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DOI:
10.1016/j.ahj.2015.05.011

Document Version
Peer reviewed version

Citation for published version (Harvard):
Pastori, D, Pignatelli, P, Farcomeni, A, Cangemi, R, Hiatt, WR, Bartimoccia, S, Nocella, C, Vicario, T, Bucci, T, Carnevale, R, Lip, GYH & Violi, F 2015, 'Urinary 11-dehydro-thromboxane B2 is associated with cardiovascular events and mortality in patients with atrial fibrillation', American Heart Journal, vol. 170, no. 3, pp. 490-497.e1. https://doi.org/10.1016/j.ahj.2015.05.011

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PII: S0002-8703(15)00362-2
DOI: doi: 10.1016/j.ahj.2015.05.011
Reference: YMHJ 4907

To appear in: *American Heart Journal*

Received date: 17 October 2014
Accepted date: 4 May 2015

Please cite this article as: Pastori Daniele, Pignatelli Pasquale, Farcomeni Alessio, Cangemi Roberto, Hiatt William R., Bartimoccia Simona, Nocella Cristina, Vicario Tommasa, Bucci Tommaso, Carnevale Roberto, Lip Gregory Y.H., Violi Francesco, Urinary 11-dehydro-thromboxane B_2 is associated with cardiovascular events and mortality in atrial fibrillation patients, *American Heart Journal* (2015), doi: 10.1016/j.ahj.2015.05.011

Urinary 11-dehydro-thromboxane B_2 is associated with cardiovascular events and mortality in atrial fibrillation patients.

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Running title: Urinary thromboxane B2 and Atrial fibrillation.

Key words: atrial fibrillation, 11-dehydro-thromboxane B2, cardiovascular diseases, mortality

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Abstract

BACKGROUND: Non-Valvular Atrial Fibrillation (AF) patients show high residual cardiovascular risk despite oral anticoagulants. Urinary 11-dehydro-thromboxane B2 (TxB_2) is associated with an increased risk of cardiovascular events (CVEs), but its predictive value in anticoagulated AF patients is unknown.

METHODS: Prospective single-center cohort study, including 837 AF patients. Mean time of follow-up was 30.0 months yielding 2062 person-years of observation. Urinary 11-dehydro-TxB_2 was measured at baseline. The primary end-point was the occurrence of a CVE including fatal/nonfatal myocardial infarction (MI) and ischemic stroke, transient ischemic attack, cardiac revascularization and cardiovascular death.

RESULTS: Mean age of patients was 73.1 years, and 43.6% were **women**. Median 11-dehydro-TxB_2 levels were 100 [IQR 50 -187] ng/mg of urinary creatinine. Overall, the anticoagulation control was adequate (63.9% of mean time in therapeutic range). A CVE occurred in **99** (11.8%) patients, 55 were CV deaths. At baseline, 11-dehydro-TxB_2 levels were higher in patients with a CVE compared to those without (186 [107-400] vs. 98 [52-170], p<0.001). An increased rate of CVEs (Log-Rank test, p<0.001) and CV deaths (p<0.001) was observed across tertiles of 11-dehydro-TxB_2.

CVEs were associated with age (Hazard Ratios [HR]: 1.72 per 1SD, 95% Confidence Interval [CI] 1.33 -2.21, p<0.001), diabetes mellitus (HR: 1.89 95%CI 1.20-2.96, p=0.005), heart failure (HR: 1.60, 95%CI 1.01-2.54, p=0.044), history of stroke/transient ischemic attack (HR: 1.96, 95%CI 1.25-3.06, p=0.003) and 11-dehydro-TxB_2 (HR: 1.64 per 1 SD, 95%CI 1.42-1.89, p<0.001).

CONCLUSIONS: Urinary 11-dehydro-TxB_2 levels are associated with a residual risk of CVEs and CV mortality in AF patients despite anticoagulant treatment.

Author Contributions

Violi, Pignatelli and Pastori had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: All authors

Acquisition of data: Pastori, Bartimoccia, Carnevale, Vicario, Bucci, Nocella

Adjudication of outcomes: Pignatelli, Violi, Cangemi

Analysis and interpretation of data: Pastori, Farcomeni

Drafting of the manuscript: Pastori, Pignatelli, Hiatt; Lip, Violi

Critical revision of the manuscript for important intellectual content: All authors

Statistical analysis: Farcomeni, Pastori

Obtained funding: Pignatelli

Study supervision: Hiatt, Lip, Violi

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia that is associated with a high risk of cardiovascular events and increased morbidity and mortality.[1] Ischemic cardiovascular events are primarily localized in the cerebral circulation, where AF is particularly associated with ischemic stroke[2]. Indeed, ischemic stroke related to AF is more severe, with a greater mortality and morbidity due to disability and longer hospital stays[3].

