Left atrial thrombus resolution in non-valvular atrial fibrillation or flutter

Biomarker substudy results from a prospective study with rivaroxaban (X-TRA)

Miyazawa, Kazuo; Pastori, Daniele; Hammerstingl, Christoph; Cappato, Riccardo; Meng, Isabelle Ling; Kramer, Frank; Cohen, Ariel; Schulz, Anke; Eickels, Martin van; Lip, Gregory Y H; Marin, Francisco; X-TRA study investigators

Published in:
Annals of Medicine

DOI (link to publication from Publisher):
10.1080/07853890.2018.1495337

Publication date:
2018

Document Version
Accepted author manuscript, peer reviewed version

Annals of Medicine

ISSN: 0785-3890 (Print) 1365-2060 (Online) Journal homepage: http://www.tandfonline.com/loi/iann20

Left atrial thrombus resolution in non-valvular atrial fibrillation or flutter: Biomarker substudy results from a prospective study with rivaroxaban (X-TRA)

Kazuo Miyazawa, Daniele Pastori, Christoph Hammerstingl, Riccardo Cappato, Isabelle Ling Meng, Frank Kramer, Ariel Cohen, Anke Schulz, Martin van Eickels, Gregory Y H Lip, Francisco Marin & on behalf of the X-TRA study investigators

To cite this article: Kazuo Miyazawa, Daniele Pastori, Christoph Hammerstingl, Riccardo Cappato, Isabelle Ling Meng, Frank Kramer, Ariel Cohen, Anke Schulz, Martin van Eickels, Gregory Y H Lip, Francisco Marin & on behalf of the X-TRA study investigators (2018): Left atrial thrombus resolution in non-valvular atrial fibrillation or flutter: Biomarker substudy results from a prospective study with rivaroxaban (X-TRA), Annals of Medicine, DOI: 10.1080/07853890.2018.1495337

To link to this article: https://doi.org/10.1080/07853890.2018.1495337

Accepted author version posted online: 29 Jun 2018.

Left atrial thrombus resolution in non-valvular atrial fibrillation or flutter: Biomarker substudy results from a prospective study with rivaroxaban (X-TRA)

Kazuo Miyazawa[1]; Daniele Pastori[1,2]; Christoph Hammerstingl[3]; Riccardo Cappato[4]; Isabelle Ling Meng[5]; Frank Kramer[5]; Ariel Cohen[6]; Anke Schulz[7]; Martin van Eickels[5]; Gregory Y H Lip[1,8*], Francisco Marin[9*]; on behalf of the X-TRA study investigators

[*joint senior authors]

[1]Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, United Kingdom; [2]Department of Internal Medicine and Medical Specialties, I Clinica Medica, Atherothrombosis Centre, Sapienza University of Rome, Italy; [3]Department of Medicine II, Heart Centre Bonn, University Hospital Bonn, Bonn, Germany; [4]Humanitas Clinical and Research Centre, Via Manzoni 56, 20089 Rozzano (MI), Italy; [5]Global Medical Affairs, Bayer AG, Berlin, Germany; [6]Cardiology Department, Assistance publique-Hôpitaux de Paris and Université Pierre-et-Marie-Curie, Saint-Antoine University and Medical School, Paris, France; [7]Research and Clinical Sciences Statistics, Bayer AG, Berlin, Germany; [8]Aalborg Thrombosis Research Unit, Department of Clinical Medicine, Aalborg University, Aalborg, Denmark; [9]Department of Cardiology, Hospital Clínico Universitario Virgen de la Arrixaca, IMIB-Arrixaca, CIBER-CV, Murcia, Spain

Running Head: Biomarker and thrombus resolution with rivaroxaban

Address for correspondence

Professor Gregory Y.H. Lip, Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, England UK

Phone: +44 121 507 5080; Fax: +44 121 507 5503; E-mail: g.y.h.lip@bham.ac.uk

ABSTRACT

***Background*:** Non-vitamin K antagonist oral anticoagulants including rivaroxaban are widely used for stroke prevention in patients with atrial fibrillation (AF). We investigated the relationship between plasma biomarkers (indicative of thrombogenesis, fibrinolysis and inflammation) and left atrial thrombus resolution after rivaroxaban treatment.

***Methods*:** This was an ancillary analysis of the X-TRA study, which was a prospective interventional study evaluating the use of rivaroxaban for left atrial/left atrial appendage (LA/LAA) thrombus resolution in AF patients. We assessed various biomarkers of thrombogenesis/fibrinolysis [D-dimer, plasminogen activator inhibitor-1 (PAI-1), prothrombin fragment 1+2 (F1,2), thrombin–antithrombin (TAT) complexes, von Willebrand factor (vWF)] and inflammation [high-sensitivity interleukin-6 (hsIL-6), and high-sensitivity C-reactive protein (hsCRP)], measured at baseline and after 6 weeks' of rivaroxaban treatment.

***Results*:** There were significant decreases in the mean levels of hsCRP, D-dimer, vWF, and TAT from baseline to end of treatment with rivaroxaban. Although none of the thrombogenesis/fibrinolysis biomarkers showed a significant relationship with thrombus resolution, high inflammatory biomarkers at baseline were significantly associated with an increased chance of the thrombus being completely resolved (hsIL-6) or reduced/resolved (hsCRP).

