Atrial Fibrillation: Biophysics, Molecular Mechanisms, and Novel Therapies

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Editorial

Atrial Fibrillation: Biophysics, Molecular Mechanisms, and Novel Therapies

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Atrial fibrillation (AF) affects millions of people worldwide and is associated with increased morbidity and mortality [1]. The incidence of AF is expected to rise with aging of the population. Research over the past decades has identified a multitude of pathophysiological processes contributing to the initiation, maintenance, and progression of AF. Therefore, a comprehensive understanding of AF pathophysiology is needed to foster the development of improved diagnostic, pharmacological, and nonpharmacological therapeutic approaches to improve clinical management. The focus of this special issue of this journal is to capture most recent advances in the study of AF with the aim of directing further research.

Conventional mechanisms linked to AF are diverse and expertly reviewed in various manuscripts [2, 3]. However, progression in the field of AF research may come from an unconventional view-angle. For example, M. Miragoli and A. V. Glukhov reviewed the role of myofibroblasts as novel targets for cardiac arrhythmias with the aim of describing and evaluating the implications of noncardiomyocyte view in the context of AF. B. Weil and C. Ozcan discussed the pathophysiological remodelling in AF in comparison with that occurring in hibernating myocardium, attempting to identify common molecular mechanisms and proposing possible future therapeutic implications of this emerging paradigm.

In addition, the issue also contains the study of F. Cacciani and M. Zaniboni who used a computational approach to study the source-sink relationship between the sinoatrial node and surrounding atrium in control conditions and under different simulated chronotropic interventions. The authors defined a safety-factor for pacemaker-to-atrial action potential conduction and tested the effects of different antiarrhythmic-like interventions. Analytical study of C. Loardi et al. found that there is an association between postoperative left atrium dimensions and “kick” restoring and an influence of right atrium contraction on left ventricle function in patients that underwent radiofrequency (Maze) procedure. Their study also highlights the deleterious influence of arrhythmia “chronic state” on procedural success and importance of the cardiac right side, an entity somehow overlooked.

The initial presentation of undiagnosed AF is often a stroke. Therefore, methods for early detection of AF are highly demanded. In this special issue, M. D. Zink et al. investigated whether heart beat cycle length measurement assessed by a novel algorithm for a ballistocardiography
(BCG) foil is feasible during sinus rhythm and AF. The latter might offer new possibilities for noninvasive heart rate and thereby AF monitoring. Another approach to detect AF would be the use of biomarkers. P. J. Howlett et al. summarized the available evidence for electrophysiological, molecular, and morphological biomarkers to improve the detection of paroxysmal AF with reference to the underlying mechanisms for the conduction. F.-C. Su et al. performed a meta-analysis to investigate whether serum level of high-sensitivity C-reactive protein (hs-CRP), a protein produced by the liver during infection, tissue injury, and chronic inflammation, can predict the efficacy of AF treatment with atorvastatin.

The coagulative state in patients with chronic AF is higher than that in patients with sinus rhythm [4]. As a result, the incidence of thromboembolism is significantly higher in patients with AF [5] which makes the latter an important risk factor of cerebral embolism. In this issue, F. Shamoun et al. reviewed the new generation of anticoagulants, along with their efficacy and safety data. Their study suggests that the novel oral anticoagulants indeed can reduce the relative incidence of stroke in patients with AF. Similarly, A. Panisello-Tafalla et al. examined the effectiveness of the use of oral anticoagulation medication, recommended by national guidelines for stroke preventions. They suggest that the low efficiency of oral anticoagulants results from the association between their underuse and undiagnosed AF. The treatment of platelet aggregation in patients with AF requires a specific approach as highlighted by A. M. Martischnig et al. who found that dabigatran as compared to phenprocoumon had no impact on adenosine diphosphate (ADP) induced platelet aggregation in AF patients neither with nor without concomitant clopidogrel therapy.

The extensive work reviewed in this special issue highlights the enormous advances achieved in understanding AF pathophysiology over the past 20 years and suggests an importance of the mechanism-based approaches for therapeutic breakthroughs. We believe that the future of AF management will be determined by the application of new scientific insights into the underlying mechanistic determinants.

Acknowledgment

As the guest editors of this special issue we would like to acknowledge all authors and reviewers who contributed either by reviewing recent literature or by including original experimental and clinical studies, making this issue valuable for diverse audience of researchers interested in the AF pathophysiology and therapy.

References

Novel Anticoagulants in Atrial Fibrillation: Monitoring, Reversal and Perioperative Management

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Atrial fibrillation (AF) is one of the most common tachyarrhythmias in clinical practice. It accounts for about 35% of hospital admissions from cardiac arrhythmias. AF prevalence is projected to increase from 5.2 million in 2010 to 12.1 million cases in 2030 [1]. AF increases the risk of stroke 4-5-fold, independent of other cardiac or noncardiac morbidities [2]. At least 15–20% of all ischemic strokes are due to AF. Also, AF is an independent risk factor for stroke recurrence [3]. Penado et al. showed that the hazard ratio for recurrent stroke among those with AF who were not treated with anticoagulants was 2.1 (95% confidence interval (CI): 1.4 to 2.9; P < 0.001), whereas the hazard ratio for recurrent severe stroke was 2.4 (95% CI: 1.6 to 3.6; P < 0.001) [3].

2. Warfarin

Warfarin has been the most common medication used for anticoagulation [4]. It has established its effectiveness in preventing thromboembolic events in patients with AF. At least 1% of the population in the United Kingdom is taking warfarin, as well as 8% of those aged over 80 years.

Warfarin use is associated with many undesired side effects that could significantly affect patients’ well-being. The challenges associated with warfarin therapy often outweigh its benefits [5].

A study by Birman-Deych et al. shows that about one-third of AF patients who are ideal candidates for warfarin therapy are not offered the treatment [6]. That is especially true for the black and Hispanic population. Another study by Hylek et al. published in 2007 shows that 26% of patients 80 years of age or older stop taking warfarin within 1 year of treatment despite ongoing indication [7]; 81% of those patients stopped warfarin due to safety concerns.

A study to assess the prevalence of hospital admissions due to adverse drug reactions in the adult population concluded that warfarin is the leading drug causing these hospitalizations with a rate of 33.3% of all admissions due to adverse drug events [8].

Of all types of bleeding associated with warfarin therapy, intracranial hemorrhage (ICH) is the most significant [9]. ICH is mainly responsible for the majority of deaths and disabilities caused by warfarin-related bleeding.

Monitoring of warfarin is easily achievable by testing prothrombin time (PT) and measuring the international
normalized ratio (INR) values. The target INR that is required in AF patients is between 2 and 3. Home monitoring of INR has proven to reduce the risk of thromboembolism, bleeding event, and death [10].

The major side effect of warfarin is bleeding; the risk of bleeding increases when the INR is higher than 3. When INR is within therapeutic range and an elective surgery is needed, warfarin should be stopped for at least 5 days. For patients who are bleeding or require rapid reversal of warfarin due to serious bleeding or emergency surgery, vitamin K should be given at 10 mg with prothrombin complex concentrate (PCC) (25–50 IU/kg) or fresh frozen plasma (15–30 mL/kg) [11].

3. Aspirin versus Warfarin

Since the risk of bleeding increases with age, some have suggested that using aspirin in elderly patients could be a suitable alternative to warfarin; however, the Birmingham Atrial Fibrillation Treatment of the Aged (BAFTA) study confirmed that aspirin was associated with the same rate of bleeding events (1.9% versus 2.0% risks per year; 0.97 relative risk (RR), 95% CI: 0.53–1.75), and worse primary outcomes, ICH, arterial embolism, or stroke (yearly risk 1.8% in the aspirin group versus 3.8% in the aspirin group, RR 0.48, 95% CI: 0.28–0.80, P = 0.003) [12].

4. Novel Oral Anticoagulants (NOACs)

The novel oral anticoagulants (NOACs) appear to be a good alternative to traditional anticoagulation with vitamin K antagonists (VKAs). They have better oral bioavailability with less food and drug interactions. They do not require frequent INR monitoring and seem to be well tolerated in the long-term use. A systematic review and meta-analysis of 5 phase 3 clinical trials that studied the efficacy and safety of NOACs was published in 2012 [13]. It compared warfarin to NOACs in AF patients. NOAC use was associated with decreased stroke and systemic embolism (RR: 0.82; 95% CI: 0.69–0.98; P = 0.03) as well as all-cause mortality (RR: 0.91; 95% CI: 0.85–0.96; P = 0.0026) compared with warfarin. The analysis showed better safety outcomes associated with NOACs; the RR of major bleeding was 0.83 (95% CI: 0.69–1.002; P = 0.055). Also, the RR of hemorrhagic stroke was significantly low (RR: 0.51; 95% CI: 0.41–0.64; P ≤ 0.001).

The 4 medications that are currently available in the market are dabigatran (a direct thrombin inhibitor), rivaroxaban, edoxaban, and apixaban (factor Xa inhibitors). All of these agents (except edoxaban) are approved in the United States, the European Union, and Canada for the indication of nonvalvular AF. Currently, edoxaban is under evaluation. Rivaroxaban is the most commonly prescribed NOAC in the United States.

There are still many questions about the NOACs that require more randomized data relating to perioperative use, particularly relating to cessation of anticoagulant therapy in surgical patients who need emergent procedures. A systematic review by Harel et al. examined the efficacy and safety of NOACs compared to VKAs by studying the data from 8 randomized controlled trials that included patients with AF or venous thromboembolism, and associated chronic kidney disease (creatinine clearance (CrCl) = 30–50 mL/min) [14]. It is concluded that there was no major difference in the primary efficacy outcomes or the primary safety outcomes with NOACs compared to VKAs; however, dose adjustments in renal failure as well as choice of optimal NOAC in this high-risk group remain important clinical questions.

The combination of antiplatelet agents, specifically the use of dual antiplatelet agents, remains an important question. At this point, there are no clear guidelines to help understand the risk of bleeding in patients who require the combination of antiplatelet agents.

Finally, it is important to note that with the newer anticoagulants, we are seeing different types of bleeding with less retroperitoneal and ICHs compared to warfarin. That is believed to be due to abundance of factor VII on the blood-brain barrier that is affected by warfarin and not the newer anticoagulants [16].

5. Dabigatran

Dabigatran is the only direct thrombin inhibitor currently approved for stroke prevention in patients with nonvalvular AF. The doses approved in the United States are 150 mg or 75 mg twice daily; 80% is renally cleared [17]. The 75 mg dosage is indicated when the patients have poor renal function (CrCl 15–30 mL/min) or are on P-glycoprotein inhibitors with poor CrCl 30–50 mL/min. Dabigatran is contraindicated when CrCl is <15 mL/min.

The RE-LY trial compared warfarin and dabigatran for the prevention of stroke and systemic embolism in AF patients [18]. Patients were divided into 3 groups. The first group received warfarin; the second received dabigatran 110 mg; and the third received dabigatran 150 mg. Low-dose dabigatran was found to be noninferior to warfarin (RR 0.91; 95% CI: 0.74 to 1.11; P < 0.001). High-dose dabigatran was superior to warfarin (RR 0.66; 95% CI: 0.53 to 0.82; P < 0.001). Major bleeding was lower in the 110 mg group (P = 0.003) and similar to warfarin in the 150 mg group (P = 0.31).

5.1. Dabigatran Monitoring. Although there is no need to monitor dabigatran routinely when given to AF patients, there are certain clinical settings when monitoring becomes a necessity [19], such as in the setting of urgent surgery, where elevated dabigatran plasma levels can raise the risk of bleeding. Supratherapeutic levels in patients who are experiencing adverse effects due to decreased clearance of dabigatran (possibly because of deteriorating renal function) are a real concern.

Dabigatran prolongs the activated partial thromboplastin time (aPTT) more than the PT (it affects the intrinsic
coagulation pathway more than the extrinsic coagulation pathway) [20]. A recent study comparing different tests for monitoring dabigatran levels in patients with AF found a strong correlation between the total and free dabigatran plasma levels measured by liquid chromatography-tandem mass-spectrometry (LC-MS/MS) and indirect measurements by Hemoclot Thrombin Inhibitor (HTI) and ecarin clotting time (ECT) assays (\(P < 0.001\)) [21]. This correlation suggests that HTI and ECT assays are highly sensitive for the assessment of dabigatran activity when compared to standard coagulation tests (aPTT, PT).

In another study by Hapgood et al., investigators measured the dabigatran concentrations by the Hemoclot assay and correlated the results with aPTT and thrombin time (TT) [22]. They found that TT was very sensitive to the presence of the drug and that aPTT is useful as a qualitative test (to determine whether dabigatran is having an anticoagulant effect in the patient), but both TT and aPTT had only moderate correlation with the drug levels. This could be useful in preparing patients for surgery in settings where HTI or ECT assays are not available. The study recommended measuring aPTT and TT before elective surgery in patients taking dabigatran.

5.2. Dabigatran Reversal. The half-life of dabigatran is between 12 and 14 hours [20] and up to 18 hours when the CrCl drops to 30–50 mL/min and 27 hours when the CrCl is less than 30 mL/min. In patients with normal renal function, the steady-state trough level should be diminished by 75% after discontinuation of dabigatran for 24 hours. Therefore, stopping dabigatran administration is simply enough for most of the cases that require reversal of its effect. Patients in the perioperative period are recommended to stop dabigatran at least 24 hours prior to low-risk surgery if kidney function is normal, and at least 48 hours before surgeries with a high risk of bleeding [23]. If the CrCl is 31 to 50 mL/min, the last dose should be at least 48 hours before the procedure for low-risk surgery and 4 days before a procedure that poses a high risk of bleeding [20]. If the CrCl is less than 30 mL/min, dabigatran should be permanently discontinued, and any surgical intervention should be deferred for at least 5 days.

In the case of an emergent surgical procedure or severe bleeding, stopping the drug may not be sufficient. Limited data and studies are available to identify the best reversal method. Transfusion of packed red blood cells, fresh frozen plasma, and surgical interventions to stop the bleeding are suggested as a supportive therapy. Administration of activated charcoal could be useful to inhibit the absorption of dabigatran from the gastrointestinal tract if a recent ingestion has been reported. Dabigatran can also be dialyzed in patients with renal impairment. A study that enrolled 23 patients with different stages of renal impairment investigated the fraction of dabigatran that could be eliminated from the blood after hemodialysis [24]. It concluded that hemodialysis removed 62% of dabigatran after 2 hours and 68% after 4 hours.

Non-specific therapies (activated factor VIIa or PCC) can also be considered. A randomized controlled study by Eerenberg et al. compared the effect of nonactivated PCC versus saline to reverse the anticoagulation of either dabigatran or rivaroxaban in 12 healthy subjects [25]. In this trial, dabigatran was administered at a dose of 150 mg, and it increased aPTT, ECT, and TT. This was followed by administration of a single bolus of 50 IU/kg PCC; the PCC failed to restore these coagulation tests to their normal value. The study concluded that PCC is not effective as an antidote for dabigatran; however, Bernstein et al. have noticed that the PCC administered in the previous study was not activated and proposed an activated form of PCC as an alternative to reverse the dabigatran effect [26]. This proposal was made based on a trial by van Ryn et al. [27], which concluded that FEIBA (which is an activated PCC) reversed the prolonged bleeding time in rats treated with dabigatran (Figure 1).

In May 2013, Schiele et al. reported for the first time a specific antidote for dabigatran [28]. They generated an antibody fragment (aDabi-Fab (idarucizumab)) that could
bind to the dabigatran molecule and reverse its effect in vitro and in vivo. aDabi-Fab mimics the thrombin molecule and is able to bind to dabigatran with an affinity that is 350 times more than the affinity of dabigatran to thrombin, but it has no functional thrombin mimicking activity, and it does not induce coagulation. Schiele et al. infused rats with dabigatran until they reached a 4-fold prolongation of TT and 2-fold prolongation of aPTT. They found that a single bolus injection of aDabi-Fab was able to restore TT and aPTT to normal within 1 minute. In April 2014, another trial on pigs showed that aDabi-Fab was able to reverse the effect of dabigatran even when it was given in supratherapeutic levels and when severe bleeding was induced by trauma [29].

Van Ryn et al. presented a study on 35 healthy volunteers which showed that dabigatran inhibited the fibrin formation after a small scratch, and idarucizumab was able to completely reverse this effect and restored fibrin formation [30]. Idarucizumab is currently investigated in real life bleeding events in patients who are receiving dabigatran. This study (RE-VERSE AD) is going to take place in 35 different countries including the United States.

PER977 is another synthetic small molecule under development that has shown to reverse dabigatran as well as other NOACs ex vivo in human blood and decreased bleeding in a standard rat tail bleeding model [31]. This new antidote is currently undergoing more clinical trials.

6. Rivaroxaban

Rivaroxaban is the first direct factor Xa inhibitor. It is dosed once daily; 40% is excreted through the kidney and the remaining one-third is metabolized in the liver and excreted in the feces. The recommended dose is 20 mg once a day for patients with CrCl >50 mL/min and 15 mg once daily for those with CrCl <50 mL/min. Rivaroxaban was noninferior to warfarin in the prevention of stroke and systemic embolism in ROCKET AF [32, 33], but it had better outcomes in terms of life-threatening bleeding events (ICH and fatal bleeding).

Even though the hepatic metabolism of rivaroxaban could help eliminate the drug in cases of renal failure, further studies should be conducted to make sure it is effective and safe in this patient population. Thus, rivaroxaban is contraindicated in patients with CrCl <15 mL/min for the treatment of AF. It is also contraindicated in deep venous thrombosis and pulmonary embolism prophylaxis when with the CrCl <30 mL/min. Its use should be avoided in patients with moderate or severe hepatic impairment (Child-Pugh B and Child-Pugh C, resp.) [34].

6.1. Rivaroxaban Monitoring. As with dabigatran, rivaroxaban does not require monitoring except in certain circumstances. The PT has a linear correlation with rivaroxaban concentrations in the plasma [35]; however, PT results may vary with different reagents. For example, when using Neoplastin Plus (a thromboplastin reagent), PT doubles when rivaroxaban concentration is 301 \( \mu g/L \). When using Innovin (a different reagent), PT doubles when rivaroxaban concentration is 700 \( \mu g/L \). This result variation is mainly caused by different sensitivities of these reagents to rivaroxaban. This variation cannot be fixed by conversion of PT to INR, therefore, INR should not be used to evaluate rivaroxaban activity. A normal PT indicates no rivaroxaban activity [35].

Samama et al. proposed that anti-Factor Xa chromogenic assays are the best way for the estimation of rivaroxaban concentrations (when using standard calibration curves generated with the use of rivaroxaban calibrators and controls) [35]; however, there are 2 points that should be considered when using the chromogenic assays. The first one is that the assay measures the drug concentration in the plasma, not its activity, which means that a high level in the plasma does not necessarily indicate higher activity and, therefore, a higher risk of bleeding. The second point is that the results will be different depending on the time of blood sampling after rivaroxaban administration. For example, the plasma level of rivaroxaban will be higher after 2–4 hours of intake when compared to after 12 hours of intake. This should be considered when evaluating the treatment with rivaroxaban.

6.2. Rivaroxaban Reversal. Rivaroxaban has a half-life of 7–11 hours in patients with normal kidney function [24]. If an elective surgery is planned, rivaroxaban should be stopped for at least 24 hours before low-risk surgery or 48 hours before high-risk surgery. It can be resumed after 6–10 hours if the patient has normal kidney function (CrCl >30 mL/min) and hemostasis has been achieved. If CrCl is below 30 mL/min, it should be stopped 2 days prior to low-risk surgery and 4 days prior to high-risk surgery. It is important to note that, unlike dabigatran, rivaroxaban cannot be diazayed due to the high plasma protein binding capacity of this medication (95% is bound to plasma proteins).

The previously mentioned study by Bernstein et al. that evaluated Co-fact© (a nonactivated PCC) for the reversal of anticoagulation showed that rivaroxaban effect (prolongation of PT) was immediately and completely reversed by PCC (PT came back to normal) [26]. The endogenous thrombin potential was inhibited by rivaroxaban and normalized after PCC bolus as well. Since this study was performed on healthy individuals, more studies should be done to confirm the benefit of PCC in real-life bleeding situations.

The recombinant protein, PRT064445, was suggested by Lu et al. as a specific antidote for all direct and indirect factor Xa inhibitors [36]. It has the ability to reverse the effect of rivaroxaban in rabbits, by binding to the free factor Xa inhibitor concentration in plasma, and, therefore, decreasing its activity. It also succeeded in the management of blood loss induced in rats after administration of enoxaparin and fondaparinux.

A recent phase 2 study was designed to test another agent, andexanet alfa (PRT4445) [37]. The study reported that this antidote was able to dose-dependently reverse the effect of rivaroxaban in healthy volunteers. Also, it was well tolerated and did not cause any significant adverse effects. Andexanet alfa is now being studied in a phase 3 clinical trial known as
ANNEXA-R to evaluate its efficacy and safety in reversing rivaroxaban [38].

7. Apixaban

Apixaban is the second direct factor Xa inhibitor. It is dosed twice daily and mainly excreted through the liver. The dose is 5 mg twice daily and could be reduced to 2.5 mg twice daily if patients meet 2 of 3 criteria: age 80 years, body weight 60 kg, or serum creatinine level 1.5 mg/dL.

Apixaban is superior to aspirin in the phase 3 AVERROES clinical trial [39]. It reduced significantly the stroke and pulmonary embolism events (hazard ratio with apixaban, 0.45; 95% CI: 0.32 to 0.62; \( P < 0.001 \)). The risk of major bleeding appeared to be similar compared to aspirin in that trial (hazard ratio with apixaban, 1.13; 95% CI: 0.74 to 1.75; \( P = 0.57 \)). Another phase 3 clinical trial, ARISTOTLE, showed apixaban to be superior to warfarin in prevention of stroke and systemic embolism in patients with AF (hazard ratio with apixaban, 0.79; 95% CI: 0.66 to 0.95; \( P < 0.001 \) for noninferiority; \( P = 0.01 \) for superiority) [40]. The risk of major bleeding was also lower in the apixaban group compared to the warfarin group (hazard ratio, 0.69; 95% CI: 0.60 to 0.80; \( P < 0.001 \)).

Thus far, apixaban appears to be probably the safest option in case of chronic kidney disease [41]. Hohnloser et al. [42] evaluated the outcomes of the ARISTOTLE trial in relation to renal function. They concluded that apixaban reduced the rate of stroke, death, and major bleeding, when compared to warfarin, regardless of renal function. Patients with estimated glomerular filtration rate of \( \leq 50 \) mL/min (as determined by the Cockcroft–Gault and Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] equations) seemed to have greater relative risk reduction in major bleeding with apixaban (hazard ratio 0.50 (95% confidence interval: 0.38–0.66), \( P = 0.005 \)). There are still limited data that compare NOACs to each other in specific populations such as patients with renal failure. For now, what we know is that apixaban is certainly a very promising anticoagulation treatment for this population.

PT, INR, and aPTT tests are not ideal to monitor apixaban; however, a normal PT value indicated no activity of apixaban and can be useful when other tests are not available. Becker et al. proved that there is a strong linear correlation between apixaban plasma concentration and apixaban activity when measured using a standard laboratory chromogenic anti-Xa assay with either low molecular weight heparin or apixaban calibrators [43]. Hence, measurement of apixaban antifactor Xa activity using chromogenic laboratory assays appears to be the most accurate method.

Just like rivaroxaban, apixaban should be stopped for 24 hours at least before low-risk surgery or 48 hours before high-risk surgery when CrCl is \( > 30 \) mL/min [24]. If CrCl is below 30 mL/min, it should be stopped 2 days prior to low-risk surgery and 4 days prior to high-risk surgery. Apixaban cannot be dialyzed due to the high plasma protein binding capacity of this medication (87% is bound to plasma proteins).

A recent phase 2 study was designed to test the new specific antidote for the factor Xa inhibitors, andexanet alfa (PRT4445) for apixaban reversal [44]. The study administered 5 mg of apixaban to 54 healthy volunteers for 6 days. Then, the volunteers were given intravenous andexanet. The effect of apixaban was reversed within 2 minutes after the administration of the new antidote, by decreasing the concentration of the unbound apixaban in plasma. Currently, a phase 3 clinical trial is ongoing to study the efficacy and safety of andexanet alfa to reverse apixaban effect [38].

8. Edoxaban

Edoxaban is the third direct factor Xa inhibitor. It is dosed once daily and excreted through the liver. Edoxaban is not yet approved by the food and drug administration for the management of AF. One phase 3 clinical trial was conducted to evaluate its efficacy and safety as well as the best dosing regimen [45]. The study had 3 groups: the first group received warfarin; the second one received edoxaban 30 mg once daily; and the third group received edoxaban 60 mg once daily. High-dose edoxaban was noninferior to warfarin in the prevention of stroke and systemic embolism (HR 0.79; 97.5% CI: 0.63 to 0.99; \( P < 0.001 \)) but had a higher rate of major bleeding (HR 0.80; 95% CI: 0.71 to 0.91; \( P < 0.001 \)). The low-dose edoxaban was noninferior to warfarin as well (HR 1.07; 97.5% CI: 0.87 to 1.31; \( P = 0.005 \)) and had a lower rate of major bleeding (HR 0.47; 95% CI: 0.41 to 0.55; \( P < 0.001 \)).

Similar to other factor Xa inhibitors, chromogenic antifactor Xa assays can be used to measure the plasma concentrations of edoxaban when drug-specific calibrators are available [46]. A trial that evaluated the use of the reversal agent PER977 was published in November 2014 [47]. The study was on 80 healthy volunteers who were randomized into 8 cohorts (each cohort was assigned to a different dose of PER977 ranging from 5 to 300 mg). Eight persons in each cohort received PER799 intravenously, alone and after administration of edoxaban, and 2 persons in each cohort received placebo. The trial used whole-blood cloting time to measure the anticoagulant effect of edoxaban and its reversal by PER977. Whole-blood clotting time shows low variability and high reproducibility and correlates well with edoxaban plasma concentrations. The effect of edoxaban was successfully reversed and the whole-blood clotting time was restored to values close to baseline in those who received 100–300 mg of PER977 within 10–30 minutes (Table 1).

9. Conclusion

Dabigatran, rivaroxaban, and apixaban are effective and safe alternative to warfarin for the prevention of stroke and systemic emboli in patient with paroxysmal or permanent atrial fibrillation. NOACs have a wide therapeutic range with reasonable safety margin.

Dabigatran activity can be monitored using HTI and ECT assays. The best tests to monitor factor Xa inhibitors are antifactor Xa chromogenic assays when standard calibrators are available.
Table 1: Recommendation for NOACs cessation before elective procedure.

<table>
<thead>
<tr>
<th>Creatinine clearance</th>
<th>Low risk</th>
<th>High risk</th>
<th>Low risk</th>
<th>High risk</th>
<th>Low risk</th>
<th>High risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 80 mL/min</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
</tr>
<tr>
<td>50–80 mL/min</td>
<td>≥ 36 hours</td>
<td>≥ 72 hours</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
</tr>
<tr>
<td>30–50 mL/min</td>
<td>≥ 48 hours</td>
<td>≥ 96 hours</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
<td>≥ 24 hours</td>
<td>≥ 48 hours</td>
</tr>
<tr>
<td>15–30 mL/min</td>
<td>Not indicated</td>
<td>Not indicated</td>
<td>≥ 36 hours</td>
<td>≥ 48 hours</td>
<td>≥ 36 hours</td>
<td>≥ 48 hours</td>
</tr>
<tr>
<td>&lt; 15 mL/min</td>
<td>No official indication for use</td>
<td>No official indication for use</td>
<td>No official indication for use</td>
<td>No official indication for use</td>
<td>No official indication for use</td>
<td>No official indication for use</td>
</tr>
</tbody>
</table>

Table 2: Recommendations for monitoring and reversal of NOACs.

<table>
<thead>
<tr>
<th>NOAC</th>
<th>Trial name</th>
<th>Most accurate monitoring tests</th>
<th>Qualitative monitoring tests</th>
<th>Reversal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran</td>
<td>RE-LY</td>
<td>HTI</td>
<td>TT aPTT</td>
<td>(i) Activated charcoal (if a recent ingestion has been reported)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ECT</td>
<td></td>
<td>(ii) FEIBA (activated PCC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iii) Hemodialysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iv) aDabi-Fab [idarucizumab]*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(v) PER977*</td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>ROCKET AF</td>
<td>Antifactor Xa chromogenic assays</td>
<td>PT</td>
<td>(i) Recombinant activated factor VII (rFVIIa)</td>
</tr>
<tr>
<td></td>
<td>ARISTOTLE</td>
<td></td>
<td></td>
<td>(ii) Co-fact® (a nonactivated PCC)</td>
</tr>
<tr>
<td></td>
<td>ENGAGE AF-TIMI</td>
<td></td>
<td></td>
<td>(iii) FEIBA (activated PCC)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iv) Andexanet alfa (PRT4445) (ANNEXA-R)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(v) PER977*</td>
</tr>
</tbody>
</table>

*Those reversal agents are still under evaluation.

Table 3: Dosage recommendations for NOACs and contraindications [17, 34, 48].

<table>
<thead>
<tr>
<th>NOACs</th>
<th>Dosage for stroke prevention</th>
<th>Indications for a reduced dosage</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabigatran</td>
<td>150 mg twice daily</td>
<td>(i) 75 mg twice daily for those with CrCl 15–30 mL/min</td>
<td>(i) Patients with CrCl &lt; 15 mL/min</td>
</tr>
<tr>
<td></td>
<td>(ii) 75 mg twice daily for those on P-gp inhibitors in with CrCl 30–50 mL/min</td>
<td>(ii) Active pathological bleeding</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(iii) Mechanical prosthetic heart valve</td>
<td>(iii) Mechanical prosthetic heart valve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(iv) Patients on P-gp inducer rifampin</td>
<td>(iv) Patients on P-gp inducer rifampin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(v) Patients on P-gp inhibitors with CrCl &lt; 30 mL/min</td>
<td>(v) Patients on P-gp inhibitors with CrCl &lt; 30 mL/min</td>
<td></td>
</tr>
<tr>
<td>Rivaroxaban</td>
<td>20 mg once a day</td>
<td>15 mg once daily for those with CrCl 15–50 mL/min</td>
<td>(i) Severe renal impairment (CrCL &lt; 15 mL/min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(ii) Moderate or severe hepatic impairment (Child-Pugh B or Child-Pugh C) or with any degree of hepatic disease associated with coagulopathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(iii) Nursing women</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(iv) Active pathological bleeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(v) Coadministration of combined P-gp and strong CYP3A4 inhibitors and inducers</td>
</tr>
<tr>
<td>Apixaban</td>
<td>5 mg twice daily</td>
<td>(i) 2.5 mg twice daily if patients meet 2 of 3 criteria: age 80 years, body weight 60 kg, or serum creatinine level L5 mg/dL</td>
<td>(i) Active pathological bleeding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(ii) 2.5 mg twice daily if coadministration of strong dual inhibitors of CYP3A4 and P-gp</td>
<td>(ii) Pregnant and nursing women</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(iii) Coadministration of strong dual inducers of CYP3A4 and P-gp</td>
</tr>
</tbody>
</table>
Specific antidotes for direct thrombin inhibitors and Xa inhibitors are underway. Clinical studies are currently ongoing to evaluate some suggested antidotes.

Tables 1, 2, and 3 and Figure 1 summarize the key data useful in perioperative management, including NOAC dosages, reversal options, and therapeutic options in bleeding patients.

Dabigatran is reversed by the administration of activated factor VIIa or activated PCC. Hemodialysis is also effective in life-threatening emergencies.

Abbreviations

AF: Atrial fibrillation  
aPTT: Activated partial thromboplastin time  
CI: Confidence interval  
CrCl: Creatinine clearance  
ECT: Ecarin clotting time  
HTI: Hemoclot Thrombin Inhibitor  
ICH: Intracranial hemorrhage  
INR: International normalized ratio  
NOACs: Novel oral anticoagulants  
PCC: Prothrombin complex concentrate  
PT: Prothrombin time  
RR: Relative risk  
TT: Thrombin time  
VKA: Vitamin K antagonist.

Conflict of Interests

The authors have no relevant conflict of interests to disclose.

References


Clinical Study

Heartbeat Cycle Length Detection by a Ballistocardiographic Sensor in Atrial Fibrillation and Sinus Rhythm

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Background. Heart rate monitoring is especially interesting in patients with atrial fibrillation (AF) and is routinely performed by ECG. A ballistocardiography (BCG) foil is an unobtrusive sensor for mechanical vibrations. We tested the correlation of heartbeat cycle length detection by a novel algorithm for a BCG foil to an ECG in AF and sinus rhythm (SR).

Methods. In 22 patients we obtained BCG and synchronized ECG recordings before and after cardioversion and examined the correlation between heartbeat characteristics.

Results. We analyzed a total of 4317 heartbeats during AF and 2445 during SR with a correlation between ECG and BCG during AF of $r = 0.70$ (95% CI 0.68–0.71, $P < 0.0001$) and $r = 0.75$ (95% CI 0.73–0.77, $P < 0.0001$) during SR. By adding a quality index, artifacts could be reduced and the correlation increased for AF to 0.76 (95% CI 0.74–0.77, $P < 0.0001$, $n = 3468$) and for SR to 0.85 (95% CI 0.83–0.86, $P < 0.0001$, $n = 2176$).