AF patients are characterized by various atherosclerotic risk factors including arterial hypertension and coronary artery disease, resulting in a high risk of myocardial infarction (MI).[4, 5] Indeed, amongst elderly AF patients with high CHA_2DS_2-VASc score, the risk of MI is similar to that of stroke[6]. For this reason, AF patients are often treated with a combination of oral anticoagulants (OACs) and aspirin, which inhibits platelet thromboxane A_2. Despite an unclear evidence of meaningful net clinical advantage,[7] a large number of AF patients are often treated with OACs and aspirin, resulting in an associated high risk of serious bleeding.[8] Recent registry data (ORBIT-AF)[9] reported that a combination of OACs with aspirin was observed in 35% of patients but the rationale for the use of such a combination was unclear. In more than one third, there was no evidence of any atherosclerotic disease, suggesting poor definition of potentially 'high risk' candidates for combination therapy with OACs plus aspirin.

Urinary levels of 11-dehydro-thromboxane B_2 (TxB_2), a stable metabolite of thromboxane A_2, are a reflection of thromboxane A_2 body generation[10] and its inhibition by aspirin.[11] Previous studies in patients at risk, or with previous cardiovascular events (CVEs) have shown that urinary 11-dehydro-TxB_2 levels are **associated with** CVEs including stroke, MI and cardiovascular deaths, suggesting that this biomarker may identify patient categories in which suppression of thromboxane A_2

generation is of potential clinical benefit.[12] Until now, **the** value of measuring urinary 11-dehydro-TxB_2 in anticoagulated patients with AF is unknown.

We hypothesized that urinary 11-dehydro-TxB_2 levels could be useful to identify AF patients on OACs who are at high residual risk of cardiovascular events. To evaluate this hypothesis, we performed a prospective cohort study in which the relationship between urinary 11-dehydro-TxB_2 and cardiovascular events was investigated in anticoagulated AF patients, who underwent a mean follow-up of 30.0 months. Second, we also assessed the clinical factors **associated with** urinary 11-dehydro-TxB_2 levels.

Materials and Methods

Study design and patient selection.

This prospective single-center cohort study included 950 patients with AF who were referred to the Atherothrombosis Center of the Department of Internal Medicine and Medical Specialties of Sapienza-University of Rome from February 2008 to October 2013. Included patients received oral vitamin K antagonists according to the CHA_2DS_2-VASc score.[13] International normalized ratio was maintained in a therapeutic range of 2.0–3.0 and time in therapeutic range (TTR) was calculated. Patients taking warfarin and an antiplatelet drug were eligible.

Exclusion criteria at baseline included: presence of prosthetic heart valves, cardiac stent placement in the previous year, severe cognitive impairment, chronic infectious diseases, autoimmune systemic diseases and active cancer. Patients treated with antiplatelet agents alone were also excluded, as well as patients with a cardiac revascularization in the previous year.

At enrollment, medical history and anthropometric data were recorded and a sample of urine was collected from all patients. Arterial hypertension was defined as elevated blood pressure (≥140/≥90 mmHg) or taking antihypertensive therapy;[14] diabetes mellitus as a random plasma glucose ≥200 mg/dl, or fasting plasma glucose ≥126 mg/dl, or use of anti-diabetic treatment.[15] Heart failure was defined as the presence of signs and symptoms typical of heart failure or reduced ejection fraction (≤40%).[16]

Follow-up

All patients were followed-up for the entire duration of the study and outcome events were recorded. Patients were regularly seen at each control for INR monitoring every 20-40 days at the Atherothrombosis Center. Patients missing INR control were contacted and information about vital status and hospitalizations were recorded. Follow-up data were obtained by review of

hospital databases, medical records, death certificates, telephone interviews or general practitioners.

Outcome Events. The occurrence of CVEs was considered as the primary outcome of the study. CVEs included fatal and nonfatal ischemic stroke, fatal and nonfatal MI, transient ischemic attack (TIA), cardiac revascularization/coronary artery bypass surgery and cardiovascular death. Diagnosis of MI was made according to the international definition.[17] The occurrence of ischemic stroke was determined on clinical manifestations and confirmed by radiological findings; TIA was defined according to the Classification of Cerebrovascular Diseases III.[18] If a patient died within 4 weeks of stroke or MI, this event was recorded as fatal stroke or fatal MI. Cardiovascular death included sudden death; progressive congestive heart failure; procedure related death (surgical or percutaneous revascularization); and presumed cardiovascular deaths (i.e. those for which a non-cardiovascular cause had not been clearly established). Only the first event that occurred during follow-up was used in the analysis. Bleeding events were classified according to the International Society on Thrombosis and Haemostasis.[19]

Adjudication of outcomes. Data on CVEs were prospectively collected during follow-up. When a CVE occurred, a standardized form was filled in by the investigators. Details on CVEs were registered, as well as death certificates, hospital discharge letter or copy of the medical records of hospitalization, and other clinical documentation (i.e. radiology and laboratory data) were also obtained from patients, or in case of death, from relatives of patients or from general practitioner. Adjudication of cardiovascular events was performed by a committee composed by three physicians (FV, PP, RC) who did not participate to the recruitment of patients and was unaware of the clinical and laboratory characteristics of any enrolled patient. Each member of the committee independently evaluated and adjudicated CVEs in a blinded manner. **In case of discordant evaluation, adjudication of an event was made after a shared review of available clinical data.**

All patients provided a written informed consent. The study protocol was approved by the local ethical board of Sapienza-University of Rome and was conducted according to principles of the Declaration of Helsinki[20]. **This work was supported by Sapienza University of Rome, Grant Code "Pignatelli Progetto Ricerca Sapienza 2011". No extramural funding was used to support this work. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.**

Laboratory analyses. The concentration of urinary 11-dehydro-TxB_2 was measured by an ELISA commercial kit (Cayman). Data are expressed as ng/mg creatinine. Intra- and inter-assay coefficients of variation were 4.0% and 3.6%, respectively. Analyses were performed in a blinded manner. Thus, the biologist (RC) who analyzed the samples was not aware of clinical characteristics of AF patients, or of CVEs. Moreover, 20% of samples were randomly selected and re-analyzed by a second biologist (SB), and results were compared by a third one (CN).