***Conclusions*:** Biomarkers of inflammation are significantly associated with LA/LAA thrombus outcomes in AF patients prospectively treated with rivaroxaban.

Keywords: Atrial fibrillation; Non-vitamin K antagonist oral anticoagulant; Thrombus resolution; Biomarker

Key messages:

Changes in the thrombogenesis/fibrinolysis biomarker levels reflected the expected pharmacodynamics of rivaroxaban.

Higher levels of inflammation biomarkers were significantly associated with thrombus being completely resolved or reduced.

Introduction

Patients with atrial fibrillation (AF) are at high risk of left atrial (LA) thrombus formation, especially in the LA appendage (LAA).(1) The prevalence of LA/LAA thrombus detected by transesophageal echocardiography (TEE) or computed tomography varies from 0.2% to 3.6%, depending on anticoagulation status and CHA_2DS_2-VASc score.(2-5) Patients with documented LA/LAA thrombus are at risk of thromboembolism with the annual event rate of 14%.(6) This increased propensity to thrombus formation may be related to a prothrombotic or hypercoagulable state, which can be quantified by systemic assessment of various biomarkers of thrombogenesis/fibrinolysis and inflammation(7).

Oral anticoagulants (OAC) including vitamin K antagonist (VKA) and non-vitamin K antagonist oral anticoagulants (NOACs) are well-established for effective stroke prevention in AF.(8, 9) Previous studies have reported the efficacy of VKA treatment on LA/LAA thrombus resolution,(10-13) whereas data on NOAC treatment remains scarce. Single case reports and case series have shown LA/LAA thrombus resolution treated with dabigatran, rivaroxaban, and apixaban.(14-16) More recently, a randomized clinical trial was conducted for investigating safety and efficacy of dabigatran in patients with LA/LAA thrombus.(17) Although there are some reports about the effectiveness of NOACs over VKA therapy, so far current guidelines recommend that VKA is the only treatment in AF patients who developed LA/LAA thrombus.(18) Furthermore, the relationship between LA/LAA thrombus outcome and biomarkers such as thrombogenesis/fibrinolysis and inflammation with NOAC treatment has not

been rigorously examined.

The X-TRA study was the first prospective interventional study designed to explore the use of rivaroxaban, a factor Xa inhibitor, for the resolution of LA/LAA thrombi in patients with non-valvular AF or atrial flutter.(19) The main X-TRA study results demonstrated that resolved or reduced thrombus after 6 weeks' rivaroxaban treatment was identified in 60.4 % of patients and was consistent with LA/LAA thrombus resolution with VKA therapy. Additionally, no patients experienced stroke, noncentral nervous system systemic embolism, and major bleeding event during the treatment period and the 30-day follow-up.

In the present analysis, we assessed biomarkers of thrombogenesis/fibrinolysis and inflammation in the X-TRA study patient population and related these biomarkers with LA/LAA thrombus outcomes (reduced/resolved) following rivaroxaban treatment.

Methods

Study population

The study design of X-TRA (ClinicalTrials.gov identifier NCT01839357) has been previously described(19). This was a prospective, interventional, single-arm, open-label, multicenter study (Figure 1) where once-daily (OD) rivaroxaban (20 mg od, or 15 mg od in patients with creatinine clearance 15–49 mL/min) was evaluated for the resolution

of LA/LAA thrombi in patients with AF or atrial flutter. Patients were eligible for inclusion in the X-TRA study if they developed LA/LAA thrombi and had to be OAC naive or untreated within 1 month prior to enrollment (treatment of up to 72 hours with VKA, heparin, or a low molecular weight heparin was allowed before the start of rivaroxaban) or pretreated with VKA but at suboptimal or ineffective INR levels (i.e. INR < 2.0, documented with at least two consecutive measurements that were at least 24 hours apart) within the last 6 weeks.

Echocardiogram parameters at baseline and during follow-up

Echocardiogram parameters at baseline included LAA peak emptying velocity, presence of spontaneous echocontrast and LA/LAA thrombus area. The presence of LA/LAA thrombi was assessed by TEE at baseline and after 6 weeks of treatment with rivaroxaban. The efficacy of treatment was evaluated according to the modification of the thrombus, such as resolved, reduced, unchanged, larger, or newly-detected at the end of treatment.

Biomarkers

The biomarker testing was performed by Covance Laboratories on behalf of Bayer AG. The following plasma/serum biomarkers were analysed centrally at baseline and end of treatment, as follows: (i) thrombogenesis/fibrinolysis biomarkers: D-dimer, plasminogen activator inhibitor-1 (PAI-1), prothrombin fragment 1+2 (F1,2), thrombin–antithrombin (TAT) complexes, and von Willebrand factor (vWF), and (ii) inflammation: high-sensitivity interleukin-6 (hsIL-6) and high-sensitivity C-reactive protein (hsCRP).

The associations of demographic data and medical history on biomarker levels at baseline were investigated. In addition, we assessed the relationship between biomarker levels and echocardiogram characteristics as well as LA/LAA thrombus outcomes.

The X-TRA study was conducted with approval from the appropriate Independent Ethics Committee/Institutional Review Board obtained for all participating centers before the start of the study, in accordance with the ethical principles of the Declaration of Helsinki, Good Clinical Practice (GCP) and local laws, regulations, and organizations, as applicable.