Conclusion. Heartbeat cycle length measurement by our novel algorithm for BCG foil is feasible during SR and AF, offering new possibilities of unobtrusive heart rate monitoring. This trial is registered with IRB registration number EK205/11. This trial is registered with clinical trials registration number NCT01779674.

1. Introduction

Heart rate control is of importance for patients suffering from atrial fibrillation (AF) [1] or heart failure [2] to improve morbidity and mortality. Heart failure is frequently found amongst the elderly and is often associated with arrhythmias like AF. Above the age of 60, the prevalence of AF is around 5–10%, with about 6 million Europeans and more than 3 million US Americans suffering from AF [3–5]. Up to 90% of AF episodes are paroxysmal, especially in its early stage, and up to 90% are asymptomatic [6, 7]. This is of great clinical relevance since AF is responsible for up to 30% of ischemic strokes [3], for systemic embolisms [8], and for an increased perioperative risk [9].

The gold standard for heart rate diagnosis is an ECG, but, for example in cases of asymptomatic and paroxysmal AF its diagnosis with intermittent ECG recordings is difficult. Recent evidence suggests that continuous ECG monitoring with implantable pacemakers can detect all relevant AF episodes [10], but, due to the large number of patients at risk, implantable monitoring devices are not affordable. Furthermore, prolonged regular ECG monitoring seems more effective in detecting silent AF episodes than short-term continuous ECG recordings [11]. For this reason the National Heart, Lung & Blood Institute Expert Panel of the United States of America encourages the development of new methods and technologies for asymptomatic AF detection [12]. New devices such as smartphone applications [13] and wearable [14] and videoplethysmographic sensors [15] are being tested as potential candidates, but to date their clinical application remains difficult.

In this original investigation, we used a ballistocardiographic sensor in a prospective cohort of patients with AF receiving an electric cardioversion. The sensor can be positioned beneath conventional textiles and bed sheets and measured the mechanical equivalent of the heartbeat indicating bradycardia, tachycardia, and arrhythmia by the calculated cycle length.
The aim of this study was to evaluate the correlation of the heartbeat analyzed by our novel algorithm compared to an ECG as the gold standard.

2. Materials and Methods

22 patients with AF scheduled for elective electric cardioversion at the University Hospital of Aachen were recruited. After informed consent, all patients were enrolled according to the following inclusion criteria: presence of AF with scheduled, elective cardioversion and at least 18 years of age. Exclusion criteria were pregnancy or lactation, mental incapacity, or implanted electric device. Baseline demographic, clinical, laboratory, ECG, and synchronized BCG data were collected before and after electric cardioversion by trained staff. All recordings were performed in a supine position in spontaneously breathing participants.

Electric cardioversion was performed by a trained physician of the department of cardiology after exclusion of left atrial auricular thrombus by transoesophageal echocardiography.

The study was performed between January 2012 and March 2013 at the Department of Cardiology, Pneumology, Angiology and Intensive Care Medicine, University Hospital RWTH Aachen, Germany. The clinical trial was approved by the Ethics Committee of the Medical Faculty of the University Hospital Aachen (registration number: EK205/11, date: 27 May 2011; ClinicalTrials.gov: NCT01779674) and met current legal requirements (German Medical Devices Act and Code of Medical Ethics) as well as ethical principles contained in the Declaration of Helsinki and Good Clinical Practice guidelines.

2.1. Data Collection. For data collection a dedicated measuring cart with an “IntelliVue MX800 Patient Monitor” (Koninklijke Philips N. V., Amsterdam, Netherlands) connected to a personal computer was purpose-built. The electrical integrity was approved by the VDE (Verband Deutscher Elektrotechnik Elektronik Informationstechnik e.V., Frankfurt, Germany) for EN IEC 60601-1. For electronic data management an electronic case report form was programmed in OpenClinica (OpenClinica, LLC, Waltham, MA, USA).

2.2. Ballistocardiographic Sensor. Ballistocardiography (BCG) is a technique to monitor mechanical activity of the heart by recording mechanical forces on the body’s surface [16]. The basic concept has been known since the 19th century [17]. However, recent advances in sensor technologies have allowed the integration of highly sensitive mechanical sensors into beds for the purpose of unobtrusive cardiac monitoring [18, 19]. We used a thin and flexible foil, consisting of charged polymer layers containing air voids that behave in a similar way to electrical capacitors. Mechanical activity causes physical deformations of the sensor’s geometry. If the geometry of the enclosed air voids changes, their electrical charges move with respect to each other. These charge shifts can be measured by the sensor electrodes, converted to a voltage signal, and subsequently displayed as an ECG related signal (Figure 1).

2.3. Heartbeat Measurement by BCG Sensor. Every patient was measured in a supine position on a mattress with an attached BCG foil. BCGs were recorded by a ballistocardiographic sensor (Emfit Ltd., Vaajakoski, Finland). The sensor foil (30 × 60 cm) was positioned under the textile bed sheet and was invisible to the patients (Figure 1(a)). The motion signal was recorded by the sensor foil along a dorsoventral axis. There was no direct contact between the ballistocardiographic sensor and the patient.

2.4. Signal Processing. The BCG sensor acquired mechanical movement by a change of charge with 1000 Hz. The calculating time allowed an almost real-time analysis with a latency of <2 seconds. Heart contraction (Figure 1(b)), valve movement, blood flow, respiration, muscular activity [20, 21], and other mechanical activities were measured by the BCG foil and were part of the resulting BCG signal (Figure 1(c)). Depending on the subject’s position related to the sensor, the force vector of each mechanical activity produced corresponding
amplitudes. The superposition of different mechanical vectors impaired the signal analysis, so that the genuine signal had to be cleaned by filtering for the specific frequency range in question. A genuine BCG signal (Figure 2(a)) measured in a dorsoventral direction showed, along its vertical axis, slow oscillations for about 5 seconds of breathing which included smaller deflections oscillating at a higher frequency. By time-domain filtering and differencing, the breathing component was removed and the smaller, higher frequency oscillations became visible (Figure 2(b)). For filtering we used fixed and identical filters for all recordings with a cutoff frequency of 0.5 Hz and 80 dB stop-band attenuation. By a beat-to-beat analysis of local interval estimators the cycle length was calculated (Figure 2(c); Figure 3). Additional calculations such as quality index, integral, and maximal amplitude of the BCG complex were performed afterwards (Figure 2(d)). In the final step the BCG data was harmonized to a synchronized recorded ECG (Figure 2(e)). In this step the BCG peaks showed a specific sequence corresponding to the recorded ECG (Figure 2(e)).

2.5. Cycle Length Detection and Quality Index. Common techniques for automated heartbeat analysis consisted of locating relevant events, like the QRS complex, to obtain beat-to-beat intervals. Prior knowledge of the characteristics for the events of interest was necessary. Due to the variability of the inter- and intrasubject BCG deflection depending on the vector of interest related to the sensor and artifacts, these kinds of algorithms did not seem applicable for beat-to-beat analysis using the BCG signal [22]. We used a novel approach for heart rate analysis inspired by the so-called pitch-tracking method for speech processing [23].

The first window of interest was of constant size by a prior defined frequency of interest. The specific sequence of a heartbeat was not known but the assumption was that consecutive heartbeats consisted of a corresponding sequence of amplitudes. The algorithm analyzed the BCG signal for repeated patterns of deflections and identified these events as heartbeats (Figure 3(a)). A sliding window of interest moved 200 ms forward and an adaptive threshold measurement was performed of the window location. If the thresholds were violated, the presence of a high-energy artifact was assumed. The location was marked as corrupted and the algorithm restarted. In the case of no threshold violation the algorithm continued. The window of interest was more than twice the length of the estimated cycle length and identified two consecutive heartbeats for their specific amplitude pattern (Figure 3(b)). Three local interval estimators compared the isolated sequences to each other and each of them estimated a cycle length. The quality index defined the match of these three local interval estimators. The higher the accordance between the three estimators, the higher the quality index and the more precise the calculated cycle length (Figure 3(c)). Finally the cycle length and the corresponding quality index were defined and the window of interest moved on (Figure 3(d)). After computing the algorithm the results were displayed in less than two seconds (Figure 4).

By means of the quality index it was possible to identify artifacts or hampered signals by filtering the whole recorded signal for a specific quality. Subsequently, we added the synchronized ECG signal and analyzed the beat-to-beat interval in ECG using the "Open Source Arrhythmia Detection Software" (EP Limited, 35 Medford St., Somerville, MA, USA). At least 10 consecutive heartbeats were used for signal analysis.

2.6. Statistical Analysis. For correlation analysis we used Pearson’s correlation coefficient and the Bland-Altman Plot for visual analysis. For qualitative analysis all values are expressed in percentages and absolute numbers. Values of \( P < 0.05 \) were considered as statistically significant. Statistical analysis was performed with SPSS 21 (IBM Corp., Released 2012, IBM SPSS Statistics for Windows, Version 21.0., Armonk, NY, IBM Corp.) and MedCalc Statistical Software version 13.3.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014).

3. Results

The average age of patients was 72; 75% were male. Participants had a significantly higher heart rate before electric cardioversion (AF 88 ± 21 versus SR 67 ± 21 beats per minute). Two participants suffered serious medical problems during cardioversion so that due to many artifacts and a short measuring time these data were excluded. One patient converted spontaneously to sinus rhythm (SR) prior to cardioversion and his data were included in the SR group only. After successful cardioversion two patients showed premature ventricular beats after every normal sinus heartbeat (bigeminus) and thus were not considered as SR data. In five patients cardioversion was not successful; therefore their data after cardioversion were included in the AF group. Overall, we analyzed the data of 20 patients.

Cardioversion converted AF to SR and increased the mean ECG cycle length significantly \( (P < 0.001) \) from 729 ± 280 ms to 1004 ± 180 ms. Comparably, the mean BCG cycle length increased significantly \( (P < 0.001) \) from 758 ± 276 ms to 983 ± 199 ms (Table 1). After cardioversion, the BCG amplitude and integral of BCG complex decreased

| Table 1: ECG and BCG interval characteristics before and after cardioversion. |
|---------------------------------|-----------------|---------|
|                                 | Atrial fibrillation | Sinus rhythm |
| ECG interval [ms]               | Mean (±SD)       | Mean (±SD) | \( P \) value |
| BCG interval [ms]               | 729 (±280)       | 1004 (±180) | <0.001 |
| Quality index [AU]\*            | 0.41 (±0.21)     | 0.52 (±0.27) | <0.001 |
| BCG amplitude [AU]\*            | 0.088 (±0.047)   | 0.059 (±0.03) | <0.001 |
| Integral BCG complex [AU]\*     | 0.018 (±0.011)   | 0.011 (±0.006) | <0.001 |

\*AU: arbitrary units.
Figure 2: Signal processing of BCG data: (a) the raw signal includes its highest deflections in inhalation and exhalation; (b) after time-domain filtering the breathing component is removed and repeating oscillations as a surrogate for the heart contraction are visible; (c) the local interval estimator defines the cycle length by beat-to-beat analysis (Figure 3); (d) additional calculations for the integral of the BCG complex and the maximal amplitude deflections are carried out; (e) the BCG signal is synchronized to the ECG.
FIGURE 3: Estimating the heartbeat cycle length: (a) the window of interest analyzes the signal for repeated amplitude patterns and estimates the cycle length; (b) a sliding window of interest performs basic threshold measurements and identifies two consecutive heartbeats; (c) three local interval estimators analyze the signals and each estimates a cycle length: the match between the three estimators is the quality index; (d) the window of interest moves forward and the estimated cycle length and quality index are displayed. *BCG: ballistocardiogram; CL: cycle length; QI: quality index.

significantly (P < 0.0001) with a narrow standard deviation indicating a more consistent heartbeat signal complex in BCG during SR (Table 1).

We analyzed 4317 heartbeats between BCG and ECG during AF resulting in a correlation coefficient of 0.7 (0.68–0.71, P < 0.0001, n = 4317) (Figure 5(a)). 2445 heartbeats during SR were analyzed; here we found a correlation coefficient between BCG and ECG of 0.75 (95% CI 0.73–0.77, P < 0.0001, n = 2445) (Figure 5(b)). By filtering the AF signal with the quality index >0.25 (Table 2), the number of analyzable heartbeats was reduced to 80%, and the correlation coefficient increased to 0.76 (95% CI 0.74–0.77, P < 0.0001, n = 3468) (Figure 5(c)). The correlation in SR increased to 0.85 (95% CI 0.83–0.86, P < 0.0001, n = 2176) (Figure 5(d)) by filtering with the quality index >0.25, with 89% of heartbeats remaining analyzable data. For higher quality indexes the resulting correlation coefficient increased with a decrease of analyzable heartbeat intervals. Thus, a quality index >0.4 resulted in a high correlation coefficient during AF with 0.89 (95% CI 0.88–0.90, P < 0.0001, n = 1606) (Figure 5(e)) and a near-perfect correlation coefficient of 0.95 (95% CI 0.95–0.96, P < 0.0001, n = 1410) (Figure 5(f)) during SR.

Figures 6(a)–6(d) document examples of BCG analysis corresponding to quality index and synchronized ECG. For AF with normal heart rate (Figure 6(a)) the BCG interval
Table 2: Filtering of the measured BCG during AF and SR by the quality index with remaining analyzable episodes and corresponding correlation coefficient.

<table>
<thead>
<tr>
<th>Quality index</th>
<th>Atrial fibrillation</th>
<th></th>
<th>Sinus rhythm</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>r</td>
<td>95% CI</td>
</tr>
<tr>
<td>&gt;0.1</td>
<td>4317</td>
<td>100</td>
<td>0.70</td>
<td>0.68 to 0.71</td>
</tr>
<tr>
<td>&gt;0.15</td>
<td>4301</td>
<td>100</td>
<td>0.70</td>
<td>0.69 to 0.72</td>
</tr>
<tr>
<td>&gt;0.2</td>
<td>4071</td>
<td>94</td>
<td>0.72</td>
<td>0.71 to 0.74</td>
</tr>
<tr>
<td>&gt;0.25</td>
<td>3468</td>
<td>80</td>
<td>0.76</td>
<td>0.74 to 0.77</td>
</tr>
<tr>
<td>&gt;0.3</td>
<td>2711</td>
<td>63</td>
<td>0.83</td>
<td>0.82 to 0.84</td>
</tr>
<tr>
<td>&gt;0.35</td>
<td>2088</td>
<td>48</td>
<td>0.87</td>
<td>0.86 to 0.88</td>
</tr>
<tr>
<td>&gt;0.4</td>
<td>1606</td>
<td>37</td>
<td>0.89</td>
<td>0.88 to 0.90</td>
</tr>
</tbody>
</table>

Figure 4: ECG (black signal) synchronized BCG (blue signal), heartbeat count, ECG cycle length, and corresponding estimated BCG cycle length and quality index are simultaneously displayed. Beat 2 is a premature ventricular contraction resulting in a minor accordance of ECG and BCG cycle length. Also heartbeat 3 is affected by premature ventricular contraction; the following heartbeats show near-perfect accordance to the ECG cycle length with a high corresponding quality index. *BCG: ballistocardiogram; QI: quality index.

Detection showed a good correlation to ECG. In heartbeats with inaccurate correlation of BCG and ECG cycle length the corresponding calculated quality index of the BCG signal decreased too.

In SR (Figure 6(b)) after cardioversion the ECG and BCG cycle length correlation was near perfect resulting in a high quality index for each heartbeat.

Premature atrial contraction in SR (Figure 6(c)) after cardioversion resulted in a decreased BCG quality index and poor correlation to the ECG for the premature contraction and the following heartbeat. However, a good correlation of BCG signals was observed during normal sinus beats (without premature contraction) with a corresponding high quality index.

Figure 6(d) depicts the effect of a movement artifact. The match between BCG and ECG was interrupted by a high-energy artifact resulting from patient movement. The episode was marked as corrupt and could be used for filtering the BCG signal. After the artifact the first heartbeat showed a poor correlation of cycle length detection due to a changed amplitude pattern. However, the algorithm needed no training phase and the consecutive heartbeats showed again a good cycle length correlation and an improving quality index.

4. Discussion

The present study demonstrates that heartbeat interval detection by a ballistocardiographic sensor during SR and AF is feasible using a novel pitch-tracking inspired algorithm. Heartbeat analysis is currently mainly performed by ECG or by photoplethysmographic sensors. The ECG represents the gold standard for heart rate measurement. Although new sensor technologies are the focus of research to deal with the upcoming problems of an aging society and an increasing demand for outpatient diagnostic tools, none have proven clinically useful so far. The pitfalls of these new sensor technologies are compliance of the patient, operability, availability, and accuracy.

In our study participants were placed in a supine position with their chests above the BCG foil. The sensor is unobtrusive and has no direct contact to the skin. This offers the possibility of integrating the sensor foil into any bed sheet. Other measuring situations such as a prone or sideways position are theoretically possible. In particular, the prone position might offer a better signal for the heartbeat analysis owing to direct contact of the BCG foil and the apical impulse of the heart. However, this position was not tested due to the study setting of cardioversion.

There seems to be a circadian distribution of arrhythmias with peaks at different times during the day [24, 25]. Thus the proposed technology, which can be easily integrated into a mattress, may potentially be suited for large scale and long-term recording of the heart rate and rhythm during sleep. The measurement system needs the BCG foil and a computer for the algorithm. Excluding the attached computer, the costs for the system remain below $100.

The BCG signal measures any mechanical vibration. To receive the best results the longitudinal axis of the movement of interest should be positioned perpendicular to the measuring foil and other movements have to be excluded because the signal is hampered by any other movement which puts pressure on the foil. In real life conditions this will not be possible so a robust and flexible algorithm is needed.
to exclude artifacts and filter the signal of interest. Due to the ambiguous nature of the BCG deflection our approach does not search for a specific or defined signal appearance but looks for repeating signal deflections. For this reason no training is needed and a change of BCG deflection, for example, after a body movement, does not affect the analysis.

In clinical practice patients are advised to remain motionless during ECG recording; this would probably also improve the BCG signal quality but was not tested. The algorithm at this point does not offer a qualitative analysis of the heartbeat characteristics and is not able to distinguish between SR and AF.
Figure 6: Examples of cycle length detection by synchronized ECG and BCG with corresponding quality index. ECG (black signal) and synchronized BCG (blue signal). (a) AF shows a good correlation of the ECG and synchronized BCG interval detection. BCG#2 indexing a change in heartbeat cycle length resulting in an inaccurate BCG cycle length detection with corresponding decreased quality index. (b) SR after cardioversion with a near-perfect ECG and BCG cycle length correlation resulting in a high quality index for each heartbeat above 0.4. (c) SR after cardioversion with a premature atrial contraction (BCG#3). The corresponding quality index indicates a poor BCG quality for the premature contraction (BCG#3) and the following beat (BCG#4) due to a change in the deflection pattern and a good quality of BCG signal in normal SR. (d) SR after cardioversion with a good BCG cycle length detection interrupted by a high-energy artifact, most likely a moving artifact with a BCG interval marked as corrupt (BCG#3). The consecutive beats are all detected with an improving ECG and BCG cycle length correlation and an increasing quality index. Although the BCG pattern changed after the moving artifact no training phase was necessary for cycle length detection. * BCG: ballistocardiogram; QI: quality index.

In contrast to photoplethysmographic sensor technologies, BCG measures the mechanical movements of the organ of interest. Peripheral pulse deficits due to low blood pressure, increased peripheral resistance, venous return, sympathetic arousal, temperature, or centralization of circulation do not interfere with the signal as much as they do for the photoplethysmographic sensors [15]. Due to different filling conditions and an irregular heartbeat during AF the match between consecutive heartbeats in the BCG signal alternates. Thus, arrhythmia heartbeat detection is challenging for the algorithm and resulted in a decreased quality index (0.41) during AF in contrast to sinus rhythm (0.52) as described in Table 1. The lower quality index during AF or premature ventricular contraction compared to sinus rhythm could hamper...
the recognition and differentiation of true heartbeats in contrast to artifacts. However, we were able to calculate cutoffs for the quality index to differentiate between quality index values during AF and quality index values during artifacts. Thus, the algorithm remains robust in its signal detection under different filling conditions and motion sequences of the heart such as during AF or premature ventricular contractions (Figures 6(a)–6(d)).

We have seen encouraging results with a good baseline correlation of the BCG signal to the synchronized ECG. The algorithm needs no training for heartbeat detection and offers almost real-time cycle length analysis with a delay of less than 2 seconds. So in addition to the recording opportunities like a Holter ECG a bedside application seems possible too. Interestingly and in contrast to our own previous results, the baseline BCG signal during SR offers a lower quality index and correlation coefficient than expected. This is caused by the direct recording after cardioversion during the awaking period in which there is some body movement. These movement artifacts could be filtered easily by the quality index, resulting in a high correlation coefficient. In contrast to our expectations, the BCG signal also offers good interval recognition during AF even though different filling conditions and a beat-to-beat change of cycle length during AF can hamper the BCG signal. This shows the strength and flexibility of the used algorithm.

4.1. Limitations. The number of patients included in this feasibility study was low. However, the number of heartbeats analyzed in the study was high. The filter includes means to filter the organ and frequency of interest and distinguish artifacts so the algorithm works in the frequency we are interested in (for this investigation from 30 to 180 beats per minute). Other cycle lengths could have been neglected but were not present during the data collection. In addition, the algorithm presented in the study provides no qualitative assessment of the rhythm so a differentiation between SR and AF is not presented to the user. However, the aim of the study was not to distinguish between SR and AF but to assess the feasibility of cycle length analysis during SR and AF.

5. Conclusion

In conclusion, we demonstrated that the heartbeat cycle length detection by our novel algorithm with a ballistocardiographic sensor is feasible in AF and SR with a good correlation to a synchronized ECG. Artifacts can be filtered by using a quality index of each analyzed heartbeat in the BCG signal.

Acknowledgments

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References


Abbreviations

AF: Atrial fibrillation
AU: Arbitrary units
BCG: Ballistocardiogram.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.


Atrial fibrillation (AF) is the most frequently encountered cardiac arrhythmia globally, affecting 2% of the general population and rising to 10% of those aged over 80 years. One in four individuals will experience AF in their lifetime [1]. By 2050 the prevalence of AF is expected to increase by threefold and this poses a considerable public health concern [2]. Overall AF exacerbates the risk of stroke and is associated with a twofold excess mortality with 20% of all strokes being as a result of AF and 1 in 5 patients first presenting with AF in the context of cerebral ischaemia [3]. In addition AF is also linked to larger strokes based on both clinical and radiological findings [4]. Despite these risks, it has been estimated that at least 20% of cases of AF remain undiagnosed and are not prescribed appropriate stroke prevention therapy [5].

Paroxysmal atrial fibrillation (PAF) is thought to constitute between 25 and 60% of cases of AF [6] and is thought to precede sustained AF, culminating in progressive atrial electrical and structural remodelling, otherwise coined “AF begets AF.” PAF has been defined by the American Heart Association as “recurrent (two or more) episodes of AF that terminate spontaneously lasting between 30 seconds and less than seven days” [7]. An example of the initiation of PAF is seen in Figure 1.

The true prevalence of PAF is unknown due to asymptomatic episodes and a low yield afforded by conventional monitoring. However between 6 and 28% of cryptogenic strokes have been found to be secondary to PAF [8] and PAF conveys an equivalent risk of stroke to sustained forms of AF [9]. Unfortunately PAF, relative to the other forms of AF termed “persistent” and “permanent,” can be a particular challenge to diagnose due to the variable onset of the arrhythmia, potential brevity, and also frequent lack of symptoms [10].
The resting potential is maintained at about −80 mV via K+ equilibrium potential. Upon activation, rapid depolarization occurs by a large inward Na+ current ($I_{\text{Na}}$) and an inward Ca2+ current via L-type Ca2+ channels ($I_{\text{CaL}}$) resulting in the plateau phase. As the Ca2+ current declines the AP repolarizes to the resting potential.

Arrhythmia generation in the left atrium (LA) originates from a combination of abnormalities in impulse initiation, impulse conduction, or a combination of the two [11]. Abnormal impulse initiation can be subclassified further due to enhanced automaticity or triggered activity. Enhanced automaticity (ectopic arrhythmias) may be produced by irregular intracellular Ca2+ release. Similarly triggered activity, caused by a spontaneous inward current, generates secondary afterdepolarizations early in the plateau phase (EADs) or upon repolarisation (DADs) (Figure 2(b)). Alternatively due to abnormal AP conduction, reentrant arrhythmias occur, preventing the normal pattern of propagation across the myocardium. This is attributable to a combination of alteration of the electrical properties of intercellular gap junctions; structural remodelling of the myocardium; and generation of EADs/DADs that perse have slower conduction. Both mechanisms may be present simultaneously where ectopic activity begets reentrant arrhythmias resulting in self-perpetuation. The former described electrophysiological mechanisms are coupled to atrial electrical remodeling, characterised by changes in atrial refractoriness and slowed conduction time. These changes occur due to alterations in AP currents, especially Ca2+ influx and its subsequent homeostasis, and provide ongoing atrial arrhythmogenic substrates [12].

Atrial structural remodeling is typified by cardiac fibrosis, characterized by accumulation of collagenous material in the extracellular space (Figure 3), and is proposed to widen gap junctions and decrease communication between cardiomyocytes. Reduced AP conduction velocity is directly correlated to the extent of fibrosis and is linked to persistent reentrant circuit arrhythmias [13]. Angiotensin II produced by fibroblasts exacerbates the situation to increase cellular proliferation and cardiac fibrosis [14]. Alternatively structural remodeling may arise due to the release of proinflammatory cytokines after surgery or injury and the interaction of inflammatory proteins with angiotensin II promotes further atrial fibrosis [15]. This inflammatory response has been implicated in the aetiology of AF although the question remains whether this is attributable to the arrhythmia itself or another underlying disease state [16].

The ultimate consequences of AF, stroke and systemic embolism, result from the dislodgement of LA thrombi. Thrombus is most commonly located in the left atrial appendage (LAA) [17], a small pouch found in the LA (Figure 4). The generation of thrombi is triggered by atrial stasis, as a result of impaired atrial contraction due to atrial arrhythmia. Other factors promoting thrombus formation include damage to the atrial wall and a hypercoagulable state [18].

3. Current Diagnostics for Paroxysmal Atrial Fibrillation

Current methods to diagnose PAF are limited to electrocardiogram (ECG) analysis, which may fail to document an episode of AF if occurring outside the monitoring period. Recent trials have highlighted that prolonged cardiac monitoring detects significantly more cases of PAF in the cryptogenic stroke population than using a conventional 24-hour Holter monitor [19, 20]. Clinical risk factors for AF are widely recognized and models have been proposed to predict incident AF using a number of clinical variables. Such a risk score devised by the Framingham Heart Study is based on a number of simple clinical parameters including age, gender, a significant murmur, cardiac failure, systolic blood pressure, hypertension, body mass index, and PR interval. The score yielded a C-statistic of 0.78 (95% CI 0.76–0.80), with a C-statistic being a measure of a model's predictive power and a value of over 0.7 considered to be reasonable. The addition of echocardiographic measurements further improved this to 0.79 [21]. There is however a lack of diagnostic armoury to detect PAF. This is in stark contrast to other cardiovascular conditions, for example, coronary artery disease and cardiac failure, with a comparable disease prevalence and routine use of diagnostic biomarkers [22].

4. Biomarkers

A biomarker can be defined as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [23]. Biomarkers not only have the potential to identify a disease process but also provide valuable information about underlying disease mechanisms and, as a consequence, potential therapeutic targets.

Biomarkers have been proposed as a tool to predict onset of AF in a variety of settings including at initial presentation, following cardiothoracic surgery, recurrence after cardioversion and ablation, and in the event of a cryptogenic stroke. Additionally, accumulating research has shown that biomarkers could be used to predict the transition of PAF to sustained AF, as well as, alongside conventional risk scores, to determine thromboembolic risk [24, 25]. The LA has been the main focus for identifying biomarkers for PAF given the inextricable association between this chamber and the disease process itself. As such potential biomarkers to detect PAF can be broadly categorised into electrophysiological, molecular, and morphological indices.
5. Biomarkers for Paroxysmal Atrial Fibrillation

5.1. Electrophysiological Biomarkers. A number of studies have shown that simple resting ECG parameters are highly predictive of PAF. “P-maximum” is the maximum duration from the onset to the end of the P-wave deflection from all 12 ECG leads. This is seen as a marker of prolonged atrial conduction time, a hallmark of patients with AF. “P wave dispersion,” the difference between the maximum and minimum
P-wave duration in any of the 12 leads, signifies nonuniform atrial conductivity. Both parameters are representative of the underlying atrial remodelling occurring in AF and previously alluded to. One study showed that P-maximum and P-wave dispersion were highly predictive of previous episodes of PAF. A P-maximum of at least 110 ms had a sensitivity of 88% and specificity of 75% and a P-wave dispersion of at least 40 ms yielded a sensitivity of 83% and specificity of 85% for PAF [26]. These findings have been substantiated by another study showing that P-wave dispersion was an independent predictor of PAF in stroke patients. In this population a P-wave dispersion 57.5 ms and above predicted PAF with a sensitivity and specificity of 80% and 73%, respectively [27].

Likewise a J-shaped relationship was shown between the QTc interval and risk of incident AF in a large primary care population. QTc intervals at the 99th percentile and above (≥464 ms) resulted in an overall hazard ratio of 1.44 for all AF subtypes. Additionally an even more powerful relationship was seen in lone AF with a QTc interval ≥458 ms demonstrating a hazard ratio of 2.32. Conversely a QTc ≤372 ms was associated with a hazard ratio of 1.45 [28]. More specific to PAF, another group have shown that a prolonged QTc interval is an independent predictor of PAF in patients presenting with ischaemic stroke. In this instance a QTc threshold ≥438 ms generated a sensitivity of 59.4% and specificity of 83.7% [29]. It has been proposed that these associations may be on account of universal expression of ion channels in both atrial and ventricular tissue.

Atrial premature beats (APBs) precede PAF in a significant proportion of AF episodes and are a feature of enhanced automaticity, a recognised mechanism for AF (Figure 5). In one study monitoring 33 patients with documented PAF and 297 total episodes of AF, the arrhythmia was initiated by APBs in 93% of cases [30]. Consequently frequent APBs have been suggested as a marker of predisposition to PAF. One study investigating 98 stroke patients with transtelphonic monitoring determined that at least 100 APBs on a 24-hour Holter monitor equated to an odds ratio of 11 for the subsequent development of PAF after one month [31].

In another cohort of stroke patients, greater than 4 APBs per hour during an initial 24-hour Holter monitor equated to subsequent development of PAF in 19.6%, compared to 2.8% in those with 4 APBs per hour or less [32]. Using a similar study design, Wallmann et al. also deduced that frequent APBs were an independent risk factor for PAF in stroke patients. Significantly more of those patients with frequent APBs (defined as at least 70 in 24 hours) developed PAF at 22.4 months compared with those with infrequent APBs (33% versus 5%) [33].

5.2. Molecular Biomarkers. Previously validated biomarkers, currently used in standard clinical practice to diagnose cardiac failure and myocardial infarction, have also been tested in the setting of AF. Numerous studies have shown an association between AF and brain natriuretic peptide (BNP) with persistently higher plasma levels than in healthy matched controls and a reduction to that of control subjects following successful restoration of sinus rhythm [34]. It has been proposed that the main source of BNP release in AF is the atrium as a result of pressure and volume overload. It remains to be established however whether BNP is merely a marker of atrial dysfunction or active in the underlying pathological process [35]. With a particular focus on PAF, several studies have shown a relationship between natriuretic peptides and diagnosis of this arrhythmia. One study showed higher levels of plasma BNP in patients with lone PAF compared to age- and sex-matched controls [36]. A further study confirmed significantly raised N-terminal of prohormone brain natriuretic peptide (NT-proBNP, a peptide cleaved from pro-BNP to release BNP) in contrast to matched controls. Yet a similar relationship was not demonstrated for pro-ANP (atrial natriuretic peptide) in this group [37]. Likewise in the Find-AF study, BNP levels were significantly higher in those with confirmed PAF than those without [38]. A similar prospective study found that a BNP over 140 pg/mL had a sensitivity and specificity of 77.6% and 84.8%, respectively, for cardioembolic stroke [39]. Likewise NT-proBNP levels over 265.5 pg/mL conferred a sensitivity 100% and specificity 70.5% for PAF [40]. Similarly troponin, another established cardiac biomarker, is increased in PAF subjects relative to their controls. Elevation of troponin levels in cryptogenic stroke patients independently predicted new onset AF during 24-hour Holter monitoring [41]. This finding was also confirmed in a similar retrospective study [42].