Statistical analysis. Categorical variables were reported as counts (percentage). Following a test of statistical normality, continuous variables were expressed as mean ± standard deviation (SD) or median and interquartile range (IQR) unless otherwise indicated. Independence of categorical variables was tested with the $\chi 2$ test. The normal distribution of parameters was assessed by Kolmogorov–Smirnov test. Student unpaired t test and Pearson product-moment correlation analyses were used for normally distributed continuous variables. Appropriate nonparametric tests (Mann-Whitney U test and Spearman rank correlation test) were used for all the other variables. Group comparisons were performed using Fisher's F-test (ANOVA) or Kruskall-Wallis test when needed. After dividing the AF population into tertiles according to urinary 11-dehydro-TxB_2 values, univariate analyses comparing clinical characteristics among tertiles of 11-dehydro-TxB_2 was performed. The cumulative incidence was estimated using a Kaplan–Meier product-limit estimator. Survival curves were formally compared using the log-rank test. The association

between tertiles of 11-dehydro-TxB_2 levels and outcomes was examined for both CVEs and CV deaths separately. Cox proportional hazards analyses were used to calculate the adjusted relative hazards of CVEs and CV deaths by each clinical variable. **The multivariable analyses were determined including pre-specified variables and using urinary 11-dehydro-TxB_2 as continuous variable. Thus, in addition to 11-dehydro-TxB_2 levels, the following variables were entered as covariates**: **age, smoking, arterial hypertension (treated), diabetes, history of MI, history of stroke/TIA, heart failure, treatment with antiplatelet agents and statins.**

Risk prediction metrics. Calibration was assessed by comparing predicted and expected events at each time point. Discrimination and risk reclassification were assessed by means of C-index, difference in C-index with a model without TxB_2, and Integrated Discrimination Improvement (IDI) index for censored data.[21, 22] **The proportionality of hazards assumption for the Cox model has been checked using plots of Schoenfeld residuals.**

Finally, we used B-splines to identify a threshold according to Molinari et al.[23], but no threshold was detected.

P values <0.05 were considered as statistically significant. All tests were two-tailed and analyses were performed using computer software packages (SPSS-18.0, SPSS Inc. and R version 2.15.2).

Sample size calculation. The sample size was planned using a log-rank test for comparing the first and third tertile of urinary 11-dehydro-TxB_2. We planned a power of 80% and a type-I error rate of 5% and a difference in the rate from 0.93 to 0.85. Therefore, 53 events in the first and last tertile combined were planned in order to guarantee the prescribed power.

Results

Of the 950 AF patients screened, 20 patients refused to be included in the study. Based on the above-mentioned exclusion criteria, 71 patients were excluded and 22 patients were lost at follow-up. Thus, 837 AF patients were finally included in the study cohort. All patients were followed for a mean time of 30.0 (±21.5) months, yielding 2064 person-years of observation. Mean age was 73.1±8.6 years, 43.6% were women. Median CHA_2DS_2-VASc score was 3 [2-4] (table 1). Overall, the mean TTR was adequate (63.9 ± 17.1%). In the whole cohort, median 11-dehydro-TxB_2 levels were 100 [IQR 50 -187] *ng/mg of urinary creatinine*.

***Correlates** of urinary* 11-dehydro-TxB_2 *levels*

To better stratify AF patients, we divided our study cohort into three groups based to tertiles of 11-dehydro-TxB_2 levels: <70 ng/mg creatinine for first tertile, 70-150 ng/mg creatinine for the second tertile and >150 ng/mg creatinine for the third tertile.

As shown in table 2, a significant difference among tertiles of urinary 11-dehydro-TxB_2 was found for CHA_2DS_2-VASc score, HAS-BLED score and use of β blockers agents. No difference in TTR was detected among *the tertiles of urinary* 11-dehydro-TxB_2.

Multivariable stepwise linear regression analysis of **factors associated with** logarithm of urinary 11-dehydro-TxB_2 demonstrated that history of MI was **positively** associated with 11-dehydro-TxB_2 levels (β=0.11, p=0.006), whilst antiplatelet use was inversely associated (β=-0.10, p=0.012). **All other factors were not significant.**

Relationship to outcomes

A CVE occurred in 99 patients (11.8%): non-fatal MI in 11, stent/CABG in 15, ischemic non-fatal stroke in 16, TIA in 2, and cardiovascular deaths in 55 (including 12 fatal MI and 6 fatal ischemic strokes). Table 1 depicts the clinical characteristics of the study cohort, in relation to

presence/absence of CVEs. Mean TTR was non-significantly lower in patients with and without CVEs (Table 1). Those sustaining CVEs were older, with more diabetes, heart failure, a clinical history complicated by cardiac and cerebrovascular events and higher CHA_2DS_2-VASc score (Table 1).

