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Title

Influence of age on the relationship between apixaban concentration and anti-factor Xa activity in older patients with non-valvular atrial fibrillation

Permalink

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Journal

International Journal of Cardiology, 331

ISSN

0167-5273

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Publication Date

2021-05-01

DOI

10.1016/j.ijcard.2021.01.025

Peer reviewed

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Highlights:

- The apixaban concentration vs anti-Factor Xa activity relationship demonstrated followed a linear relationship across all age groups
- Increasing age was not associated with a clinically relevant change in the apixaban vs. anti- Factor Xa activity response relationship
- The data suggest that target apixaban concentrations to produce equivalent anti-Factor Xa activity as measured in the laboratory are not influenced by increasing older age.

Abstract

Background/Objectives: Despite lower major bleeding rates associated with direct oral anticoagulants (DOACs) as compared to conventional warfarin therapy, bleeding rates remain higher in older patients compared to younger patients suggesting a potential role for DOAC measurements. The objective of this study is to examine the effect of age on the relationship between apixaban concentrations and anti-Factor Xa activity in patients with non-valvular atrial fibrillation (NVAF).

Methods: This is a retrospective analysis based on a database created using data from the ARISTOTLE study. Outpatient, stable adult patients with NVAF receiving apixaban were included in this study. Data collection consisted of apixaban concentration, anti-Factor Xa activity, age, weight, creatinine, and co-medications.

Results: The database composed of 2,058 patients receiving apixaban. Distribution of race, NVAF subtype, and aspirin use was fairly similar across each age quantile. Older patients were observed with a higher number of co-medications and received the 2.5 mg apixaban dose as compared to younger patients (22% vs. < 1%). Linear regression demonstrated that the unadjusted slope for apixaban concentration effect on anti-Factor Xa activity was similar across each age quantile. Although, the overall adjusted linear regression analysis demonstrated that the age by concentration interaction was statistically significant, relative differences in anti-Factor Xa (< 8%) were not clinically meaningful.

Conclusion: Data on apixaban concentrations and anti-Factor Xa activity from a pivotal randomized double-blind study of apixaban for the prevention of stroke in NVAF patients have confirmed that the chromogenic anti-factor Xa assay can accurately assess apixaban concentrations in patients regardless of age. Age was not associated with a clinically relevant change in the apixaban vs. anti-Factor Xa activity response relationship and target ranges are unchanged.

Influence of Age on the Relationship between Apixaban Concentration and Anti-Factor Xa Activity in Older Patients with Non-valvular Atrial Fibrillation

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Abstract

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Results: The database composed of 2,058 patients receiving apixaban. Distribution of race, NVAF subtype, and aspirin use was fairly similar across each age quantile. Older patients were observed with a higher number of co-medications and received the 2.5 mg apixaban dose as compared to younger patients (22% vs. < 1%). Linear regression demonstrated that the unadjusted slope for apixaban concentration effect on anti-Factor Xa activity was similar across each age quantile. Although, the overall adjusted linear regression analysis demonstrated that the age by concentration interaction was statistically significant, relative differences in anti-Factor Xa (< 8%) were not clinically meaningful.

Conclusion: Data on apixaban concentrations and anti-Factor Xa activity from a pivotal randomized double-blind study of apixaban for the prevention of stroke in NVAF patients have confirmed that the chromogenic anti-factor Xa assay can accurately assess apixaban concentrations in patients regardless of age. Age was not associated with a clinically relevant change in the apixaban vs. anti-Factor Xa activity response relationship and target ranges are unchanged.

