grabowska J, Watala C, Kozubski W. Upregulation of CD40 ligand and enhanced monocyte-platelet aggregate formation are associated with worse clinical outcome after ischaemic stroke. Thromb Haemost 2012;107:346–355.
25. Pfuecke C, Berndt K, Wydra S, Tarnowski D, Barthel P, Quick S, Ulbrich C, Christoph M, Waessing N, Speiser U, et al. Atrial fibrillation is associated with high levels of monocyte-platelet-aggregates and increased CD11b expression in patients with aortic stenosis. Thromb Haemost 2016;115(5):993–1000.
26. Pfuecke C, Tarnowski D, Plichta L, Berndt K, Schumacher P, Ulbrich S, Ferkmann M, Christoph M, Poitz DM, Wunderlich C, et al. Monocyte-platelet aggregates and CD11b expression as markers for thrombogenicity in atrial fibrillation. Clin Res Cardiol 2015;4:314–322.
MPAs are associated with AF recurrence

27. Morgan E, Varro R, Sepulveda H, Ember JA, Apgar J, Wilson J, Lowe L, Chen R, Shivraj L, Agadir A, et al. Cytometric bead array: A multiplexed assay platform with applications in various areas of biology. Clin Immunol 2004;110:252–266.

28. Hayashi M, Takeshita K, Inden Y, Ishii H, Cheng XW, Yamamoto K, Murohara T. Platelet activation and induction of tissue factor in acute and chronic atrial fibrillation: Involvement of mononuclear cell-platelet interaction. Thromb Res 2011;128:e113–e118.

29. Kopjar T, Petricic M, Gasparovic H, Svetina L, Milicic D, Biocina B. Postoperative atrial fibrillation is associated with high on-aspirin platelet reactivity. Ann Thorac Surg 2015;100:1704–1711.

30. Makowski M, Smorag I, Bissinger A, Grycewicz T, Masiarek K, Makowska J, Grabowicz W, Lubinski A, Baj Z. Effect of sinus rhythm restoration on platelet function in patients with lone atrial fibrillation. Int J Cardiol 2014;172:e22–e23.

31. Lim HS, Willoughby SR, Schultz C, Chakrabarty A, Alasady M, Lau DH, Roberts-Thomson KC, Worthley MI, Young GD, Sanders P. Successful catheter ablation decreases platelet activation and improves endothelial function in patients with atrial fibrillation. Heart Rhythm 2014;11:1912–1918.

32. Mahe I, Drouet L, Chassany O, Mazoyer E, Simonneau G, Knellwolf AL, Caulin C, Bergmann JF. D-dimer: a characteristic of the coagulation state of each patient with chronic atrial fibrillation. Thromb Res 2002;107:1–6.

33. Morris AE, Remmele RL, Jr., Klinke R, Macluff BM, Fanslow WC, Armitage RJ. Incorporation of an isoleucine zipper motif enhances the biological activity of soluble CD40L (CD154). J Biol Chem 1999;274:418–423.

34. Andre P, Nannizzi-Alaimo L, Prasad SK, Phillips DR. Platelet-specific markers are associated with monocyte-platelet aggregates after valve replacement for aortic stenosis: Relation to p-selectin and antithrombotic therapy. Chest 2007;131:809–815.

35. Schonbeck U, Libby P. The role of monocytes in thrombotic disorders. Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms. Thromb Haemost. 2009;102:916–924.

36. Conway DS, Buggins P, Hughes E, Lip GY. Relationship of interleukin-6 and C-reactive protein to the prothrombotic state in chronic atrial fibrillation. Circulation 2002;106:896

37. Badr ER, Gremmel T, Schneller A, Stegfellner M, Kaider A, Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman AL, Caulin C, Bergmann JF. D-dimer: a characteristic of the coagulation state of each patient with chronic atrial fibrillation. Thromb Res 2002;107:1–6.