Urinary 11-dehydro-TxB_2 levels were higher in patients with CVEs during the follow-up (p<0.001) (Table 1). Similarly, urinary 11-dehydro-TxB_2 levels were significantly higher in patients with cardiovascular death (186 [120-387] vs. 100 [55-180] ng/mg of urinary creatinine, p<0.001). Kaplan-Meier analysis demonstrated a significantly increased rate of CVEs (Log-Rank test: p<0.001. Figure 1) and cardiovascular deaths (Log-Rank test: p<0.001) across tertiles of 11-dehydro-TxB_2. Thus, 13 CVEs occurred in the first, 24 in the second, and 61 in the third tertile of 11-dehydro-TxB_2.

Using a Cox proportional hazards analysis (table 3, panel A), CVEs were associated with age (Hazard Ratios [HR]: 1.72 per 1SD, 95% Confidence Interval [CI] 1.33 -2.21, p<0.001), diabetes mellitus (HR: 1.89 95%CI 1.20-2.96, p=0.005), heart failure (HR: 1.60, 95%CI 1.01-2.54, p=0.044), history of stroke/TIA (HR: 1.96, 95%CI 1.25-3.06, p=0.003) and urinary 11-dehydro-TxB_2 (HR: 1.64 per 1 SD, 95%CI 1.42-1.89, p<0.001).

When we analyzed only CV deaths (table 3, panel B), we found that age (p<0.001), diabetes mellitus (p=0.004), history stroke/TIA (p=0.022), and urinary 11-dehydro-TxB_2 (p<0.001) were positively associated with the outcome. Conversely, statin use was inversely associated with CV death (p=0.037).

Risk prediction metrics

Cardiovascular events. In order to assess calibration we compared observed vs. predicted CVEs at each time point (Figure 1, supplemental material). The goodness of fit is very good, and the line is not significantly different from the 45 degrees line (p=0.64). The C-index for the Cox model

without TxB_2 is 0.70, while including TxB_2 increased it to 0.76. IDI for TxB_2 was computed as 0.06 (p=0.007, 95%CI: 0.01 - 0.13).

Cardiovascular deaths. In order to assess calibration we compared observed vs. predicted CV deaths at each time point (Figure 2, supplemental material). The goodness of fit is very good, and the line is not significantly different from the 45 degrees line (p=0.86). The C-index for the Cox model without TxB_2 is 0.75, while including TxB_2 increased it to 0.80. IDI for TxB_2 was computed as 0.08 (p=0.019, 95%CI: 0.01 - 0.17).

Bleeding events

During follow-up, 105 hemorrhages (27 major and 78 minor) occurred. Patients who experienced a bleeding event had *lower values* of urinary 11-dehydro-TxB_2 (87 [49-136] vs. 100 [59-183]; p=0.025) and, as expected, a higher mean HAS-BLED score (2.0±0.96 vs. 1.69±0.94; p=0.02). A significant inverse correlation between HAS-BLED score and 11-dehydro-TxB_2 levels was found (r: -0.11, p=0.002). After dividing population according to tertiles of urinary 11-dehydro-TxB_2 values, a non-significant trend in the proportion of patients who bled was observed: 21.4% patients in the first, 19.9% in the second and 12.6% in the third tertile (p=0.052 among groups). The difference was significant, when we compared the first to the third tertile (p=0.013). Kaplan-Meier analysis showed a significant inverse rate of bleeding events across tertiles of urinary 11-dehydro-TxB_2 **(Log-Rank test, p=0.024).**

Discussion

This study provides novel data that urinary 11-dehydro-TxB_2 is associated with cardiovascular events in anticoagulated AF patients.

Previous studies have shown that urinary 11-dehydro-TxB_2 levels are predictive of cardiovascular events in patients at risk of athero-thrombotic complications.[12] In a secondary prevention trial,[12] a nested case-control study comparing patients who experienced or not cardiovascular events found that urinary 11-dehydro-TxB_2 levels were predictive of the risk of MI and cardiovascular death. This finding was confirmed in the CHARISMA trial,[24] which found that urinary 11-dehydro-TxB_2 levels were associated with an increased risk of stroke, MI or cardiovascular death.[24]

In our study, we measured baseline urinary 11-dehydro-TxB_2 levels and analyzed the relationship between this variable and vascular outcomes during a follow-up of about 3 years. All patients included in the study were at high risk of stroke as evident by the high CHA_2DS_2-VASc score, and were on good quality anticoagulation control, as reflected by the average TTR.[25] As expected, a residual vascular risk remained, and the annual rate of cerebrovascular events in our study was 1.1% per year, which is similar to that reported in recent clinical studies.[9, 26] Furthermore, 1.7% patients/year suffered from MI and cardiac revascularization, which is consistent with Roldàn et al.[27], who found a rate MI of 1.83%/year, and apparently in contrast with recent clinical trials with non-vitamin K oral anticoagulants (NOACs). In particular, the annual rate of MI is about 1% (ranging from 0.53%/year in the RE-LY trial[28], to 1.1%/year in the ROCKET AF study[29]) in AF patients treated with warfarin, and even lesser in those treated with NOACs (from 0.53%/year in the ARISTOTLE trial[30] to 0.9%/year in the ROCKET AF study[29]). The reason for this difference cannot be fully established at the moment, but different populations' age may account for this. Thus, the average age of our population is relatively higher than that reported in the trials with

NOACs, which is consistent with a previous study indicating that age >75 years is a risk factor for MI in AF[31].