Markers of inflammation, implicated in the pathogenesis of AF, have yielded mixed results. Raised serum IL-18 was significantly associated with both PAF and persistent AF with a twofold concentration in all AF subtypes compared...
to controls [43]. Another study confirmed that patients with PAF had significantly higher levels of CRP (C-reactive protein) than their controls, in a graded fashion according to arrhythmia burden [44]. Similarly a raised white blood cell count predisposed to incident AF after 5 years in the Framingham Study [45]. In stark contrast another study demonstrated no difference in the inflammatory markers CRP, IL-6, and IL-8 between PAF patients and their controls when attending for radiofrequency ablation [46]. Glucose haemostasis and lipid metabolism also appear to be associated with the development of AF. In the Atherosclerosis Risk in Communities (ARIC) cohort a positive linear relationship was seen between haemoglobin Alc (HbAlc) and incident AF in patients both with and without type 2 diabetes [47]. Interestingly in the Women's Health Study however, although HbAlc was positively correlated with the incidence of nonparoxysmal AF, it was inversely related to PAF [48]. Additionally the ARIC study demonstrated that both higher LDL and total cholesterol resulted in a lower incidence of all AF subtypes [49]. This, somewhat unexpected, inverse relationship was replicated by another group [50] yet an explanation for this repeated finding is not clear.

Parameters reflecting thrombogenesis, the source of cardioembolism and stroke in PAF, have also been investigated. For example, plasma von Willebrand factor and fibrinogen were significantly elevated in patients with PAF compared to their matched controls [51]. Similarly fibrinogen and fibrin D-dimer were significantly increased in PAF relative to age- and sex-matched controls [52]. Additionally regulators of extracellular turnover, the culprits of the previously described fibrotic remodelling, were all increased in lone PAF subjects compared to their controls. These markers include CICP (C-terminal propeptide of collagen type-I), CITP (C-terminal telopeptide of collagen type-I), MMP-1 (matrix metalloproteinase-1), and TIMP-1 (tissue inhibitor of matrix metalloproteinase) [53]. Likewise CITP was also raised in PAF cases compared to healthy subjects in another study [54]. Both of these studies were limited by a younger control group however, introducing potential confounding.

A genetic predisposition has been shown to contribute to the development of AF. For example, the Framingham Study showed a heightened risk of AF in the children of a parent with AF; independent of other risk factors (odds ratio of 1.85) [55]. Genetic analysis of over 14,000 European and Japanese individuals with a history of AF noted susceptibility signals on chromosome 4q25, upstream of PTIX2, a gene previously implicated in AF [56]. Furthermore six further susceptibility loci (1q24, 7q31, 14q23, 9q22, 15q24, and 10q22) were identified in a meta-analysis comprising over 10,000 individuals with AF of European descent [57]. These findings prompted the Women's Health Study to validate a prediction score for incident AF incorporating 12 single-nucleotide polymorphisms in nine loci. Genetic risk markers were shown to improve a conventional clinical risk score from a C-statistic of 0.718 to 0.741 ($P = 0.001$) [58]. Whether genetic markers will aid in the diagnosis of PAF in particular is, as yet, to be definitively answered. There is however promising research suggesting that microRNAs, stable noncoding RNAs (ribonucleic acid) found in serum and plasma which modulate RNA transcription, may play such a role. There is a relative paucity of research in this area to date and studies investigating a link to PAF are even scarcer. However promisingly, several preliminary studies have shown that plasma miRNA-150 expression, already implicated in the regulation of genes associated with atrial remodeling, is significantly reduced in individuals with PAF [59, 60].

5.3. Morphological Biomarkers. It is widely recognised that LA size is a powerful predictor of cardiovascular mortality, conferring an independent 2.3-fold excess risk of cardiovascular mortality at 13 years [61]. Specific to AF, each increase in LA diameter by 5 mm increased the risk of new AF by 39% in the Framingham Heart Study [62]. Likewise subjects with PAF had increased LA size relative to healthy controls with an inferosuperior dimension of at least 50 mm, yielding a sensitivity and specificity for PAF of 66% and 80%, respectively [63]. These observations are a direct result of the structural remodelling occurring in the LA in the course of the disease process.

With the advent of increasingly sophisticated techniques to assess the LA, echocardiographic indices of function are now widely thought to be more robust predictors of outcomes than size alone [64, 65]. Methods to assess LA function include Doppler analysis of transmural flow and tissue Doppler assessment of LA myocardial velocities. Not only have alterations in LA function been shown to be a hallmark for PAF, but also they may predict the onset of AF following cardiothoracic surgery, cardioversion, and ablation, as well as being an independent marker of thromboembolic risk [66–69]. Variables of LA function, as assessed using transoesophageal echocardiography (TOE), were impaired in PAF patients following stroke. LAA peak velocity was markedly reduced in the PAF group compared to the control group. Additionally LA spontaneous echo contrast, graded from 0 (none) to 4+ (severe), was higher in the PAF group. Both of these measurements reflect atrial stasis and were more powerful predictors of PAF than LA dimension alone [70].

These results have been replicated using transthoracic echocardiography where, LA function as assessed by myocardial Doppler, is thought to predict PAF more precisely than LA dimensions. Toli et al. used a novel marker of both LA size combined with LA pump function [LAVI/a'] to discriminate between patients with PAF in a group of hypertensive patients. LAVI (left atrial volume index) is calculated from the LA volume corrected for body surface area. Left atrial velocity in late diastole, or a', is derived from myocardial tissue Doppler measured from the mitral annulus (Figure 6). In this population a LAVI/a' threshold of 2.7 conferred a sensitivity 82% and specificity 91% for PAF [71]. The Find-AF group used the same method to distinguish between stroke or TIA patients with underlying PAF. It was deduced that LAVI/a' of 2.3 had a 93% sensitivity and 55.8% specificity. Again this parameter predicted PAF more powerfully than measures of LA size, including LA diameter and LAVI [72].

Mitrval valve disease is commonly associated with a concurrent diagnosis AF, attributed to atrial remodeling and
dilatation as a result of volume and pressure overload. For example, over 40% of individuals with rheumatic mitral valve disease and between 44 and 48% of those with degenerative mitral regurgitation (MR) develop AF in the long-term [73, 74]. One observational study has proposed a form of “functional MR” as a direct consequent of AF, possibly induced by atrial wall dyssynchrony, which improves following restoration of sinus rhythm [75]. As a predictive tool, one group proposed that, in patients with at least moderate MR, the rate of left ventricular pressure rise in early systole ($dP/dt_{\text{max}}$) independently predicted new onset AF or ischaemic stroke. The study was significantly limited by a small sample size with only 9 patients having this combined endpoint [76]. To our knowledge no large studies assessing the use of MR as a diagnostic marker for PAF specifically have been undertaken to date.

6. Conclusions

Despite several decades of plausible research suggesting that biomarkers might be used to improve the diagnosis of PAF (summarised in Table 1) these techniques have not as yet entered clinic practice. This begs the question why this might be the case. There appears to have been a shift in clinicians’ and scientists’ attitudes to AF in recent years and PAF is no longer seen as a benign entity. This and the impending “AF epidemic” have highlighted the importance of optimal arrhythmia detection. This had been somewhat neglected previously with the 24-hour Holter monitor remaining commonplace as a first-line investigation. Critically the lack of a universal definition for PAF has hindered research in the field. AF research is riddled with conflicting definitions primarily dependent on the duration of the arrhythmia and tendency to self-terminate. Furthermore a lack of consensus may be due to oversimplified classification systems. AF is a heterogeneous condition likely to represent a number of underlying pathologies and a lack of aetiological classification may have hindered the quest for improved diagnostic markers.

The majority of the literature in this review suggests that biomarkers might be a valuable addition to current investigational techniques. However these findings are admittedly subject to inherent publication bias. Furthermore recurrent methodological flaws are encountered. Many of the papers published in the area consist of small populations. Additionally a number of groups relied on self-reported AF as opposed to objective screening. Crucially many of the control groups were younger than those with confirmed PAF. Given that age is an independent risk factor for AF and with age comes increasing comorbidity, this introduces confounding. Lastly a number of the techniques described are time-consuming and some echocardiographic techniques are hindered by difficult patient anatomy.

In summary it remains an open question if biomarkers will add to conventional diagnostic techniques for PAF. If the role of biomarkers is to be established then future research requires refinement. We propose that an accurate future biomarker is likely to feature a combination of electrophysiological, molecular, and morphological indices therefore encompassing the heterogeneity seen in this condition. A reliable diagnostic marker to improve the diagnosis of undetected PAF and ultimately reduce risk of consequent thromboembolism certainly warrants further investigation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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Table 1: A summary of the main advantages and disadvantages of current diagnostic techniques for PAF (not bold) including potential biomarkers for PAF based on the evidence detailed in this review (bold) (unpublished).

<table>
<thead>
<tr>
<th>Diagnostic class</th>
<th>Technique</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Automated analysis</th>
<th>Remote analysis</th>
<th>Cost</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECG rhythm monitoring</td>
<td>Continuous long-term ECG monitoring using implantable devices</td>
<td>High</td>
<td>High</td>
<td>+</td>
<td>+</td>
<td>High</td>
<td>High sensitivity and specificity</td>
<td>Costly, invasive equipment required</td>
</tr>
<tr>
<td></td>
<td>Medium-term noninvasive ECG monitoring &gt;24 hours</td>
<td>Moderate</td>
<td>High</td>
<td>+/-</td>
<td>+/-</td>
<td>Moderate</td>
<td>Moderate sensitivity and specificity</td>
<td>Patient inconvenience, some cases missed</td>
</tr>
<tr>
<td></td>
<td>Short-term ECG monitoring -24 hours</td>
<td>Low</td>
<td>High</td>
<td>+/-</td>
<td>+/-</td>
<td>Low to moderate</td>
<td>Relatively inexpensive</td>
<td>Low diagnostic yield</td>
</tr>
<tr>
<td>Electrophysiological Biomarkers</td>
<td>Analysis of ECG indices, for example, P wave dispersion, QTc interval</td>
<td>Low to high</td>
<td>Moderate to high</td>
<td>–</td>
<td>–</td>
<td>Low</td>
<td>Cost-effective test already in routine clinical practice</td>
<td>Relatively labour intensive without automated analysis. Room for potential error</td>
</tr>
<tr>
<td></td>
<td>Frequency of atrial premature beats on 24-hour Holter monitor</td>
<td>Moderate</td>
<td>Low-moderate</td>
<td>+/-</td>
<td>+/-</td>
<td>Low to moderate</td>
<td>Noninvasive test which the patient may already be undergoing</td>
<td>Low specificity</td>
</tr>
<tr>
<td>Molecular biomarkers</td>
<td>Proteins</td>
<td>Moderate to high</td>
<td>Moderate</td>
<td>+</td>
<td>–</td>
<td>Low</td>
<td>Mass screening possible</td>
<td>Relatively low specificity</td>
</tr>
<tr>
<td></td>
<td>microRNAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Expected to be equivalent to protein biomarkers</td>
<td></td>
</tr>
<tr>
<td>Morphological biomarkers</td>
<td>Echocardiography LA size</td>
<td>Low</td>
<td>Moderate</td>
<td>–</td>
<td>–</td>
<td>Moderate</td>
<td>Noninvasive test which the patient may already be undergoing, LA size is a standard measurement</td>
<td>Highly trained specialists and costly equipment required</td>
</tr>
<tr>
<td></td>
<td>Echocardiography LA function, for example, myocardial tissue Doppler</td>
<td>High</td>
<td>Moderate</td>
<td>–</td>
<td>–</td>
<td>Moderate</td>
<td>Noninvasive test which the patient may already be undergoing</td>
<td>Highly trained specialists and costly equipment required. Potential room for error in patients with suboptimal image quality</td>
</tr>
</tbody>
</table>
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S. M. Vaziri, M. G. Larson, E. J. Benjamin, and D. Levy, “Echocardiographic predictors of nonrheumatic atrial


Review Article

Atrial Fibrillation and Fibrosis: Beyond the Cardiomyocyte Centric View

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Atrial fibrillation (AF) associated with fibrosis is characterized by the appearance of interstitial myofibroblasts. These cells are responsible for the uncontrolled deposition of the extracellular matrix, which pathologically separate cardiomyocyte bundles. The enhanced fibrosis is thought to contribute to arrhythmias “indirectly” because a collagenous septum is a passive substrate for propagation, resulting in impulse conduction block and/or zigzag conduction. However, the emerging results demonstrate that myofibroblasts also promote arrhythmogenesis due to direct implications upon cardiomyocyte electrophysiology. This electrical interference may be considered beneficial as it resolves any conduction blocks; however, the passive properties of myofibroblasts might cause a delay in impulse propagation, thus promoting AF due to discontinuous slow conduction. Moreover, low-polarized myofibroblasts reduce, via cell-density dependence, the fast driving inward current for cardiac impulse conduction, therefore resulting in arrhythmogenic uniformly slow propagation. Critically, the subsequent reduction in cardiomyocyte membrane potential significantly increases the likelihood of ectopic activity. Myofibroblast densities and the degree of coupling at cellular border zones also impact upon this likelihood. By considering future in vivo studies, which identify myofibroblasts “per se” as a novel targets for cardiac arrhythmias, this review aims to describe the implications of noncardiomyocyte view in the context of AF.

1. Introduction

The normal function of the heart is a painstaking cooperation between cardiomyocytes and fibroblasts. It is well known that cardiomyocytes provide the “pumping” function of the organ, whereas fibroblasts are responsible for organizing the cellular scaffold and maintaining the proper 3D-network and thus the normal mechanical function. Moreover, fibroblasts contribute importantly to the uniformity of the excitable substrate and to the continuous and rapid electrical activation of the myocardium. In the healthy normal heart, fibrosis-related arrhythmia is normally absent, which indicates that although fibroblasts outnumber cardiomyocytes roughly three-to-one [1], they do not exert any arrhythmogenic effect. Though there is a general assumption that cardiomyocytes play the crucial role in atrial arrhythmogenesis, little is known regarding an “active” role of the connective tissue in this respect.

A variety of pathological conditions, including pressure overload, volume overload, infarction, and aging [2], induces structural remodelling of the heart leading to heart failure and cardiac arrhythmias. This structural remodelling involves changes in the 3D organization of the heart and is based on complex and diverse responses to injury; as a result, all types of cardiac cells are involved. Histopathologically, cardiac remodelling typically involves changes in myocytes size (hypertrophy), the activation and proliferation of fibroblasts, uncontrolled deposition of the extra cellular matrix (ECM), and cell death [3]. This is in favour of the beginning and perpetuation of supraventricular and ventricular arrhythmias.
due to the presence of collagenous septa, which physically separate regions of cardiomyocytes, thus inducing structural discontinuities at cellular and tissue levels. This can result in conduction block and zigzag conduction, both of which permit structurally determined reentrant propagation of cardiac impulse.

Functionally, cardiac remodelling leads to mechanical dysfunction which increases the likelihood of life-threatening cardiac arrhythmias [10]. Consequently, arrhythmias arising from structurally remodelled hearts are caused by changes in electrical properties of cardiomyocytes and/or by the remodelling of the ECM.

Electrically, remodelling of cardiomyocytes affects a large number of ion channels, ionic pumps, and proteins [11, 12]. Furthermore, redistribution and regulation of gap junction proteins (connexins) alter the physiological anisotropic ratio, which causes abnormal impulse propagation, thus enabling reentrant electrical activity [13].

2. Role of Myofibroblasts in Perpetual Atrial Fibrillation

Atrial interstitial fibrosis has been shown to increase with age in humans and has been observed in patients with atrial fibrillation (AF) [14, 15], in animal models of aging [16, 17] and in congestive heart failure [18]. Through these studies, it has been shown that atrial fibrosis creates a substrate that promotes AF. Increased collagen deposition has been documented in patients with AF secondary to mitral valve disease versus those in sinus rhythm [19]. Extracellular matrix volume and composition correlate with AF persistence [20]. These findings highlight the association between atrial fibrosis and AF, although determining the causal importance of tissue fibrosis in AF occurrence and persistence remains an important challenge.

AF is also capable of enhancing atrial fibrosis. In human lone AF, it has been shown that long-term assessment of patients diagnosed with AF, which had normal sized atria upon diagnosis, does lead to structural remodeling of the atria causing atrial enlargement and dilatation over a subsequent period of 20 months [21]. The studies suggest that atrial fibrosis acts as both a trigger and a by-product of AF, potentially through a mechanism affecting signaling pathways associated with atrial dilatation [22, 23].

The mechanism of AF that is associated with an increased level of fibrosis is still under debate as both focal and reentrant mechanisms have been observed in patients and animal models of AF. In the dog model of ventricular tachypacing induced congestive heart failure, atrial fibrosis causes localized regions of conduction slowing, increasing conduction heterogeneity and providing an AF substrate [18]. Conduction abnormalities provide a basis for unidirectional conduction block and macroreentry [24].

In contrast, in the study by Stambler et al. on dogs with rapid ventricular pacing-induced congestive heart failure, AF was shown to be focal in origin caused by triggered activity [25]. This triggered activity was shown to be produced by delayed afterdepolarizations initiated by intracellular Ca$^{2+}$ overload. Drugs that reduced intracellular Ca$^{2+}$ levels (verapamil, flunarizine, and ryanodine) all terminated AF. Fenelon et al. expanded on this study by performing biatrial mapping in dogs with heart failure and showed that the majority of AF episodes had a focal mechanism [26].

There is evidence that atrial fibrosis is associated with a profound remodelling of the atrial pacemaker complex. It has been shown that the function of the SAN declines in AF [27], heart failure [28–30], and with age [31], conditions associated with an increased level of fibrosis. A strong correlation between these conditions and the incidence of sick sinus syndrome has been observed [31, 32]. It should be noted that, histologically, the healthy SAN is distinguished from the surrounding atrial muscle by a remarkably large amount of interstitium (e.g., up to 75%–90% of SAN volume in cat) [33]. It allows SAN electrical insulation from the surrounding atrial myocardium, except for several critical conduction pathways. Indeed, the SAN as a leading pacemaker requires both anatomical (fibrosis, fat, and blood vessels) and/or functional (paucity of connexins) barriers to protect it from the hyperpolarizing influence of the surrounding myocardium [34–36]. The presence of conduction barriers and pathways explain how a small cluster of pacemaker cells in the SAN pacemaker complex manages to depolarize widely distributed areas of the right atria. An increased level of interstitial fibrosis can further insulate the SAN thereby altering the delicate balance between depolarized cells (source) and the resting tissue ahead (sink) [37].

On the other hand, an increased fibrosis can unmask the latent pacemakers by forming specialized, isolated clusters of pacemakers located throughout the atrial pacemaker complex [4, 38]. It has been known for over a century that pacemaker cells are widely distributed throughout the entire region located between the superior and inferior vena cava and between the crista terminalis and intra-atrial septum [33, 39]. Canine and human studies [40–44] have revealed an extensive distributed system of atrial pacemakers, the atrial pacemaker complex, which extends well beyond the anatomically defined SAN and includes primary and subsidiary pacemakers located within the right atrium. Functional anatomy of the atrial pacemaker complex has been extensively studied in mouse models of sick sinus syndrome. Recently, in calsequestrin 2 null mice which were characterized by an increased susceptibility to AF, we have shown, using a high-resolution optical mapping and 3D atrial immunohistology a selective interstitial fibrosis in the atrial pacemaker complex [4], Figure 1. Deletion of calsequestrin 2 depressed primary SAN activity and conduction, but enhanced atrial ectopic activity and AF associated with macro- and micro-reentry during autonomic stimulation (Figure 2). It depressed primary SAN activity and conduction but enhanced atrial ectopic activity and AF associated with macro- and microreentry during autonomic stimulation (Figure 2). Thus, the latent pacemakers will be more stable compared to the primary pacemaker, SAN, probably due to protective electrical insulation role of fibrosis. Such aberrantly isolated clusters of latent pacemakers could become activated and take over the role of the leading pacemaker which can be exaggerated during the abnormal response to autonomic stimulation.
Figure 1: Continued.
Similar results have been observed in other genetically engineered mouse models. Deletion of some structural proteins (such as Cx40 [45, 46], ankyrin-B [38], liver kinase B1 (LKB1) [47], natriuretic peptide receptor C [48], or overexpression of tumor necrosis factor- (TNF-) α [49]) has been linked to enhanced fibrosis, depression of the SAN function, and increased atrial arrhythmogenesis. Shift of the leading pacemaker outside of the SAN structure and a beat-to-beat competition between different pacemakers have been revealed in these mouse models and resulted in heart rate irregularities, tachy-brady arrhythmias, and AF. Interestingly, autonomic stimulation [4] or consecutive thermal ablation of such ectopic sites [45] resulted in leading pacemaker shift back to the SAN but at a prolonged intrinsic cycle length.

In addition to the concept that enhanced interstitial fibrosis contributes to cardiac arrhythmias “indirectly” by affecting passive properties of impulse conduction, recent studies demonstrate that at least a paracrine interaction, or likely a direct electrical coupling, exists between the cardiomyocytes and (myo)fibroblasts (MFBs, see paragraphs below). It has been suggested that structural remodelling including fibrosis of the SAN complex could be attributable to abnormal Ca²⁺ handling in the pacemaker cells [28]. Enhanced diastolic Ca²⁺ could directly lead to increased fibrosis within the SAN complex as well as in the latent pacemaker areas by favouring downstream activation of apoptosis due to cytosolic Ca²⁺ overload. In fact, it has been suggested that chronic Ca²⁺ leak from the sarcoplasmic reticulum can directly stimulate cell damage and fibrogenesis [50]. Increased intracellular [Ca²⁺] could also stimulate activation of the multifunctional Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) which in turn promotes myocardial dysfunction [51] and heart failure [52], SAN cell apoptosis, increased fibrosis and alternating atrial arrhythmogenesis [28].

Finally, the emerging results demonstrate that MFBs in vitro also promote cardiac arrhythmogenesis due to direct implications upon cardiomyocyte electrophysiology [9, 53]. When coupled to cardiomyocytes, MFBs have a depolarizing effect on cardiomyocyte resting membrane potential, which can lead to partial or total sodium channel inactivation. Recent studies have indicated that depolarization in the
AF, CL = 33 ms (1818 bpm), duration = 28 s

Macoreentry

(a) Pacing-induced AF in Casq2−/− heart

Microreentry

(b) Spontaneous AF in Casq2−/− heart

Figure 2: Continued.
resting membrane potential of fibroblasts is the most critical factor promoting cardiomyocyte early after depolarizations ectopic activity [8, 54].

3. Role of Myofibroblasts in the Heart

Under the pathological conditions like hypertension, fibrosis, and infarction, MFBs appear in the myocardium. These cells have an important role in reparative fibrosis; they share a phenotype with fibroblasts and smooth-muscle cells and were first identified years ago in skin wound tissue [55] and granulation tissue [56]. However, it is not yet known if these cells are resident changed-phenotype-fibroblasts, endothelial-mesenchymal derived cells or from fibrocytes [57, 58]. Their role is merely reparative and they disappear following programmed cell death. Currently, the most reliable marker for MFBs is alpha-smooth-muscle actin (αSMA), which is expressed in smooth-muscle cells but not in fibroblasts. It has also been shown that MFBs participate in the process of reparative fibrosis in the lung [59], liver [60], and pancreas [61], where they produce excessive ECM, a process similar to that of fibrotic heart remodelling. The trigger for recruitments of MFBs to the diseased heart is not fully understood. The local upregulation of cytokines including foremost TGF-B1 seems to play a prominent role. However it has been demonstrated that tissue stiffening following excessive ECM deposition drives transdifferentiation of precursor cells into forming fibrogenic MFBs [62]. Myofibroblasts themselves produce uncontrolled ECM; hence, a vicious circle ensues. It has also been shown that variation in oxygen (O₂) concentration plays a key role in the proliferation of cardiac MFBs. Adult mouse cardiac fibroblasts cultured at 21% O₂ express de novo αSMA [63]; in contrast the same was observed when human fetal cardiac fibroblasts were exposed to low percentage O₂ [64].

4. Electrical Communications between MFBs and Adjacent Parenchymal Cells

MFBs form gap junctions with the resident parenchymal cells and can exist in different organs like skin [65], intestines [66], and bladder walls [67]. In the healthy heart, MFBs are present only in the valve leaflets; postinfarct MFBs appear in large numbers a few days after injury at the site of infarction.
These MFBs differ from those in skin wounds as they can persist in the infarct area for 20 years [68, 69] whilst maintaining intimate contact with the surviving cardiomyocytes.

It is assumed that MFBs primarily differentiate from resident fibroblasts. This process is initiated by transforming growth factor β (TGFβ), followed by an activation of several “canonical” cellular pathways (Smad, ERK, P38 kinase, AP-1 but not JNK) [59]. In culture, neonatal rat cardiac fibroblasts undergo transdifferentiation into MFBs. De novo expression of αSMA increases in parallel with the expression of connexin43 (Cx43) [70]; thus, cutback in expression growth factor normally encountered in the infarcted heart.

In vivo our previous investigations into a variety of histoechnology CM cell-cell contacts (Figure 3) [7]. The successful establishment of MFB-MFB cell-cell contacts and importantly also at MFB-cardiomyocyte remain unanswered. It is essential to note that it is not yet reported whether MFBs in vivo establish a heterocellular electronic coupling with cardiomyocytes. We have successfully engineered the heterocellular contact in vitro by coculturing neonatal rat cardiomyocytes and MFBs from cardiac origin (Figure 3). As expected [73], these fibroblasts became MFBs when cultured on rigid substrates (glass coverslips). This was confirmed by observed de novo expression of αSMA (Figure 3(a)). We have also demonstrated that in vitro MFBs express gap junction proteins Cx43 and 45 (not Cx40) at MFB-MFB cell-cell contacts and importantly also at MFB-CM cell-cell contacts (Figure 3(c)) [7]. The successful establishment of this heterocellular contact in vitro together with our previous investigations into a variety of histoarchitecture in vivo now allows for the study of two different situations normally encountered in the infarcted heart.

Immunohistochemical images of chronic infarct in rat cardiac tissue (37 weeks) shown in Figure 4 demonstrate that there is an intimate contact between the areas heavy populated with MFBs (αSMA, brown) and the areas of surviving cardiomyocytes (white). We hypothesised that an area of MFBs might (i) interrupt or affect impulse propagation and (ii) induce ectopic activity, due to electrical coupling.

5. Areas of Myofibroblasts Linking Up Separate Bundles of Cardiomyocytes

The general assumption is that cardiac impulse conduction is blocked at site where cardiomyocyte areas are in contact with collagenous septa or with fibrotic tissue (like infarct regions or sutures site follow heart transplantation). This latter case sporadically reports an unexpected synchronization between donor and recipient heart [74]. Because MFBs are present in the fibrotic tissue, we tested this hypothesis, by engineering the situation represented in Figure 4(a) top, in vitro, by seeding cardiomyocytes in a geometrical defined pattern, and interrupted them with an insert of MFBs (Figure 4(a), bottom).

Details of the experiments are represented in Figure 5(a) [6]. Strands were stimulated from the left hand side and the characteristics of impulse propagation were assessed optically after being exposed to a voltage sensitive dye [75]. For the final analysis, we took into consideration only the insert of MFBs without “cardiomyocytes contamination” (Figure 5(a), lower panel). Each photodiode recorded an optical action potential upstroke, which was correlated with the activation time. Whereas activation time was rapid in the cardiomyocyte area, a passive local electrotonic transmission induced a delay of 30 ms across the MFB insert (Figure 5(c)). As shown in Figure 5(d), the delay is strictly related to the length of the insert. Under these experimental conditions MFBs can support impulse propagation up to ~320 μm; at lengths greater than this, propagation invariably failed. These experiments demonstrate that the heterocellular electrical coupling between the two cell populations can reinstate conduction across an interrupted network of cardiomyocytes resulting in a discontinuity of propagation. In consequence, one has to take into consideration that patchy fibrosis as encountered in the remodelled atria (i.e., ageing) [76] may alter the normal pattern of propagation by inducing discontinuous slow conduction, playing a key role in the context of reentrant circuits.

6. Myofibroblasts Overlaid as to Completely Cover an Area of Cardiomyocytes

To further investigate any direct electrical coupling consequences, MFBs were also cultured in order to overlay the cardiomyocytes. In Figure 4(b), the situation is such that the fibrotic area is heavily populated with MFBs, which infiltrate and diffuse throughout the area of cardiomyocytes, thus increasing the heterotissue interaction (as compared with Figure 4(a)). Due to the depolarized resting membrane potential of MFBs [7], we hypothesized that in this circumstances, together with the increased electrical cell-to-cell interaction area, might produce a large depolarized region which can affect local impulse propagation. This, in turn, might reduce the conduction velocity for the cardiac tissue, which is in contact with MFBs. Using the same pattern growth technique we engineered 80 μm wide strands of neonatal rat ventricular cardiomyocytes, to which a layer of MFBs is seeded on top of [7] (Figure 4(b), bottom). The conduction velocity (θ) in control preparations, which are virtually devoid of MFBs, is high (~43 cm/s, Figure 6(a), left). However, the presence of MFBs drastically reduces θ by up to 25 cm/s (Figure 6(a), right). An overall analysis of θ dependence on MFB coverage area is represented in Figure 6(b) left; θ denotes biphasic behaviour towards the number of MFBs per measured area. This behaviour is highly reminiscent of the phenomena of supernormal conduction in cardiac tissue investigated both in vivo [77] and in vitro [78]. Both demonstrated that θ is biphasically dependent on the gradual increment of extracellular potassium concentration. Similar, but in a MFBs density-dependent manner, our results show a similar behaviour, suggesting that MFBs may directly depolarize cardiomyocytes resembling the well-known depolarizing effect of $[K^+]_\text{out}$. This hypothesis was proved by conventional intracellular microelectrode techniques for measuring diastolic resting membrane potential ($V_{m}$). We found that MFBs gradually depolarized cardiomyocytes in a density-dependent manner (Figure 6(b), right) where recorded $V_{m}$
dropped from $\sim -80$ mV at a MFB density less than 5% to $\sim -55$ mV when more than 40% of the cardiomyocytes area is covered by MFBs. This data propose that, assuming the same effect in vivo at the epicardial border zone where the minimal wall thickness is comparable to a two-dimensional layer, infiltrated laminae of MFBs might induce epicardium slow conduction velocity. In contrast, in a 3D architecture (ventricular wall) the coupling of cardiomyocytes bordering infarct area might counterbalance this depolarizing effect. The consequences in the context of AF are clear: MFBs can directly depolarize the surrounding cardiomyocytes tissue and thus lead to local conduction slowing and enhance the likelihood of arrhythmia (cf. paragraph below).

7. Myofibroblasts Induce Ectopic Activity in Cardiac Tissue

The last part of the study sought to investigate the intimate contact between cardiomyocytes and MFBs (Figure 4(b),
Figure 4: Characteristics of heterocellular interaction between myofibroblasts and cardiomyocytes in regionally infarcted rat heart. (a) Top. Immunohistochemistry picture of rat heart slices after 37 weeks of coronary occlusion. A region of myofibroblasts (brown) stained for αSMA physically separates two bundles of cardiac myocytes. Bottom. Schematic representation of heterocellular culture mimics the in vivo situation. (b) Same as A with a MFBs stratum infiltrated into cardiac tissue (top) with schematic represented situation (bottom) (with courtesy: Dr. Alex Lyon, NHLI, Imperial College, London. Unpublished).

Figure 5: Myofibroblasts act as passive electrical paths for impulse conduction. (a) Heterocellular construct of a left-stimulated strand of cardiomyocytes (top) interrupt by a pure region (length = 134 μm) of myomesin-deficient myofibroblasts (bottom). Circles indicate the optical mapped area detected from each photodetector. (b) Optical action potential upstrokes recorded in a detected length of ∼50 μm. (c) Activation times reconstruct from each action potential upstrokes; in both cardiomyocytes areas, the activation is almost immediate whereas the propagation into myofibroblast area exhibits a delay of 30 ms. (d) Summary of propagated delays related to the inserts’ length, therefore indicating a passive electronic transmission of up to ∼320 μm (modified with permission from [6]).
right) and how ectopic electrical activity was elicited following heterocellular coupling [8]. An in vitro fibrotic situation was created by coating strands of neonatal rat ventricular myocytes with increasing densities of MFBs (Figure 7). Spontaneous electrical activities were recorded for 4 seconds. The overview of preparation in Figure 7(a) corresponds to a single frame taken from such recording. In this point in time the quasicompletely coverage of MFB elicits spontaneous activity in two strands; the recording shows action potentials occurred regularly with a frequency of 75 bpm. Crucially, the presence of spontaneous activity is strictly proportional to MFBs densities (Figure 7, top). When MFB coverage was
Figure 7: Myofibroblasts induce ectopic activity. (a) Upper row: overview of a preparation consisted of 24 myofibroblasts coated with cardiomyocyte strands (0.6 × 4.5 mm). Right: details of the microarchitecture (a phase contrast photo) and of the MFB cover layer (vimentin immunostaining) of an individual strand. Lower row: propagated action potential recorded optically at specific sites (blue and red circles in the overview). (b) Left: ectopic spontaneously active strands and spontaneous frequency correlate with myofibroblast density. Spontaneous activity is invariably absent when myofibroblast density is less than 15.7% of the total area examined. Right: electrical spontaneous activity correlated with maximal diastolic potential. Similar to active strands where membrane potential threshold for elicit automaticity is −65.9 mV, single cardiomyocytes elicit spontaneous action potentials at −67.4 mV (modified with permission from [8]).

below 16% all the preparations were invariably quiescent; however, when coverage was above ~80%, all preparations exhibited spontaneous activity. In contrast, we found beat frequency reduced from ~80 bpm (20% MFBs density) to ~40 bpm (60% MFBs density) due to progressive reduction in the diastolic membrane potential (Figure 6(b)). A comparison of cardiac membrane potential between isolated cardiomyocytes and heterocellular strands is demonstrated in Figure 7(b), right. Gradual reduction of resting $V_{m}$ in single cardiomyocytes was examined using a patch clamp technique. Stepwise depolarization during injection of 30 sec long current pulses exhibits electrical spontaneous activity elicited at membrane potential less negative than −67 mV. These results were similar to those found in the heterocellular strands where spontaneous impulse initiation was induced with a minimal density of MFBs corresponding to ~−66 mV.
These findings indicate that the heterocellular coupling between MFBs and cardiomyocytes might structurally form an ectopic focus. The firing area is preferentially generated from MFBs and not from the injured cardiomyocytes. In our experiments, the cardiac network appears healthy but ectopic activity could be as well induced.