Introduction

Optimizing anticoagulation with warfarin, a narrow therapeutic index drug, in older patients has been a challenge due to the variability in responses related to diet, dietary supplements, co-medications, genetic variation, increased risks for bleeding and falls, as well as increased sensitivity to warfarin in older patients compared to younger patients.[1-5] Direct-acting oral anticoagulants (DOACs) are replacing vitamin K antagonists for anticoagulation due to fewer food and medication interactions and simplified dosing and monitoring regimens.[6-8] Despite lower major bleeding rates with DOACs as compared to warfarin in randomized trials and emerging real world data, bleeding rates are higher in older patients compared to younger patients with non-valvular atrial fibrillation (NVAF) and remain a significant clinical concern.[9-11] Major bleeding rates have been shown to be directly related to DOAC concentrations and anti-Factor Xa activity.[12]

Routine DOAC concentration measurements are not currently recommended but are available in clinical laboratories. Measurement of DOAC concentrations may be useful in selected situations, including confirming minimal anticoagulant effect prior to invasive/surgical procedures, when drug distribution or clearance may be altered due to marked obesity, chronic kidney disease, or concomitant administration of medications with drug-drug interactions, altered drug absorption, or to assess adherence.[1-3] Most current clinical laboratory measurements are done by chromogenic assays of anti-Factor Xa activity that is calibrated to the DOAC concentration. For apixaban, the ranges reported by laboratories are based on concentration data determined by mass spectrometry collected during multiple clinical studies and modeled using population pharmacokinetic techniques.[13] However, anti-factor Xa activity in addition to was measured during the ARISTOTLE trial but has not been previously reported. Our goal was to examine the relationship

between apixaban concentrations and anti-Factor Xa activity with previously unreported data collected during the pivotal randomized double-blind study of apixaban and warfarin in patients with non-valvular atrial fibrillation.

Methods

Study Design.

The design and overall results of the ARISTOTLE trial have been published previously.[14,15] In brief, this multicenter, double-blind, double-dummy randomized clinical trial included 18,201 patients with AF presenting with at least 1 additional risk factor for stroke or systemic embolism. Patients were randomized to apixaban (n =9,120) or warfarin (n = 9,081). The standard dose of apixaban was 5 mg twice daily; however, patients with $\geq$2 dose-adjustment criteria (age 80 years or older, weight 60 kg or less, and creatinine 1.5 mg/dl (133 mmol/l) or higher) received 2.5 mg twice daily. The present analysis is of the subset of apixaban patients with prespecified single time-matched blood samples for determination of apixaban concentrations and anti-Factor Xa activity at the 2-month visit.

Pharmacokinetic Sampling and anti-Factor Xa Activity Measurements

Population pharmacokinetic models of apixaban concentration data from ARISTOTLE have been reported.[13] Concentrations were determined using a validated liquid chromatography assay coupled with atmospheric pressure ionization mass spectrometry method with a lower limit of quantification of 1 ng/mL. Anti-Factor Xa activity results in human plasma were generated by Rotachrom Heparin (low molecular weight heparin) assay on the STA Compact ® analyzer at Esoterix Clincal Trials Services, Cranford, NJ 07016. In brief, study samples are incubated with a chromogenic substrate; a fixed amount of exogenous Factor Xa is added to the plasma-substrate

mixture which triggers two reactions simultaneously: inhibition of FXa by LMWH or apixaban and hydrolysis of substrate by FXa to cleave the chromophore. The color generated is measured at 405 nm and is inversely proportional to the concentration of LMWH or apixaban. Absorbance values are interpolated against a 5 point linear LMWH curve and reported in LMWH units (IU/ml = U/mL). The LLOQ and the ULOQ (IU/mL) were 0.10 and approximately 18.5, respectively. Between run % CV was $\leq$ 7.2 Anti-FXa unit of IU/mL is equivalent to U/mL.[16]

Statistical Analyses

Patients in the pharmacokinetic-pharmacodynamic (PK/PD) analytical dataset were categorized into age quantiles, where each quantile bin represented approximately 20% of the PK/PD sample. Summary statistics for patient characteristics for each age quantile are presented as mean and standard deviation (SD) for continuous variables and as number of patients and percentage of sample or median and range for categorical variables. Concentrations versus response relationships across each age quantile were characterized by an unadjusted linear regression model to compare the concentration effect. In addition, a linear regression model was developed using the overall analytical dataset and was adjusted for apixaban concentration, age (as a continuous variable), apixaban concentration and age interaction, gender, race, weight, creatinine clearance calculated using the Cockcroft-Gault formula, and number of co-medications (with at least 80% adherence during the trial period).