38. Mahe I, Drouet L, Chassany O, Mazoyer E, Simonneau G, Knellwolf AL, Caulin C, Bergmann JF. D-dimer: a characteristic of the coagulation state of each patient with chronic atrial fibrillation. Thromb Res 2002;107:1–6.

39. Gremmel T, Ay C, Riedl J, Eichelberger B, Koppensteiner R, Panzer S. Platelet-specific markers are associated with monocyte-platelet aggregate formation and thrombin generation potential in advanced atherosclerosis. Thromb Haemost 2015;115(3):615–621.

40. Choudhury A, Chung I, Blann AD, Lip GY. Elevated platelet microparticle levels in nonvalvular atrial fibrillation: Relationship to p-selectin and antithrombotic therapy. Chest 2007;131:809–815.

41. Smyth SS, Monroe DM, III, Wysokinski WE, McBane RD, Whiteheart SW, Becker RC, Steinhubl SR. Platelet activation and its patient-specific consequences. Thromb Res 2008;122:435–441.

42. Shantsila E, Lip GY. The role of monocytes in thrombotic disorders. Insights from tissue factor, monocyte-platelet aggregates and novel mechanisms. Thromb Haemost. 2009;102:916–924.

43. Kopp CW, Gremmel T, Steiner S, Seidinger D, Minar E, Maurer G, Huber K. Platelet-monocyte cross talk and tissue factor expression in stable angina vs. unstable angina/non-ST-elevation myocardial infarction. Platelets 2011;22:530–536.

44. Singh MV, Davidson DC, Kiebala M, Maggirwar SB. Detection of circulating platelet-monocyte complexes in persons infected with human immunodeficiency virus type-1. J Virol Methods 2012;181:170–176.

45. Lip GY, Frison L, Halperin JL, Lane DA. Identifying patients at high risk for stroke despite anticoagulation: A comparison of contemporary stroke risk stratification schemes in an anticoagulated atrial fibrillation cohort. Stroke 2010;41:2731–2738.

46. Evangelista V, Manarini S, Sideri R, Rotondo S, Martelli N, Piccoli A, Totani L, Piccardoni P, Vestweber D, de GG, Cerletti C. Platelet/polymerized ATC leukocyte interaction: P-selectin triggers protein-tyrosine phosphorylation-dependent CD11b/CD18 adhesion: Role of PSGL-1 as a signaling molecule. Blood 1999;93:876–885.

47. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. J Clin Invest 1995;95:2297–2303.

48. Wu Q, Ren J, Hu D, Wu X, Li G, Wang G, Gu G, Chen J, Li R, Li Y, et al. Monocyte subsets and monocyte-platelet aggregates: implications in predicting septic mortality among surgical critical illness patients. Biomarkers 2016;21(6):509–516.

49. Dopheide JF, Rubrech J, Trumpp A, Geissler P, Zeller GC, Bock K, Dunschede F, Trinh TT, Dorweiler B, Munzel T, et al. Leukocyte-platelet aggregates: A phenotypic characterization of different stages of peripheral arterial disease. Platelets 2016; [epub ahead of print].

50. Sarma J, Laan CA, Alam S, Jha A, Fox KA, Dransfield I. Increased platelet binding to circulating monocytes in acute coronary syndromes. Circulation 2002;105:2166–2171.

51. Passacquale G, Vamaev I, Pereira L, Hamid C, Corrigall V, Ferro A. Monocyte-platelet interaction induces a pro-inflammatory phenotype in circulating monocytes. PLoS One 2011;6:e25595.

52. Suzuki A, Fukuzawa K, Yamashita T, Yoshida A, Sasaki N, Emoto T, Takei A, Fujiwara R, Nakanishi T, Yamashita S, et al. Circulating intermediate CD44++CD16+ monocytes are increased in patients with atrial fibrillation and reflect the functional remodelling of the left atrium. Europace 2016 doi:10.1093/europace/euv432

53. Christersson C, Johnell M, Siegbahn A. The influence of direct thrombin inhibitors on the formation of platelet-leukocyte aggregates and tissue factor expression. Thromb Res 2010;126:e327–e333.