Statistical analysis

Relationships between biomarker values and variables of interest were analysed by linear and logistic regression models. Multivariate models were built under consideration of all clinical variables. The present analysis included 3 steps: (1) identify relevant covariates per each endpoint (model for each endpoint with all baseline demographic and medical history data) based on the Akaike information criteria (AIC), (2) build a base model for each endpoint with the relevant covariates (from step 1) and one of the biomarkers as an independent variable “univariate model”, (3) build a final multivariate model with the base model variables as well as all biomarkers as independent variables and select the best variable set based on the AIC “multivariate model”. Models to find the relevant covariates were built not only for the biomarkers

but also for the clinical endpoints. These covariates were used for further models for the respective biomarker and clinical endpoints. In each step, the coefficient of determination (R^2, goodness-of-fit measure) of the model was calculated to assess the add-on value of the clinical variables by looking at the improvement in R^2. Models for each biomarker separately are called univariate in this context, whereas models with all biomarkers included are called here multivariate. The analyses assessing the relationship of biomarkers to echocardiogram characteristics and primary endpoint-related variables were performed on the modified intention to treat (mITT) population who had both TEE results at baseline and the end of treatment; all other analyses were performed on the ITT population if the patients provided at least one biomarker measurement. The number of actual measurements analysed varied by biomarker depending upon missing data, which were due to sample not taken or not qualified for testing or other reasons. A significant alpha-level of 0.05 was used for all models. Log-transformation of biomarker level was decided upon as appropriate. All statistical analyses were performed using SAS system version 9.4 (SAS Institute Inc.; Cary, NC, USA) and R software (version 3.1.2; R Core Team 2013).

Results

The X-TRA study was conducted between August 2013 and December 2014. A total of 60 patients were enrolled in the present study (ITT analysis), and 53 patients with complete TEE data available were included. The remaining 7 patients were excluded

from the mITT population as 4 had no TEE data at baseline available (1 patient withdrew from the study because of an adverse event), and 3 had no TEE data at end of treatment (1 patient died of acute heart failure, 1 patient emigrated to another country because of a war, and 1 patient was hospitalized for heart transplantation).

Baseline patient characteristics, echocardiography and biomarkers

For biomarker data descriptive statistics are given for different subgroups based on demographic data including sex, age, and body mass index (BMI) (Supplementary Table S1).

Multivariate models, adjusted by selected covariates based on the AIC, demonstrated that demographic data had limited influence on baseline levels of the biomarkers examined (up to 14.9 % of explained variability of log PAI-1 at baseline, see Table 1). The levels of hsCRP and PAI-1 were significantly reduced for patients with arterial hypertension (hsCRP: geometric mean of 3.25 mg/L vs. 8.53 mg/L, t-statistic of -2.061, p=0.045, PAI-1: geometric mean of 11.24 mg/L vs. 22.83 mg/L, t-statistic of -3.099, p=0.003). The levels of hsCRP were significantly increased for patients with diabetes mellitus (8.87 mg/L vs. 3.15 mg/L, t-statistic of 2.369, p=0.022) (Supplementary Table S2).

Table 2 shows the relationship between echocardiogram characteristics assessed by TEE and biomarker levels. On univariate analysis, there were no significant associations between echocardiogram parameters and biomarkers at baseline (Supplementary

Table S3). Multivariate analysis (Table 2) shows that low hsIL-6 levels were significantly associated with increased LAA peak emptying velocity at baseline (regression coefficient with 95% CI of -9.311 [-16.58;-2.04], p=0.021). No significant association between LA/LAA thrombus area and biomarker levels at baseline was found, but high D-dimer levels were associated with an increased chance for the presence of LAA spontaneous echocontrast at baseline (OR with 95% CI of 33.212 [1.09;1015.30], p=0.045).

Changes in biomarker levels between baseline and end of treatment

Figure 2 shows comparisons of the biomarker levels between baseline and end of rivaroxaban treatment. There were significant baseline-adjusted decreases in the mean levels of hsCRP, D-dimer, vWF, and TAT from baseline to end of treatment with rivaroxaban (hsCRP: -39.83%, p=0.005, D-dimer: -41.49%, p<0.001, vWF: -32.061 percentage points, p<0.001, TAT: -35.85%, p=0.022). No significant changes were observed in the mean PAI-1, hsIL-6 and F1,2 levels.

LA/LAA thrombus outcomes and biomarker levels

In the present study, LA/LAA thrombi were completely resolved in 41.5% of subjects and were reduced or resolved in 60.4% of subjects. For the biomarker analyses, Table 3 shows the relationship between thrombus outcomes and biomarker levels.

On univariate analysis, there were no significant associations between thrombus outcomes and biomarker levels at baseline, as well as change in biomarker levels from

baseline to end of treatment (Supplementary Tables S4 and S5). On multivariate analysis (Table 3), high levels of inflammation biomarkers, i.e. hsIL-6 and hsCRP at baseline were significantly associated with thrombus being completely resolved or reduced (OR with 95% CI of 4.909 [1.27;19.00], *p*=0.021, for hsIL-6 and thrombus completely resolved, OR with 95% CI of 9.120 [1.16;72.00], *p*=0.036, for hsCRP and thrombus reduced or resolved). None of the thrombogenesis/fibrinolysis biomarkers at baseline showed a significant relationship to thrombus being completely resolved or reduced.