8. Is the Myofibroblast a Possible Target for Atrial Fibrillation?

Recently we have investigated the possibility to target MFB in order to suppress electrical disorders in the heart. Thanks to the collaboration with Professor Gorelik and Professor Williamson at Imperial College London, we have discovered that MFBs transiently appear during heart development and they can be responsible for fetal arrhythmias [79]. Clinically, it has been associated with a pregnant disease called intrahepatic cholestasis and the prognosis ameliorates after administration of ursodeoxycholic acid (UDCA). Because MFBs tend to depolarize the coupled cardiomyocytes, we sought to investigate whether UDCA may directly target MFBs. We found that UDCA hyperpolarizes MFB membrane potential by targeting the sulphonylurea receptor of $I_{K1}$ channel [9], reestablishing the normal conduction velocity and terminating reentrant arrhythmia (Figure 8). A double-blind randomized placebo-controlled crossover trial is under investigation by administering UDCA in patient with chronic heart failure [80].

9. Outlook

Electrical communication between the stromal and parenchyma tissue has been the focus of much research over the last 50 years. Certain “myths,” like that we are using
Noncardiomyocyte centric view: myofibroblasts in AF

**Figure 9:** Schematic representation of a “well-known” (indirect) and “proposed” (direct) involvement of myofibroblasts in atrial fibrillation (AF).

a total of 10% of our brain, have been dispelled, including the discovery that indeed only 10% of the human brain is made of neurons, with the rest comprised of “Glia,” identified as stromal “glue” or nerve “putty,” which merely fills the spaces within the parenchyma tissue. In the last two decades researchers have shown that Glia cells express gap junctions and interact directly with neurons \[81, 82\]. In vitro, stromal cardiac MFBs are electrically and mechanically coupled with cardiomyocytes and this pairing disturbs the electrical homeostasis of the parenchymal cardiac tissue \[83, 84\]. If the same situation in pathological cardiac tissue will be observed \textit{in vivo}, MFBs could be considered as a new cellular target for cardiac arrhythmia (Figure 9). Strategies might focus on (i) inducing the MFBs “inactive” (overturn the phenotype back to fibroblast and thus circumvent the electrical coupling) or (ii) targeting these cells, pharmacologically or genetically, for radically hyperpolarization \[9, 85\]. Evidence obtained so far requires further characterization in order to fully understand the impact(s) of heterocellular interactions upon the complex 3D remodelling of the cytoarchitecture, which occurs during heart failure.

Interestingly, there is speculation that MFBs might appear transiently during heart development and follow the partial state of fetal hypoxia \[64\] and in the aged heart \[76, 86\]. Additional studies are necessary to understand if MFBs are not only proarrhythmogenic in heart failure but also during heart development and aging; these are frequently subjected to other pathological “MFB-triggering” situations (diabetes, autoimmune disorder, and metabolic diseases).

Problems regarding engraftment for tissue regeneration have also been investigated and reveal that if other cells, less polarised than cardiomyocytes, were to form gap junctions with cardiomyocytes, spontaneous activity could be induced \[87\]. This was highlighted by our study where coating cardiomyocytes with Cx43-transfected-HeLa cells (\[8\], data not shown) give rise to spontaneous activity. Further studies are necessary in order to understand and predict an accurate arrhythmogenic mechanism following cell engraftment in heart failure models using possibly or conductive patches \[88\], embryonic cardiomyocytes \[89\], and progenitor stem cells \[90\]. Regarding the cell therapy, it is also unclear whether engraftment will perturb the electrical homeostasis of cardiac tissue due to an intrinsic resting membrane potential, and there exists the possibility that the engrafted cells might electrically couple with MFBs. Both of these may cause the regenerated cardiac tissue engraftment to become an unexpected source of arrhythmia.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Research Article

Results from the Registry of Atrial Fibrillation (AFABE): Gap between Undiagnosed and Registered Atrial Fibrillation in Adults—Ineffectiveness of Oral Anticoagulation Treatment with VKA


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Objective. This study aimed to examine the effectiveness of the use of oral anticoagulation (OAC) medication, recommended by national guidelines for stroke prevention but reportedly underused in AF patients with moderate to high stroke risk. Method. A multicentre and cross-sectional study of undiagnosed AF among out-of-hospital patients over 60 years old was carried out, visiting 3,638 patients at primary health centres or at home for AF diagnosis using the IDC-10 classification. The main outcome measures were CHA<sup>2</sup>DS<sup>2</sup>VASC scores, cardiovascular comorbidity, pharmacological information, TTR, and SAMe-TT2R2 scores. Results. The main findings were undiagnosed AF in 26.44% of cases; 31.04% registered with AF but not using OAC despite 95.6% having a CHA<sup>2</sup>DS<sup>2</sup>VASC ≥ 2 score; a risk of bleeding in important subgroups using OAC without indication (37.50% CHA<sup>2</sup>DS<sup>2</sup>VASC < 2 score); the use of OAC with TTR < 60% (33.1%), of whom 47.6% had a HAS-BLED score ≥ 3. Thus, 35.4% of the expected AF prevalence achieved an optimal time in the therapeutic range. Conclusions. The expected AF prevalence was 10.9% (n 5267), but the registered prevalence was 7.5% (n 3638). Only 35.04% (CI = 95%, 33.7–36.3) of AF patients treated with vitamin K antagonists (VKAs) achieve the goal of TTR > 60%.
1. Introduction

Atrial fibrillation (AF) is a common cardiac arrhythmia [1] that affects 1-2% of the general population and accounts for one-third of hospitalizations for heart rhythm disturbances. The risk increases with age [2]. With the aging of the population, the number of patients with AF is expected to increase 150% in the next four decades, with more than 50% of patients being over the age of 80. This increasing burden from AF will lead to a higher incidence of stroke, as patients with AF have a five- to sevenfold greater risk of stroke than the general population [3, 4]. Strokes secondary to AF have a worse prognosis than in patients without arrhythmia. In addition, the costs of managing AF patients and its complications have been well documented and are high [5]. This will have serious implications for the planning of health and welfare systems, not only because of predictions of a continuous increase in AF prevalence [3, 6, 7] given the close association between arrhythmia and aging, but also because of the current cost constraints due to the economic context.

Due to the associated increased morbidity, mortality, and cost, challenges in the identification of patients at risk for thromboembolic events from AF must be addressed. AF is often only detected with the onset of severe AF-related complications such as stroke or heart failure [8, 9]. Although national guidelines recommend the use of oral anticoagulation (OAC) medication for stroke prevention and there is clear evidence of the effectiveness of vitamin K antagonist (VKA) therapy in patients with AF [10], the literature consistently reports its underuse in AF patients with moderate to high stroke risk [1, 11]. This underutilization imposes a substantial clinical and economic burden on healthcare systems. Finally, the percent time in therapeutic INR range (TTR) has been used to evaluate the effectiveness of VKA therapy as a quality measure, but there is a general lack of quality measurement in OAC use. Data show that if the TTR is < 50%, the result is actually worse than not using any warfarin at all, whereas when the therapeutic range is at least 70%, the likelihood of stroke or systemic embolism is very small [12]. This paper highlights the results of clinical practice in patients with AF, focusing on the assessment of results in the rates of appropriate use of and patient adherence to OAC treatment plans administering VKAs (warfarin/acenocoumarol) beyond simply examining the percentage of AF patients treated with OAC.

The aim of the study is to document the quality of anticoagulant control in primary care, considering the potential impact of undiagnosed AF, the underutilization of VKAs, and results related to TTR. The challenges include compliance with performance measures, adherence to guidelines, adequate prevention, and early control of comorbidities that affect the progression of AF and associated risks, early initiation of treatment, and successful evaluation of the associated risks of bleeding, primary or recurrent stroke, and patient awareness and compliance [1, 8, 13].

2. Materials and Methods

The AFABE [8, 13] study is a cross-sectional, multicentre study of undiagnosed AF among out-of-hospital patients over 60 years old attending primary care teams in the Terres de l’Ebre health area in Catalonia, north-eastern Spain, on 31 July 2014. The patients in the sample were registered with health centres and were visited there or at home for AF diagnosis according to the routine ICD-10 classification used in the primary care dataset for a revision of the electronic medical history. The variables for which data were collected are as follows.

(1) Patient Identification Code. It includes individualized TIS number (individual health card used in Catalonia).

(2) Sociodemographic Information. It includes age, gender, and place of residence.

(3) Cardiovascular Information. We described clinical comorbidities included in the cardioembolic CHA2DS2-VASc rule [14, 15] (congestive heart failure; hypertension; age ≥ 75 years [doubled]; type 2 diabetes; previous stroke, transient ischemic attack, or thromboembolism [doubled]; vascular disease; age 65–75 years; sex category) and HAS-BLED (hypertension, abnormal renal/liver function, stroke, bleeding history or predisposition, labile INR, elderly, and drugs/alcohol concomitantly) [16, 17] codistribution representing bleeding risk among AF patients treated with VKAs. We considered them “previous” when they had been diagnosed and registered at least one month before the AF diagnosis and “later” when they had been diagnosed and registered simultaneously to or after AF diagnosis. Patients with a CHA2DS2-VASc Score ≥ 2 were categorized as high stroke risk and those with a HAS-BLED score ≥ 3 were categorized as high bleeding risk. We studied the age-specific incidence of all AF-related annual stroke rates, extrapolating average CHA2DS2-VASc score values to the population and projecting future numbers [18, 19].

(4) Pharmacological Information. It includes drugs assigned as clinical treatment for all conditions including AF; antiarrhythmic agents received as a rhythm control strategy (class I/class III), with or without rate control strategy (class II/class IV, digital) and/or antithrombotic treatment; OAC treatment with VKAs (warfarin/acenocoumarol) or NOAC therapy and/or antiplatelet treatment and/or angiotensin-converting enzyme (ACE) inhibitors and/or statins. We used the concept “polymedication” (the prescription of at least 10 different medications simultaneously) to seek a possible relation with the percent time in therapeutic INR range.

(5) Diagnosis Dates. AF incidence, cardiovascular comorbidities, and death dates (all-cause mortality) are registered in patients’ electronic medical PC. All the diagnostics were defined in the patient dataset using the ICD-10 classification. As this was a retrospective study of confirmed AF, we did not include cases with a changed diagnosis of AF or with unconfirmed AF. The registered AF prevalence included people who were a case with a diagnosed and registered AF in their public health primary care electronic medical history according to the ICD-10 routine classification used in the primary care dataset for a revision of the electronic medical history. Based on the census of 2011, the expected
AF prevalence was calculated using the data obtained in the AFABE study [8, 13]. The AFABE (Baix Ebre) study was a sample of the current population (Figure 1).

(6) INR Control. The TTR for individual patients was estimated by Rosendaal method [20], using linear interpolation to assign an INR value to each day between two successively observed INRs. If the sampling interval exceeded 60 days, values were not interpolated. Patients with less than three consecutive INRs were excluded to achieve a meaningful estimation of the TTR. Likewise, the first two weeks of INRs were excluded from the analysis for patients who began warfarin treatment. Adult patients with AF who used warfarin for a 12-month period with no gap of > 60 days between visits were identified and the records collected were analyzed. We considered the average time in therapeutic range to be lower if it was < 60%. VKAs (warfarin/acenocoumarol) are the anticoagulant therapy of choice in Catalonia for patients with AF who are at risk of stroke. The patients were stratified according to their proportion of time in range.

(7) SAME-TT$_2$R$_2$ Score [21]. It includes sex, age (<60 years), medical history (at least two of the following: hypertension, diabetes, coronary artery disease/myocardial infarction, peripheral arterial disease, congestive heart failure, previous stroke, pulmonary disease, and hepatic or renal disease), and treatment (interacting drugs, e.g., amiodarone for heart rhythm control) [all 1 point], as well as current tobacco use (2 points) and race (non-Caucasian; 2 points). The SAME-TT$_2$R$_2$ score was calculated for all patients with a diagnosis of AF, but as it makes a simple prediction of which AF patients are likely to do well on VKAs (with an average time in therapeutic range ≥ 60%), the TTR percentage included just those patients using VKAs. We tested the hypothesis that the new SAME-TT$_2$R$_2$ score was a predictor for good average time in therapeutic range and, second, that this would translate into adverse events in a “real-world” cohort of patients with AF.

2.1. Statistical Analysis. In the descriptive analysis, the data for categorical variables are expressed as number of cases and percentages and the data for continuous variables are expressed as means with standard deviations and/or IC95%. Categorical variables were compared using a $\chi^2$ test or Fisher’s exact test depending on the application conditions. Continuous variables were compared using Student’s $t$-test or the Mann-Whitney $U$ test depending on the normal distribution assumption. Normal distribution was checked using the Shapiro-Wilk test. A logistic regression analysis was performed to find possible risk factors that characterize the population with AF. A $P$ value of less than 0.05 was considered to indicate statistical significance in all statistical tests. The analysis was carried out using the SPSS statistical software package (version 19).

3. Results

The Ebro Lands study population (Figure 1) comprised 48,325 ≥ 60 years old in the census of the territory. Of these, 92% use primary care services. Their mean age was 78.7 years (SD = 7.3) and 53.6% were men. We examined 3,638 (1,689
TABLE 1: Subjects’ baseline data and cardiovascular risk factors in AF patients and the average CHA2DS2-VASc and HAS-BLED scores.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Registered AF prevalence (N 3638)</th>
<th>CHA2DS2-VASc score ≥2 (95.6%)</th>
<th>HAS-BLED score ≥3 (95.6%)</th>
<th>TTR ≥60 (%)</th>
<th>Vitamin K antagonism (VKA) therapy (95.6%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (%)</td>
<td>1688 (46.4)</td>
<td>95.6 (CI = 95%, 92.9–98.2)</td>
<td>47.6 (CI = 95%, 45.7–49.4)</td>
<td>67.0 (CI = 95%, 65.2–68.8)</td>
<td>68.9 (CI = 95%, 67.3–70.4)</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>78.7 ± 7.30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ≥75 y (%)</td>
<td>74.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (% CI = 95%)</td>
<td>77.1 (CI = 95%, 71.9–82.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus (% CI = 95%)</td>
<td>26.5 (CI = 95%, 21.1–32.01)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vascular disease (% CI = 95%)</td>
<td>14.7 (CI = 95%, 10.35–19.16)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous stroke/TIA (% CI = 95%)</td>
<td>17.4 (CI = 95%, 12.65–22.03)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart failure (% CI = 95%)</td>
<td>22.8 (CI = 95%, 17.7–20.06)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thromboembolism (% CI = 95%)</td>
<td>2.2 (CI = 95%, 0.27–4.15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The registered AF prevalence was 7.5% (CI = 95%, 7.3–7.7); when stratified by gender and age (Figure 2), the groups progressively increased. The average age at AF diagnosis was 73.65 ± 8.0 years; 75% were ≥ 75 years.

Table 1 shows the group baseline data and cardiovascular risk factors in AF patients and the average CHA2DS2-VASc and HAS-BLED scores; these are stratified by age in Table 2. A high prevalence of cardiovascular risk factors (CVRF) was found for hypertension (HTA, 77.1%) and diabetes mellitus type 2 (DM2, 26.5%). Men had significantly more prevalence of DM2, previous stroke, vascular diseases, and smoking. The average CHA2DS2-VASc score was 3.6 and 95.6% of subjects had a CHA2DS2-VASc score ≥ 2. The older the patient (increasing up to 85 years), the higher the CHA2DS2-VASc score.

The average HAS-BLED score was registered in 69.5% of patients. The average score was 2.3 and 47.6% of subjects treated with VKAs had HAS-BLED ≥ 3. The proportions of subjects with abnormal renal function and abnormal hepatic function were 14.4% and 15.1%, respectively. Risk factors related to a history of or predisposition to bleeding (2.3%) and chronic concomitant use of antiplatelet and/or anti-inflammatory (5.2%) treatment were less frequent. The older the patient (increasing up to 85 years), the higher the HAS-BLED score.

Before the AF diagnosis, 36.8% (CI = 95%, 30.8–42.7) had been diagnosed with some cardiovascular complication (CVC). Almost half of the overall CVCs were ischemic cardiomyopathy (24.2%) and ischemic stroke (23.2%). The incidence of ischemic cardiomyopathy was significantly higher among men (P = 0.031), while the incidence of ischemic stroke was similar among men and women (P = 0.612). There were no differences in the overall incidence of CVC by gender. Patients who had suffered ischemic stroke or ischemic cardiomyopathy previously were at greater risk (OR = 2.63) of suffering AF than those who had not. Mortality was higher (P = 0.05) among those ones who had been diagnosed with ≥ 2 CVCs before the AF. On other hand, mortality was significantly lower among those who were treated with statins (P = 0.032).

Simultaneous to or after AF diagnosis, 28.6% (CI = 95%, 38.1–50.3) were diagnosed with new vascular complications. The most frequent vascular complication was congestive heart failure (CHF, 46.7%), the incidence of which was significantly higher among women (P = 0.037). The five-year survival rate with a diagnosis of CHF is lower (0.69 ± SD 0.09) than when there is no CHF present (0.96 ± DE 0.01). The main predictor of mortality is a nontreatment with OAC, with significantly lower mortality in patients treated with OAC (P = 0.003) versus antiplatelet treatment (Figure 3). There were no differences in the overall incidence of cardiovascular complications by gender.
Table 2: AF prevalence, TTR, and average CHA<sub>2</sub>DS<sub>2</sub>VAS<sub>C</sub> and HAS-BLED scores stratified by age.

<table>
<thead>
<tr>
<th>Age</th>
<th>60–64</th>
<th>65–69</th>
<th>70–74</th>
<th>75–79</th>
<th>80–84</th>
<th>&gt;85</th>
<th>Total/mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (N)</td>
<td>9840</td>
<td>9690</td>
<td>7993</td>
<td>7146</td>
<td>6864</td>
<td>6792</td>
<td>48325</td>
</tr>
<tr>
<td>Men</td>
<td>4918</td>
<td>4756</td>
<td>3868</td>
<td>3331</td>
<td>2870</td>
<td>2543</td>
<td>22286 (46.1%)</td>
</tr>
<tr>
<td>Women</td>
<td>4922</td>
<td>4934</td>
<td>4125</td>
<td>3815</td>
<td>3994</td>
<td>4249</td>
<td>26039 (53.8%)</td>
</tr>
</tbody>
</table>

Registered cases
Prevalence of AF

<table>
<thead>
<tr>
<th>N (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>(1.88)</td>
<td>(3.09)</td>
<td>(5.51)</td>
<td>(9.61)</td>
<td>(13.57)</td>
<td>(16.09)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Expected cases (AFABE)
Prevalence of AF

<table>
<thead>
<tr>
<th>N (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>(2.4)</td>
<td>(3.4)</td>
<td>(10.9)</td>
<td>(14.0)</td>
<td>(19.6)</td>
<td>(24.4)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
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</table>

Absolutedifference

<table>
<thead>
<tr>
<th></th>
<th>−51</th>
<th>−29</th>
<th>−430</th>
<th>−313</th>
<th>−413</th>
<th>−564</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>−1630</td>
</tr>
</tbody>
</table>

Average CHA<sub>2</sub>DS<sub>2</sub>VAS<sub>C</sub>score

<table>
<thead>
<tr>
<th></th>
<th>3.3%</th>
<th>8.9%</th>
<th>12.6%</th>
<th>24.5%</th>
<th>29.4%</th>
<th>21.2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>1.22</td>
<td>2.20</td>
<td>2.76</td>
<td>3.92</td>
<td>4.06</td>
<td>4.07</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.60</td>
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</tbody>
</table>

HAS-BLED ≥ 3 (%)

<table>
<thead>
<tr>
<th></th>
<th>1.12%</th>
<th>14.6%</th>
<th>13.48%</th>
<th>24.71%</th>
<th>29.21%</th>
<th>16.85%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47.6%</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total AF and no OAC (%)

<table>
<thead>
<tr>
<th></th>
<th>27.5%</th>
<th>15.8%</th>
<th>51.2%</th>
<th>32.8%</th>
<th>31.9%</th>
<th>36.1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>(21.6–33.4)</td>
<td>(11.7–19.9)</td>
<td>(47.8–54.5)</td>
<td>(29.8–35.9)</td>
<td>(29.4–34.5)</td>
<td>(33.8–38.4)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TTR ≥ 60%

<table>
<thead>
<tr>
<th></th>
<th>66.2</th>
<th>65.3</th>
<th>69.1</th>
<th>66.3</th>
<th>68.1</th>
<th>66.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>67.03%</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

SAME-TT<sub>R</sub> ≥2

<table>
<thead>
<tr>
<th>N (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>(36.8%)</td>
<td>(40.0%)</td>
<td>(44.2%)</td>
<td>(49.1%)</td>
<td>(50.4%)</td>
<td>(57.6%)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Registered prevalence of stroke and AF

<table>
<thead>
<tr>
<th>N (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>(9.7%)</td>
<td>(13%)</td>
<td>(10.4%)</td>
<td>(14.9%)</td>
<td>(15.0%)</td>
<td>(20.0%)</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Registered AF incidence/1000/year

<table>
<thead>
<tr>
<th>n/1000/year</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>2.8</td>
<td>4.1</td>
<td>8.4</td>
<td>11.6</td>
<td>13.1</td>
<td>19.1</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>438</td>
</tr>
</tbody>
</table>

The prevalence of ischemic stroke and AF was 15.07%. There were 438 incidents of AF per year, 9.1 (CI = 95%, 8.2–10.0)/1000 patients ≥ 60 years old per year. Of 565 incidents of ischemic stroke, 359 (63.5%) occurred at ≥ 80 years (Table 2). Overall, the incidence of AF-related strokes was 1.11 (CI = 95%, 0.9–1.5)/1000 AF patients ≥ 60 years old per year. The numbers of AF-related strokes at age ≥ 80 years were double the average incidence (Table 1). In the Cox regression, after adjusting for age, gender, number of cardiovascular complications before and after AF diagnosis, OAC treatment, antiplatelet treatment, and other specific treatments, the only variable with a protective value against mortality was antithrombotic treatment (HR = 0.344, CI = 95%, 0.163–0.728) (Figure 3). The overall percentage of patients not treated with OAC was 26.9% (CI = 95%, 22.7–30.9). In all, 4.2% were treated with NOACs (apixaban, dabigatran, or rivaroxaban) and 18.9% with antiplatelet drugs. In terms of the expected AF prevalence [9, 10], the percentage of undiagnosed AF rises with age. Approximately 1,630 AF patients could have remained undiagnosed and the overall average percentage without OAC treatment was 31.0% (CI = 95%, 29.7–32.3). It
is notable that the greatest proportion of AF cases was in patients aged 70–74 years (51.2%; CI = 95%, 47.8–54.5) with unknown AF and no OAC treatment. The percentage of AF with no OAC treatment rises with age (Figure 4).

The percentage of patients with time in therapeutic range (TTR) < 60% was 33.1% (CI = 95%, 30.5–35.6) for those using VKAs. This research identified a high rate of patients with anticoagulant therapy in primary healthcare (> 90%). Most patients take coumarins and the quality of OAC control is reasonably high. In all, 50.5% had a SAME-\textsuperscript{TT\textsubscript{2}}R\textsubscript{2} score ≥ 2 and the percentage gradually increased in patients from 60 to 64 years (36.8%) up to > 85 years (57.6%). Of these, 54.6% had TTR < 60% and the ROC curve results were 0.48 (CI = 95%, 0.46–0.50), sensitivity 0.62, and specificity 0.29.

It is possible to improve patients’ control of their VKA serum levels in various ways, but clearly it is necessary to address risk conditions reflecting poor anticoagulation control and labile INRs among patients with AF given that OAC treatment seems to depend solely on these. This study has found a gap (30.9%) between expected and registered AF prevalence and registered AF with no use of OAC treatment (26.9%) despite 95.6% having a score of CHA\textsubscript{2}DS\textsubscript{2}VAS\textsubscript{C} ≥ 2 and there being a group at special risk (70–74 years) of whom 47.6% had a score ≥ 3 for HAS-BLED. Furthermore, there is a risk of bleeding in important subgroups using OAC without indication (37.50%, CHA\textsubscript{2}DS\textsubscript{2}VAS\textsubscript{C} < 2). In this study, one-third (33.1%) of AF patients using OAC showed a time in therapeutic INR range < 60%; the older the patient, the higher the risk of TTR < 60%. According to the panel data [1], only 35.4% (CI = 95%, 33.7–37.3) of AF patients treated with VKAs achieved the goal of optimal effectiveness in order to secure clinical benefits (Figure 5).

4. Discussion

This paper focuses in particular on the overall prevalence of AF increasing with age and its related possible consequences, that is, more undiagnosed AF. Furthermore, the potential increase in the percentage of AF not treated with OAC carries the foreseeable threat of an increase in cardiovascular morbidity and associated costs, primarily caused by ischemic stroke, stroke, and CHF with the requirement for chronic use of medication. Our main conclusion, unlike other studies, is not much the relative underuse of the VKA treatment in high risk AF patients but the low efficiency resulting from the association between its underuse and undiagnosed AF.

The rate of undiagnosed AF for subjects over 60 years old was 3.4%, representing 31% of the overall AF prevalence in our study, compared to a percentage between 25% and 35% in other studies [22–26]. Although it is practically impossible to reduce the prevalence of undiagnosed AF to zero, it is possible try to reduce it. New external devices that register prolonged intermittent arrhythmia have substantially improved the detection of silent paroxysmal AF in patients with recent ischemic stroke/transient ischemic attack [29]. Nevertheless, until these new external devices can be used more widely, ECG combined with reviews of medical history
The prevalence of AF is high and rising. This work has paid particular attention to the alarming increase in overall AF prevalence, especially in people $>70$ years, the prevalence of AF being $>20\%$ at age $\geq80$ years. Similar results have been found [30] suggesting that the total number of people with AF in a practice could be around 10\% of the number of people aged 60 and over. From the sixties to over the eighties the prevalence increases by 10 times [13]. The population over 80 in the last 30 years has grown by approximately 66\%, representing an increase from 3.5\% to 5.9\% of the total population. At the current incidence rates, the numbers of AF-related embolic events at age $\geq80$ years will treble by 2050, with most events occurring in this age group. Approximately 15\% to 25\% of ischemic strokes are attributed to AF [3, 4], a similar proportion to that in our results. Among patients $\geq80$ years with AF, the effectiveness of anticoagulation treatments should be a major public health priority [31], although the impact of population aging on rates of AF-related ischemic events is uncertain.

The percentage of undiagnosed AF should be added to the percentage of known AF not treated with OAC, representing 40–50\% [28] of the overall AF. Clearly, if we add the TTR results, this percentage lowers even more. The reasons for the underuse of anticoagulation are poorly understood. There is a complex interaction between patient-, physician-, and healthcare system-related factors.
In addition, polypharmacy observed for increasing bleeding risk within cardioembolic patients treated with VKAs scored had one or more additional morbidities present was almost of treatment. Of the people with a stroke, the proportion that is an important marker of both multimorbidity and burden strokes occurred at an increase in patients on warfarin and 40% of haemorrhagic statistically significant difference (percentage of haemorrhagic stroke can be observed, progressing falls should be evaluated thoroughly to determine the causes. Looking at the NOACs together, there is evidence of a significant reduction in intracranial haemorrhage [47] and also in stroke or systemic embolism. These are safer and less expensive socially [48] and facilitate management in the geriatric population with AF: no INR monitoring is needed, there is easier bridging during interventions, and there are fewer risks and better results. Furthermore, based on the data available, they exhibit a better benefit-risk ratio compared to VKAs. Drugs with predominantly nonrenal elimination are safer in geriatric patients and should be preferred [39]. We should consider NOACs an interesting option in slowing down the current evolution of approximately 30% fewer strokes every year. This management decision is often complex and involves taking into account contraindications, financial constraints, patient preferences, and cost-benefit analyses. NOACs are more likely to be cost-effective options in settings with poor warfarin management than in settings with better anticoagulation control, where they may not represent good value for money [49].

Our regional findings reflect the care provided by a limited set of investigators in any geographic region. These differences may explain, in part, the current divergence of anticoagulation treatment decisions from guideline recommendations, but we believe that it is essential to achieve quality assurance information on anticoagulation therapy at a local level beyond just the treatment coverage. In particular, we consider it very important to determine the expected number of AF patients according to the demographic characteristics of the beneficiary population as undervaluation results from solely describing AF patient numbers with OAC treatment. Also, access to electronic support tools in clinics using TTR for monitoring could lead to an increase in quality and would allow for ascertaining in detail how the GPs managed all the aspects of the treatment [50]. The recent approvals of several new, novel OAC agents with a benefit/risk profile that represents an important advance over VKA prophylaxis [51] have given rise to great expectations in the management of these patients but also new doubts. The main limitations to their general use are the lack of data for some subgroups of frail patients and the lack of availability of specific antidotes and especially their high cost.

5. Study Limitations

As this is a study of subjects requesting primary care attention (at health centres, their own homes, or their care or nursing
homes), it is possible that a higher frequency of AF patients or patients with AF risk factors could have produced an artificial increase in the prevalence described. The strengths of the present study include a population-based design and its reflection of routine clinical practice. Minimal exclusions were employed as the exclusion of patients with missing data would potentially have introduced selection bias. The risk of referral bias was low as it can be assumed that all patients with acute symptoms of stroke are referred to the public healthcare system if hospitalized. The weaknesses of the present study include the retrospective design and the risk of misclassifications during data collection in routine clinical settings.

6. Conclusions

The expected AF prevalence was 10.9% (n 5267), but the registered prevalence was just 7.5% (n 3638). Although the “gold standard” for anticoagulation is warfarin, only 68.9% (n 2506) of patients were treated with VKAs and only 67.3% attained a TTR > 60%. A relatively high rate of patients with anticoagulant therapy in primary healthcare has been found in this research, but the INR control remains suboptimal. Thus, only 35.4% of the expected AF prevalence achieved an optimal TTR. It seems clear that the providers of care and the systems within which they work have a profound effect on the quantity and quality of anticoagulation treatment.

Ethical Approval

The project presented here was evaluated and approved by the Clinical Research Ethics Committee of the Primary Care Research Institute, IDIAP Jordi Gol, Barcelona, on 12 June 2009 (Ref. 501/011) and by the Clinical Research Ethics Committee of the Hospital Universitari de Tarragona Joan XXIII (Ref. 35/2011). The authors of this paper have certified that they comply with the Ethical Guidelines of the International Committee of Medical Journal Editors.

Conflict of Interests

The authors declare that they have no competing interests.

Acknowledgment

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References


Atorvastatin Treatment for Atrial Fibrillation Reduces Serum High-Sensitivity C-Reactive Protein Levels

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We investigated whether serum hs-CRP levels predict the efficacy of atrial fibrillation (AF) treated with atorvastatin. Bibliographic databases were exhaustively searched for studies relevant to the research topic. Newcastle-Ottawa Scale (NOS) criteria, combined with the Quality Assessment of Diagnostic Accuracy Studies (QUADAS), were applied for study quality assessment. Our meta-analysis identified seven cohort studies (2006–2013), providing information on the change in serum hs-CRP levels in AF patients receiving atorvastatin therapy. After atorvastatin treatment, hs-CRP level in AF patients decreased significantly (SMD = 1.02, 95% CI: 0.58–1.47, \( P < 0.001 \)). Subgroup analysis by country and hs-CRP detection methods suggested a negative relationship between atorvastatin treatment and hs-CRP levels among Chinese AF patients (SMD = 1.34, 95% CI: 1.00–1.69, \( P < 0.001 \)) and by using ELISA method (SMD = 1.11, 95% CI: 0.51–1.71, \( P < 0.001 \)), but not among Turkish population and using INA method (all \( P > 0.05 \)). Egger’s test showed no publication bias (\( P = 0.450 \)). hs-CRP was clearly lowered in AF patients treated with atorvastatin, which may be helpful in the choice of statin agents for AF treatment. However, longer follow-ups are necessary to assess the clinical value of lowering hs-CRP in the clinical setting of AF treatment outcomes.