While we confirm previous data on the association between urinary 11-dehydro-TxB_2 levels and cardiovascular events, the novelty of the present study is our demonstration that urinary 11-dehydro-TxB_2 levels were associated with an increased risk of CVEs and cardiovascular death in an AF cohort already on OACs. Whilst urinary 11-dehydro-TxB_2 levels do not fully reflect platelet-thromboxane A_2 formation,[32] this finding may suggest a potential role for platelets promoting vascular complications in some AF patients despite being on adequate OACs. Among the predictors of urinary 11-dehydro-TxB_2 levels, previous history of cardiac events was associated with urinary 11-dehydro-TxB_2 levels suggesting a potentially major interplay between coronary heart disease (CHD) and platelet activation in AF.

Analysis of the relationship between 11-dehydro-TxB_2 levels and bleeding showed that higher urinary 11-dehydro-TxB_2 levels were associated with lower risk of bleeding; accordingly an inverse association between HAS-BLED and urinary 11-dehydro-TxB_2 was detected. This is in contrast with a previous finding showing a direct relationship between urinary 11-dehydro-TxB_2 and bleeding, but different populations and designs do not allow a direct comparison of these data.[24] The biologic plausibility of our finding is in the probable inverse association between platelet activation and bleeding risk.

The study has clinical implications and limitations. **The present study would suggest that in AF on adequate oral anticoagulation, persistent platelet activation, as assessed by urinary 11-dehydro-TxB_2, is associated with vascular events. Patients with a clinical history of CHD were associated with enhanced urinary 11-dehydro-TxB_2** suggesting that AF patients with previous CHD is a sub-set which could be benefit from warfarin in combination with antiplatelet treatment. However, a

recent retrospective study on this specific sub-setting denied this possibility as no beneficial effect but a higher risk of bleeding has been observed in AF patients treated with warfarin plus aspirin[33]**.** Statins may represent an interesting option to be investigated as they possess antithrombotic effects by interfering with clotting system and, overall, by lowering platelet 11-dehydro-TxB_2[34, 35]. Notably, statins reduce the risk of cardiovascular events in primary and secondary prevention trials[36, 37] and could be therefore useful in combination with warfarin to reduce the residual cardiovascular risk in patients with AF. Randomized study is, however, necessary to investigate the effectiveness of warfarin plus statins in patients with AF. Finally, inhibitor of TxB_2 receptors[38, 39] may represent another option in which this drug category could be investigated on top of oral anticoagulants.

Whilst this was a prospective study, it was conducted in a single Italian center, including patients with average age >70 years and a coexistence of a serious vascular risk profile. **Other limitations of this study include the relatively low number of patients and CVEs, the potential for residual confounding, the observational study design with the resulting inability to establish causal relations, the uncertain generalizability to other races/ethnicities.** A last limitation is the lack of a control group of patients with similar characteristics but without AF.

In conclusion, urinary 11-dehydro-TxB_2 levels is associated with residual risk of cardiovascular events in anticoagulated AF patients. Such association is particularly evident in AF patients with a history of coronary heart disease, who, therefore, may be potential candidates for additional antithrombotic strategies as part of the holistic approach to managing this common arrhythmia[40].

Conflict of Interest/Disclosures: None.