Discussion

The present analysis extends data of the X-TRA study with new findings relating biomarker levels to LA/LAA thrombus outcomes in AF patients prospectively treated with rivaroxaban. First, we demonstrate that some biomarkers of thrombogenesis/ fibrinolysis and inflammation are favourably affected by rivaroxaban treatment. Second, higher levels of inflammation biomarkers, i.e. hsIL-6 and hsCRP at baseline were significantly associated with thrombus being completely resolved or reduced.

There is growing evidence to support a relationship between inflammation and AF-related thrombogenesis.(20, 21) Inflammatory biomarkers are independent predictors of myocardial infarction, stroke, and sudden cardiac death,(22, 23) as well as incident AF and AF-related thrombosis.(24-28) The underlying mechanisms linking

inflammation and the prothrombotic state in AF include endothelial activation/damage, production of tissue factor from monocytes, increased platelet activation, and increased expression of fibrinogen.(29, 30) Endothelial dysfunction contributes to the development of a pro-inflammatory and pro-thrombotic environment through the production of cytokines.(31) Similarly, activated inflammatory cells such as monocytes can trigger and sustain thrombosis through regulation of monocyte-derived gene expression, including interleukin-8 and monocyte chemoattractant protein-1.(32, 33)

However, to the best our knowledge, the relationship between LA/LAA thrombus outcomes and inflammatory biomarkers has not been previously examined. The present study indicates that high levels of inflammatory biomarkers in AF patients with LA/LAA thrombus were associated with thrombus being completely resolved or reduced with rivaroxaban treatment. This finding is of difficult explanation, but raises some issues regarding the relationship between inflammation and coagulation activity. Recently, in addition to the well-established anticoagulant activity, direct factor Xa and thrombin inhibitors have been explored for their potential effects beyond anticoagulation, including atherosclerotic plaque stabilization through anti-inflammatory activities.(34, 35) There is evidence suggesting that coagulation protease such as factor Xa and thrombin are involved in non-hemostasis cellular activities like inflammation. Previous studies have demonstrated that factor Xa exhibits pro-inflammatory activity by stimulation of interleukin (IL)-6, IL-8, and monocyte chemotactic protein 1 expression in endothelial cells and leukocytes.(36) Moreover, in primary human umbilical endothelial cells, inhibition of factor Xa downregulated the

expression of thrombin-mediated pro-inflammatory cytokine through prevention of thrombin generation.(37) Therefore, treatment with rivaroxaban may be particularly beneficial in patients with high-grade inflammation.

D-dimer is well known as a marker of fibrin turnover and an index of thrombogenesis.(38) Regarding thrombus formation in patients with AF, D-dimer levels are a surrogate markers for a hypercoagulable state, and has been reported to increase with the accumulation of clinical risk factors for thromboembolism or by the presence of LAA thrombus.(39) Clinical utility of D-dimer level as a reflection of the thrombus state and burden in patients with AF has been further supported by the findings that high D-dimer levels in AF were reduced by both anticoagulation and cardioversion to the sinus rhythm.(40) In the present study, D-dimer level at baseline was associated with the presence of LAA spontaneous echocontrast at baseline; however, this result may not be applicable to the general population, as there was a selection bias due to no control group without LA/LAA thrombi. Furthermore, changes in D-dimer levels were consistent with the expected pharmacodynamics of rivaroxaban,(41) though there were no significant associations between change in D-dimer levels and thrombus outcomes. Among the other biomarkers of thrombogenesis/fibrinolysis, there were also no significant associations with thrombus outcomes. There are limited data on biomarkers of thrombogenesis/fibrinolysis under anticoagulation therapy, but the findings in the present study can identify specific biomarkers that warrant further investigation to predict their clinical characteristics and prognosis in AF patients with treatment of LA/LAA thrombus with rivaroxaban.

Limitations

The present study was an exploratory study with a small patient population, leading to insufficient number to perform multivariate analysis. Effect sizes were small and associations between biomarkers and clinical parameters were generally weak. Second, oxidative stress has been reported to play an important role in the pathogenesis of AF and thromboembolism.(42, 43) The present study did not include the data on biomarkers of oxidative stress such as GDF-15 and Nitrotyrosine, but extensive survey including such biomarkers must be needed in order to comprehensively assess the pathophysiology of AF. Third, there were no control groups without LA/LAA thrombus and no placebo treatment arm. Therefore, the results should be interpreted with caution and regarded to as hypothesis generating.

Conclusions

The present study is an ancillary analysis of the X-TRA study, which was the first prospective, interventional, and multicentre study examining the relationship between biomarkers and LA/LAA thrombus resolution with rivaroxaban. We observed a significant decrease of some biomarkers of thrombogenesis/fibrinolysis and inflammation (i.e hsCRP, D-dimer, vWF, and TAT) in AF patients with LA/LAA thrombus after treatment with rivaroxaban. Furthermore, biomarkers of inflammation are associated with LA/LAA thrombus outcomes in AF patients prospectively treated with

rivaroxaban.