1. Introduction

Atrial fibrillation (AF) refers to extremely rapid and disorganized cardiac rhythm which may result in elevated afterload, increased filling pressures, and left atrial enlargement [1, 2]. The clinical manifestation of AF is a rapid heart rate usually associated with palpitations, exercise intolerance, anginal chest pain, and congestive heart failure [3]. The annual prevalence of AF per 1000 person-years is 1.9 in females and 3.1 in males under the age of 65. AF influences 5% of population over 65 years and 71% of octogenarians [2, 4]. The incidence of AF in the US is projected to reach 5.6 to 12 million in 2050. AF confers 1.5–2.0-fold greater relative risk of mortality [5, 6]. Clinically, electrophysiological abnormalities, surgical interventions, increase in atrial pressure, pharmacological drugs, inflammation or infiltrative atrial disease, cardiac atrium ischemia, and endocrine diseases may cause AF [7]. AF is a major public health problem and impairs patients’ quality of life, and various antiarrhythmic drugs have been utilized in the clinical management of AF patients [8, 9]. In this context, AF patients treated with atorvastatin showed decreased levels of high-sensitivity C-reactive protein (hs-CRP), a protein produced by the liver during infection, tissue injury, and chronic inflammation, indicating that atorvastatin may have significant clinical benefits in AF treatment and in prevention of AF recurrence [9, 10].

Atorvastatin belongs to a class of drugs known as the statins, routinely prescribed to reduce blood cholesterol and to prevent adverse events related to cardiovascular diseases [11]. Statins inhibit the expression of tissue factors and cell adhesion proteins, prevent monocyte adhesion with the vascular endothelium and subsequently the subendothelial space, inhibit the release of inflammatory cytokines and the formation of foam cells, and decrease the levels of C-reactive
protein (CRP) [7]. Atorvastatin, similar to the other statins, has been shown to reduce hs-CRP levels [12]. CRP is an acute-phase plasma protein that binds to complement proteins commonly assembled on apoptotic cells, on the surfaces of pathogens, and is implicated in the systemic response to inflammation [13]. CRP synthesis is rapidly elevated within hours after infection or tissue injury, indicating that it may be conducive to supporting host defense and participates in innate immune response [14]. CRP and SP pathways converge due to the fact that inflammation, endothelial/endocardial dysfunction, and oxidative stress play a crucial role in AF [15, 16]. As a sensitive indicator of the inflammation state in the body, hs-CRP levels are significantly increased in AF patients, suggesting that upregulated hs-CRP level is closely linked to AF pathogenesis [17]. Several previous studies have demonstrated the relatively high efficacy of statins in improving endothelial function and decreasing oxidative stress, while they also possess an anti-inflammatory and antithrombotic effect [18, 19]. More importantly, hs-CRP levels in AF patients treated with atorvastatin were lowered compared to the control untreated group, implying that atorvastatin suppressed inflammation by reducing the damage due to atrial electrical and structural remodeling, and prevented AF persistence, thereby reducing hs-CRP levels [20, 21]. Evidence, supporting the notion that atorvastatin therapy may impact hs-CRP levels in AF patients, is available [22, 23]; however, other studies contradict these findings [10, 21]. In order to address this issue, we used a meta-analysis approach and focused on the hs-CRP levels in AF patients before and after atorvastatin treatment.

2. Materials and Methods

2.1. Data Sources and Keywords. Bibliographic databases, (MEDLINE and EMBASE, Web of Science, Cochrane Library, PubMed, Google Scholar, China BioMedicine (CBM), and China National Knowledge Infrastructure (CNKI)), were exhaustively searched to identify published studies that assessed the change in hs-CRP levels in adult AF subjects who were administrated with atorvastatin. The search included studies available from the inception to June 2014. We used medical subject heading (MeSH) and keywords for atorvastatin and AF as follows: “atorvastatin” or “liptonorm” or “lipitor” and “Atrial Fibrillation” or “atrial fibrillations” or “fibrillation, atrial” or “familial atrial fibrillation” or “auricular fibrillation.” The search was limited to human studies and without restrictions to the language of the paper. In addition to the above electronic search, relevant articles were checked manually to identify additional potential papers.

2.2. Selection Criteria. This meta-analysis focused on observational studies where monitoring of hs-CRP was used to predict AF patients treated with atorvastatin. To be included in our meta-analysis, published studies fulfilled the following selection criteria: (1) patients with AF and the opportunistic screening for AF by pulse palpation, followed by recording of an electrocardiogram (ECG) to verify diagnosis [24]; (2) human-associated clinical trials focusing on AF and atorvastatin; (3) providing data on hs-CRP levels before and after atorvastatin medication; (4) providing information on the adjusted standard mean differences (SMDs) and 95% confidence intervals (CI) for hs-CRP level; (5) supplying the sample number; and (6) having sample sizes greater than 44. When the chosen studies included subjects that overlapped more than 50% in two or more studies, we only included the study whose sample population was the most comprehensive. Furthermore, only the most recent study of papers published by the same authors was included.

2.3. Data Extraction. In order to reduce the bias and enhance the confidence, two investigators separately extracted information from the selected studies based on the selection criteria and arrived at a consensus on all the items through discussion and reexamination. The following relevant data was extracted from the final selected studies for analysis: surname of first author, time of publication, source of publication, study type, study design, ethnicity and country of subjects, sample size, gender and age information, and detection method for hs-CRP levels in the human subjects. All authors approved the selected studies.

2.4. Quality Assessment. To determine whether the study in question was of high quality, two investigators independently scored the studies based on the Newcastle-Ottawa Scale (NOS) criteria [28]. The NOS criteria are as follows: (1) selection of the cohort: representativeness of the exposed cohort (NOS1), selection of the nonexposed cohort (NOS2), ascertainment of exposure (NOS3), and demonstration of the outcome of interest being not present at start of study (NOS4); (2) comparability of the cohort: whether the study was selected and analyzed according to the most important factor (NOS5) and whether the study controlled other confounding factors (NOS6); (3) assessment of outcome: follow-up long enough for outcomes to occur (NOS8) and adequacy of follow-up of cohort (NOS9). Discrepancies between the investigators on NOS scores were resolved by a third reviewer, through discussions with the two investigators. In addition, a validated tool for quality assessment of diagnostic accuracy, known as the Quality Assessment of Diagnostic Accuracy Studies (QUADAS), was applied to assess the methodological quality of the selected studies [29].

2.5. Statistical Analysis. Pooled odd ratios (ORs) with a 95% confidence interval (CI) were calculated, along with a Z test, to determine the effect size for each study. The ORs were calculated utilizing the STATA software, version 12.0 (Stata Corp., College Station, TX, USA) by two separate investigators. In order to supply quantitative data from all of the selected studies and to minimize the variance of the summary SMDs and 95% CI, we performed the current statistical analysis by utilizing a random-effects model (DerSimonian and Laird method) or a fixed-effects model (Mantel-Haenszel method). The random-effect model was applied when heterogeneity existed among the studies, while fixed-effects model was applied when there was no statistical heterogeneity. The
Articles identified through electronic database searching (N = 309)

Additional articles identified through a manual search (N = 2)

Articles reviewed for duplicates (N = 311)

Studies were excluded due to being:
(N = 76) letters, reviews, and meta-analysis
(N = 87) nonhuman studies
(N = 90) not related to research topics

Studies were excluded due to being:
(N = 5) not case-control or cohort study
(N = 18) not relevant to atorvastatin
(N = 22) not relevant to atrial fibrillation and
hs-CRP serum level

Full-text articles assessed for eligibility (N = 55)

Studies excluded due to being:
(N = 5) not case-control or cohort study
(N = 18) not relevant to atorvastatin
(N = 22) not relevant to atrial fibrillation and
hs-CRP serum level

Studies included in qualitative synthesis (N = 10)

Studies included in quantitative synthesis (meta-analysis) (N = 7)

Figure 1: Flowchart of literature search and study selection. Seven clinical case-control studies were included in this meta-analysis.

3. Results

3.1. Included Studies. Our present meta-analysis identified a total of 7 cohort studies, published between 2006 and 2013, that provided information on the changes in hs-CRP level in AF patients before and after administration of atorvastatin [10, 21–23, 25–27]. Demographic information, other characteristics, and methodological quality of the extracted studies included in our meta-analysis are presented in Table 1. All studies were performed in populations of Asian descent (n = 726) (369 patients were treated with atorvastatin and 357 patients with no statin treatment). The countries where the studies were performed were China (n = 5) and Turkey (n = 2). Gender data was also available from the majority of the included studies, with more males present in this meta-analysis than females; however, two studies [Wang (2011) and Guo (2011)] failed to report this information. The screening steps and study selection procedure are shown in Figure 1. Initially, a total of 311 papers were retrieved through the electronic database search and a manual search. Among
<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>Total</th>
<th>Sample size</th>
<th>Gender (M/F)</th>
<th>Age (days)</th>
<th>Disease</th>
<th>Method</th>
<th>NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhuo [22]</td>
<td>2013</td>
<td>China</td>
<td>Asians</td>
<td>206</td>
<td>104 102</td>
<td>61/43 55/47</td>
<td>64.0 ± 10.0 61.0 ± 13.0</td>
<td>AF</td>
<td>ELISA</td>
<td>8</td>
</tr>
<tr>
<td>Wang [25]</td>
<td>2011</td>
<td>China</td>
<td>Asians</td>
<td>98</td>
<td>50 48</td>
<td>— —</td>
<td>55.6 ± 10.7 55.6 ± 10.7</td>
<td>PAF</td>
<td>ELISA</td>
<td>6</td>
</tr>
<tr>
<td>Guo [23]</td>
<td>2011</td>
<td>China</td>
<td>Asians</td>
<td>87</td>
<td>45 42</td>
<td>— —</td>
<td>60.2 ± 8.3 60.2 ± 8.3</td>
<td>PAF</td>
<td>ELISA</td>
<td>6</td>
</tr>
<tr>
<td>Demir [21]</td>
<td>2011</td>
<td>Turkey</td>
<td>Asians</td>
<td>44</td>
<td>22 22</td>
<td>10/12 13/9</td>
<td>62.0 ± 9.0 60.0 ± 10.0</td>
<td>PAF</td>
<td>INA</td>
<td>7</td>
</tr>
<tr>
<td>Dong [26]</td>
<td>2009</td>
<td>China</td>
<td>Asians</td>
<td>81</td>
<td>41 40</td>
<td>25/16 26/14</td>
<td>56.0 ± 15.2 56.0 ± 15.2</td>
<td>AF</td>
<td>ELISA</td>
<td>7</td>
</tr>
<tr>
<td>Yuan [27]</td>
<td>2008</td>
<td>China</td>
<td>Asians</td>
<td>162</td>
<td>83 79</td>
<td>50/33 51/28</td>
<td>58.0 ± 11.2 58.0 ± 11.2</td>
<td>AF</td>
<td>INA</td>
<td>8</td>
</tr>
<tr>
<td>Ozaydin [10]</td>
<td>2006</td>
<td>Turkey</td>
<td>Asians</td>
<td>48</td>
<td>24 24</td>
<td>17/7 12/12</td>
<td>61.0 ± 13.0 64.0 ± 9.0</td>
<td>AF</td>
<td>ELISA</td>
<td>7</td>
</tr>
</tbody>
</table>

M: male; F: female; NOS: Newcastle-Ottawa Scale; PAF: paroxysmal atrial fibrillation; AF: atrial fibrillation; ELISA: enzyme linked immunosorbent assay; INA: immunoturbidimetry.
Figure 2: The methodological quality of included studies was evaluated by Newcastle-Ottawa Scale criteria.

<table>
<thead>
<tr>
<th>Included study</th>
<th>Serum level of hs-CRP (control versus atorvastatin)</th>
<th>SMD (95% CI)</th>
<th>Weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zhuo et al. (2013)</td>
<td></td>
<td>1.19 (0.89, 1.48)</td>
<td>15.73</td>
</tr>
<tr>
<td>Wang et al. (2011)</td>
<td></td>
<td>1.75 (1.29, 2.22)</td>
<td>14.27</td>
</tr>
<tr>
<td>Guo (2011)</td>
<td></td>
<td>1.90 (1.39, 2.41)</td>
<td>13.87</td>
</tr>
<tr>
<td>Demir et al. (2011)</td>
<td></td>
<td>0.33 (−0.26, 0.93)</td>
<td>13.00</td>
</tr>
<tr>
<td>Dong et al. (2009)</td>
<td></td>
<td>0.80 (0.35, 1.25)</td>
<td>14.40</td>
</tr>
<tr>
<td>Yuan (2008)</td>
<td></td>
<td>1.19 (0.86, 1.53)</td>
<td>15.44</td>
</tr>
<tr>
<td>Ozaydin et al. (2006)</td>
<td></td>
<td>−0.14 (−0.71, 0.42)</td>
<td>13.29</td>
</tr>
</tbody>
</table>

Heterogeneity test \( (I^2 = 86.4\%, \ P < 0.001) \)
Z test \( (Z = 4.49, \ P < 0.001) \)
Random effects analysis

Figure 3: Forest plots for the change of hs-CRP level between AF patients and controls.

those papers, three articles were duplicates and therefore excluded. Furthermore, letters, reviews, meta-analyses, non-human studies, and studies not related to the present research topics were excluded \((n = 256)\). The remaining 55 studies were reviewed and an additional 45 studies were excluded since they were not case-control or cohort designed studies, not relevant to atorvastatin or not relevant to AF and hs-CRP levels. After the remaining 10 papers were fully reviewed, 7 papers were finally selected for our meta-analysis, and the other 3 articles were excluded for lack of data integrity. The quality scores of the studies were higher than 7 (high quality), as shown in Figure 2.

3.2. Change of hs-CRP Level in AF Patients. As shown in Figure 3, the results of our present meta-analysis revealed that after treatment with atorvastatin, hs-CRP level in AF patients decreased significantly \((SMD = 1.02, 95\% CI: 0.58–1.47, \ P < 0.001)\). Subgroup analysis based on country and detection methods showed that atorvastatin treatment reduced serum hs-CRP levels in AF patients in the Chinese populations \((SMD = 1.34, 95\% CI: 1.00–1.69, \ P < 0.001)\), and by using the ELISA detection method of hs-CRP level \((SMD = 1.11, 95\% CI: 0.51–1.71, \ P < 0.001)\), but not among Turkish populations \((SMD = 0.09, 95\% CI: −0.38–0.55, \ P = 0.718)\) and using the INA method for detection of serum hs-CRP \((SMD = 0.80, 95\% CI: −0.04–1.64, \ P = 0.062)\) (Figure 4).

3.3. Sensitivity Analysis and Publication Bias. Sensitivity analysis to evaluate the stability of the results was performed by removal of each study one by one. The corresponding
<table>
<thead>
<tr>
<th>Included study</th>
<th>Serum level of hs-CRP (country: control versus atorvastatin)</th>
<th>Weight (%)</th>
<th>Heterogeneity test ($I^2 = 72.7%, P = 0.006$)</th>
<th>$Z$ test ($Z = 7.71, P &lt; 0.001$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td></td>
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<tr>
<td>Zhuo et al. (2013)</td>
<td>1.19 (0.89, 1.48)</td>
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<td>0.80 (0.35, 1.25)</td>
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<tr>
<td>Yuan (2008)</td>
<td>1.19 (0.86, 1.53)</td>
<td>15.44</td>
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<tr>
<td>Heterogeneity test ($I^2 = 72.7%, P = 0.006$)</td>
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<td>1.34 (1.00, 1.69)</td>
<td>73.70</td>
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<tr>
<td>Z test ($Z = 7.71, P &lt; 0.001$)</td>
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<tr>
<td>Turkey</td>
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<tr>
<td>Demir et al. (2011)</td>
<td>0.33 (−0.26, 0.93)</td>
<td>13.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozaydin et al. (2006)</td>
<td>−0.14 (−0.71, 0.42)</td>
<td>13.29</td>
<td></td>
<td></td>
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<tr>
<td>Heterogeneity test ($I^2 = 22.0%, P = 0.258$)</td>
<td></td>
<td>0.09 (−0.38, 0.55)</td>
<td>26.30</td>
<td></td>
</tr>
<tr>
<td>Z test ($Z = 0.36, P = 0.718$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneity test ($I^2 = 86.4%, P &lt; 0.001$)</td>
<td></td>
<td>1.02 (0.58, 1.47)</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Z test ($Z = 4.49, P &lt; 0.001$)</td>
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<td></td>
<td></td>
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<tr>
<td>Random effects analysis</td>
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</table>

**Figure 4:** Subgroup analyses by country and method for the differences of hs-CRP level between AF patients and controls.
pooled ORs in overall comparison and stratified analyses were not significantly altered, indicating a stable and credible outcome (Figure 5). As shown in Figure 6, no obvious visual asymmetry was observed from the graphical funnel plots and Egger’s test, showing no publication bias ($t = -0.82, P = 0.450$).

### 4. Discussion

In order to investigate the relationship between atorvastatin treatment and the serum hs-CRP levels in AF patients, an extensive meta-analysis was undertaken, and our main result shows that a significant reduction in serum level of hs-CRP was achieved after atorvastatin treatment in AF patients. Statins inhibit HMG-CoA reductase and are used in dyslipidemia patients to reduce blood cholesterol levels and prevent cardiovascular diseases [33]. Statins influence inflammation and oxidative stress by regulating the expression of iNOS, TNF-α, and MMPs [7]. Furthermore, as an anti-inflammatory agent, statins improve endothelial cell function, inhibit vascular smooth muscle cell proliferation, stabilize atherosclerotic plaque, and reduce serum level of hs-CRP [34]. Atorvastatin, known widely as Lipitor, is effective as an antilipemic drug used to reduce lipoprotein-rich cholesterol, thus significantly lowering the risk of cardiovascular diseases [35]. Atorvastatin was also shown to influence the cellular activities of components of the PI3/AKT signaling molecules such as AKT, P2X7, pERK, RhoA, cyclin D1, and β-catenin to inhibit proliferation and induce apoptosis in pancreatic cancer cells [36]. Atorvastatin’s cellular effect on proliferation and apoptosis was found to be beneficial in reducing the risk of development of advanced prostate cancer [37]. Additionally, atorvastatin suppressed the activation of ERK and AKT and disrupted Kras/PI3K and Kras/Raf complex formation in the NSCLC cells through its inhibitory action on HMG-CoA reductase [38]. It has been reported that atorvastatin
treatment in AF patients, when accompanied by a reduction in serum level of hs-CRP, is associated with clinical improvement in AF patients [39]. After treatment with atorvastatin, AF patients recovered sinus rhythm and electrical cardioversion [7]. The lower CPR levels due to atorvastatin treatment suggest that atorvastatin helps in preventing remodeling of atrial electricity and structure and inhibits the inflammatory process to prevent the development of AF [21]. Atorvastatin could inhibit inflammation by lowering proinflammatory cytokine levels, such as that of IL-6 and hs-CRP, inhibiting the complementary system, and increasing the NO release from the endothelial cells [40]. As a result, atorvastatin is linked to reduction of endothelial cell interaction with neutrophils and other anti-inflammatory mechanisms, inhibition of degeneration and fibrosis of myocardial cells, and eventually limiting the occurrence of atrial structural remodeling in AF patients, suggesting an excellent therapeutic effect on AF through inhibiting inflammation [21]. Consistent with this, atorvastatin-treated animals also had lower hs-CRP levels and relatively shorter AF duration compared to the control group [20]. Based on our results, we propose that atorvastatin treatment decreased hs-CRP serum levels in AF patients due to the anti-inflammatory role of atorvastatin. In line with our conclusion, Höglund et al. also found a lower serum level of hs-CRP after atorvastatin treatment in the AF patient group compared to the placebo group over time [41].

To further analyze the influence of other related factors such as country and serum hs-CRP detection methods on the relationship between atorvastatin treatment and lower hs-CRP serum level, a subgroup analysis was performed. From the result of country-stratified analysis, we observed that the relationship was not affected in Chinese population but was not statistically significant in Turkish population. One possible explanation could be different life styles and genetic backgrounds of individuals from different countries. Based on the detection method for hs-CRP, the ELISA method did not affect the observed relationship between atorvastatin therapy and hs-CRP serum level. However, the INA method showed no statistically significant relationship and might be explained by technical differences between the different detection methods. In summary, our results of the effect of atorvastatin treatment in lowering hs-CRP serum levels in AF patients are in agreement with other studies, suggesting atorvastatin therapy is effective in AF patients.

There are several limitations in our study. First, we did not take into consideration the viral load or treatment efficacy of atorvastatin over time, and the long-term follow-up of patients was not completed. Second, our study was single-center, cross-sectional retrospective study, with relatively small number of articles and a smaller number of patients. This may have resulted in article and patient selection bias. Another restriction may be that we only evaluated the role of hs-CRP in AF patients treated with atorvastatin, while comparison with other anti-inflammatory indexes such as IL cytokines are lacking. Lastly, since only paroxysmal AF was analyzed, patients with persistent AF or postoperative AF may have responded differently to atorvastatin but were not investigated.

5. Conclusions
In conclusion, our meta-analysis investigated the hs-CRP levels in AF patients treated with atorvastatin. We observed that hs-CRP levels decreased significantly in AF patients who used atorvastatin during the study period. Nevertheless, this study did not follow up patients over time, so the predictive values of hs-CRP levels may be limited. Therefore, further investigation is necessary.

Conflict of Interests
The authors have declared that no competing interests exist.

Acknowledgments
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References


Research Article

Chronotropic Modulation of the Source-Sink Relationship of Sinoatrial-Atrial Impulse Conduction and Its Significance to Initiation of AF: A One-Dimensional Model Study

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Initiation and maintenance of atrial fibrillation (AF) is often associated with pharmacologically or pathologically induced bradycardic states. Even drugs specifically developed to counteract cardiac arrhythmias often combine their action with bradycardia and, in turn, with development of AF, via still largely unknown mechanisms. This study aims to simulate action potential (AP) conduction between sinoatrial node (SAN) and atrial cells, either arranged in cell pairs or in a one-dimensional strand, where the relative amount of SAN membrane is made varying, in turn, with junctional resistance. The source-sink relationship between the two membrane types is studied in control conditions and under different simulated chronotropic interventions, in order to define a safety factor for pacemaker-to-atrial AP conduction (SASF) for each treatment. Whereas antiarrhythmic-like interventions which involve downregulation of calcium channels or of calcium handling decrease SASF, the simulation of Ivabradine administration does so to a lesser extent. Particularly interesting is the increase of SASF observed when downregulation $G_{Kr}$, which simulates the administration of class III antiarrhythmic agents and is likely sustained by an increase in $I_{CaL}$. Also, the increase in SASF is accompanied by a decreased conduction delay and a better entrainment of repolarization, which is significant to anti-AF strategies.

1. Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia, characterized by high morbidity and mortality; the mechanisms underlying its initiation, known to be complex and multifactorial, are still largely unexplained [1]. The most generally recognized causes of initiation and maintenance of AF are conduction abnormalities along interatrial accessory pathways [2, 3], abnormal interaction between sinoatrial node (SAN) cells and cardiac tissue nearby the insertion of pulmonary veins [4], increased fibrotic deposition within atrial tissue [5], electrical and calcium handling remodeling per se [6, 7] or secondary to atrial tachycardia (AT) [8, 9], and altered connexins ratio (Cx40/Cx43) leading to heterogeneity of conduction velocity [10, 11]. This complex scenario basically underlies two main pro-AF mechanisms, that is, triggered activity and reentry [12]. Furthermore, the crucial role of bradycardia in initiating and maintaining AF is well documented, either when associated with pathological conditions, like the sick sinus syndrome [13, 14], or brought about experimentally by cholinergic hyperactivation [15, 16]. Importantly, SAN spontaneous activity plays an active role in controlling atrial arrhythmias by its ability to terminate or convert atrial flutter to AF during cholinergic withdrawal [17].

A factor that makes the treatment of AF particularly complex is the paradoxical proarrhythmic effect of some of the most commonly used antiarrhythmic drugs. A typical case is the administration of Adenosine which, meant to terminate AT, frequently triggers AF by increasing potassium conductance via $I_{K,ACH}$ [18]. Analogous adverse effect is commonly found with Dobutamine and with other antiarrhythmic
agents, whose mechanism of action involves shortening of ECG Effective Refractory Period (ERP) and/or of atrial Action Potential Duration (APD) by increasing repolarizing potassium currents [18–21].

An increasingly adopted and promising bradycardic agent is Ixabradine (Ixa), which slows heart rate without significantly affecting inotropy [22], and thus it is widely used in the treatment of angina [23, 24]. Unlike beta-blockers, Ixa significantly affecting inotropy [22], and thus it is widely used.

Potassium currents [18–21]. ECG Effective Refractory Period (ERP) and/or of atrial models (32 and 100 pF, resp.), $I_{ion,sa}$ and $I_{ion,at}$ are the total ionic current (nA), and $V_{m,sa}$ and $V_{m,at}$ are the membrane potential (mV) in both models. The left term of both equations represents the electrotonic current (nA) flowing, through the $R_f$, and with opposite sign, across SAN and atrial membrane, respectively. $S$ is a scaling factor that allows the control of the size (membrane surface) of the SAN cell model. $S = 2$, for example, simulates electrical coupling of two parallel connected SAN cells firing synchronously, that is, a single SAN cell with twice the membrane surface and same channel densities, to the same atrial cell. $S$ was made varying throughout all simulations between 1 and 20, in order to specifically investigate an ($S$ and $R_f$) region where the 3 pacing conditions (NP&ND, P&D, and P&ND) were all represented together with all the possible transitions between them. Sinoatrial AP waveforms, either in control or under any simulated intervention, were integrated starting with initial conditions taken after 120 s of spontaneous beating. Similarly, atrial AP waveforms were obtained by using initial conditions taken after 100 simulated electrically driven APs at a pacing cycle length (CL) intrinsic for the rabbit heart (355 ms).

2.2. Integrating AP Propagation in a One-Dimensional Cell Strand. Action potential propagation along a strand made of a variable number ($N_{SA}$) of SAN cells and 10 atrial cells (Sev-eri and Lindblad model, resp.), longitudinally connected with each other with an electrical resistance $R_f$, was simulated by solving, for each kth cell, the following differential equation:

$$\frac{V_{k-1} - V_k}{R_f} - \frac{V_k - V_{k+1}}{R_f} = C_{m,k} \frac{dV_k}{dt} + I_{ion,k}.$$  

(2)

Source-sink properties were investigated by varying $N_{SA}$ between 1 and 10 and $R_f$ from 5 up to 200 MΩ, step 5 MΩ. In some cases, in the last cell of the atrial side of the cable, a 2 ms suprathreshold current injection was simulated in order to elicit an AP propagated in atrial-SAN direction.

2.3. Simulated Experimental Conditions

2.3.1. Autonomic Modulation of Rate. The effect of autonomic agonists acetylcholine (ACh) and isoproterenol (Iso) on the SAN AP model is built-in functions of the original Severi et al. formulation, and we refer to it [31] for the description of both treatments on ion channels, membrane transporters, and calcium dynamics.

2.3.2. Modulation of the Membrane and Calcium Clocks. As in the Severi et al. original paper, we simulated the application of 3 µM Ixa, which corresponds to the block of 66% of $I_f$ conductance [31, 33]. Since a univocal formulation for Ryanodine application in order to silence calcium clock [34, 35] is not provided for the Severi et al. model (whose CL barely changes after complete block of sarcoplasmic reticulum (SR) calcium release), we followed the indications by Maltsev and Lakatta [36] and simulated a Ryanodine-like (Rya") application by turning off SR calcium up-take and simultaneously downregulating SAN $C_{gel}$ by 34%, the aim being, as in all bradycardic treatments under study, to match the 28% CL prolongation found with Ixa.

2. Materials and Methods

2.1. Integrating Single Cell and Cell Pairs Action Potentials. All simulations reported in the present study have been performed by means of two mathematical models of rabbit cardiac action potentials (APs); the Severi et al. sinoatrial model and the Lindblad et al. atrial model [32]. Both models were downloaded and recompiled in their MATLAB version by means of COR facility at http://cor.physiol.ox.ac.uk/. The “ode15s” solver built into the R2010b version of MATLAB (The MathWorks, Inc., USA) was used to integrate the models equations. All simulations were run on a PC with Intel Core 2, 3 GHz CPU. SAN-atrial electrical cell coupling was simulated by solving the following system of differential equations:

$$\frac{V_{m,sa} - V_{m,at}}{R_f} = S \left( C_{m,sa} \frac{dV_{m,sa}}{dt} + I_{ion,sa} \right),$$

$$\frac{V_{m,at} - V_{m,sa}}{R_f} = C_{m,at} \frac{dV_{m,at}}{dt} + I_{ion,at},$$

(1)

where $R_f$ is the global electrical gap junctional resistance (MΩ) between the SAN and atrial elements, $C_{m,sa}$ and $C_{m,at}$ are the electrical capacitance of the original sinoatrial and atrial models (32 and 100 pF, resp.), $I_{ion,sa}$ and $I_{ion,at}$ are the total ionic current (nA), and $V_{m,sa}$ and $V_{m,at}$ are the membrane potential (mV) in both models. The left term of both equations represents the electrotonic current (nA) flowing, through the $R_f$, and with opposite sign, across SAN and atrial membrane, respectively. $S$ is a scaling factor that allows the control of the size (membrane surface) of the SAN cell model. $S = 2$, for example, simulates electrical coupling of two parallel connected SAN cells firing synchronously, that is, a single SAN cell with twice the membrane surface and same channel densities, to the same atrial cell. $S$ was made varying throughout all simulations between 1 and 20, in order to specifically investigate an ($S$ and $R_f$) region where the 3 pacing conditions (NP&ND, P&D, and P&ND) were all represented together with all the possible transitions between them. Sinoatrial AP waveforms, either in control or under any simulated intervention, were integrated starting with initial conditions taken after 120 s of spontaneous beating. Similarly, atrial AP waveforms were obtained by using initial conditions taken after 100 simulated electrically driven APs at a pacing cycle length (CL) intrinsic for the rabbit heart (355 ms).

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2.3.3. Effects of Classes III and IV Antiarrhythmic Agents. The action of a class III antiarrhythmic agent like Dofetilide was simulated in single SAN cells and in cell pairs by a 74% \( G_{Kr} \) downregulation [37] and that of a typical class IV agent like Verapamil via 61.5% reduction of \( I_{Cat} \) conductance [38]. In cable simulations only 10% downregulation of both conductances was applied.

3. Results

Aim of the present study is to compare the effect of different simulated chronotropic interventions on the source-sink relationship between a SAN and an atrial rabbit AP model, either arranged in cell pairs or in a cells strand, in order to study differences in the electrical strength of the pacemaking source and discuss the corresponding significance to the initiation of AF.

3.1. Estimating the Strength of the Source. Figure 1(a) shows spontaneous firing of the original Severi et al. rabbit SAN AP model, whereas Figure 1(b) shows a single AP obtained by the Lindblad et al. atrial model by simulating a 2 ms depolarizing current injection at a physiological pacing rate. The two AP models were then electrically coupled as in System(1) in Methods. The dynamical behavior of the cell pair can be summarized in 3 different types of response: high values of \( R_J \) correspond to a condition when SAN pacing occurs but does not provide enough electrotonic current to drive the atrial cell at its own frequency (“pace and not drive,” P&ND, red in panel (d)). This is shown in panels (c1) and (c2): in (c1) \( R_J \) is set to be infinitely high, cells are practically uncoupled, and SAN cell displays its intrinsic spontaneous rhythm whereas atrial cell is quiescent at its resting potential (\( V_r = -75 \) mV). In (c2) \( R_J \) is high but small enough to allow depolarizing electrotonic current to flow across it and induce subthreshold depolarizations into the atrial model cell. By further decreasing \( R_J \), we find a window of its values where, despite the electrical load exerted by the more polarized atrial membrane, SAN cell keeps firing and provides enough electrotonic current to synchronously drive the atrial cell (“pace and drive,” P&D, blue in figure). Finally, when \( R_J \) is decreased below a certain value, the electrical load of the atrial cell prevails, preventing SAN firing and, with that, its own pacing (“not pace and not drive,” NP&ND, white in figure). It is found experimentally that these three conditions are not necessarily present for any cell pair but depend on the relative amount of membrane surface of the source and the sink cells [39]. This latter property is reproduced by the scaling parameter \( S \) of the first equation of System (1) reported in Methods. Thus the source-sink behavior of the SAN-atrial cell pair can be summarized in a graphic like that reported in panel (d), previously described by Joyner and van Capelle [40], and obtained here by varying \( S \) (step = 1) and \( R_J \) (step = 50 MΩ). Given an excitable pacemaking source, electrically coupled to an excitable resting sink, there is, in general, an \( R_J \) value separating NP&ND from P&ND or from P&D conditions. Also, only above a given value of \( S \), P&D condition can take place (\( S > 4 \) in panel (d)). The ensemble of (\( S \) and \( R_J \)) values which allow P&D condition fills the blue area, whose surface, as we will show, is a measure of the safety factor with which the SAN membrane can drive the atrial sink; we refer to the numerical value of such a factor as SASF. SASF surface scales dimensionally as \( M^2 \). The conductance value of a single gap junctional channel (50 pS [41]) sets a nonlinear discretization for the \( Y \)-axes of Figure 1(d), which would allow us to convert the measured surface into a given number of gap junctional channels. Nevertheless, for the sake of the relative quantification of the strength of the source in different pacing conditions, our assumption of continuity for the \( Y \)-axes (and therefore for SASF) serves well our scope. The ability of an excitable current source to sustain a safe AP conduction is usually measured as safety factor (SF), and slightly different methods have been proposed in order to estimate it [42]. For each value of \( S \) we could derive the \( R_J \)-dependency of SF, which we calculated according to the Leon and Roberge formulation:

\[
SF_L = \frac{\int_A I_{\text{son}} \, dt}{\int_B I_{\text{m}} \, dt},
\]

with \( I_{\text{son}} \) the ionic current and \( I_{\text{m}} \) the total membrane current flowing, respectively, across the atrial cell membrane and integrated over the time when they are negative (A and B time windows, resp.). In so doing, we found that, for each value of the scaling factor \( S \), \( SF_L \) is greater than 1 only for \( R_J \) values falling within the P&D area (see representative example in lower inset of Figure 1(d)). SASF surface gives, in other words, a compact representation of source-sink properties when both \( R_J \) and membrane scaling \( S \) are subject to changes.

