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Drug interactions and pharmacogenetic factors contribute to variation in apixaban concentration in atrial fibrillation patients in routine care

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Key Points

- Cytochrome P450 (CYP)3A-metabolism and ATP-binding cassette (ABC) efflux transporters influence the pharmacokinetics of apixaban.
- *ABCG2* c.421C>A and *CYP3A5**3 genotypes were shown to associate with apixaban exposure in small cohorts of Japanese patients with atrial fibrillation (AF).
- The effect of concomitant amiodarone, a moderate ABCB1/CYP3A4 inhibitor, on apixaban exposure is controversial.
- This study identified concomitant amiodarone, *ABCG2* c.421C>A, and female sex as being independently associated with higher apixaban concentration in a large cohort of Caucasian AF patients.
- Consideration of interacting medications and genetic markers modulating ABCG2 and/or ABCB1 transport in addition to known clinical factors may improve apixaban dosing.

Abstract

Factor Xa-inhibitor apixaban is an oral anticoagulant prescribed in atrial fibrillation (AF) for stroke prevention. Its pharmacokinetic profile is known to be affected by cytochrome P450 (CYP)3A metabolism, while it is also a substrate of the efflux transporters ATP-binding cassette (ABC)B1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein, BCRP). In this study, we assessed the impact of interacting medication and pharmacogenetic variation to better explain apixaban concentration differences among 358 Caucasian AF patients. Genotyping (*ABCG2*, *ABCB1*, *CYP3A4**22, *CYP3A5**3) was performed by TaqMan assays, and apixaban quantified by mass spectrometry. The typical patient was on average 77.2 years old, 85.5 kg, and had a serum creatinine of 103.1 µmol/L. Concomitant amiodarone, an antiarrhythmic agent and moderate CYP3A/ABCB1 inhibitor, the impaired-function variant *ABCG2* c.421C>A, and sex predicted higher apixaban concentrations when controlling for age, weight and serum creatinine (multivariate regression; R^2=0.34). Our findings suggest that amiodarone and *ABCG2* genotype contribute to interpatient apixaban variability beyond known clinical factors.

Keywords: atrial fibrillation, apixaban, pharmacokinetics, breast cancer resistance protein genotype, concomitant amiodarone

Introduction

Apixaban is a direct factor Xa inhibitor prescribed to reduce stroke risk in patients with non-valvular atrial fibrillation (AF) [1]. Direct anticoagulants (DOACs) such as apixaban have been rapidly replacing warfarin therapy due to an improved risk-benefit profile shown in clinical trials, a simple dosing strategy and no need for routine therapeutic monitoring [2-4]. However, we previously reported up to 50-fold interpatient variation in apixaban plasma concentration in routine care, where 13% of patients exceeded the 95th percentile for the predicted maximum plasma concentration observed in clinical trials of AF patients [5]. Since the factor Xa inhibitory effects of apixaban are known to closely correlate with plasma concentration [6-8], increased exposure may result in augmented bleeding risk, highlighting the need to identify associated factors for risk prediction [9, 10].

Apixaban is eliminated through renal and non-renal pathways, including hepatic cytochrome P450 (CYP) 3A4/5 metabolism, biliary excretion and intestinal secretion, and was shown to be a substrate of the efflux transporters ATP-binding cassette (ABC) B1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein or BCRP) [11-14]. Co-prescribed ketoconazole, a strong inhibitor of CYP3A4/ABCB1, leads to clinically significant increased apixaban exposure, whereas moderate inhibitors such as amiodarone, diltiazem and cyclosporine are thought to have smaller effects [15-17]. Similarly, concomitant rifampicin, a strong inducer of CYP3A4/ ABCB1, significantly reduces exposure [18], and is therefore not recommended during apixaban therapy [19]. Various clinical factors are also known to influence the pharmacokinetics of apixaban, including age, weight and renal function [20-24]. Accordingly, a dose reduction from the standard dose of 5 mg to 2.5 mg twice daily is recommended in an AF patient with at least two of the

following characteristics: age ≥ 80 years, weight ≤ 60kg, and serum creatinine ≥ 133 μmol/L [19]. Female sex and Asian race were also reported to be associated with moderately increased apixaban exposure, while mild or moderate hepatic impairment does not seem to have an effect [21, 22].

The efflux transporters ABCG2 and ABCB1 are widely recognized for their relevance in drug disposition [25]. Expressed at the apical membrane of enterocytes, hepatocytes, and kidney proximal tubules these carriers mediate intestinal secretion, biliary and renal excretion of drug substrates, respectively. Altered transporter function can arise from genetic polymorphisms or interacting medication, and often underlies variable drug exposure. We and others previously reported decreased transport activity *in vitro* for *ABCG2* c.421C>A (Q141K), a non-synonymous single nucleotide polymorphism (SNP) [26-28]. Moreover, we observed increased exposure of the anti-inflammatory agent sulfasalazine, an ABCG2 substrate, in *ABCG2* c.421A variant carriers compared to wildtype in 17 normal individuals [28]. Recently, *ABCG2* c.421A/A and *CYP3A5**3 genotypes were shown to predict increased apixaban concentration in two small cohorts of Japanese patients with AF, while there was no effect of known *ABCB1* markers [29, 30]. This study aimed to assess the effects pharmacogenetic markers and interacting medication on apixaban plasma concentration in 358 Caucasian AF patients to better explain interpatient differences.