Results

Time-matched apixaban concentrations and anti-Factor Xa activity were available for 2,058 patients randomized to apixaban. Ninety-five percent received a dosing regimen of 5 mg orally twice daily (n=1,965) while 5% received an adjusted dose of 2.5 mg twice daily (n=94). Of

participants who received the reduced dose, 17% (n=16) did not meet the stated dose reduction criteria and 0.2% of patients (n=5) who received the 5 mg twice daily dose met the dose reduction criteria. Patient baseline demographic and clinical characteristics are presented in Table 1. Three Asian and one Caucasian male participant over age 65 years and receiving 5 mg twice daily had anti-Factor Xa activity above the upper limit of detection of 18 IU/m and were removed from final analyses. Apixaban concentrations in these outliers ranged from 416 to 1860 ng/mL.

The majority of the sample were men with the proportion of women increasing with age. Average weight, BMI, and creatinine clearance decreased with increasing age. Distribution of race, NVAF type as well as aspirin use was fairly similar across each age quantile. The median $CHADS_2$ stroke risk score was similar from ages 26-77 years but was highest in patients older than >77 years (2 vs. 3). The number of co-medications in older patients was larger than in younger NVAF patients, likely reflecting a larger number of co-morbid medical conditions. The percentage receiving the 2.5 mg apixaban dose was larger in the highest age quantile as compared to the lower age quantiles (22% vs. < 1%).

Exploratory analysis of the time course of apixaban concentration and anti-Factor Xa activity demonstrated that the trend was similar and that no pharmacodynamic delay was apparent. Visual inspection of apixaban concentration vs anti-Factor Xa activity demonstrated that the two measures were highly correlated and followed a linear relationship (Figure 1), thus showing no delay in pharmacologic response. Figure 1 also provides the apixaban concentration vs. anti-Factor Xa activity relationship by age quantile. Linear regression demonstrated that the unadjusted slope for apixaban concentration effect on anti-Factor Xa activity was similar across each age quantile. The concentration slope effect ranged from 0.0126 to 0.0159. However, the overall adjusted linear regression analysis demonstrated that age, apixaban concentration, age by concentration

interaction, and aspirin use were statistically significant (Supplementary Table 1). To understand the clinical meaningfulness and potential clinical impact of the statistically significant covariates, anti-Factor Xa activity was estimated for ages 50-90 years for apixaban concentrations ranging from 100-400 ng/mL and compared between the unadjusted base model (Figure 1) with the adjusted reduced linear regression model (Supplementary Table 1). The results indicate that the relative difference in anti-Factor Xa activity was at maximum, less than 8 percent. Relative difference in anti-Factor Xa activity in patients at the age of 80 and 90 years was less than 5% and 8 percent, respectively.

Discussion

These are the first data to our knowledge showing that neither the concentration versus anti-Factor Xa activity relationship of apixaban nor the degree of anti-Factor Xa activity is influenced by advancing age in patients with NVAF. Monitoring of apixaban concentrations or anti-Factor Xa activity were not analyzed as an endpoint or reported with the main results of the pivotal trial that led to the approval of apixaban for prevention of stroke in patients with NVAF.[13,14] The data were, however, collected with the apixaban concentrations incorporated into population pharmacokinetic models for apixaban.[15] The current analysis reports anti-Factor Xa activity observed in the pivotal randomized double-blind controlled trial and may have implications for clinical care.