Acknowledgments

The authors very appreciated the excellent work done by the X-TRa investigators (see Appendix B. List of investigators in *Am Heart J 2016; 178:133)* [18], the Study Outcome Committee (chair: Martin Prins; members: Trang Ding and Bas Kietselaer) and Bayer study team during the clinical trial. We thank the Covance Laboratories for the measurement of all biomarkers. We also would like to thank Adam Skubala and Sebastian Voss (Fa. Chrestos Concept GmbH & Co. KG, Essen, Germany) for performing the data analysis and Sonja Schiffer (Clinical Sciences - Experimental Medicine, Bayer AG, Wuppertal, Germany) for her support in data analysis and interpretation.

Disclosures

KM and DP has received no funding. FM has received funding for research, acted as a consultant for and has lectured on behalf of Abbott, Boston Scientific, Bayer, AstraZeneca, Daiichi Sankyo, Bristol-Myers Squibb/Pfizer and Boehringer Ingelheim. CH has received research grants from Sanofi-Aventis and has received speaker's honoraria from Bayer, Boehringer Ingelheim and Pfizer. RC has received consultancy fees or research funding from Boston Scientific, Medtronic, St. Jude, Biosense Webster,

Boehringer Ingelheim, Bayer, Abbott, ELA Sorin, Pfizer and BARD medical, and has equity and intellectual property rights in Cameron. ILM, AS, FK and MvE are employees of Bayer AG. AC has received a research grant for research nurses (RESICARD) and consultant and lecture fees from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol-Myers Squibb, Daiichi Sankyo, GlaxoSmithKline and Sanofi-Aventis. GYHL is a member of various guideline and position statement committees (including ESC, EHRA, NICE); a member of steering committees for various phase II, phase III and health economics and outcomes research studies; an investigator in various clinical trials in cardiovascular disease, including those on antithrombotic therapies in atrial fibrillation, acute coronary syndrome; has been a consultant for Bayer/Janssen, Astellas, Merck, Sanofi, Bristol-Myers Squibb/Pfizer, Biotronik, Medtronic, Portola, Boehringer Ingelheim, Microlife and Daiichi Sankyo; and a speaker for Bayer, Bristol-Myers Squibb/Pfizer, Medtronic, Boehringer Ingelheim, Microlife, Roche and Daiichi Sankyo. The X-TRA study was supported by Bayer AG.

References

1. Odell JA, Blackshear JL, Davies E, Byrne WJ, Kollmorgen CF, Edwards WD, et al. Thoracoscopic obliteration of the left atrial appendage: potential for stroke reduction? Ann Thorac Surg. 1996;61(2):565-9.
2. Wallace TW, Atwater BD, Daubert JP, Voora D, Crowley AL, Bahnson TD, et al. Prevalence and clinical characteristics associated with left atrial appendage thrombus in fully anticoagulated patients undergoing catheter-directed atrial fibrillation ablation. J Cardiovasc Electrophysiol. 2010;21(8):849-52.
3. Wasmer K, Kobe J, Dechering D, Milberg P, Pott C, Vogler J, et al. CHADS(2) and CHA(2)DS (2)-VASc score of patients with atrial fibrillation or flutter and newly detected left atrial thrombus. Clin Res Cardiol. 2013;102(2):139-44.
4. Wyrembak J, Campbell KB, Steinberg BA, Bahnson TD, Daubert JP, Velazquez EJ, et al. Incidence and Predictors of Left Atrial Appendage Thrombus in Patients Treated With Nonvitamin K Oral Anticoagulants Versus Warfarin Before Catheter Ablation for Atrial Fibrillation. Am J Cardiol. 2017;119(7):1017-22.
5. Bertaglia E, Anselmino M, Zorzi A, Russo V, Toso E, Peruzza F, et al. NOACs and atrial fibrillation: Incidence and predictors of left atrial thrombus in the real world. Int J Cardiol. 2017;249:179-83.
6. Stoddard MF, Singh P, Dawn B, Longaker RA**.** Left atrial thrombus predicts transient ischemic attack in patients with atrial fibrillation. Am Heart J. 2003;145(4):676-82.
7. Watson T, Shantsila E, Lip GY**.** Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited. Lancet. 2009;373(9658):155-66.
8. Lip G, Freedman B, De Caterina R, Potpara TS**.** Stroke prevention in atrial fibrillation: Past, present and future. Comparing the guidelines and practical decision-making. Thromb Haemost. 2017;117(7):1230-9.
9. Li YG, Pastori D, Lip GYH**.** Fitting the right non-vitamin K antagonist oral anticoagulant to the right patient with non-valvular atrial fibrillation: an evidence-based choice. Ann Med. 2018;50(4):288-302.
10. Corrado G, Tadeo G, Beretta S, Tagliagambe LM, Manzillo GF, Spata M, et al. Atrial thrombi resolution after prolonged anticoagulation in patients with atrial fibrillation. Chest. 1999;115(1):140-3.