References

1. Benjamin EJ, Wolf PA, D'Agostino RB, Silbershatz H, Kannel WB, Levy D. Impact of atrial fibrillation on the risk of death: the Framingham Heart Study. Circulation 1998;98(10):946-52.
2. Wolf PA, Abbott RD, Kannel WB. Atrial fibrillation as an independent risk factor for stroke: the Framingham Study. Stroke; a journal of cerebral circulation 1991;22(8):983-8.
3. Lin HJ, Wolf PA, Kelly-Hayes M, Beiser AS, Kase CS, Benjamin EJ, et al. Stroke severity in atrial fibrillation. The Framingham Study. Stroke; a journal of cerebral circulation 1996;27(10):1760-4.
4. Wilke T, Groth A, Mueller S, Pfannkuche M, Verheyen F, Linder R, et al. Oral anticoagulation use by patients with atrial fibrillation in Germany. Adherence to guidelines, causes of anticoagulation under-use and its clinical outcomes, based on claims-data of 183,448 patients. Thrombosis and haemostasis 2012;107(6):1053-65.
5. Lam CC, Tse HF, Siu CW. Transient atrial fibrillation complicating acute myocardial infarction: a nuisance or a nemesis? Thrombosis and haemostasis 2012;107(1):6-7.
6. Marijon E, Le Heuzey JY, Connolly S, Yang S, Pogue J, Brueckmann M, et al. Causes of death and influencing factors in patients with atrial fibrillation: a competing-risk analysis from the randomized evaluation of long-term anticoagulant therapy study. Circulation 2013;128(20):2192-201.
7. Lane DA, Raichand S, Moore D, Connock M, Fry-Smith A, Fitzmaurice DA, et al. Combined anticoagulation and antiplatelet therapy for high-risk patients with atrial fibrillation: a systematic review. Health technology assessment 2013;17(30):1-188.
8. Hansen ML, Sorensen R, Clausen MT, Fog-Petersen ML, Raunso J, Gadsboll N, et al. Risk of bleeding with single, dual, or triple therapy with warfarin, aspirin, and clopidogrel in patients with atrial fibrillation. Archives of internal medicine 2010;170(16):1433-41.
9. Steinberg BA, Kim S, Piccini JP, Fonarow GC, Lopes RD, Thomas L, et al. Use and associated risks of concomitant aspirin therapy with oral anticoagulation in patients with atrial fibrillation: insights from the Outcomes Registry for Better Informed Treatment of Atrial Fibrillation (ORBIT-AF) Registry. Circulation 2013;128(7):721-8.
10. Catella F, Healy D, Lawson JA, FitzGerald GA. 11-Dehydrothromboxane B2: a quantitative index of thromboxane A2 formation in the human circulation. Proceedings of the National Academy of Sciences of the United States of America 1986;83(16):5861-5.
11. Tohgi H, Konno S, Tamura K, Kimura B, Kawano K. Effects of low-to-high doses of aspirin on platelet aggregability and metabolites of thromboxane A2 and prostacyclin. Stroke; a journal of cerebral circulation 1992;23(10):1400-3.
12. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. Circulation 2002;105(14):1650-5.
13. Camm AJ, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, et al. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation. Developed with the special contribution of the European Heart Rhythm Association. European heart journal 2012;33(21):2719-47.
14. Mancia G, De Backer G, Dominiczak A, Cifkova R, Fagard R, Germano G, et al. 2007 Guidelines for the management of arterial hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). European heart journal 2007;28(12):1462-536.
15. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabetic medicine : a journal of the British Diabetic Association 1998;15(7):539-53.
16. Dickstein K, Cohen-Solal A, Filippatos G, McMurray JJ, Ponikowski P, Poole-Wilson PA, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2008: the Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association of the ESC (HFA) and

endorsed by the European Society of Intensive Care Medicine (ESICM). European heart journal 2008;29(19):2388-442.
17. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. Journal of the American College of Cardiology 2012;60(16):1581-98.
18. Special report from the National Institute of Neurological Disorders and Stroke. Classification of cerebrovascular diseases III. Stroke; a journal of cerebral circulation 1990;21(4):637-76.
19. Schulman S, Kearon C, Subcommittee on Control of Anticoagulation of the S, Standardization Committee of the International Society on T, Haemostasis. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. Journal of thrombosis and haemostasis : JTH 2005;3(4):692-4.
20. World Medical A. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bulletin of the World Health Organization 2001;79(4):373-4.
21. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. Circulation 2009;119(17):2408-16.
22. Uno H, Tian L, Cai T, Kohane IS, Wei LJ. A unified inference procedure for a class of measures to assess improvement in risk prediction systems with survival data. Statistics in medicine 2013;32(14):2430-42.
23. Molinari N, Daures JP, Durand JF. Regression splines for threshold selection in survival data analysis. Statistics in medicine 2001;20(2):237-47.
24. Eikelboom JW, Hankey GJ, Thom J, Bhatt DL, Steg PG, Montalescot G, et al. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. Circulation 2008;118(17):1705-12.
25. Wan Y, Heneghan C, Perera R, Roberts N, Hollowell J, Glasziou P, et al. Anticoagulation control and prediction of adverse events in patients with atrial fibrillation: a systematic review. Circulation Cardiovascular quality and outcomes 2008;1(2):84-91.
26. Dans AL, Connolly SJ, Wallentin L, Yang S, Nakamya J, Brueckmann M, et al. Concomitant use of antiplatelet therapy with dabigatran or warfarin in the Randomized Evaluation of Long-Term Anticoagulation Therapy (RE-LY) trial. Circulation 2013;127(5):634-40.
27. Roldan V, Marin F, Manzano-Fernandez S, Fernandez H, Gallego P, Valdes M, et al. Does chronic kidney disease improve the predictive value of the CHADS2 and CHA2DS2-VASc stroke stratification risk scores for atrial fibrillation? Thrombosis and haemostasis 2013;109(5):956-60.
28. Connolly SJ, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, et al. Dabigatran versus warfarin in patients with atrial fibrillation. The New England journal of medicine 2009;361(12):1139-51.
29. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. The New England journal of medicine 2011;365(10):883-91.
30. Granger CB, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, et al. Apixaban versus warfarin in patients with atrial fibrillation. The New England journal of medicine 2011;365(11):981-92.
31. Soliman EZ, Safford MM, Muntner P, Khodneva Y, Dawood FZ, Zakai NA, et al. Atrial fibrillation and the risk of myocardial infarction. JAMA internal medicine 2014;174(1):107-14.
32. Patrono C, Ciabattoni G, Davi G. Thromboxane biosynthesis in cardiovascular diseases. Stroke; a journal of cerebral circulation 1990;21(12 Suppl):IV130-3.
33. Lamberts M, Gislason GH, Lip GY, Lassen JF, Olesen JB, Mikkelsen AP, et al. Antiplatelet therapy for stable coronary artery disease in atrial fibrillation patients taking an oral anticoagulant: a nationwide cohort study. Circulation 2014;129(15):1577-85.
34. Pignatelli P, Carnevale R, Pastori D, Cangemi R, Napoleone L, Bartimoccia S, et al. Immediate antioxidant and antiplatelet effect of atorvastatin via inhibition of Nox2. Circulation 2012;126(1):92-103.
35. Violi F, Calvieri C, Ferro D, Pignatelli P. Statins as antithrombotic drugs. Circulation 2013;127(2):251-7.