3.2. Chronotropic Interventions on the SAN Firing. In Figure 2 we summarize a number of different maneuvers (see Methods) we have performed on the SAN AP model in order to achieve increase (only one case) and decrease of its intrinsic beating rate. The simulated application of 1 μM Ito leads to a 22% decrease in pacing CL by dramatically increasing the rate of diastolic depolarization (DDR, V/s) and leaving AP amplitude (APA, mV) unaltered (panels (b) and (h)). In contrast, the simulated application of 11 nM ACh leads to a 28% increase in CL (panel (c)), which is very close to that expected from the linear ACh-dose dependency for CL predicted by Rocchetti et al. in their study on real rabbit sinoatrial cells [43] and recently confirmed by our own study on guinea pig SAN cells [35]. We then simulated other interventions, like downregulating \( G_{Cat} \) by 61%, downregulating \( G_{Kr} \) by 74%, downregulating \( I_f \) conductance by 66% via simulation of 3 μM Iva application [31, 33], and simultaneously downregulating SR rate of calcium uptake by 100% and \( G_{Cat} \) by 34% (panels (d)–(g)) in order to simulate Rya application (Rya*, see Methods). In all these instances we fine-tuned parameters in order to achieve exactly the same bradycardic effect, that is, the same 28% CL increase obtained with ACh. Corresponding relative changes in APA and DDR are shown in the histogram of panel (h). Absolute values of AP parameters can be found in Table 1.

3.3. Relative Strength of the Sinoatrial Source. For each one of the simulated SAN APs which underwent the chronotropic
Figure 1: SAN-atrial AP conduction. (a) Severi et al. rabbit SAN simulated APs sequence. (b) Lindblad et al. rabbit atrial electrically driven AP, simulated at the physiological pacing rate of 355 ms. (c1–c4) Electrical coupling of the two AP models (SAN in red and atrial in black) according to equations system (1), for $S = 5$, and $R_J =$ co, 850, 700, and 300 MΩ, respectively. (d) Points of the $(R_J, S)$ plan correspond to different conditions concerning SAN-atrial AP conduction: not pace and not drive (NP&ND, white), pace and drive (P&D, blue), and pace and not drive (P&ND, red). Lower inset: SF calculated with the Leon and Roberge formulation (see Section 3) for $S = 5$. The green double arrows label the same $R_J$ windows in panel (d) and lower inset.
Figure 2: Simulated chronotropic interventions on Severi et al. SAN AP firing. (a–g) 1 s long APs sequences reported in their steady state conditions under the following treatments: physiological condition (NT), 1 μM isoproterenol (Iso), 11 nM acetylcholine (ACh), 61.5% $G_{CaL}$ reduction, 74% $G_{Kr}$ reduction, 3 μM Ivabradine (Iva), and 100% reduction of SR calcium uptake + 34% $G_{CaL}$ reduction (Rya$^*$). (h) Treatments-induced changes in 3 AP parameters, as compared to their control values (NT).
interventions described in Figure 2, we simulated electrical coupling with a Lindblad et al. atrial cell model by solving the equation system (1) reported in Methods and varying, in turn, coupling resistance $R_j$ and scaling factor $S$, as explained for Figure 1(d). Each simulation resulted, as shown above, in NP&ND, P&D, and P&ND configurations, all summarized in the color panels of Figure 3. In the case of downregulation of $G_{Kr}$ and $G_{CaL}$, we also report in Figure 4, superimposed to the surface profiles shown in Figure 3 (black dotted lines), the color contour plots of the corresponding CL values. Our hypothesis that the P&D area of each graphic (SASF) is a measure of the strength of the SAN source is confirmed by the simulated experiment reported in Figure 5. In each beating SAN cell, taken in steady state conditions (after 120 s, see Methods), we simulated a hyperpolarizing constant current injection of increasing amplitude in order to find the amount of current needed to stop pacemaking within an arbitrarily chosen interval of time (5 s). Longer intervals were also tried and gave qualitatively identical results (not shown). The correlation between the constant current value needed to switch off pacemaking and the corresponding SASF value derived from Figure 3 clearly appears in the histogram of Figure 5(b) and is statistically quantified by the correlation analysis in panel (c) of the same figure. Whether we consider, for each treatment in the uncoupled condition and for the corresponding coupled configuration, the hyperpolarizing current needed to turn off pacemaking and the SASF value, respectively, we find that both parameters significantly correlate with DDR and with no other AP parameter (see panel (d)).

3.4. Transitory Changes in Electrical Coupling. We hypothesize here that the source-sink SAN-atrial system is set, in physiological conditions, at a given value of $(S, R_j)$, say $(11, 590 \text{ MO})$; that is, 11 SAN cells are spatially arranged around a single atrial cell and are cumulatively coupled to it with a total of 34 gap junctional channels (assumed to have, as pointed out above, a single channel conductance of 50 pS). We further assume that none of the antiarrhythmic interventions that we simulate changes this set point (none of them has known effects on geometrical nor on gap junctional coupling). On the other hand, we know from the literature [10, 11] that AF is often accompanied by gap junctional remodeling. We show in Figure 6 what a transitory (500 ms) closure of only 2 gap junctional channels (yellow path in panels (a) and (c) and in the magnified details of panels (b) and (d)) is expected to cause on the safety factor of SAN pacing. Whereas in control conditions (NT) such slight junctional change leaves SAN-atrial intercellular conduction within the “safe” area (blue) of P&D behavior, the same modification transiently (horizontal double arrow in panel (d)) brings the $G_{CaL}$ downregulated system into the P&ND region (red), where a single beat fails to be conducted from the SAN to the atrial membrane (bottom of panel (d)).

3.5. Relative Changes in Ion Currents for Each Treatment. In order to identify the ion currents primarily involved in determining the strength of the pacemaking source when coupled to a atrial sink, we performed a series of simulations like that reported in Figure 7(a) for the cell pair in NT. For each treatment, the minimum values of $S$ and $R_j$ (taken in this order), corresponding to the first permissive P&D configuration, were used to simulate cell coupling. Coupled (dotted lines) and uncoupled (solid lines) AP waveforms are reported for both cells (SAN in red and atrial in black) in the top left panel. The other panels represent the main SAN ion currents in the uncoupled and coupled conditions. The histogram in panel (b) represents corresponding percent coupling-induced changes in peak ion current of the SAN AP for the 7 treatments under study. Figure 8 shows how SAN-atrial conditions characterized by a larger “safety factor” correspond to a larger increase in $I_f$ when passing from uncoupled to coupled conditions (panel (a)). Similarly, they correspond to a larger decrease of $I_{Kr}$ when coupled (panel (b)). Correlation coefficients were 0.77 and 0.93, respectively. Positive significant correlations were also found for $I_{CaL}$ and $I_{NaK}$, though involving much smaller changes (data not shown).

3.6. Conduction Delay and Entrainment of Repolarization. We have shown, up to this point, that the area of the blue surfaces in Figure 3 measures the safety factor SASF associated with spread of pacemaker activity from SAN to atrial cells. We have also observed that SASF is different among bradycardic interventions which lead to the same decrease in SAN beating rate. We also wanted to test how

| Table 1: Sinoatrial AP parameters under chronotropic interventions. Beating parameters were measured after 100 s of simulated spontaneous firing, with AP waveform in its steady state conditions. Cycle length (CL), maximum diastolic potential (MDP), upstroke $V_{m}$ value ($V_{peak}$), action potential amplitude (APA), action potential duration as measured at $-40 \text{ mV}$ (APD$_{-40 \text{ mV}}$), diastolic depolarization rate (DDR), maximum value of the first time derivative of AP ($dV/dt_{max}$), and threshold value as take-off potential (TOP) are reported. |

<table>
<thead>
<tr>
<th>CL (ms)</th>
<th>MDP (mV)</th>
<th>$V_{peak}$ (mV)</th>
<th>APA (mV)</th>
<th>APD$_{-40 \text{ mV}}$ (ms)</th>
<th>DDR (V/s)</th>
<th>$dV/dt_{max}$ (V/s)</th>
<th>TOP (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT 355</td>
<td>$-58.01$</td>
<td>22.32</td>
<td>80.33</td>
<td>125.7</td>
<td>0.79869</td>
<td>7.22</td>
<td>-18.42</td>
</tr>
<tr>
<td>Iso 276</td>
<td>$-57.97$</td>
<td>22.04</td>
<td>80.01</td>
<td>127.5</td>
<td>1.2862</td>
<td>11.13</td>
<td>-25.02</td>
</tr>
<tr>
<td>$I_{Kr}$ 456</td>
<td>$-45.51$</td>
<td>31.71</td>
<td>77.22</td>
<td>274.7</td>
<td>0.91447</td>
<td>5.75</td>
<td>-19.08</td>
</tr>
<tr>
<td>Iva 454</td>
<td>$-59.19$</td>
<td>24.53</td>
<td>83.72</td>
<td>132</td>
<td>0.80923</td>
<td>6.05</td>
<td>-18.46</td>
</tr>
<tr>
<td>ACh 449</td>
<td>$-58.58$</td>
<td>14.81</td>
<td>73.39</td>
<td>103.6</td>
<td>0.60802</td>
<td>7.79</td>
<td>-19.32</td>
</tr>
<tr>
<td>$I_{CaL}$ 453</td>
<td>$-52.54$</td>
<td>$-2.567$</td>
<td>49.973</td>
<td>84.4</td>
<td>0.19244</td>
<td>1.47</td>
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<tr>
<td>Pup 456</td>
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<td>96.2725</td>
<td>0.33101</td>
<td>3.76</td>
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Figure 3: Passive and active properties of SAN source-atrial sink. $R_J$ versus $S$ plots, like that reported in Figure 1, were derived for each chronotropic intervention described in Figure 2. Iso corresponded to a 22% CL decrease, whereas all bradycardic maneuvers increased CL by 28%. Color code as in Figure 1. SASF values in MΩ were 8400 (NT), 11300 (Iso), 9550 ($G_{Kr}$), 4850 (Iva), 3400 (ACh), 2950 (Rya$^*$), and 2550 ($G_{CaL}$).
3.7. Simulations of a Linear SAN-Atrial Cell Strand. In order to test whether SASF evaluation as a measure of source strength could be applied also in a cable-like AP propagation, we performed simulations on a SAN-atrial chain (see scheme, top panel of Figure 10) of cells, electrically connected with a variable junctional resistance (see Methods). For small $R_f$ values, the more polarized atrial side of the cable completely depressed spontaneous firing in the left SAN side (NP&ND condition in Figure 10(a1) and white area in panel (a)). As $R_f$ increased, and in analogy with what is observed for cell pairs reported in Figure 3, spontaneous APs were generated in the SAN side of the cable and conducted to the atrial side (P&D condition in Figure 10(a2) and blue area in panel (a)). A further $R_f$ increase prevented APs generated in the SAN side from being conducted to the atrial side (P&D condition in Figure 10(a3) and red area in panel (a)). The same is shown in panels (b) and (c) of Figure 10 in the case of 10% downregulation of $G_{Kr}$ and $G_{CaL}$, respectively. These results confirm, in a cable-like model, the effects reported above on cell pairs, where $G_{Kr}$ downregulation increases and $G_{CaL}$ downregulation decreases the strength of the source, that is, the safety factor for AP conduction (see also Figure 10(d)).

Given the relevance of unidirectional block (UB) in the initiation of AF, we tested for it the cable-like model described in Figure 10, with the additional aim of relating UB to source-sink properties as measured with SASF surfaces. UB developed when AP propagation was simulated in control conditions for $N_{SA}=10$ and $R_f = 110 \, \text{M} \, \Omega$ (yellow circle in the SASF representation); that is, conduction failed when the SAN side of the cable was let free of beating at its own intrinsic frequency ($CL = 341 \, \text{ms}$) but succeeded when the end of the atrial side of the cable was electrically paced at the same frequency (Figure 11(a)). This was indeed expected, since the yellow circle is located into the P&ND (red) region of the graph. As we have shown in Figure 10 and reported here in the inset of panel (b), $G_{Kr}$ downregulation extends the P&D (blue) area, which now includes the yellow circle. Accordingly, conduction becomes permissive also in the SAN-atrial direction, making $G_{Kr}$ downregulation effective, in this case, also in removing UB.

4. Discussion

The present modeling study aims to investigate, at the cellular level, the source-sink SAN-atrial relationship in conditions which are critical for the development of AF. We first define a graphical representation which quantifies the safety factor for impulse conduction from SAN to atrial cell membrane into a single parameter (SASF). We then use SASF in order to compare identical bradycardic effects on SAN firing, which follow pacemaker autonomic regulation or antiarrhythmic-like treatments targeting AF.

Representations of the source-sink properties of SAN-atrial electrical coupling in graphics like those of Figures 1 and 3 have been previously described by Joyner and van Capelle [40], who showed that some degree of electrical uncoupling is...
Figure 5: Strength of the pacemaker as a current source. (a) A simulation is shown where, during the NT steady state AP firing, a constant hyperpolarizing current of increasing amplitude was injected, until a critical value (in red) was reached, when firing stopped within 5 s. The same critical value was derived for all the treatments and reported in the histogram of panel (b), normalized to the value derived in NT (orange bars). In the same histogram, normalized values of SASF are reported as well (black bars). (c) Linear correlation between the absolute values of the two parameters reported in panel (b) ($R = 0.97$). (d) Normalized (to NT) values of SASF (left panel) and $i_{\text{OFF}}$ (right panel) versus normalized values of DDR. In both instances a linear correlation ($R = 0.90$) was found.
Figure 6: Deviation from P&D set point. SAN-atrial system is initially set to \( S = 11 \) and \( R_J = 590 \, \text{M} \Omega \) (magnified detail in panel (b)) and transitorily displaced into \( S = 11 \) and \( R_J = 620 \, \text{M} \Omega \) for 500 ms. (a, b) The transition is simulated under NT conditions, where it does not affect P&D dynamics (horizontal double arrow in lower panel (b)). (c, d) When the same transition is simulated under 61.5% \( G_{CaL} \) reduction, it transiently brings the system into the P&ND area (top panel (d)), leading to a single beat conduction block (horizontal double arrow in lower panel (d)).

an essential design for proper SAN-atrial conduction. Several other simulation studies have been performed on this same issue [44–46], though not addressing its potential relevance for the initiation of AF. Basically it has been demonstrated that some degree of SAN to atrial interdigitation improves reliability of conduction and that the critical junctional resistance allowing AP entrainment in SAN-atrial cell pairs can easily fall within the \( G_{CaL} \)s range. Though the geometry of SAN is far to be fully elucidated and the possible arrangement of mutual intercellular coupling still needs to be clarified, nevertheless it is assumed that SAN ability to electrically drive the larger atrial volume is based on the presence of different cell types within its interior [47, 48], on a complex gradient of gap junctional distribution [49, 50], and on some favorable intercellular geometry [47]. This latter is achieved by interdigitation of different cell types [46, 50], which will likely result into a many-to-one SAN-atrial cells connection. Graphics (Figures 1(d) and 3) reporting \( R_J \) versus \( S \) (number of SAN cells coupled to a single atrial cell) compactly summarize these properties, by defining, for each \( S \), the \( R_J \) range that allows SAN membrane to electrically drive the atrial cell (P&D blue area). The same type of representations can be applied also to cable-like AP propagation (Figures 10 and 11).

Autonomic modulation, which is known to shift the leading pacemaker site within the SAN [51], is involved into the initiation and maintenance of AF [52]. Particularly, cholinergic stimulation is indicated as one of the main factors for the initiation of spontaneous AF, even though the exact knowledge of underlying mechanism is unknown [52]. Our simulations of cholinergic (equivalent to 11 nM ACh) and adrenergic (equivalent to 1 \( \mu \)M Iso) modulation of the Severi
et al. SAN AP model show that the two treatments lead to a large decrease and increase of SASF, respectively (Figures 3 and 5(b)), which is likely underlying the increased propensity to AF under cholinergic hyperstimulation [53] and related to the dramatic differences in coupling induced-Δ$I_f$ in the two instances (Figure 7(b)). Indeed, the correlation between SASF and Δ$I_f$ (Figure 8(a)) suggests that coupling-induced changes in $I_f$ play a major role in determining SASF.

Recent studies have attributed the molecular mechanism of SAN rhythm to the interplay between two clocks, one...
involving the hyperpolarization activated cation current \( I_f \) (membrane clock) [54] and the second attributable to activation of the electrogenic NaCa exchanger by spontaneous SR releases of calcium (calcium clock) [36]. The two clocks have been shown to possess different intrinsic dynamic properties, which can contribute to the physiopathology of the heart rhythm [35]. Thus, it is of interest to investigate the source-sink changes induced into the SAN when the two clocks are separately downregulated in order to obtain the same bradycardic effect. Whereas both maneuvers lead to a decrease in SASF; we note that the simulated application of Iva reduces SASF to a lesser extent when compared to Rya (Figures 3 and 5(b)). If, from one hand, the downregulation of the membrane clock appears to be safer than that of the calcium clock, on the other hand the Iva-induced reduction in SASF might be the mechanism underlying the slight increase in AF development found when Iva is used, as it is frequently, instead of beta-blockers in the treatment of ischaemic diseases [26].

Sinus node dysfunction is often associated with initiation and maintenance of AF [13], and an electrophysiologically weaker SAN, even in the absence of structural, electrical, or junctional remodeling, is more prone to transient SAN blocks, leading to a substrate favoring arrhythmias. This is shown, for instance, in the case of \( I_{CaL} \) downregulation (Figure 6), where even a very rapid and transient 5% increase in \( R_f \), ineffective in physiological conditions (panel (b)), brings the system out of the P&D area, leading to a 1-beat failure in SAN-atrial conduction (panel (d)). A comprehensive study of the SASF associated with pharmacological treatments of AF is beyond the scope of the present study. We limit our analysis to two ion channels modulatory effects involved in the action of classes III and IV antiarrhythmic drugs, like Dofetilide and Verapamil, respectively, known to decrease \( I_{Ks} \) and \( I_{CaL} \) conductance [38, 55]. Furthermore, since our aim was to compare bradycardic effects of the same extent (28% reduction of CL), we did not try to match, in our simulations, the therapeutic doses clinically administered for each one of these agents.

\( I_{CaL} \) downregulation, which results from class IV antiarrhythmics administration, is also a common finding in conditions when atrial tachycardia develops into AF [30]. It is interesting, at this regard to observe that a decrease in \( G_{CaL} \) brings about a dramatic reduction of SASF both in cell pairs (Figures 3 and 5(b)) and in cable propagation (Figures 10(c) and 10(d)), which might explain the transition to AF due to (1) a decreased safety factor for AP conduction, (2) an increased conduction delay, and (3) a diminished control in the entrainment of repolarization (Figures 9(b) and 9(c)). This is in agreement with the experimental findings of Hondeghem and Hoffmann in isolated rabbit hearts [29], and with documented Verapamil-induced SAN asystole [28] as well. An opposite effect on SASF is brought about in SAN-atrial cell pairs by the downregulation (−74%) of \( G_{Ks} \) in order to simulate the action of pure anti-\( I_{Ks} \) class III antiarrhythmic agents, like Dofetilide and Nifekalant [56, 57]. In this case, the decrease of \( G_{Ks} \) leads to (1) an increase in SASF, likely mediated by the coupling-induced greater increase in \( I_f \) and in \( I_{CaL} \) (Figures 7(b) and 8(a)), (2) a decrease in conduction delay, and (3) a better entrainment of repolarization, all synergic with a reduced proneness to develop AF. Furthermore, the larger coupling-induced reduction in \( I_{Ks} \) (Figures 7(b) and 8(b)) is going to reduce the likelihood of AF initiation by further increasing APD [58].

The role of \( I_{CaL} \) in granting safety of conduction can be summarized as follows. The depolarizing (negative) \( I_{ion} \) exceeding the depolarizing (negative) electrotonic \( I_m \) (see (3)), which makes AP spread safe (\( S_{F_L} > 1 \)) or, in other words, makes the source sink system fall into the P&D area, is almost entirely due to the contribution of \( I_{CaL} \), which is far the larger depolarizing current in SAN membrane. When this current is reduced, SASF gets reduced as well (Figure 3) and, with it, do the strength of the SAN source and the beating frequency (bottom panel of Figure 4). When, on the opposite,
Figure 9: Conduction delay and entrainment of repolarization. (a) A single conducted beat (SAN in red and atrial in black), measured in steady state conditions and for $S = 14$ and $R_J = 550 \, \text{M} \Omega$ (see Section 3), is shown for all simulated treatments. (b) Delay between the time-to-peak of the first $V_m$ time derivative for the source and sink APs is reported against normalized (to NT) values of P&D area. (c) Same representation for the delay between APD$_{-40 \, \text{mV}}$ measured in the 2 APs for each treatment.
Figure 10: AP propagation in a SAN-atrial cell strand. A chain made of a variable number ($N_{SA}$, ranging between 1 and 10) of SAN cells and 10 atrial cells intercellularly connected with an $R_J$ ranging from 5 up to 200 MΩ was simulated and reported as a scheme in the top panel. (a–c) Source-sink properties measured as in cell pairs simulations (see Methods) are reported for control conditions (NT), 10% $G_{Kr}$ downregulation, and 10% $G_{CaL}$ downregulation. (a1–a3) Three-dimensional representations of NP&ND, P&D, and P&ND conditions for NT. (d) Normalized (to NT) values of SASF (blue areas in a–c) as measured for the 3 conditions.
Figure 11: Unidirectional block. (a) In control (NT) conditions, and for cable properties defined by $N_{SA} = 10$ and $R_J = 110 \text{ M}\Omega$ (yellow dot in the inset. Note that it falls into the red P&ND area), AP conduction succeeds in atrial-SAN direction, when atrial side electrical stimulation is simulated, but fails in SAN-atrial direction (no stimulus applied); see also colored three-dimensional representations. (b) When $G_{Kr}$ downregulation is applied in the same cable conditions (yellow dot in the inset. Note that now it falls into the blue P&D area), AP conduction succeeds in both ways.
$I_{CaL}$ increases, like during $G_{Kr}$ reduction (see Figure 7(b)), the safety of conduction increases as SASF does (Figure 3); beating frequency decreases (middle panel of Figure 4) more than it does under $G_{CaL}$ downregulation.

Finally, in the cable simulation of Figure 11, SASF calculation predicts UB for a given set of passive parameters ($N_{CA}$ and $R_P$, yellow dots in the insets) in control conditions, and its removal after $G_{Kr}$ downregulation following SASF increases. Unidirectional block, together with decrease in conduction velocity, is a well-recognized cause of reentry, which, in turn, as mentioned above, is one of the two main mechanisms underlying initiation of AF [12]. The fact that, as we show, decreasing $G_{Kr}$ prevents UB induced by electrical uncoupling, might point to an unexplored property of class III antiarrhythmic agents, which are usually mainly described for their AP prolonging action.

To summarize, it is accepted that the successful SAN drive of the atria is based on a combination of active and passive electrical properties, and on a given geometrical arrangement between the two cell types within the SAN node and at its border [47]. The three factors determine a set point which, in physiological conditions, belongs to the P&D area of the graphic representations of Figure 3. The surface of such area can be used to quantify (SASF) the strength of the SAN as an electrotonic source. We find that (1) parasympathetic and orthosympathetic modulations exert opposite effects on SASF; (2) class III antiarrhythmic agents increase it and class IV decreases it, and (3) the same bradycardic effect obtained by separately downregulating the membrane and calcium clock corresponds to a smaller and larger SASF reduction, respectively.

5. Conclusions

Among the several conditions leading to AF, the role of safety in SAN-atrial AP conduction in terms of source-sink relationship has not been previously explored. Our simulation study shows that such relationship should be taken into account, together with usually recognized active, passive, and geometrical factors, in order to reconstruct the mechanism underlying the initiation and maintenance of AF. Particularly, a parameter like SASF compactly describes the strength of SAN source and thus might draw light on further SAN modeling and on the mechanism of action of antiarrhythmic agents.

Limitations of the Study

Despite the critical role that the morphological structure of SAN-atrial arrangement, including the complex interaction between several recognized cell types [47, 59, 60], is going to play into the atrial transition to fibrillation, such information could not possibly be considered in our simulations, given the still incomplete and sparse literature on the subject, and the much simpler geometry of our simulation setting. On the other hand, our simplified approach is meant to provide a more general background on SAN source-atrial sink properties, which can be improved as the underlying structure will be elucidated. A limitation of the Severi et al. SAN AP model is the lack of sensitivity of beating rate to reduction of SR calcium release. Thus, as mentioned above, in order to simulate Rya application, we followed a procedure already in use by other authors [36]. Finally, unfortunately the measure of SASF cannot possibly be pursued in any thinkable experimental preparation, which confines its use to the theoretical in silico explanation of experimentally observed phenomena.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References


Clinical Study

Impact of Dabigatran versus Phenprocoumon on ADP Induced Platelet Aggregation in Patients with Atrial Fibrillation with or without Concomitant Clopidogrel Therapy (the Dabi-ADP-1 and Dabi-ADP-2 Trials)

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Background. A relevant number of patients receive triple therapy with clopidogrel, aspirin, and oral anticoagulation. Clopidogrel's efficacy on ADP induced platelet function may be influenced by concomitant antithrombotic therapies. Data regarding the effect of dabigatran on platelet function is limited to in vitro studies and healthy individuals. Methods. The "Dabi-ADP-1" and "Dabi-ADP-2" trials randomized patients with atrial fibrillation to either dabigatran or phenprocoumon for a 2-week period. In Dabi-ADP-1 (n = 70) patients with clopidogrel therapy were excluded and in Dabi-ADP-2 (n = 46) patients had to be treated concomitantly with clopidogrel. The primary endpoint was ADP-induced platelet aggregation between dabigatran and phenprocoumon at 14 days. Secondary endpoints were ADPtest HS-, TRAP-, and COL-induced platelet aggregation. Results. There was no significant difference regarding the primary endpoint between both groups in either trial (Dabi-ADP-1: Dabigatran: 846 [650–983] AU × min versus phenprocoumon: 839 [666–1039] AU × min, P = 0.90 and Dabi-ADP-2: 326 [268–462] versus 350 [214–535], P = 0.70) or regarding the secondary endpoints, ADPtest HS-, TRAP-, and COL-induced platelet aggregation. Conclusion. Dabigatran as compared to phenprocoumon has no impact on ADP-induced platelet aggregation in atrial fibrillation patients neither with nor without concomitant clopidogrel therapy.

1. Introduction

Dabigatran is at least as effective as vitamin K antagonists in the prevention of stroke and systemic embolism in patients with atrial fibrillation [1]. Dabigatran etexilate (Pradaxa) is an oral prodrug that is converted by serum esterases to dabigatran, a potent, direct, competitive inhibitor of thrombin. Thrombin has multiple roles in hemostasis. It converts fibrinogen to fibrin which is necessary to form the fibrous matrix of blood clots and it also has a direct action on cells [2]. Thrombin has an impact on shape and vascular permeability of vascular endothelium and is the most potent agonist for platelet activation and aggregation [2]. By inhibiting thrombin, platelet signaling pathways are also blocked and therefore platelet function may be affected.

Data regarding the effect of dabigatran on platelet function is limited to in vitro studies [3–5] and studies in healthy individuals of modest size [6] and has so far not been tested in real life patients.

Triple therapy, the combination of aspirin, clopidogrel, and oral anticoagulation, is necessary in patients with coronary stent implantation who also have atrial fibrillation to reduce ischemic events [7]. With the advent of the direct oral anticoagulants (DOAC), substances such as dabigatran...
etexilate are also given concomitantly with clopidogrel [8]. Platelet function testing is more and more emerging in the clinical routine because it has been shown that in patients who are treated with dual antiplatelet therapy (DAT) with aspirin and clopidogrel, both low and high on-treatment platelet reactivity (HPR) are associated with adverse clinical events [9]. One factor that may predispose to HPR is the patients’ comedication that may interfere with clopidogrel metabolism. In fact, there is some evidence that traditional anticoagulants such as the vitamin K antagonist phenprocoumon attenuate the antiplatelet effects of clopidogrel [10].

In the setting of concomitant clopidogrel therapy, it was already demonstrated that the parenteral direct thrombin inhibitor bivalirudin was able to further inhibit ADP induced platelet aggregation [11, 12]. Whether this is due to a direct interaction with the platelets or has an impact on clopidogrel metabolism is unclear. The oral direct thrombin inhibitor dabigatran is currently challenging the role of vitamin K antagonists in patients with atrial fibrillation and in those treated with triple therapy [8]. Therefore evaluating its role on platelet function and clopidogrel metabolism in real life patients is imperative. It is therefore our aim to evaluate whether dabigatran as compared to phenprocoumon alters ADP mediated platelet signaling pathway in clopidogrel naive patients or in patients concomitantly treated with clopidogrel. We therefore initiated two randomized trials to study the impact of dabigatran as compared to phenprocoumon (i) on ADP induced platelet aggregation in patients with atrial fibrillation (Dabi-ADP-1) and (ii) on clopidogrel mediated ADP induced platelet aggregation in patients with atrial fibrillation who are concomitantly treated with clopidogrel (Dabi-ADP-2).

2. Methods

2.1. Study Population. The “Impact of DABIGatran and phenprocoumon on the ADP induced platelet aggregation in patients with atrial fibrillation” (DABI ADP 1) study and the “Impact of DABIGatran and phenprocoumon on the clopidogrel mediated ADP induced platelet aggregation in patients with atrial fibrillation” (Dabi-ADP-2) study were two single centre randomized open label trials designed to compare the impact of dabigatran etexilate (Pradaxa) versus the vitamin K antagonist phenprocoumon on platelet function (ClinicalTrials.gov identifiers: NCT01339819 and NCT01352702). Patients were enrolled at the Deutsches Herzzentrum Munich between April 2011 and February 2013.

Both trials shared the same inclusion and exclusion criteria with the main difference that in Dabi-ADP-1 patients with clopidogrel therapy were excluded and in Dabi-ADP-2 had to be treated concomitantly with clopidogrel.

Patients were eligible if they were 18 years of age, if they had atrial fibrillation with an indication for oral anticoagulation, and if written informed consent by the patient or her/his legally authorized representative for participation in the study was obtained. Exclusion criteria included patients with a recent thromboembolic event (defined as severe disabling stroke in the last 6 months or any stroke in the last 14 days) or a high thromboembolic risk such as a history of mechanical valve, pulmonary embolism, deep vein thrombosis, or LV thrombus. In addition, patients with a contraindication for oral anticoagulation, active bleeding or high bleeding risk, cardiogenic shock, severe renal insufficiency (Creatinine Clearance < 30 mL/min), or moderate or severe hepatic impairment (Child-Pugh class B or C) were excluded. The studies were conducted in accordance with the provisions of the Declaration of Helsinki and with the International Conference on Harmonization Good Clinical Practices. The trial protocols were approved by the institutional ethics committee responsible for the Deutsches Herzzentrum Munich (Germany) and the Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM, Germany).

2.2. Study Protocol. Patients who met all of the inclusion criteria and none of the exclusion criteria were randomized in the order that they qualify. Allocation to treatment was made by means of sealed envelopes containing a computer-generated sequence. Patients were randomized according to a 1×1 factorial design to a dabigatran versus a phenprocoumon therapy for 2 weeks. Time zero was defined as the time of randomization. Patients were considered enrolled in the study and eligible for the final intention to treat analysis at the time of randomization. Commercially available drugs were given according to the protocol.

Oral anticoagulation with phenprocoumon or dabigatran was given on the day of randomization and was administered orally at the dosage recommended by current guidelines [13, 14]. Phenprocoumon was given with a target INR of 2.0–3.0 and dabigatran was administered at a dosage of 110 mg or 150 mg twice daily. Study drugs were provided by the sponsor (Deutsches Herzzentrum Munich). Other cardiac medications were given according to the judgment of the patients’ physician (e.g., ASA, β-blockers, ACE-inhibitors, statins, proton pump inhibitors, etc.).

Patients were scheduled for an outpatient visit at 14 days after randomization for clinical follow-up and laboratory testing (see below).

2.3. Laboratory Testing. Whole blood for platelet function testing on the Multiplate analyzer (Roche Diagnostics, Basel, Switzerland) was obtained from the cubical vein. Blood was placed in 4.5 mL plastic tubes containing the anticoagulant lepirudin (25μg/mL; Refludan, Dynabyte, Munich, Germany). Platelet aggregation was assessed with multiple electrode aggregation (MEA) using an impedance aggregometer (Multiplate analyzer). ADP (6.4 μmol/L), ADPtest HS (6.4 μmol/L ADP in the presence of 9.4 nmol/L prostaglandin E1), TRAP-6 (32 μmol/L), and Collagen (3.2 μg/mL collagen (COLtest)) served as agonists. Details of this method have been reported previously [15, 16]. Aggregation measured on the Multiplate device is quantified as area under the curve of aggregation units (AU) (area under the curve = AU × min). All material used for platelet function testing was obtained from the manufacturer.