11. Jaber WA, Prior DL, Thamilarasan M, Grimm RA, Thomas JD, Klein AL, et al. Efficacy of anticoagulation in resolving left atrial and left atrial appendage thrombi: A transesophageal echocardiographic study. Am Heart J. 2000;140(1):150-6.
12. Saeed M, Rahman A, Afzal A, Agoston I, Jammula P, Birnbaum Y, et al. Role of transesophageal echocardiography guided cardioversion in patients with atrial fibrillation, previous left atrial thrombus and effective anticoagulation. Int J Cardiol. 2006;113(3):401-5.
13. Fukuda S, Watanabe H, Shimada K, Aikawa M, Kono Y, Jissho S, et al. Left atrial thrombus and prognosis after anticoagulation therapy in patients with atrial fibrillation. J Cardiol. 2011;58(3):266-77.
14. Vidal A, Vanerio G**.** Dabigatran and left atrial appendage thrombus. J Thromb Thrombolysis. 2012;34(4):545-7.
15. Hammerstingl C, Potzsch B, Nickenig G**.** Resolution of giant left atrial appendage thrombus with rivaroxaban. Thromb Haemost. 2013;109(4):583-4.
16. Kawakami T, Kobayakawa H, Ohno H, Tanaka N, Ishihara H**.** Resolution of left atrial appendage thrombus with apixaban. Thromb J. 2013;11(1):26.
17. Ferner M, Wachtlin D, Konrad T, Deuster O, Meinertz T, von Bardeleben S, et al. Rationale and design of the RE-LATED AF--AFNET 7 trial: REsolution of Left atrial-Appendage Thrombus--Effects of Dabigatran in patients with Atrial Fibrillation. Clin Res Cardiol. 2016;105(1):29-36.
18. Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. Eur Heart J. 2016;37(38):2893-962.
19. Lip GY, Hammerstingl C, Marin F, Cappato R, Meng IL, Kirsch B, et al. Left atrial thrombus resolution in atrial fibrillation or flutter: Results of a prospective study with rivaroxaban (X-TRA) and a retrospective observational registry providing baseline data (CLOT-AF). Am Heart J. 2016;178:126-34.
20. Engelmann MD, Svendsen JH**.** Inflammation in the genesis and perpetuation of atrial fibrillation. Eur Heart J. 2005;26(20):2083-92.
21. Guo Y, Lip GY, Apostolakis S**.** Inflammation in atrial fibrillation. J Am Coll Cardiol. 2012;60(22):2263-70.
22. Ridker PM, Hennekens CH, Buring JE, Rifai N**.** C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women.

N Engl J Med. 2000;342(12):836-43.

23. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation. 2000;101(15):1767-72.

24. Aviles RJ, Martin DO, Apperson-Hansen C, Houghtaling PL, Rautaharju P, Kronmal RA, et al. Inflammation as a risk factor for atrial fibrillation. Circulation. 2003;108(24):3006-10.

25. Marott SC, Nordestgaard BG, Zacho J, Friberg J, Jensen GB, Tybjaerg-Hansen A, et al. Does elevated C-reactive protein increase atrial fibrillation risk? A Mendelian randomization of 47,000 individuals from the general population. J Am Coll Cardiol. 2010;56(10):789-95.

26. Schnabel RB, Larson MG, Yamamoto JF, Kathiresan S, Rong J, Levy D, et al. Relation of multiple inflammatory biomarkers to incident atrial fibrillation. Am J Cardiol. 2009;104(1):92-6.

27. Smith JG, Newton-Cheh C, Almgren P, Struck J, Morgenthaler NG, Bergmann A, et al. Assessment of conventional cardiovascular risk factors and multiple biomarkers for the prediction of incident heart failure and atrial fibrillation. J Am Coll Cardiol. 2010;56(21):1712-9.

28. Conway DS, Buggins P, Hughes E, Lip GY. Relationship of interleukin-6 and C-reactive protein to the prothrombotic state in chronic atrial fibrillation. J Am Coll Cardiol. 2004;43(11):2075-82.

29. Akar JG, Jeske W, Wilber DJ. Acute onset human atrial fibrillation is associated with local cardiac platelet activation and endothelial dysfunction. J Am Coll Cardiol. 2008;51(18):1790-3.

30. Kaski JC, Arrebola-Moreno AL. [Inflammation and thrombosis in atrial fibrillation]. Rev Esp Cardiol. 2011;64(7):551-3.

31. Yacoub D, Hachem A, Theoret JF, Gillis MA, Mourad W, Merhi Y. Enhanced levels of soluble CD40 ligand exacerbate platelet aggregation and thrombus formation through a CD40-dependent tumor necrosis factor receptor-associated factor-2/Rac1/p38 mitogen-activated protein kinase signaling pathway. Arterioscler Thromb Vasc Biol. 2010;30(12):2424-33.

32. Campbell RA, Vieira-de-Abreu A, Rowley JW, Franks ZG, Manne BK, Rondina MT, et al. Clots Are Potent Triggers of Inflammatory Cell Gene Expression: Indications for Timely Fibrinolysis. Arterioscler Thromb Vasc Biol.

2017;37(10):1819-27.