The data suggest that target apixaban concentrations to produce equivalent anti-Factor Xa activity as measured in the laboratory are not influenced by increasing older age. Our data also suggest that while unwanted bleeding events are more frequent with increasing age in NVAF patients receiving apixaban it is likely due to patient-related factors and not altered relationships between apixaban concentrations and anti-Factor Xa activity. Furthermore, the lack of a detectable effect of creatinine clearance or weight on anti-Factor Xa activity with apixaban supports similar

pharmacodynamic targets to achieve clinically relevant effects across a wide range of patient weight and renal function.

It is important to note that the assay used for measuring anti-Factor Xa activity in the ARISTOTLE trial and reported here was calibrated to low molecular weight heparin (LMWH) anti-Factor Xa activity using one manufacturer's chromogenic method and results for heparin based anti-Factor Xa activity are reported in IU/mL.[17] A number of hospital and commercial laboratories are measuring and reporting Factor Xa inhibition by chromogenic methods (https://testdirectory.questdiagnostics.com/test/test-detail/94223/?cc=MASTER; https://www.labcorp.com/tests/117085/apixaban) but use calibrators and standards specific to individual DOACs. Results are reported to clinicians as the individual or requested DOAC concentration in ng/mL. The chromogenic measurement of anti-Factor Xa activity however does not distinguish between DOACs or heparin. Most, if not all, clinical laboratories have established and validated assays for anti-Factor Xa activity calibrated to LMWH. As data on anti-Factor Xa activity with apixaban for stroke prevention in NVAF were generated with the LMWH calibrated anti-Factor Xa assay, it suggests that this may be an appropriate and more economical and efficient approach to assessing anti-Factor Xa activity in patients with NVAF receiving apixaban.

Current guidelines for management of patients with NVAF receiving apixaban (or other DOACs) do not recommend routine monitoring of concentrations due to the lack of standardization of tests, and lack of data correlating measures with clinical outcomes.[18] However, this statement may be somewhat misleading as anti-factor Xa activity has been related to bleeding outcomes for all DOACs during drug development and during clinical use and the relationship of anti-factor Xa activity and stroke prevention has been reported from the pivotal trial of edoxaban in patients with

NVAF (notably the only DOAC trial for which sufficient concentration or anti-factor Xa activity data were collected to analyze stroke outcomes in addition to bleeding outcomes).[19,20] Real world data has also associated higher stroke rates in NVAF patients with the lowest DOAC concentrations compared to higher DOAC concentrations.[21] Guidelines do state that concentration data may be useful in patients such as those with chronic kidney disease (CKD) or undergoing renal dialysis, the obese (BMI > 40 kg/m^2), receiving potentially interacting drugs, undergoing surgical procedures, and to evaluate adherence.[20,22-23] Many older patients have CKD as in the oldest quantiles in this study would be classified, many receive potentially interacting medications, and adherence may be a concern. Therefore, some authors consider older age a potential reason to measure DOAC levels.[24] Others suggest that if drug levels or anti-factor Xa activity is measured, the purpose should be to confirm that the drug is being absorbed and that levels are not excessive. Higher than expected plasma concentrations of apixaban in older patients with NVAF measured using research intensive methods have been reported.[18,25] Bleeding has been related to high peak concentrations in real world DOAC use and levels as much as two-hold higher in NVAF patients over 80 years of age using DOAC calibrated anti-Factor Xa activity assays have been reported.[26] Measurements of anti-Factor Xa activity with widely available tests that have been standardized and that can be related to data from pivotal trials might be a reasonable step to facilitate avoidance of excessively high inhibition of Factor Xa and potentially decrease the bleeding risk during management of older patients with NVAF receiving apixaban.

Our data have limitations. Enrollment of racial minorities in ARISTOTLE was low and the relationship between apixaban concentrations and anti-Factor Xa activity could not be examined. Although, a limited number of patients received the reduced dose of apixaban, there appeared to be adequate data at the lower end of the apixaban concentration vs. anti-Factor Xa activity

relationship. While LMWH anti-Factor Xa activity assay results may vary with the method used, our data are only from one assay that is widely available. Finally, the concentration vs. anti-Factor Xa activity relationship analyses were limited to concentrations observed in the trial and precluded nonlinear regression analyses.