36. Zheng H, Xue S, Hu ZL, Shan JG, Yang WG. The use of statins to prevent postoperative atrial fibrillation after coronary artery bypass grafting: a meta-analysis of 12 studies. Journal of cardiovascular pharmacology 2014.
37. Cho KI, Kim BJ, Cha TJ, Heo JH, Kim HS, Lee JW. Impact of duration and dosage of statin treatment and epicardial fat thickness on the recurrence of atrial fibrillation after electrical cardioversion. Heart and vessels 2014.
38. Santilli F, Mucci L, Davi G. TP receptor activation and inhibition in atherothrombosis: the paradigm of diabetes mellitus. Internal and emergency medicine 2011;6(3):203-12.
39. Patrono C. Biosynthesis and pharmacological modulation of thromboxane in humans. Circulation 1990;81(1 Suppl):I12-5; discussion I22-3.
40. Kirchhof P, Lip GY, Van Gelder IC, Bax J, Hylek E, Kaab S, et al. Comprehensive risk reduction in patients with atrial fibrillation: Emerging diagnostic and therapeutic options. Executive summary of the report from the 3rd AFNET/EHRA consensus conference. Thrombosis and haemostasis 2011;106(6):1012-9.

Figure legend

Figure 1. Kaplan-Meier curves estimate of cardiovascular events according to tertiles of 11-dehydro-thromboxane B_2 levels.

Table 1. Baseline characteristics of the study cohort and according the occurrence of cardiovascular events.

	Whole cohort n=837	Cardiovascular events		p value
		No (n=738)	Yes (n=99)	
Age (years)	73.2±8.5	72.9±8.6	75.5±7.4	0.001
Women (%)	43.6	44.0	40.4	0.52
Body mass index (kg/m^2)	27.2±4.7	27.2±4.7	27.3±4.2	0.88
Smokers (%)	9.9	9.8	11.1	0.72
CHA_2DS_2-VASc score[#]	3 [2-4]	3 [2-4]	4 [3-5]	<0.001
Time in therapeutic range (%)	63.8±17.2	64.2±17.2	59.3±16.5	0.09
Urinary 11-dehydro-TxB_2[#]	100.0 [56.5-181.0]	98.0 [51.0-170.0]	185.0 [109.0-400.0]	<0.001
HAS-BLED score[#]	2 [1-2]	1 [1-2]	2 [1-2]	0.60
Arterial Hypertension (treated) (%)	92.8	92.5	94.9	0.53
Diabetes mellitus (%)	19.8	18.4	30.3	0.007
Heart failure (%)	17.7	15.7	32.3	<0.001
History of stroke/TIA (%)	16.7	15.0	29.3	0.001
History of MI/ Cardiac Revascularization (%)	24.0	22.4	36.4	0.004
Anti-platelets (%)	7.0	7.3	5.1	0.53
Statins (%)	37.4	37.4	37.4	0.99

CVEs: cardiovascular events, MI: myocardial Infarction, TIA: transient ischemic attack, TxB_2: thromboxane B_2

[#]data expressed as median and interquartile range.

Table 2. Baseline characteristics according to tertiles of urinary 11-dehydro-thromboxane B_2

	1st tertile (<70 ng/mg creatinine)	2nd tertile (70-150 ng/mg creatinine)	3rd tertile (>150 ng/mg creatinine)	P value 1st vs. 3rd
Age (years)	72.8±8.0	73.6±8.8	73.2±8.6	0.56
Women (%)	39.1	45.7	45.8	0.19
Body mass index (kg/m^2)	27.1±4.5	27.2±4.6	27.4±4.9	0.74
Smokers (%)	12.2	7.9	9.8	0.24
CHA_2DS_2-VASc score[#]	3 [2-4]	3 [2-4]	3 [2-5]	0.038
Time in therapeutic range (%)	65.5±17.3	62.2±17.3	63.6±16.7	0.21
HAS-BLED score[#]	2 [1-2]	2 [1-2]	1 [1-2]	0.001
Arterial Hypertension (treated) (%)	91.5	91.8	95.1	0.18
Diabetes mellitus (%)	19.9	20.0	19.6	0.99
Heart failure (%)	15.5	18.2	19.2	0.49
History of stroke/TIA (%)	15.5	14.6	19.9	0.20
History of MI/ Cardiac Revascularization (%)	21.4	23.6	26.9	0.31
Anti-platelets (%)	9.6	6.1	5.6	0.14