33. Shahid F, Lip GYH, Shantsila E. Role of Monocytes in Heart Failure and Atrial Fibrillation. J Am Heart Assoc. 2018;7(3).
34. Hara T, Fukuda D, Tanaka K, Higashikuni Y, Hirata Y, Nishimoto S, et al. Rivaroxaban, a novel oral anticoagulant, attenuates atherosclerotic plaque progression and destabilization in ApoE-deficient mice. Atherosclerosis. 2015;242(2):639-46.
35. Pingel S, Tiyerili V, Mueller J, Werner N, Nickenig G, Mueller C. Thrombin inhibition by dabigatran attenuates atherosclerosis in ApoE deficient mice. Arch Med Sci. 2014;10(1):154-60.
36. Borensztajn K, Peppelenbosch MP, Spek CA. Factor Xa: at the crossroads between coagulation and signaling in physiology and disease. Trends Mol Med. 2008;14(10):429-40.
37. Ellinghaus P, Perzborn E, Hauenschild P, Gerdes C, Heitmeier S, Visser M, et al. Expression of pro-inflammatory genes in human endothelial cells: Comparison of rivaroxaban and dabigatran. Thromb Res. 2016;142:44-51.
38. Lip GY, Lowe GD. Fibrin D-dimer: a useful clinical marker of thrombogenesis? Clin Sci (Lond). 1995;89(3):205-14.
39. Habara S, Dote K, Kato M, Sasaki S, Goto K, Takemoto H, et al. Prediction of left atrial appendage thrombi in non-valvular atrial fibrillation. Eur Heart J. 2007;28(18):2217-22.
40. Lip GY, Rumley A, Dunn FG, Lowe GD. Plasma fibrinogen and fibrin D-dimer in patients with atrial fibrillation: effects of cardioversion to sinus rhythm. Int J Cardiol. 1995;51(3):245-51.
41. Mueck W, Stampfuss J, Kubitza D, Becka M. Clinical pharmacokinetic and pharmacodynamic profile of rivaroxaban. Clin Pharmacokinet. 2014;53(1):1-16.
42. Xie W, Santulli G, Reiken SR, Yuan Q, Osborne BW, Chen BX, et al. Mitochondrial oxidative stress promotes atrial fibrillation. Sci Rep. 2015;5:11427.
43. Wallentin L, Hijazi Z, Andersson U, Alexander JH, De Caterina R, Hanna M, et al. Growth differentiation factor 15, a marker of oxidative stress and inflammation, for risk assessment in patients with atrial fibrillation: insights from the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trial. Circulation. 2014;130(21):1847-58.

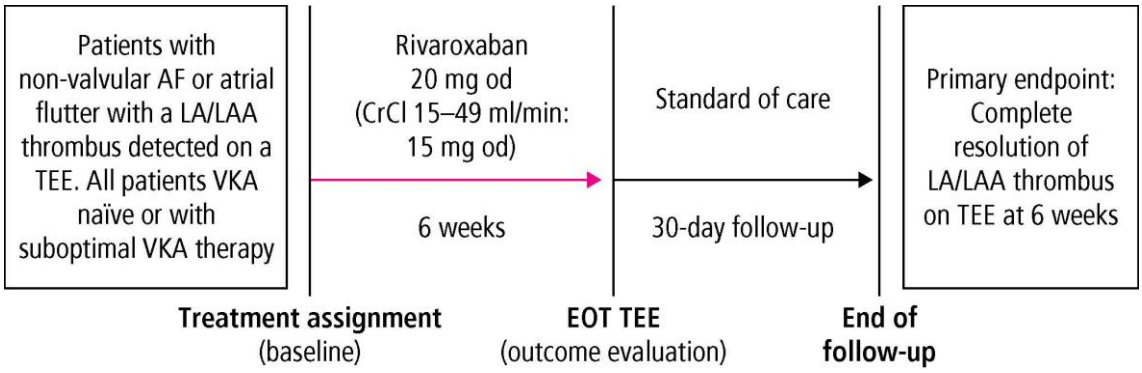
Figure legends

Figure 1. X-TRA study design.

CrCl, creatinine clearance; EOT, end of treatment

Figure 2. Boxplots of biomarker levels. Values at baseline and end of treatment (descriptive). The red circles are baseline values and the blue crosses are values for the end of treatment. The median is indicated as a solid horizontal line in bold; "+" indicates the mean; the bottom and top of the box are the first and third quartiles (25th and 75th percentile), therefore 50% of the observations lie within this box and the box length is the interquartile range; the upper and lower whiskers are at the lowest and highest observation if they do not exceed 1.5 times the box length (interquartile range).

F1,2, prothrombin fragment 1+2; hsCRP, high-sensitivity C-reactive protein; hsIL-6, high-sensitivity interleukin-6; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin–antithrombin complexes; vWF, von Willebrand factor.