In summary, we report data on apixaban concentrations and anti-Factor Xa activity from the pivotal randomized double-blind study of apixaban for the prevention of stroke in NVAF patients in which 1) increasing age was not associated with a clinically relevant change in the apixaban vs. LMWH-calibrated anti- Factor Xa activity response relationship and 2) ranges of anti-Factor Xa activity from the trial are established. These data may provide a basis for use of anti-Factor Xa activity with readily available LMWH standards to reflect apixaban exposure that could be directly compared to the pivotal randomized trial data regardless of age.

Acknowledgments

This project was supported in part by an appointment to the Research Participation Program at the U.S. Food and Drug Administration administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration. We wish to acknowledge the contributions of the investigators who conducted the ARISTOTLE and the sponsor of the trial, Bristol Meyers-Squibb, who generated the data for analysis and provided insightful comments on this manuscript. We would also like to acknowledge and thank Dr. Robert Temple, Dr. Norman Stockbridge, and Dr. Martin Rose from the Office of New Drugs at Center for Drug Evaluation and Research/US Food and Drug Administration for reviewing and contributing suggestions to improve this manuscript.

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This article reflects the views of the authors and should not be constructed to represent FDA's views or policies.

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Table 1:

Characteristics	Age Bin				
	26-62 (N=454)	**62-68 (N=413)**	**68-73 (N=443)**	**73-77 (N=343)**	**77-93 (N=404)**
Age (y) – mean (SD)	56.5 (5.2)	65.7 (1.7)	71.0 (1.4)	75.4 (1.1)	81.7 (3.2)
Weight (kg) – mean (SD)	93.8 (22.6)	90.6 (21.9)	83.3 (18.2)	81.0 (17.1)	77.1 (16.3)
BMI – mean (SD)	31.4 (6.4)	30.9 (6.2)	29.4 (5.4)	28.2 (4.8)	27.5 (4.8)
Gender – n (%)					
Male	351 (77)	279 (68)	273 (62)	226 (66)	252 (62)
Female	103 (23)	134 (32)	170 (38)	117 (34)	152 (38)
Race – n (%)					
Asian	74 (16)	54 (13)	49 (11)	34 (10)	19 (5)
African Descent	7 (2)	5 (1)	2 (1)	2 (1)	2 (1)
Caucasian	370 (81)	354 (86)	392 (88)	305 (88)	383 (94)
Other	3 (1)	0	0	2 (1)	0
CrCL (mL/min) – mean (SD)	107.9 (33.9)	88.9 (30.2)	75.8 (21.5)	65.0 (20.3)	55.8 (17.9)
$CHADS_2$ score *– median (range)	2 (0-5)	2 (1-5)	2 (0-5)	2 (1-6)	3 (1-6)
AF Type – n (%)					
Paroxysmal	81 (18)	67 (16)	88 (15)	60 (17)	55 (14)
Persistent/permanent	373 (82)	346 (84)	375 (85)	283 (83)	349 (86)
Aspirin Use – n (%)	91 (20)	90 (22)	98 (22)	71 (21)	89 (22)
Number of co-medications – n (range)	5 (1-30)	6 (1-37)	6 (1-34)	6 (1-24)	7 (1-30)
Apixaban Dose – n (%)					
2.5 mg twice daily	1 (<1)	1 (<1)	2 (<1)	2 (<1)	88 (22)
5 mg twice daily	453 (>99)	412 (>99)	441 (>99)	341 (>99)	316 (78)

BMI=body mass index, CrCL= estimated creatinine clearance by Cockcroft Gault equation(17) , $CHADS_2$= $CHADS_2$ score for atrial fibrillation stroke risk.
*Gage BF, Waterman AD, Shannon W, Boechler M, Rich MW, Radford MJ. Validation of clinical classification schemes for predicting stroke: results from the National Registry of Atrial Fibrillation. JAMA 2001; 285(22):2864-7