2.4. Study Endpoints and Definitions. The primary endpoint of both studies was the ADP induced platelet aggregation in patients treated with dabigatran versus phenprocoumon...
treatment at 14 days. ADP was chosen as primary endpoint because there is a multitude of studies that have linked alterations in the ADP value associated with antithrombotic therapy with clinical events [9].

The secondary endpoints were ADPtest HS, TRAP, and COL induced platelet aggregation in patients treated with dabigatran versus patients with phenprocoumon treatment at 14 days.

Patients were further monitored throughout the two-week study period for the occurrence of adverse events such as death, stroke, myocardial infarction (MI) according to TIMI criteria [17], stent thrombosis, TIMI major, or TIMI minor bleeding [17]. The diagnosis of ischemic or hemorrhagic stroke required confirmation by computed tomography or magnetic resonance imaging of the head. Adjudication of bleeding events according to BARC criteria [18] was done in a retrospective manner.

2.5. Follow-Up. Detailed information regarding the occurrence of adverse events was obtained in this population during follow-up at 14 days in the outpatient clinic. Patients who could not come to the hospital were interviewed by phone. Those with cardiac complaints underwent a complete clinical, electrocardiographic, and laboratory check-up. General practitioners, referring cardiologists, patients, or their relatives were contacted for additional information if necessary. Relevant data were collected from source documents and prospectively entered into a computerized database by specialized personnel of the data coordinating Intracoronary Stenting and Antithrombotic Research (ISAR) center.

2.6. Statistical Methods. There is some evidence that the parenteral direct thrombin inhibitor bivalirudin results in further inhibition of ADP induced platelet aggregation [10] while phenprocoumon attenuates clopidogrel mediated ADP aggregation [10]. Sample size calculation was therefore based on the assumption that administration of dabigatran as compared to phenprocoumon results in a 25% absolute decrease of maximal ADP. Choosing a power of 80% and a two-sided α value of 0.05 a sample size of at least 29 per group was required. To compensate for losses to follow-up, each study was designed to enroll a total of 70 patients (35 per group).

In Dabi-ADP-2 a blinded interim review performed in February 2013 demonstrated that more than 1000 patients would need to be included to show a significant difference between groups. On the basis of these data, the steering committee decided to terminate Dabi-ADP-2 prematurely for futility.

A comparison of categorical variables, expressed as counts (percentages), was performed using the Fisher exact or the χ² test, as appropriate. Continuous variables were expressed as means (±SD) and compared with the unpaired, 2-sided Student's t-test if normally distributed; otherwise, they were expressed as medians (25th–75th percentile) and statistically analysed by the Wilcoxon rank-sum test.

A P value <0.05 was considered to indicate statistical significance. All statistical analyses were performed with the software R (version 2.15.0; The R Foundation for Statistical Computing).

3. Results

3.1. Populations. In Dabi-ADP-1, 70 patients were enrolled and randomized to receive dabigatran (n = 35) or phenprocoumon (n = 35). There was no significant difference in terms of baseline patient characteristics (Table 1). Median INR levels (interquartile range) at baseline were 1.1 (1.0–1.1) in the dabigatran group and 1.1 (1.1–1.2) in the phenprocoumon group. According to intention to treat analysis the primary and secondary endpoints could be analyzed in 30 patients in the dabigatran group and 32 patients in the phenprocoumon group (Figure 1).

In Dabi-ADP-2, 46 patients concomitantly treated with clopidogrel were enrolled and randomized to receive dabigatran (n = 22) or phenprocoumon (n = 24). There was no significant difference in terms of baseline patient characteristics (Table 1). Median INR levels (interquartile range) at baseline were 1.2 (1.1–1.2) in the dabigatran group and 1.2 (1.1–1.4) in the phenprocoumon group. According to intention to treat analysis the primary and secondary endpoints could be analyzed in 20 patients in the dabigatran group and 20 patients in the phenprocoumon group (Figure 2).

3.2. Platelet Function Tests. There was no significant difference regarding the primary endpoint of ADP induced platelet function between patients treated with dabigatran as compared to patients with phenprocoumon treatment in either trial at 14 days (Dabi-ADP-1: 846 [650–983] AU × min versus 839 [666–1039] AU × min, P = 0.90 (Figure 3) and Dabi-ADP-2: 326 [268–462] AU × min versus 350 [214–535] AU × min, P = 0.70 (Figure 4)). There was also no significant difference regarding the secondary endpoints ADPtest HS, TRAP, and COL in either trial (Figures 3 and 4). Median INR values at follow-up were 1.2 (1.1–1.3) in the dabigatran group and 2.6 (1.9–3.3) in the phenprocoumon group in Dabi-ADP1 and 1.2 (1.1–1.4) in the dabigatran group and 3.0 (1.9–4.4) in the phenprocoumon group in Dabi-ADP-2.

In Dabi-APD-1 there was one patient in the dabigatran group who was switched to phenprocoumon and two patients in the phenprocoumon group who were switched to either rivaroxaban or enoxaparin by their primary physician during the study period (Figure 1). We therefore also analyzed patients according to the treatment they received. In the as treated analysis there was no significant difference regarding ADP induced platelet aggregation between patients treated with dabigatran (n = 29, as treated analysis) as compared to patients with phenprocoumon treatment (n = 31, as treated analysis) (850 [658–988] AU × min versus 842 [653–1044] AU × min, P = 0.94). There was also no significant difference regarding the secondary endpoints ADPtest HS (646 [529–760] AU × min versus 652 [457–855] AU × min, P = 0.96), TRAP (1195 [1049–1428] AU × min versus 1192 [1001–1399] AU × min, P = 0.57), and COL (804 [682–981] AU × min versus 752 [670–874] AU × min, P = 0.42).
### Table 1: Baseline characteristics of the Dabi-ADP-1 and Dabi-ADP-2 study population.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dabigatran n = 35 (%)</th>
<th>Dabigatran n = 22 (%)</th>
<th>Phenprocoumon n = 35 (%)</th>
<th>Phenprocoumon n = 24 (%)</th>
<th>P value</th>
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<tr>
<td>Age, years</td>
<td>69 ± 11</td>
<td>70 ± 13</td>
<td>71 ± 7</td>
<td>76 ± 8</td>
<td>0.06</td>
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<td>Woman (%)</td>
<td>14 (40.0)</td>
<td>10 (28.6)</td>
<td>4 (18.2)</td>
<td>3 (12.5)</td>
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<td>Arterial hypertension (%)</td>
<td>26 (74.3)</td>
<td>24 (68.6)</td>
<td>21 (95.5)</td>
<td>24 (100)</td>
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<td>Diabetes mellitus (%)</td>
<td>3 (8.6)</td>
<td>9 (25.7)</td>
<td>5 (22.7)</td>
<td>9 (37.5)</td>
<td>0.28</td>
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<td>Hypercholesterolemia (%)</td>
<td>23 (65.7)</td>
<td>23 (65.7)</td>
<td>&gt;0.99</td>
<td>18 (75.0)</td>
<td>0.57</td>
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<td>Prior myocardial infarction (%)</td>
<td>0 (0)</td>
<td>3 (8.6)</td>
<td>—</td>
<td>5 (22.7)</td>
<td>0.17</td>
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<tr>
<td>Prior bypass surgery (%)</td>
<td>1 (2.9)</td>
<td>1 (2.9)</td>
<td>&gt;0.99</td>
<td>3 (13.6)</td>
<td>0.70</td>
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<td>CHA2DS2-VASc score</td>
<td>2.8 ± 1.5</td>
<td>3.2 ± 1.6</td>
<td>4.0 ± 1.3</td>
<td>4.6 ± 1.0</td>
<td>0.13</td>
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<td>Prior stroke (%)</td>
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<td>1 (2.9)</td>
<td>—</td>
<td>1 (4.5)</td>
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<td>Prior TIA (%)</td>
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<td>1 (2.9)</td>
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<td>Prior bleeding (%)</td>
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<td>Aspirin (%)</td>
<td>12 (34.3)</td>
<td>13 (37.1)</td>
<td>20 (90.9)</td>
<td>24 (100)</td>
<td>0.13</td>
</tr>
<tr>
<td>Clopidogrel (%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>22 (100)</td>
<td>24 (100)</td>
<td>0.13</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>11 (31.4)</td>
<td>18 (51.4)</td>
<td>20 (90.9)</td>
<td>24 (100)</td>
<td>0.13</td>
</tr>
<tr>
<td>PPI (%)</td>
<td>4 (11.4)</td>
<td>7 (20.0)</td>
<td>5 (25.0)</td>
<td>8 (33.3)</td>
<td>0.55</td>
</tr>
</tbody>
</table>
3.3. **Safety.** In the phenprocoumon group of Dabi-ADP-2 there was one patient who suffered from stent thrombosis on day 13 due to clopidogrel discontinuation. In this patient ADP induced platelet aggregation was 1417 AU × min when he presented with STEMI to our emergency department. After clopidogrel loading and PCI of the culprit vessel he could be discharged and ADP values on follow-up under treatment with aspirin, clopidogrel, and phenprocoumon were 307 AU × min.

In both trials, no patient suffered from death, stroke, or TIMI minor or TIMI major bleeding during the follow-up period. The number of BARC Types 1 and 2 bleeding was low. In Dabi-ADP-1, there was one BARC Type 1 bleeding and 3 BARC Type 2 bleeding in the dabigatran group and none
in the phenprocoumon group. In Dabi-ADP-2, one BARC Type 1 bleeding occurred in the dabigatran group and 2 in the phenprocoumon group.

4. Discussion

The main finding of our two randomized trials is that dabigatran as compared to phenprocoumon has no impact on ADP induced platelet aggregation in atrial fibrillation patients neither with nor without concomitant clopidogrel therapy. Furthermore there were no significant differences regarding the other mediators of platelet aggregation such as TRAP or collagen.

The hemostatic process is a dynamic, highly interwoven array of multiple processes [19] and antithrombotic agents inhibit specific steps in the coagulation cascade or in platelet aggregation. There have been some antithrombotic substances in the past, however, which have shown to induce a prothrombotic state through modification of pathways which differ from those who are primarily targeted, with the consequence of increasing adverse clinical events [20].
In our current Dabi-ADP-1 trial in patients with atrial fibrillation with no concomitant clopidogrel therapy, we could show that the direct thrombin inhibitor dabigatran has no impact on ADP, ADPtest HS, TRAP, and COL induced platelet aggregation as compared to phenprocoumon. As it is known, that phenprocoumon itself does not alter ADP induced platelet aggregation in patients solely treated with the vitamin K antagonist [21], our data suggests that dabigatran has no impact on ADP induced platelet function.

Our results are in line with the finding of former in vitro studies which could demonstrate no impact of dabigatran on ADP induced platelet aggregation in platelet rich plasma [3, 5]. Furthermore the values obtained for platelet function in our population treated with either dabigatran or phenprocoumon are similar to the normal reference intervals which were established in 117 healthy individuals [22].

Clinically, there is still an ongoing discussion regarding a prothrombotic effect of dabigatran. In the large multicenter RE-LY trial [1], concerns were raised that the rates of myocardial infarction were significantly increased in both tested dosages, which was debilitated after publication of a subsequent analysis of the RE-LY trial, where also silent MI...
were included [23, 24]. In contrast to this data, several meta-analysis of randomized trials with dabigatran came to the conclusion that dabigatran is associated with an increased risk of MI or ACS in a broad spectrum of patients [25–27]. The underlying pathological mechanism for this observation is still unknown.

Clopidogrel’s efficacy may be hampered by genetic factors associated with clopidogrel metabolism as well as nongenetic factors such as patients’ characteristics, comorbidities, and comedication [28]. One important example is the coumarin derivate phenprocoumon which is known to effectively reduce coagulation parameters but does not alter ADP induced platelet aggregation in patients solely treated with the vitamin K antagonist [21]. It is of interest however that concomitant treatment of phenprocoumon with DAT significantly attenuated the antiplatelet effects of clopidogrel in a previous study [10]. This is thought to be induced by a drug-drug interaction at the level of hepatic CYP metabolization, leading to a significant alteration of the vivo biotransformation of clopidogrel into its active thiol metabolite.

On the other hand, the parenteral direct thrombin inhibitor bivalirudin, as compared to unfractionated heparin, was able to further inhibit clopidogrel mediated ADP induced platelet aggregation [11, 12]. We therefore hypothesized that concomitant therapy with the oral direct thrombin inhibitor dabigatran might show a similar effect on ADP induced aggregation.

In fact, however, in our Dabi-ADP-2 trial in patients with atrial fibrillation with concomitant clopidogrel and aspirin therapy, we could show that the direct thrombin inhibitor dabigatran has no impact on ADP, ADPtest HS, TRAP, and COL induced platelet aggregation as compared to phenprocoumon.

These findings may be explained in several ways: Both phenprocoumon and dabigatran attenuate clopidogrel mediated ADP induced platelet aggregation in the same way or none of these substances have an impact on the platelet function even in the setting of clopidogrel therapy.

Data regarding the influence of dabigatran on clopidogrel mediated ADP induced platelet aggregation in patients is limited. Recently one small study with 12 healthy male individuals has evaluated concomitant therapy with dabigatran and clopidogrel on the pharmacokinetic and pharmacodynamic effect of either agent [6]. It was shown that neither ADP induced platelet aggregation nor the bioavailability of either agent was significantly altered by the combined therapy which is in line with our findings.

Our knowledge of the clinical role of new oral anticoagulants as part of triple therapy is still limited. So far one post hoc analysis of the RE-LY trial revealed that in the setting of concomitant single or dual antiplatelet therapy, dabigatran is associated with fewer bleeding events than warfarin [8] which are promising results. In our randomized study, duration of triple therapy with dabigatran was only 2 weeks and the number of patients was low. This does not allow for a meaningful assessment of the safety of this therapy. However, it is known that most of the bleeding events occur early after initiation of therapy [29]. We observed only few cases of Type 1 BARC bleeding.

Study Limitations. Limitations of Dabi-ADP-2 include the small sample size and the premature termination of the study. However results of our interim analysis implicated that in Dabi-ADP-2 more than 1000 patients needed to be enrolled and such a study is not feasible in such a patient population receiving triple antithrombotic treatment.

Secondly, baseline platelet function tests at randomization without any concomitant antithrombotic therapy were not available in all patients. Therefore our trial does not answer whether platelet function may have been affected between baseline and at two weeks when the primary endpoint was assessed.

Thirdly, aggregation was not tested with other agonists, such as thrombin-induced platelet aggregation, where a concentration-dependent inhibition with dabigatran has already been shown [3].

5. Conclusion

In conclusion, in these two randomized trials in patients with atrial fibrillation we could not find an impact on ADP induced platelet aggregation in patients treated with dabigatran as compared to phenprocoumon neither with nor without concomitant clopidogrel therapy.

Clinical Perspective. Traditional antithrombotic agents such as vitamin K antagonists as well as clopidogrel are currently challenged and will possibly be replaced by direct oral anticoagulants (apixaban, dabigatran, edoxaban, rivaroxaban) and newer P2Y12 inhibitors (prasugrel, ticagrelor). In the setting of triple therapy, choosing the right combination of antiplatelet and anticoagulation therapy therefore becomes more demanding as more options exist. Platelet function studies are useful to evaluate potential medication interactions which may attenuate their antithrombotic efficacy.

Clinically, limited data suggests that reductions in bleeding complications in this population may be achieved with the omission of aspirin [30], shorter therapy duration [31], or the use of dabigatran [8]. Newer more potent P2Y12 blocker are currently not recommended in the setting of triple therapy [32] as they may increase bleeding without reducing ischemic events [33]. Ongoing randomized studies such as PIONEER AF-PCI (ClinicalTrials.gov identifier: NCT01830543) or REDUAL PCI (ClinicalTrials.gov identifier: NCT02164864) evaluate also the role of newer agents and will further help to define the optimal treatment combination and duration in this challenging population.

Abbreviations

AU: Aggregation units
DAT: Dual antiplatelet therapy
DOAC: Direct oral anticoagulants
HPR: High on-treatment platelet reactivity
MEA: Multiple electrode aggregation
MI: Myocardial infarction.
Ethical Approval
The studies were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national), the provisions of the Declaration of Helsinki of 1975, revised 2000, and with the International Conference on Harmonization Good Clinical Practices and were in accordance.

Consent
Informed consent was obtained from all patients before being included in the study.

Disclosure
No animal studies were carried out by the author of this paper.

Conflict of Interests
Dr. Sibbing has been a consultant for Verum Diagnostica and Eli Lilly, receiving payment for lectures from Eli Lilly, Daiichi Sankyo, Astra Zeneca, CSL Behring, Roche Diagnostics, and Verum Diagnostica and achieved research grants from Roche Diagnostics. Dr. Mehilli indicates having received lecture fees from Daiichi Sankyo, Eli Lilly, Terumo, Abbott Vascular, and Biotronik. Dr. Saraoff reports having received lecture fees from Eli Lilly, Boehringer Ingelheim, Astra Zeneca, Roche Diagnostics, and Bayer Healthcare. Dr. Martischnig, Janina Pollak, Dr. Petzold, Dr. Fiedler, Dr. Mayer, Dr. Schulz-Schüpke, Dr. Massberg, and Dr. Kastrati declare that they have no conflict of interests relevant to this paper.

References


Clinical Study

Surgical Treatment of Concomitant Atrial Fibrillation: Focus onto Atrial Contractility

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Background. Maze procedure aims at restoring sinus rhythm (SR) and atrial contractility (AC). This study evaluated multiple aspects of AC recovery and their relationship with SR regain after ablation. Methods. 122 mitral and fibrillating patients underwent radiofrequency Maze. Rhythm check and echocardiographic control of biatrial contractility were performed at 3, 6, 12, and 24 months postoperatively. A multivariate Cox analysis of risk factors for absence of AC recuperation was applied. Results. At 2-years follow-up, SR was achieved in 79% of patients. SR-AC coexistence increased from 76% until 98%, while biatrial contraction detection augmented from 84 to 98% at latest stage. Shorter preoperative arrhythmia duration was the only common predictor of SR-AC restoring, while pulmonary artery pressure (PAP) negatively influenced AC recuperation. Early AC restoration favored future freedom from arrhythmia recurrence. Minor LA dimensions correlated with improved future A/E value and vice versa. Right atrial (RA) contractility restoring favored better left ventricular (LV) performance and volumes. Conclusions. SR and left AC are two interrelated Maze objectives. Factors associated with arrhythmia “chronic state” (PAP and arrhythmia duration) are negative predictors of procedural success. Our results suggest an association between postoperative LA dimensions and “kick” restoring and an influence of RA contraction onto LV function.

1. Introduction

AF presents with different frequencies in patients affected by structural heart diseases requiring surgery, showing a peak incidence of up to 60–80% in mitral subjects [1]. It causes an increased risk of systemic embolism, cardiac failure development, and higher limitations due to dyspnea on exercise. Cox-Maze technique for surgical AF treatment started being used in 1987 [2] and it has suffered numerous modifications since then. Nowadays, most centers have replaced the “cut and sew” technique by other methods using several power sources to achieve the same target in a much easier way [3]. One of the alternative energy sources currently used is radiofrequency (RF) ablation which has been demonstrated to be a simple, safe, and reproducible procedure with an acceptable success rate in terms of SR restoration [4].

The original Maze was designed with three specific goals in mind: (1) the permanent AF ablation, (2) the restoration of atrioventricular synchrony, and (3) the preservation of atrial transport function [2]. If, by one side, the efficacy of the procedure in reaching the first two goals is widely known [5, 6], the restoration of the SR does not always accompany the corresponding recuperation of atrial mechanical “kick” [7, 8]. Atrial contraction is effective when A waves are found in tricuspid and/or mitral transvalvular flow using Doppler echocardiography [9], allowing the patient to fully profit from hemodynamic and clinical advantages of an organized atrial contraction. If the atrial transport function fails to recover, benefits deriving from arrhythmia abolition might only be marginal, since, by one side, blood stasis in the atria persists, thus maintaining unchanged thromboembolic risk and, by the other side, heart hemodynamic performance is still impaired resulting from the loss of atrial contribution to cardiac output [10].

Despite its relevant role for judging Maze comprehensive success, biatrial contractility restoration and its clinical importance have not been deeply investigated. In more
Table 1: Patients characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M:F)</td>
<td>59/63</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62 ± 8.5</td>
</tr>
<tr>
<td>Chronic AF</td>
<td>79 (64.7%)</td>
</tr>
<tr>
<td>Paroxysmal AF</td>
<td>53 (35.3%)</td>
</tr>
<tr>
<td>AF duration (months)</td>
<td>28.3 (range 1–220)</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>56 ± 12</td>
</tr>
<tr>
<td>LAA (cm²)</td>
<td>34 ± 7</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>34 ± 9</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>33 ± 15</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>57 ± 9</td>
</tr>
<tr>
<td>Mitral stenosis</td>
<td>35 (39%)</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td>87 (71%)</td>
</tr>
<tr>
<td>Mitral valve repair</td>
<td>76 (62%)</td>
</tr>
<tr>
<td>Mitral valve replace</td>
<td>46 (38%)</td>
</tr>
</tbody>
</table>

AF, atrial fibrillation; LAD, left atrial diameter; LAA, left atrial area; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; M : F, males : females.

2. Methods

2.1. Patients’ Characteristics. We analyzed a total of 122 consecutive patients with mitral valve disease scheduled for cardiac surgery who underwent a modified RF Maze procedure between January 2005 and June 2012 at our center (Table 1). There were 59 males and 63 females, with a mean age of 62 ± 8.5 years (range 30–82 years) at the time of operation. 64.7% of patients had permanent or persistent AF with a mean preoperative duration of 28.3 months (range 1–220 months); in the others our indication to perform the concomitant RF was an at least double occurrence of paroxysmal AF refractory to medical treatment (arrhythmia definition based on ACC/AHA Guidelines 2011) [11].

Mean preoperative LA dimensions were 56 ± 12 mm and 34 ± 7 cm². Mean basal PAP was 33 ± 15 mmHg. Patients experiencing mitral repair with residual regurgitation higher than 1 degree (scale 1 to 4) were excluded from the study.

Preoperative clinical and echocardiographic variables are shown in Table 1.

This study was approved by our institution’s ethical committee/institutional review board.

2.2. Surgical Procedures. RF energy was used to create continuous endocardial and epicardial lesions mimicking most of the left atrial incisions set as described in the Cox-Maze III procedure [12]. We did not perform any ablation line in the right atrium (Figure 1). In all patients a bipolar device was used (Cardioblate BP2 Irrigated RF Surgical Ablation System, Medtronic Inc., Minneapolis, MN, USA). The tip of the probe was irrigated with saline at room temperature at a flow rate of 4–6 mL/min.

Cardiopulmonary bypass was used with standard aortic cannulation, bicaval cannulation, and moderate hypothermia. After epicardial isolation of both pulmonary veins and of the left atrial appendage performed before aortic cross-clamping, some of the additional LA incisions sets currently used in the “cut and sew” technique were replaced by RF ablation lines, in particular represented by a double union line between the left and right pulmonary islands and a lesion connecting the right pulmonary one to the posterior annulus of the mitral valve at P2 or P3 portion (according to circumflex artery anatomy). In all patients the LA appendage was internally or externally closed.

After completion of the RF ablation, mitral valve repair or replacement was performed (Table 1).

2.3. Postoperative Management. Postoperative general care was similar to routine cardiac surgical procedures. Modality of amiodarone prophylaxis protocol treatment was the same in all patients, represented by iv amiodarone 1200 mg/24 h in the first postoperative day, followed by 200 mg per os every 8 hours until hospital discharge and then 200 mg daily for six months. In case of amiodarone intolerance or contraindications, propafenone was employed.

Patients showing SR or an ectopic atrial rhythm with rate inferior to 70 bpm in the first 48–72 postoperative hours were treated with temporary atrial pacing fixed at 80 bpm with the aim of avoiding supraventricular ectopic beats occurrence and consequently AF onset and of favoring atrial electrical activity recovery. Postoperative atrial tachyarrhythmias recurrences not responsive to medical treatment were submitted to direct-current (DC) shock; in this case, a loading
dose of amiodarone was administrated before and until 24 hours after DC shock and then interrupted.

2.4. Clinical Follow-Up. Early postoperative rhythms were checked daily by standard 12-channel surface electrocardiogram. Follow-up 24-hour Holter monitoring was checked postoperatively at 3, 6, 12, and 24 months after the intervention.

2.5. Echocardiographic Follow-Up. Contemporary to clinical follow-up, all patients were evaluated with 2-dimensional transthoracic echocardiography by the same cardiologist at 3, 6, 12, and 24 months in order to specifically monitor the evolution of cardiac chambers dimensions and systolic performance and to record left and RA contractility presence. In more detail, bilateral transport function was checked in all patients, independently from the original (paroxysmal or persistent) AF type.

All echocardiographic examinations were performed with a Philips ultrasound system (iE33, Andover, MA, USA). LA diameter was measured at the time of aortic valve closure on the M-mode echocardiogram in the parasternal long-axis view. End-diastolic and end-systolic internal LV diameters were determined from the M-mode echocardiogram and fractional shortening was obtained.

Systolic PAP was calculated by adding transtricuspid pressure gradient to mean right atrial pressure estimated from inferior vena cava diameter and motion during respiration as follows: mean right atrial pressure was estimated to be 5 mmHg if there was complete collapse of a normal inferior vena cava during inspiration, 10 mmHg if a normal inferior vena cava collapse was >50%, 15 mmHg if a dilated inferior vena cava collapsed by >50% with inspiration, and 20 mmHg if there was no visible evidence of dilated inferior vena cava collapse.

Transmitral flow velocity was measured with pulsed Doppler echocardiography, with a sample volume positioned at the level of the mitral tip in the apical four-chamber view, and was recorded on a strip chart at a paper speed of 100 mm/s. Peak velocity and the time-velocity integral of the early filling wave (E wave) and of the late filling wave (A wave) were determined. A/E ratio, representing atrial contribution to ventricular diastolic filling, was obtained. Each measurement was obtained as an average of 6 to 8 consecutive beats. We considered that a peak A wave velocity ≥10 cm/s indicated echocardiographic evidence of effective atrial contraction [9].

2.6. Statistical Analysis. Data were collected and managed in Microsoft Excel 2003 and analyzed with SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). Continuous variables were presented as mean ± SD and categorical variables as percentages or numbers. Seventeen variables were evaluated univariately to identify predictors of arrhythmia recurrence and of AC restoration. Univariate analysis was performed for all relevant categorical variables by means of \( \chi^2 \) analysis and Student’s t-tests when indicated. A multivariate Cox proportional hazard model (including factors positive at univariate analysis) was used to determine the independent predictors of AC recuperation and of AF reappearance. A multiple events Cox model was applied too for taking into account the chance of multiple arrhythmia and transport loss recurrences.

Categoric and continuous variables associations were, respectively, investigated with \( \chi^2 \) test and Spearman rank correlation coefficient. McNemar test was employed for evaluating LA and RA contractility concordance. A value of \( p \) less than 0.05 was considered statistically significant.

3. Results

3.1. Early Postoperative Data and 2-Year Rhythm Follow-Up. Mean aortic cross-clamp and cardiopulmonary bypass time were, respectively, 95 ± 38 and 121 ± 43 minutes. In all patients who underwent mitral valve repair, a residual valvular regurgitation lower than 1 degree (scale 1 to 4) was achieved.

One patient (0.8%) died in the Intensive Care Unit for unresponsive cardiac failure. Postoperative complications included major ischemic strokes (2/122, 1.6%) and reoperation for bleeding (4/122, 3.2%) whose origin was not related to Maze lesions.

79 patients (65%) were discharged from the hospital in SR, 29 (24%) presented with stable AF, and 5 showed an atrial flutter while 9 patients had an atrial ectopic rhythm with heart rate superior to 70 bpm.

2-year clinical follow-up was achieved in all patients. There were 2 (1.6%) late deaths (1 progressive congestive heart failure and 1 extra-cardiac cause). Stable SR was achieved in 96 patients (79%), while 18 (15%) experienced AF recurrence; most of them (15/18, 83%) were persistent arrhythmias resistant to pharmacological or electrical shock, and the others had AF episodes responsive to medical treatment or DC shock. No patients presented postincisional tachycardia. In the whole study population, 7 patients (5.7%) underwent permanent pacemaker implantation.

3.2. Contractility Restoration. At early follow-up (3 months), only 76% of patients showing a stable SR presented with a corresponding efficacious left AC. Nevertheless, such percentage of coexistence increased progressively, joining about 98% two years after the operation (Figure 2).

LA contraction failed to be accompanied in every case by a corresponding RA contraction, since, at 3 months of follow-up, only in 84% of patients with an “A” wave both atria efficaciously contracted (McNemar Kappa coefficient equal to 0.68), while 2 years after the intervention the percentage of bilateral “kick” coexistence reached 98% (McNemar Kappa coefficient 0.87) (Figure 3).

In patients who remained in persistent SR without known arrhythmia recurrence (nearly 74% of all population), left A/E ratio slightly augmented until a mean value of 2.65 at 24 months of echocardiographic follow-up (Figure 4).

3.3. Risk Factors Analysis of Maze Failure. The univariate analysis of the predictive factors of freedom from AF
recurrence at 2-years of follow-up is shown in Table 2 and identifies as significant protective variables a preoperative arrhythmia duration inferior to 36 months (meaning the period of any AF type history: for longstanding one, it was calculated from the beginning date, but for paroxysmal arrhythmia from the date of the first episode recording), 3 and 6 months of left AC restoration, and SR presence at hospital discharge. Such last feature failed to be confirmed at multivariate analysis (Table 2).

Due to uniformity of antiarrhythmic prophylaxis and treatment in case of arrhythmia recurrence the item "amiodarone use" was not included in the statistical analysis of risk factors of Maze failure.

In order to better evaluate the theoretical possibility of several AF recurrences, a multiple events Cox model was applied, again revealing the protective role played by a shorter preoperative arrhythmia duration and by early AC regain.
Table 3: Univariate and multivariate analysis for absence of LA contractility recovery at 3 months of follow-up.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p value</td>
<td>HR 95% CI p value</td>
</tr>
<tr>
<td>PAP</td>
<td>&lt;0.0001</td>
<td>1.05 1.01–1.11 0.02</td>
</tr>
<tr>
<td>LAA (cm²)</td>
<td>0.002</td>
<td>1.06 1.01–1.14 0.06</td>
</tr>
<tr>
<td>Duration of AF</td>
<td>0.003</td>
<td>1.014 1–1.03 0.06</td>
</tr>
<tr>
<td>Age</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>Rheumatic disease</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Permanent/persistent AF</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>0.47</td>
<td></td>
</tr>
<tr>
<td>CPB time</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Cross-clamp time</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>AF at discharge</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.58</td>
<td></td>
</tr>
</tbody>
</table>

Not all factors were entered into multivariate model.

3.4. Predictive Factors of LA Contractility Restoration. The same features used for uni- and multivariate analysis for freedom from AF recurrence (with the obvious replacement of 3 and 6 months of left AC by 3 and 6 months of SR presence for late AC regain evaluation) were entered into the statistical model in order to evaluate possible predictive factors for absence of left AC recovery. At multivariate analysis, significant predictors for absence of LA transport function restoring were preoperative arrhythmia duration for 6–12 and 24 months of follow-up (p value 0.005, 0.05, and 0.017, resp.) and basal PAP for 3 and 6 months of contractility (p value 0.02 and 0.03, resp.). 3- and 24-month results are shown in Tables 3 and 4. No other common clinical or operative features were highlighted in patients presenting SR without corresponding AC at different follow-up checks.

Again, a multiple events Cox model was applied, confirming the unfavorable role played by longer AF duration (p = 0.005) and by higher PAP (p = 0.029) (Table 5).

Table 4: Univariate and multivariate analysis for absence of LA contractility recovery at 24 months of follow-up.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Univariate</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p value</td>
<td>HR 95% CI p value</td>
</tr>
<tr>
<td>Duration of AF</td>
<td>&lt;0.0002</td>
<td>1.034 1.006–1.063 0.017</td>
</tr>
<tr>
<td>3-month SR presence</td>
<td>0.07</td>
<td></td>
</tr>
<tr>
<td>6-month SR presence</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>LAA (cm²)</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>PAP</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Rheumatic disease</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Permanent/persistent AF</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>CPB time</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Cross-clamp time</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>AF at discharge</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.49</td>
<td></td>
</tr>
</tbody>
</table>

Not all factors were entered into multivariate model.

3.5. Contractility and Echocardiographic Measurements. Some statistically significant associations were retrieved between cardiac chambers dimensions and function and biatrial A/E ratios (considered as markers of contractility presence) evaluated at different follow-up times; in more detail (Table 6),

(i) minor LA dimensions were related with improved future A/E value and consequently with more pronounced LA contractility;

Table 5: Multiple events Cox model for absence of LA contractility recovery.