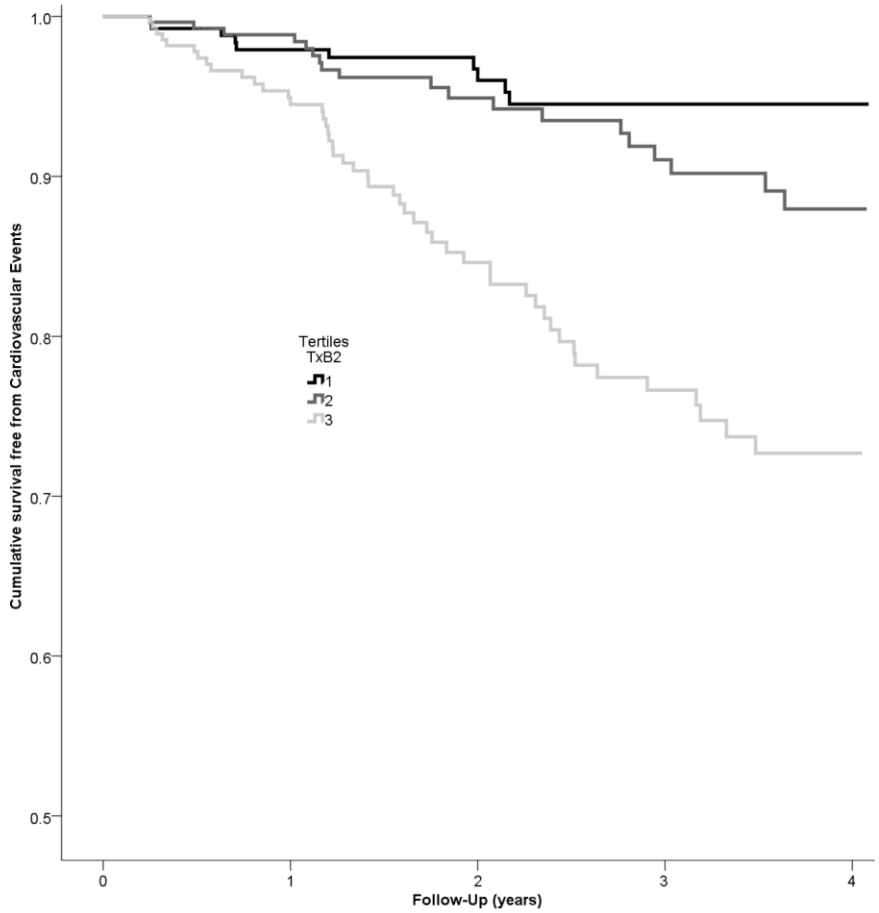
MI: myocardial Infarction, TIA: transient ischemic attack.

[#]data expressed as median and interquartile range.

Table 3. Adjusted hazard ratios, based on a Cox proportional Hazards model according to selected variables.

Panel A. CV Events	p	HR	95%CI	
Age per 1 SD	<0.001	1.72	1.33	2.21
Women	0.26	0.78	0.51	1.20
Arterial Hypertension (treated)	0.63	0.83	0.32	2.14
Diabetes Mellitus	0.005	1.89	1.20	2.96
Smoking	0.26	1.48	0.75	2.90
Heart Failure	0.044	1.60	1.01	2.54
History of Stroke/TIA	0.003	1.96	1.25	3.06
History of MI/Cardiac Revascularization	0.59	1.14	0.71	1.84
Anti-platelets	0.89	1.07	0.41	2.77
Statins	0.83	0.95	0.61	1.49
Urinary 11-dehydro-TxB_2 per 1SD	<0.001	1.64	1.42	1.89
Time in Therapeutic Range	0.96	0.99	0.98	1.02
Panel B. CV deaths	**p**	**HR**	**95%CI**	
Age per 1 SD	<0.001	2.04	1.43	2.92
Women	0.43	0.79	0.45	1.41
Arterial Hypertension (treated)	0.90	1.10	0.24	4.92
Diabetes Mellitus	0.004	2.43	1.33	4.43
Smoking	0.79	1.16	0.39	3.50
Heart Failure	0.11	1.67	0.90	3.10
History Stroke/TIA	0.022	2.00	1.10	3.63
History of MI/Cardiac Revascularization	0.39	1.33	0.70	2.54
Anti-platelets	0.56	1.44	0.42	4.99
Statins	0.037	0.49	0.25	0.96
Urinary 11-dehydro-TxB_2 per 1SD	<0.001	1.66	1.36	2.02
Time in Therapeutic Range	0.49	0.98	0.96	1.02

CI: confidence interval, CVE: cardiovascular event, HR: hazard ratios, MI: myocardial Infarction, TIA: transient ischemic attack, TxB_2: Thromboxane B_2.



1st tertile	270	209	134	90	47
2nd tertile	279	231	143	107	71
3rd tertile	285	220	130	92	66

Fig. 1