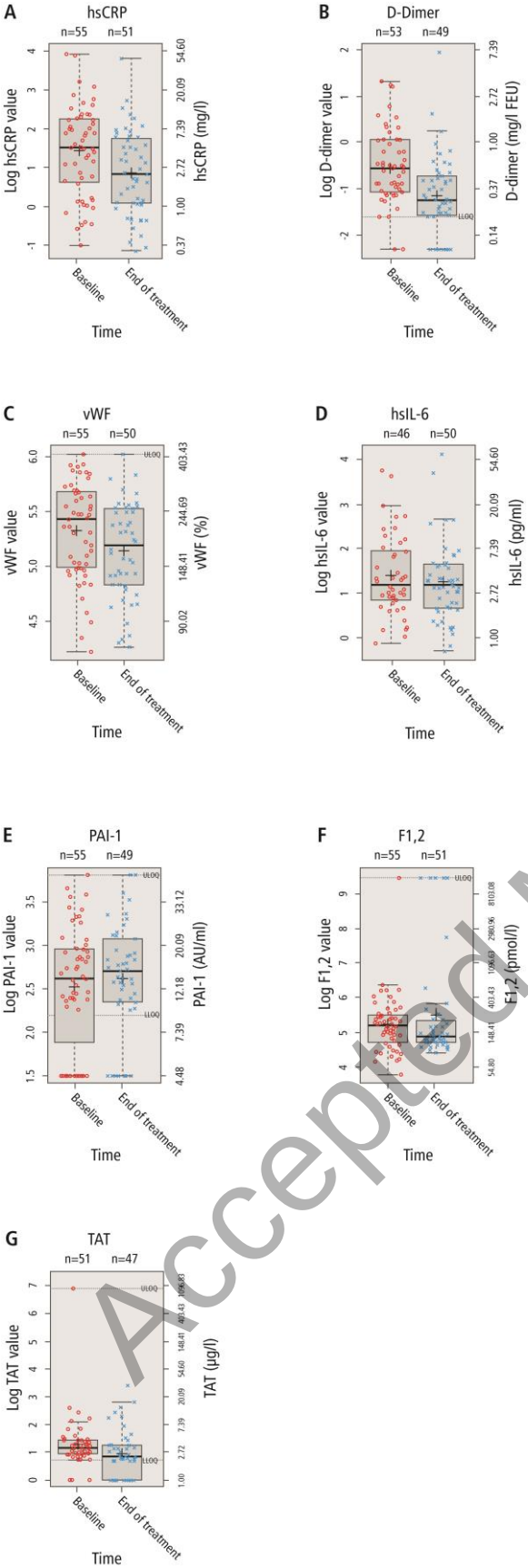


Table 1. Relationship between baseline biomarkers and demographic variables based on multivariate models.

Biomarker	Sex	Age	BMI	R^2	n
log hsCRP				0.000	55
log D-dimer			X	0.055	53
vWF			X	0.056	55
log hsIL-6				0.000	46
log PAI-1		X	X	0.149	55
log F1,2	X		X	0.130	55
log TAT		X		0.055	51

X indicates which demographic variables were used to build the model with the corresponding biomarker. R^2 is the coefficient of determination.

BMI, body mass index; F1,2, prothrombin fragment 1+2; hsCRP, high-sensitivity C-reactive protein; hsIL-6, high-sensitivity interleukin-6; PAI-1, plasminogen activator inhibitor-1; TAT, thrombin–antithrombin complexes; vWF, von Willebrand factor.

Table 2. Multivariate models of relationships between echocardiograhic characteristics and biomarker levels at baseline.

Linear models (continuous echocardiogram parameter)

Echocardiogram parameter at baseline	Biomarker (baseline)	Regression coefficient	95% CI	p-value	n
LAA peak emptying velocity	log hsCRP	4.301	[-0.41 - 9.01]	0.089	28
	log D-dimer	4.602	[-0.55 - 9.75]	0.095	28
	vWF	0.037	[-0.01 - 0.08]	0.137	28
	log hsIL-6	-9.311	[-16.58 - -2.04]	0.021	28
	log TAT	-8.964	[-17.68 - -0.25]	0.057	28
LA/LAA thrombus area	log D-dimer	42.550	[-5.49 - 90.59]	0.094	31

Logistic models (binary echocardiogram parameter)

Echocardiogram parameter at baseline	Biomarker (baseline)	Regression coefficient	OR	95% CI	p-value	n
Presence of spontaneous echocontrast	log hsCRP	-1.172	0.310	[0.05 - 1.79]	0.190	33
	log D-dimer	3.503	33.212	[1.09 - 1015.30]	0.045	33
	log TAT	6.158	472.617	[0.54 - 411305.90]	0.075	33

The table shows the results from final multivariate models for the clinical endpoints after Akaike information criteria-based parameter selection. In addition to above biomarkers other demographic data were also selected as relevant covariates in this selection (Congestive heart failure and prior stroke were relevant covariates with LAA peak emptying velocity; hypertension with LA/LAA thrombus area; BMI and diabetes mellitus with presence of spontaneous echocontrast).

LA, left atrium; LAA, left atrial appendage; OR, odds ratio; CI, confidence interval.

Table 3. Multivariate logistic models of relationships between thrombus outcomes and biomarker baseline levels.

Thrombus outcome	Biomarker (baseline)	Regression coefficient	OR	95% CI	p-value	n
Thrombus complete resolved	log hsIL-6	1.591	4.909	[1.27 – 19.00]	0.021	33
	vWF	-0.011	0.989	[0.98 – 1.00]	0.077	33
Thrombus reduced or resolved	log hsCRP	2.210	9.120	[1.16 – 72.00]	0.036	31
	vWF	-0.015	0.985	[0.97 – 1.00]	0.134	31

The table shows the results from final multivariate models for the clinical endpoints after Akaike information criteria-based parameter selection. In addition to above biomarkers other demographic data were also relevant covariates as results of this selection (diastolic blood pressure and BMI relevant with thrombus reduced or resolved).

CI, confidence interval; OR, odds ratio