<table>
<thead>
<tr>
<th>Factors</th>
<th>HR 95% CI p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of AF (&gt;36 months)</td>
<td>1.14 1.07–1.22 0.005</td>
</tr>
<tr>
<td>PAP</td>
<td>1.12 1.04–1.21 0.029</td>
</tr>
</tbody>
</table>

AC, atrial contractility; AF, atrial fibrillation; CI, confidence interval; HR, hazard ratio; PAP, pulmonary artery pressure.

(ii) 3-month augmented left A/E favored greater improvement of LA diameter evaluated 1 and 2 years after surgery;

(iii) 3 and 24 months of better RA contractility corresponded to minor contemporary decrease of LVEF with respect to basal values;

(iv) 12-month greater right A/E ratio was associated with decreased 24-month LV volumes and better LVEF.

4. Discussion

The association between AF and mitral valve disease is present in almost 60% of cases needing surgical correction. For more than 20 years, Cox-Maze procedure has been the gold standard for treating AF surgically [13]; despite its high success rate, it entails long surgery times and significant bleeding risks [14]. That is the reason why alternative
energy sources and simplifications of ablation sets have been progressively developed, including irrigated RF. Such device achieves an acceptable (although inferior to the surgical Maze) degree of SR conversion at mid-long-term follow-up, equal to 45–95% [15–17].

If arrhythmia abolition has represented the most important goal of the procedure, it was even specifically conceived for allowing the entire atrial myocardium to be activated preserving postoperative atrial transport function [10]. The importance of AC is double, since, firstly, it may contribute to an increase in stroke volume, particularly in case of fast heart rates [18], and second, it is reasonable to think that the absence of an efficacious mechanical atrial action recovery even if associated with SR detection could favor intra-atrial thrombi formation and consequent thromboembolic systemic phenomena [19], thus making the decision to interrupt anticoagulant treatment unsafe.

Based upon such considerations, we decided to focus our attention onto biatrial transport function behavior in a relatively homogeneous sample of patients affected by mitral valve disease and concomitant AF in whom RF modified left Maze procedure obtained 2-year arrhythmia abolition in about 80% of cases, in agreement with what is reported in medical literature.

Previous studies retrieved that restoration of both SR and atrial contraction varies from 21% to 95%, depending on AF etiology [20–25]. In more detail, in case of exclusively LA Maze ablation, it has been proved that, despite successful SR restoration, the progressive loss of LA function as well as LV diastolic function is more prominent in patients affected by rheumatic disease with respect to those with degenerative prolapse [26].

In our trial, AC restoring was a dynamic and raising process, a finding that perhaps translates into the practical advice of persisting in serially controlling by echocardiography also patients that early experience SR reestablishment few months after the procedure, because in about three-quarters of cases LA is still “stunned.” This consideration may assume a great empirical role, since it discourages from interrupting anticoagulant and antiarrhythmic treatment too early, even in presence of detected stable SR and low CHADS2 score.

Another element that could support this prudent attitude is the evidence of progressive increase of atrial contribution entity. A/E ratio time-course after Maze is a discussed question, as some authors [27] describe a decreasing trend but others [10] a fluctuant tendency more in line with our findings. On the contrary, Yuda and colleagues [28] demonstrated that once AC was resumed, its degree did not change during follow-up: the difference with respect to our results may lie in the major prevalence in their sample of mitral valve replacement (prosthesis could modify modality of LA transport function) and of basal LV dysfunction associated with a more aggressive lesions set performed with mixed cut and sew technique and cryoenergy. A recent report from Sayed et al. [29] highlighted the fact that the “left side only” ablation procedure, when compared to biatrial one, allowed better 6-month A/E ratio but with a mean value largely lower (0.52) than the one emerging from our trial. Nevertheless, again, enrolled patients were all affected by rheumatic disease, thus perhaps explaining worse postoperative atrial function.

Starting from the assumption that SR and AC recovery represent two inseparable arms of the same question, we tried to deeply examine possible predictors of restoration and maintenance of atrial transport function at different intervals, with the objective of screening patients who could fully benefit from the procedure. Moreover, we applied a supplementary statistical tool addressed at also evaluating the intrinsic arrhythmia nature consisting in its chance of several recurrent episodes. In fact, it is well known that many subjects experience repeated heart rhythm changes (and consequently disappearance of atrial “kick”) above all in the first postoperative months, probably due to recovery process, to postprocedural inflammation status, or to altered neurohumoral mechanisms [30].

The only shared negative predictive factor for both SR and left AC restoring highlighted by our analysis was preoperative AF duration, with a cut-off identified at 36 months. If the role of longstanding arrhythmia largely emerged in medical literature [31, 32], other issues were unexpectedly retrieved: in more detail, early atrial transport function regain was found to strictly correlate with future stable freedom from arrhythmia recurrence, while basal PAP influenced the possibility of left AC recuperation in the early postoperative phases and thus the symmetrical Maze goal of SR achievement. Concerning the former association, to the best of our knowledge, this represents the first report in medical literature, but, interestingly, the reverse interrelation failed to be retrieved. We may speculate that AC is a secondary phenomenon which follows SR recovery (and not in all cases) and which is consequently something of more definitive favoring perpetuation of SR, while early SR appearance could be a temporary event not necessarily implying rhythm and atrial transport function stability.

The discouraging role played by basal PAP onto future atrial transport function recuperation was previously reported only by Reyes et al. [33], but without statistical significance. Its real implication needs surely further analysis.
but may perhaps reflect the fact that fixed and longstanding mitral pathology associated with chronic AF which have already caused progressive augmentation of pulmonary pressures with effects onto pulmonary circulation represent a more serious and advanced disease stage implying more possibilities of Maze failure. A deeper investigation of which type of pulmonary hypertension is involved (pre- or postcapillary, in order to understand if its adverse role is more due to a pulmonary or cardiac component) is required but perhaps arises the doubt whether a more comprehensive patient’s evaluation including right heart performance should be performed.

Medical literature extensively addressed the problem of identifying predictors able to discourage the surgeon to perform ablation, including old age, larger LA diameter, lower amplitude of p-wave, having a rheumatic mitral valve disease, permanent AF, and lesion sets of Maze procedures [32, 34]. In case of analysis specifically focused onto restoration of atrial contraction, AF duration, LA diameter, an history of hypertension, rheumatic disease, and the presence of prosthetic valve failure arose as significant negative predictors [25, 26, 35]. In our opinion, all these features reflect the negative influence played by arrhythmia “chronic state” onto Maze success; the same conclusion can be suggested by our data too, since PAP and longer preoperative arrhythmia duration are intimately linked with the idea of a longstanding phenomenon. Based upon such concept, we can perhaps draw the suggestion of carefully screening patients potentially candidates to surgical ablation, being aware that those presenting “ancient” arrhythmia with augmented PAP will unlikely benefit from the procedure.

Nevertheless, in our analysis, LA dimensions did not represent a negative predictor of SR-AC restoring; however, such absence of relationship is partially mitigated by the finding of a bidirectional association between LA size and chance of future AC recovery or, at the opposite, between early atrial “kick” recuperation and more marked future atrial diameter and area decrease. To the best of our knowledge, this is the first demonstration of a strength link between these two elements which are logically and theoretically related but whose modality of respective behavior was not completely clear.

Moreover, we found that the atria seem to differently regain contraction after Maze. Available data addressing the question show various rates of RA function recovery [10, 33]. Although it is not directly touched, we can imagine that RA is somehow influenced by the ablation procedure itself. Such subject may represent an interesting matter also considering that, in our study, RA transport function recuperation appears to favor better contemporary and future LV contractile performance and decreased volumes. Even if these findings have not been found at all follow-up intervals, they may represent an intriguing hypothesis of a supplementary interrelation between right and left cardiac sides leading to the suggestion of reconsidering RA role.

An important study limitation is represented by the limited sample size which does not allow drawing definitive conclusions; we believe that our preliminary results should be confirmed in larger trials including “more ancient” mitral and fibrillating patients, in order to better describe the negative influence of longstanding arrhythmia onto Maze objectives.

5. Conclusions
We serially assessed biatrial function after RF Maze procedure in patients affected by mitral valve disease.

In the early postoperative phases, a regular rhythm with P-waves was accompanied by a coexisting atrial transport function only in three quarters of cases, but concordance joined about 100% at two years of follow-up. Once LA contraction was resumed, its degree augmented progressively and was associated with a corresponding RA transport function in an increasing percentage of cases at late follow-up. A strict interaction was detected between LA chamber postoperative evolution and LA contractility recovery, as a decrease of LA size favored atrial “kick” reappearance and vice versa.

SR and AC appear to be two intimately linked objectives of the Maze procedure, since early atrial transport function recuperation favors future stable freedom from arrhythmia recurrence.

Our study highlighted the deleterious influence of arrhythmia “chronic state” onto procedural success and the importance of the cardiac right side, an entity somehow overlooked: even if these data need further investigation in order to elucidate a possible clinical implication too, we retrieved that, first, preoperative arrhythmia duration and, more interesting, pulmonary hypertension (an element that could be associated with a latent right ventricular dysfunction) play a role in predicting Maze failure in terms of SR-AC restoring and, second, RA contractility has an influence on LV contractile performance recovery and volumes decrease.

Conflict of Interests
There is no conflict of interests.

References


Review Article

Cardiomyocyte Remodeling in Atrial Fibrillation and Hibernating Myocardium: Shared Pathophysiologic Traits Identify Novel Treatment Strategies?

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Atrial fibrillation (AF) is the most common arrhythmia and is associated with a high risk of morbidity and mortality. However, there are limited treatment strategies for prevention of disease onset and progression. Development of novel therapies for primary and secondary prevention of AF is critical and requires improved understanding of the cellular and molecular mechanisms underlying the AF disease process. Translational and clinical studies conducted over the past twenty years have revealed that atrial remodeling in AF shares several important pathophysiologic traits with the remodeling processes exhibited by hibernating myocardium that develop in response to chronic ischemia. These shared features, which include an array of structural, metabolic, and electrophysiologic changes, appear to represent a conserved adaptive myocyte response to chronic stress that involves dedifferentiation towards a fetal phenotype to promote survival. In this review, we discuss the pathophysiology of AF, summarize studies supporting a common remodeling program in AF and hibernating myocardium, and propose future therapeutic implications of this emerging paradigm. Ultimately, better understanding of the molecular mechanisms of atrial myocyte remodeling during the onset of AF and the transition from paroxysmal to persistent stages of the disease may facilitate discovery of new therapeutic targets.

1. Introduction

Atrial fibrillation (AF) is the most common sustained arrhythmia, affecting about 1% of the general US population [1]. It is an abnormal heart rhythm characterized by rapid, irregular, and heterogeneous atrial electrical activity that is associated with ineffective atrial contraction (Figure 1) [1]. There is variable and irregular conduction of atrial electrical activity to the distal electrical system and ventricles.

The incidence of AF increases with advancing age, such that 8% of adults older than 80 years are affected by the disease. It is projected that the prevalence of AF will reach 5.6 to 12.1 million individuals in 2050 [2, 3]. Lifetime risks for development of AF are 1 in 4 for men and women 40 years of age and older [4]. Also, AF is associated with an increased risk of all-cause mortality and morbidity including stroke, heart failure, dementia, embolic events, and impaired quality of life.

As a result, it is the most common cause of hospital admission for cardiac rhythm disturbances and a major public health problem, with a total annual economic burden of ~$7.9 billion [5–7]. Despite the magnitude of the disease, the precise molecular mechanisms underlying AF remain incompletely understood. Elucidating disease mechanisms at the basic and clinical level is essential to identify novel targets for prevention and treatment of AF. To date, studies have revealed that the pathophysiology of AF is complex and includes multiple components ranging from vulnerable atrial substrate to electrophysiologic triggers (Figure 2) [1]. Electroanatomical remodeling of atrial myocytes is an essential component of AF pathogenesis and exhibits features that are similar to those of another cardiac pathology, hibernating myocardium (HM). HM is characterized by an array of
AF remain unclear. While recent studies have identified a familial form of AF [8], the majority of patients with AF have the common form with no defined genetic susceptibility. Here, we summarize recent findings in AF pathogenesis, particularly those related to the structural and electrical remodeling that occurs throughout the disease process.

2.1 Atrial Morphological Changes form a Structural Substrate for AF. Several characteristic atrial architectural changes are typically observed in preclinical models of AF and in patients suffering from the disease. These include inflammation, cell hypertrophy, atrial dilation, and fibrosis, which cumulatively contribute to abnormal electrical signal formation and conduction as an arrhythmogenic substrate [9]. These changes are commonly the result of other underlying heart diseases, such as coronary artery disease, hypertension, valvular disease, and cardiomyopathies, which exert adverse effects on myocardial structure and/or function, predominantly via elevations in atrial pressure and wall stress [1]. The elevated hemodynamic load on the atria promotes cellular hypertrophy, cardiomyocyte dysfunction, and disorganization of gap junctions. This process is associated with myocyte death through apoptosis and necrosis. Myocyte loss, together with neurohumoral signaling activated by atrial stretch, prompts extensive replacement fibrosis in large part because of the greater number of fibroblasts present in the atria and its subsequent propensity for fibrotic tissue deposition [1].

Collectively, the aforementioned morphological changes contribute to the formation of a structural substrate that promotes the onset and progression of AF. For instance, loss of atrial cardiomyocyte mass with apoptosis or necrosis causes accumulation of fibrotic tissue, disruption of cell-to-cell communication, diminished conduction velocity, and heterogeneous conduction patterns [9–12]. This replacement fibrosis, along with amyloidosis, inflammation, and remodeling of the extracellular matrix, disrupts gap junctions and impairs cell coupling [13–15]. Moreover, the concomitant presence of obesity and metabolic risk factors may exacerbate structural, metabolic, and electrophysiologic changes that have been suggested to represent myocyte dedifferentiation and a conserved adaptive response to stress, which may also be apparent in atrial myocytes during AF. The adoption of a phenotype characteristic of HM by atrial myocytes may contribute to the initiation and progression of AF. Thus, further understanding of the common mechanisms underlying AF and HM may facilitate the development of novel targets for disease prevention and treatment.

The goal of this review, therefore, is to demonstrate the shared pathophysiologic traits of AF and viable dysfunctional myocardium, with particular emphasis on atrial cardiomyocyte remodeling in AF and the characteristics that are shared with myocardial hibernation. Future opportunities to investigate the molecular mechanisms and develop novel therapies for AF are also discussed. To identify relevant articles, a literature search of PubMed-indexed articles ranging from 1948 through October 1, 2014, was performed using the key words "atrial fibrillation" and "hibernation," "mechanisms," or "pathophysiology" published in English. All references in this search list were reviewed by the titles and abstracts to select relevant articles for full-text review. Retrieved relevant articles are included in this paper. Thus, the list of references reflects an overview of existing literature relevant to AF and hibernation in atrial myocardium.

2. Pathogenesis of Atrial Fibrillation

The pathophysiology of AF is complex and often multifactorial, generally involving an electroanatomical substrate, abnormal impulse formation and/or propagation, focal and dynamic triggers, reentry, and fibrosis in atrial myocardium (Figure 2). Various pathophysiologic mechanisms and multiple disease pathways have been studied in AF; however common molecular mechanisms underlying the initial development of AF and transition from paroxysmal to persistent
this process via the release of proinflammatory mediators from epicardial fat [16]. Also, a recent study demonstrated that rapid atrial pacing AF was associated with changes in atrial adipocyte/adipositas-related gene expression including upregulation of 66 genes and downregulation of 53 genes at the mRNA level [17]. The clinical importance of these genes has yet to be determined. Regardless of the initiating stimulus, paroxysmal AF promotes further AF and stretch, leading to more extensive fibrosis and increased extracellular matrix deposition that ultimately slows or blocks intra-atrial conduction as a part of the adaptation to high atrial rates. In AF, the rate of disorganized atrial contraction is over 300 beats per minute or cycle length of 130–200 milliseconds while it is only 50–100 beats per minute in sinus rhythm. This high and variable rate of atrial contraction may cause significant metabolic and oxidative stress as seen in tachyarrhythmias. Interestingly, the adverse effects of AF are not limited to the atria, as a porcine model of rapid atrial pacing-induced AF produced microcirculatory flow abnormalities in the left ventricle that were associated with elevations in markers of oxidative stress but could be reversed by administration of dronedarone [18]. Ultimately, these flow abnormalities and the structural changes that initiate AF are worsened by AF itself, forming a vicious cycle that heralds progression of disease severity (i.e., “AF begets AF” [12]).

2.2. Electrophysiological Changes Promote Initiation and Progression of AF. In parallel with structural alterations, electrophysiological changes occur in atrial myocytes that contribute to the onset and advancement of AF. Clinical studies have shown that AF can be triggered by autonomic stimulation, bradycardia, atrial premature beats, tachycardia, accessory pathways, and acute atrial stretch. Sites of focal AF triggers include the pulmonary veins, the superior vena cava, ligament of Marshall, and coronary sinus [19]. The reason for this localization is incompletely understood but could be the result of local cardiomyocyte remodeling with altered oxidative and metabolic condition.

Electrical or focal mechanisms of AF involve abnormal automaticity, triggered activity, and multiple variable reentrant circuits [1, 11]. A vulnerable atrial substrate triggers propagation of multiple reentering wavelets with heterogeneous shortening of the atrial effective refractory period (ERP) and altered conduction velocity [15]. Myocyte resting potential decreases in AF and atrial action potential morphology is altered [20]. Atrial tachycardia and stretch present in the early stages of AF alter ionic currents and promote disease progression from the paroxysmal to persistent state. Reduced L-type Ca\(^{2+}\) (\(I_{CaL}\)) current, Ca\(^{2+}\) overload, changes in K\(^+\) current (\(I_{KCa}, I_{K1}\)), Na\(^+\) current (\(I_{Na}\)), and transient outward current (\(I_{to}\)) have each been reported in AF [10]. Several pathways have been suggested to be associated with some of these changes including phosphorylation of ion channels via protein kinase A or C isoforms and effects of reactive oxygen species (ROS) [11]. However, exact molecular or subcellular mechanisms are not completely known. Increasing attention has been directed towards the role of cellular energetics and metabolism in the disease process, given the significant interaction between energetics and ionic homeostasis. Mitochondria-driven cardiomyocyte energetics and metabolism may have a significant role in regulation of ion channels [21–25]. Also, ionic currents may directly impact mitochondrial function itself, as it has been previously demonstrated that high Ca\(^{2+}\) inhibits mitochondrial respiration, dissipates membrane potential, and suppresses ATP production [26, 27]. Thus, future studies may reveal a critical role of mitochondrial-driven structural and electrophysiological changes that may have a central role in the pathophysiology of AF.

2.3. Cardiomyocyte Cellular and Molecular Remodeling in AF. Initial insight into the cellular structural remodeling associated with chronic AF was provided by Thiedemann and Ferrans [28], who used light microscopy to demonstrate fibrosis, myocyte hypertrophy, and myolysis in atrial tissue samples from patients with AF secondary to mitral valve disease. Several years later, these findings were supported in a canine model of mitral valve fibrosis characterized by left atrial enlargement and the common occurrence of atrial arrhythmias [29]. These animals exhibited reduced atrial wall thickness and substantial connective tissue between notably hypertrophied myocytes organized into disarranged cell bundles. More recent studies in patients with AF have extended these observations and shown sarcomere depletion and glycogen accumulation in remodeled atrial myocytes [30–32], though the concomitant presence of valve disease in the majority of subjects may have contributed to these results.

To address this limitation, experimental animal models of lone AF have proven useful to delineate patterns of structural remodeling in myocardies after prolonged periods of AF. For example, rapid atrial pacing-induced AF in dogs elicited characteristic electrophysiologic changes including shortened atrial ERP and structural alterations consisting of atrial chamber dilation and myocyte hypertrophy [33]. This approach was translated to a goat model [12], in which animals exhibited enlarged atrial myocytes and glycogen accumulation that progressively worsened with increasing disease severity [34, 35]. In addition, disorganization of sarcoplasmic reticulum, appearance of mini-mitochondria, reductions in T-tubular sarcosomal invaginations, and dispersion of nuclear chromatin were observed [34]. Interestingly, many of the atrial cardiomyocytes reacquired characteristics of fetal cardiomyocytes, including expression of α-smooth muscle actin, loss of cardiotin, and a punctuated titin staining pattern [35]. It was concluded that AF was associated with myocyte dedifferentiation in the absence of degeneration, perhaps representing a conserved cellular response to stress. A more recent study [36] provided additional insight into this remodeling process in AF patients. Using a genome-wide approach to compare atrial mRNA expression in AF patients versus patients with sinus rhythm, the authors identified over 1400 genes that were deregulated in chronic AF. Functional classification analysis revealed a pattern of remodeling consistent with prominent fibrosis and metabolic adaptation to long-term metabolic stress, including upregulation of genes related to extracellular matrix
composition, downregulation of contractile proteins, and coordinated transcriptional changes in metabolic enzymes favoring a shift from fatty acid oxidation to glucose utilization [36]. Additionally, atrial tissue from AF patients exhibited a general “ventricularization” characterized by substantial upregulation of ventricle-predominant genes and downregulation of atrial-specific genes, consistent with myocyte dedifferentiation and adoption of a fetal phenotype aiming at improving cell survival during extended periods of stress [36, 37].

Collectively, these results show that, aside from the atrial structural and electrical remodeling that occurs in AF, cardiomyocytes that do not succumb to apoptosis or necrosis undergo several characteristic cellular and molecular phenotypic changes. Interestingly, these alterations are reminiscent of those observed during cell dedifferentiation, including an increase in myocyte volume, myolysis, glycogen accumulation, mitochondrial changes, and chromatin redistribution [38]. Because these changes commonly occur in the absence of clear signs of degeneration, it has been suggested that atrial myocyte dedifferentiation in AF represents an adaptive, programmed cell survival response. While this idea is supported by findings in several experimental models of AF, clinical studies of AF patients that exhibit cardiomyocyte degeneration argue against this notion. These divergent observations may implicate comorbidities and/or the longer duration of disease in patients as primary factors determining the adaptive versus degenerative nature of myocyte remodeling in AF [38]. Nevertheless, our current knowledge in this area supports the notion that myocyte remodeling in AF (particularly in patients with lone AF) represents an adaptive response to stress that aims to conserve energy and promote survival, similar to what has been observed in other conditions of myocardial stress, such as the development of HM in response to chronic ischemia. Therefore, consideration of common pathophysiologic traits shared in these disease states may facilitate the development of novel therapeutic approaches by encouraging a shift in our perspective to a position that recognizes the adaptive, and potentially reversible, nature of myocyte remodeling during chronic stress.

3. Pathophysiology of Hibernating Myocardium

HM represents one entity along a pathophysiologic continuum describing the heart’s adaptive responses to ischemia. Although complete cessation of blood flow following coronary occlusion will typically begin to elicit subendocardial necrosis as early as 20 minutes after the onset of ischemia, this type of irreversible injury is fortunately less common than reversible ischemia that produces viable dysfunctional myocardium, in the absence of necrosis, which may demonstrate transient periods of contractile dysfunction [39]. The time course of recovery is variable and may occur as early as 24 hours after return of coronary perfusion in the case of acute myocardial stunning, which is defined by reduced contractile function despite normal levels of resting perfusion [40]. If these episodes of ischemia persist, regions of viable dysfunctional myocardium may transition from chronic stunning to chronic HM, characterized by contractile dysfunction with reduced resting flow in the absence of acute ischemia or significant necrosis [41]. The clinical recognition of this tissue substrate is important because, in contrast to scarred myocardium, functional recovery of HM is possible upon revascularization or elimination of hypoxic conditions.

3.1. Cellular Remodeling of Hibernating Myocardium: Evidence of Myocyte Dedifferentiation. The clinical significance of HM has motivated extensive preclinical and patient-oriented research to define the cellular and molecular changes that occur in response to chronic repetitive ischemia. This investigation has revealed a pattern of remodeling encompassing structural, metabolic, and electrophysiologic changes that are similar to that previously discussed in the context of AF [39]. Structurally, hibernating myocytes exhibit myofibrillar loss, glycogen accumulation, disorganization of the sarcoplasmic reticulum, and abnormalities in mitochondrial size and shape, in concert with reactive hypertrophy and fibrosis secondary to myocyte loss [42]. Experimental large animal studies have demonstrated that this loss of myocytes results primarily from apoptotic cell death, not necrosis, with compensatory myocyte hypertrophy sufficient to preserve wall thickness [43]. Although the degree of cell loss and myocardial dysfunction stabilizes in some animal models, suggesting an adaptive response, some patient studies show an inexorable progression of cell death and functional deterioration, likely related to factors such as ongoing neurohumoral activation or the presence of comorbidities that are common in patients (as is the case with AF, discussed above). In addition to structural remodeling, HM is characterized by metabolic alterations including a reduction in oxidative metabolism and increased dependence on anaerobic glycolysis [44]. This shift contributes to a diminution in myocardial oxygen consumption that allows maintenance of energetic balance, prevents ongoing ischemia, and protects myocyte from oxidative injury. Collectively, these changes support the notion that hibernating myocytes undergo dedifferentiation towards a fetal phenotype, consistent with the observation that several embryonic/fetal gene isoforms are expressed in myocardial tissue from adults with HM, including α-smooth muscle actin, titin, desmin, and cardiotoxin [45–47].

4. Atrial Fibrillation and Hibernating Myocardium: Shared Pathophysiologic Traits

AF and HM share similar cellular and molecular alterations. Review of the changes observed in atrial myocytes during prolonged AF and ventricular myocytes that develop a hibernating phenotype reveals several pathophysiologic traits that are shared by the two cardiac disease states. These characteristics are summarized in Section 4.1 and collectively support the hypothesis that AF and HM each elicit the activation of a conserved adaptive response to stress. This paradigm has important clinical implications in that an improved
understanding of the mechanisms driving this remodeling may facilitate the identification of therapeutic targets to promote reversion of myocytes to a mature, healthy state and restore normal structure and function. This is particularly important given the relatively recent advent of new treatment strategies for each disease, that is, revascularization of HM and restoration of sinus rhythm in AF with cardioversion. The cellular and molecular adaptations described above, and particularly their reversibility, may impact the recovery of function following these interventions, suggesting that adjunctive therapies aiming at accelerating reverse remodeling of the dedifferentiated myocyte phenotype may improve patient outcomes.

4.1. Pathophysiologic Traits Common to Atrial Fibrillation and Hibernating Myocardium. Consider the following:

(i) Apoptosis-mediated myocyte loss.
(ii) Reactive cellular hypertrophy of remaining myocytes.
(iii) Reexpression of fetal genes/gene isoforms (e.g., α-smooth muscle actin and myosin heavy chain).
(iv) Loss and/or redistribution of structural proteins (e.g., cardiotin, titin, and desmin).
(v) Myolysis.
(vi) Sarcomere depletion.
(vii) Glycogen accumulation.
(viii) Alterations in size and/or shape of mitochondria (smaller mitochondria).
(ix) Downregulation of oxidative metabolism/fatty acid utilization.
(x) Increased reliance on glycolytic metabolism.
(xi) Altered intracellular calcium handling.
(xii) Redox signaling and antioxidative response.

4.2. Potential Mechanisms Underlying Myocyte Remodeling in Atrial Fibrillation and Hibernating Myocardium. The search for the causative factor(s) driving myocyte dedifferentiation and remodeling in AF and HM has principally focused on two potential mechanisms: myocardial ischemia and elevated wall stress. A role for ischemia is plausible in both cases; HM arises as a consequence of ischemia that typically occurs as a result of a flow-limiting coronary stenosis, while demand-induced ischemia may be apparent during AF [48]. However, the observation of myocyte dedifferentiation in remote, normally perfused regions of patients and animals with HM argues against this notion, as does the finding that a similar pattern of remodeling is apparent in nonischemic cardiomyopathy [49]. Thus, myocardial stretch may be a more likely mechanism. Indeed, coculture of adult ventricular myocytes with fibroblasts, which promotes redistribution of adhesion molecules from the distal to lateral membrane and a subsequent increase in tensile force on the myocyte, induces cell dedifferentiation despite the use of normoxic culture conditions to eliminate the possibility of ischemia [50]. Furthermore, in a pig model, cardiomyocyte dedifferentiation was observed 2 weeks after the onset of coronary stenosis but was not limited to ischemic regions, as reexpression of fetal α-smooth muscle actin and the loss of structural proteins were observed throughout the heart [51]. In addition, atrial stretch has been shown to promote sustained AF by prolonging the atrial ERP, suggesting that elevations in wall stress may contribute to structural and electrical changes involved in the pathophysiology of AF.

At the cellular level, alterations in calcium handling are likely an important contributor to myocyte remodeling in AF and HM. In AF, the high rate of atrial activation elicits excessive calcium influx, causing calcium overload and depression of contractile function. Impaired contractile function subsequently causes elevated preload and atrial chamber wall stress, prompting stretch-induced remodeling. In addition, calcium overload may directly promote morphologic remodeling via the activation of proteolytic pathways [38]. Support for this notion was provided by studies in a goat model of AF, which showed that elevations in calcium are transient but coincide with the timing of atrial structural remodeling [34]. Moreover, subsequent examination of heart tissue from AF patients demonstrated elevated activity of the calcium-activated proteolytic protein calpain I that correlated with ERP shortening, atrial myocyte structural remodeling, and the reduction of K⁺ channel proteins [38]. The potential mechanistic role of calcium overload is also supported by data indicating beneficial effects of calcium channel blockade during short-term AF [52–54]. However, the transient nature of calcium elevations in chronic AF yields a narrow therapeutic window and the protective effect of these drugs is lost after a longer duration of AF [55].

4.3. Potential Therapeutic Implications. Preservation of the structural and electrical integrity of atrial myocardium is essential for the primary prevention of AF. Targeting the mechanisms that drive pathophysiologic remodeling (e.g., myocyte stretch and calcium overload) during the paroxysmal stage of AF may therefore be an effective treatment strategy if interventions can be implemented prior to myocyte loss and the concomitant deleterious structural remodeling of surviving cells that characterizes persistent AF. To decipher specific targets for treatment, it is important to develop an improved understanding of the molecular changes that contribute to the transition from paroxysmal to persistent AF. In this regard, mitochondrial dysfunction represents a promising therapeutic target given its likely role as a common pathway mediating AF and HM via metabolic and oxidative stress in association with ATP depletion, disruption of ionic currents, and increased ROS generation [56–59]. Specifically, maintenance of the functional and structural integrity of mitochondria may be efficacious to prevent AF given the important role of mitochondria in maintaining cellular energetics and ionic homeostasis [26, 27, 60]. This approach may also provide additional benefits beyond currently used catheter ablation techniques that may eliminate the source of abnormal impulse formation but do not address the myocyte remodeling that may contribute to AF generation and progression. Catheter ablation isolates pulmonary veins which
are a primary source of premature atrial contraction and triggered activities. Improved understanding and treatment of myocyte remodeling in atrial myocardium and pulmonary sleeve may prevent triggers, reentry, and ectopic activities. Future studies testing interventions targeting mitochondria are warranted to determine whether attenuation of oxidative stress and preservation of mitochondrial bioenergetics may prevent or reverse atrial electroanatomic remodeling in AF, particularly at the earlier stages of disease progression.

Particularly in persistent AF, apoptosis-mediated atrial myocyte loss may play a key role in limiting the return of normal myocyte function despite the potential reversibility of cellular and molecular changes in individual surviving myocytes. From this perspective, therapies aiming at replenishing lost myocytes may have beneficial effects in allowing reverse remodeling of atrial myocytes after cardioversion, leading to sustained restoration of sinus rhythm. This paradigm is supported by a recent study examining the effect of percutaneous revascularization of HM in a well-validated large animal model of chronic ischemia. Although revascularization stimulated myocyte proliferation in previously ischemic areas of the left ventricle, reductions in contractile and metabolic proteins persisted and newly formed myocytes appeared immature, suggesting that the delayed reversal of cellular and molecular remodeling may contribute to a protracted time course of functional recovery after blood flow restoration [61]. Thus, the implementation of therapies aiming at regenerating functional myocytes via administration of exogenous stem cells or activation of endogenous myocyte progenitors may accelerate recovery of revascularized HM. In the case of AF, these novel therapeutic approaches could be coupled with restoration of sinus rhythm to promote regression of atrial myocyte hypertrophy and reversion of remodeled cells to a normal atrial myocyte phenotype, hopefully resulting in sustained maintenance of sinus rhythm and interruption of the dangerous progression of disease. Furthermore, it is possible that AF-induced myocyte dedifferentiation may actually facilitate myocyte regeneration in light of evidence that dedifferentiated adult myocytes exhibit downregulation of cell cycle inhibitors and reexpression of cardiac progenitor cell markers, collectively contributing to an enhanced ability to proliferate and form new myocytes [62, 63].

5. Conclusions

In summary, accumulating evidence supports the notion that AF induces a program of atrial myocyte remodeling that shares several characteristics to that observed in ventricular HM, suggesting that myocyte dedifferentiation towards a fetal phenotype represents a conserved adaptive response to chronic stress. Further understanding of the molecular mechanisms underlying these remodeling processes and recognition of their potentially reversible nature, particularly at the early stages of AF, may facilitate the development of novel therapeutic approaches to effectively prevent or treat AF and reduce the significant public health burden associated with the disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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