Figure Legends

Figure 1. (A) Time paired apixaban concentration versus anti-Factor Xa activity for all patients. *Red circles* indicate values above the upper limit of detection of anti-Factor Xa activity (>18 IU/ml). *Blue solid line* represents the adjusted linear regression model predicted anti-Factor Xa activity over the observed range of apixaban concentrations. Estimation of the linear regression model did not include outliers, as identified by the *red circles*. (B) Time paired apixaban concentration versus anti-Factor Xa activity across each age quantile. *Colored solid line* represents the average predicted anti-Factor Xa activity over observed apixaban concentrations using the unadjusted linear regression model for each age quantile.

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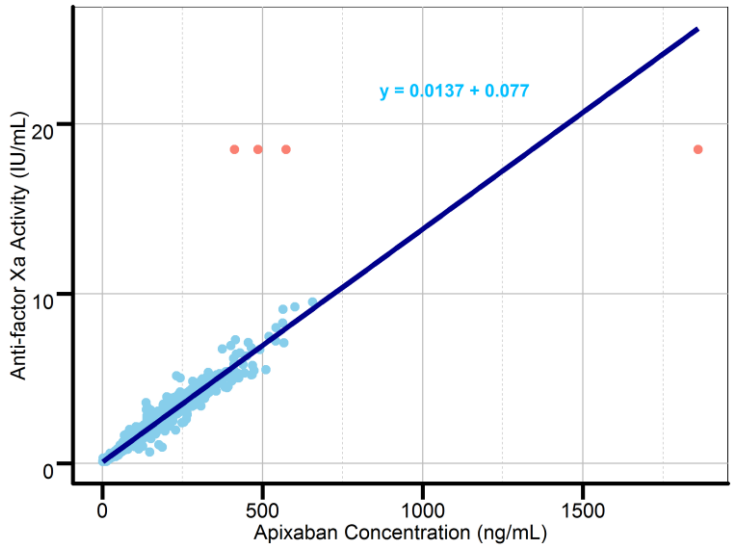
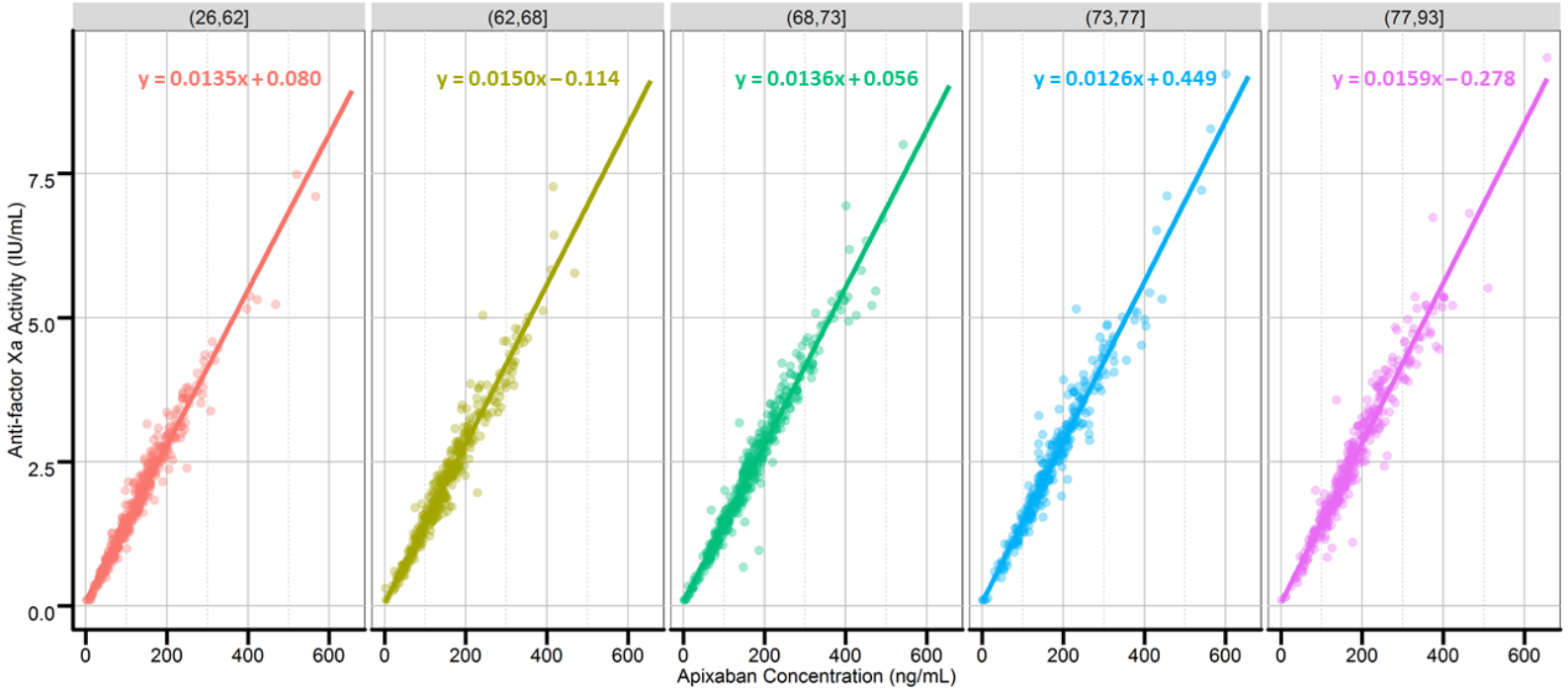
Figure 1:

A.


y = 0.0137 + 0.077
Anti-factor Xa Activity (IU/mL)
0
10
20
0
500
1000
1500
Apixaban Concentration (ng/mL)

B.


(26,62]
(62,68]
(68,73]
(73,77]
(77,93]
y = 0.0135x + 0.080
y = 0.0150x − 0.114
y = 0.0136x + 0.056
y = 0.0126x + 0.449
y = 0.0159x − 0.278
Anti-factor Xa Activity (IU/mL)
0.0
2.5
5.0
7.5
0
200
400
600
Apixaban Concentration (ng/mL)

Shamir N. Kalaria: Conceptualization, Methodology, Formal analysis, Writing-Original draft preparation, Writing-Review & Editing, Visualization; **Hao Zhu**: Conceptualization, Methodology, Writing-Review & Editing, Visualization, Supervision; **Qi Liu**: Conceptualization, Supervision; **Jeff Florian**: Conceptualization, Methodology, Software, Data Curation, Supervision; **Yaning Wang**: Conceptualization, Methodology, Supervision; **Janice Schwartz**: Conceptualization, Methodology, Writing-Original draft preparation, Writing-Review & Editing, Data Curation, Supervision

Author Agreement Form – International Journal of Cardiology

Manuscript Title: **Influence of Age on the Relationship between Apixaban Concentration and Anti-factor Xa Activity in Older Patients with Non-valvular Atrial Fibrillation**

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This statement is to certify that all authors have seen and approved the manuscript being submitted, have contributed significantly to the work, attest to the validity and legitimacy of the data and its interpretation, and agree to its submission to the *International Journal of Cardiology.*

We attest that the article is the Authors' original work, has not received prior publication and is not under consideration for publication elsewhere. We adhere to the statement of ethical publishing as appears in the International of Cardiology (citable as: Shewan LG, Rosano GMC, Henein MY, Coats AJS. A statement on ethical standards in publishing scientific articles in the International Journal of Cardiology family of journals. Int. J. Cardiol. 170 (2014) 253-254 DOI:10.1016/j.ijcard.2013.11).

On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission. Any changes to the list of authors, including changes in order, additions or removals will require the submission of a new author agreement form approved and signed by all the original and added submitting authors.

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