Methods

Subjects and study design. This is a prospective observational study that was approved by the Research Ethics Board of the University of Western Ontario (London, Canada), and written informed consent obtained from participants. A cohort of 358 patients with AF prescribed

apixaban was recruited in an outpatient hospital setting at the London Health Sciences Centre (LHSC), London, Ontario; 94 previously enrolled patients were included in this study [5].

Clinical and pharmacokinetic data. Venous blood samples were collected at steady state during regular apixaban dosing intervals. At the time of blood draw, apixaban dose, time after the last dose, age, sex, ethnicity, weight, serum creatinine, and interacting medications (ABCB1 and/or CYP3A4 inhibitors [19]) were collected. Estimated creatinine clearance (eCrCL) was calculated using the Cockcroft-Gault equation [31]. Apixaban plasma concentrations for 94 patients were obtained from our previous study, with the remaining 264 patient samples quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [5], a method considered the gold standard for DOAC quantification [32]. Apixaban calibration standards spanned 5 to 1000 ng/mL, and the lower limit of quantification was 5 ng/mL. The between-run precision (CV percentage) and accuracy bias (percentage) values for apixaban quality control samples were less than 10% and 8%, respectively.

Genotyping. Genomic DNA was extracted (Gentra Puregene Blood Kit, Qiagen, Alameda, CA). TaqMan assays (Applied Biosystems, Foster City, CA) were used to genotype patients for the following reduced-function SNPs: *ABCG2* c.421C>A (rs2231142; C__15854163_70), *ABCG2* c.34G>A (rs2231137; custom assay) [28], *ABCB1* c.3435 C>T (rs1045642; C___7586657_20), *CYP3A4**22 *C>T* (rs35599367; C__59013445_10), and *CYP3A5*3* A>G (rs776746; C__26201809_30). Hardy-Weinberg equilibrium was tested using the $\chi 2$ method. Observed allele frequencies did not deviate from Hardy-Weinberg equilibrium.

Data analysis. Statistical analysis was performed using SPSS 25 (Armonk, USA) and GraphPad Prism 8 (La Jolla, USA). For multivariate linear regression analyses, log10-transformed apixaban concentrations were used as the dependent variable since they better approximated normal distribution (**Supplemental Figure S1**). The final model included the following independent variables: age, sex, weight, creatinine, genotype, dose, time after dose, and amiodarone and diltiazem use. Different genetic models were tested for each genotype (dominant, co-dominant, recessive, additive), and the model that best described the fit of apixaban concentration was selected. Specifically, *ABCB1* c.3435C>T and *ABCG2* c.421C>A genotypes were included as ordinal variables (additive model) with homozygous wildtype coded as 0, while heterozygous and homozygous variant carriers were coded as 1 and 2, respectively. With the exception of *CYP3A5**3, the remaining polymorphisms were treated as binary variables (dominant model) with those that were homozygous wildtype coded as zero, and those that were heterozygous or homozygous variant coded as one. CYP3A5 expressers (*CYP3A5**1/*1 and *CYP3A5**1/*3) were coded as zero and non-expressers (*CYP3A5**3/*3) were coded as one. Amiodarone and diltiazem were included as ordinal variables categorized according to the daily dose of the inhibitor (**Supplemental Table S1**). Dose and sex were coded as categorical variables, while age, creatinine, weight, time after last dose were entered as continuous variables.

Results

Patients characteristics are summarized in **Table 1**. Almost half of the patients were ≥80 years (46%), 20% had a serum creatinine of ≥133 μmol/L, and 10% weighted ≤60 kg. A reduced apixaban dose of 2.5 mg twice daily was taken by 48% of patients. No patients received potent

ABCB1 and/or CYP3A4 inhibitors (ketoconazole, clarithromycin) or inducers (carbamazepine, rifampin, phenytoin, phenobarbital), while 23% were on a moderate inhibitor (diltiazem or amiodarone).

Single time-point apixaban concentrations were assessed between 3 to 12 hours after dose from times between peak and trough concentrations (**Figure 1**). There was an overall 41-fold variation (min-max) with a coefficient of variation (CV %) of 55.1% (**Figure 1a**). For 5 mg and 2.5 mg twice daily doses, the CV was 55.0% and 47.3% for apixaban peak concentration (3−4 hours post-dose assessed in 78 patients), and 71.1% and 63.0% for trough concentration (8−12 hours post-dose assessed in 67 patients), respectively (**Figure 1b**).

The relative contribution of demographic, clinical and pharmacogenetic factors to apixaban concentration was assessed by multiple regression analysis (**Table 2**). After controlling for dose and sampling time, concomitant amiodarone use was significantly associated with apixaban concentration, with the effect most pronounced in patients receiving 400 mg daily. Furthermore, *ABCG2* c.421C>A genotype was identified as a predictor of increased apixaban concentration, while there was no association between other genotypes and apixaban exposure. In addition, female sex, older age, and increased creatinine further contributed to higher apixaban concentration, together explaining 34.4% (adjusted R^2) of the observed variation. While weight was significant when entered without sex into the regression model (β=-0.002, p=0.004; model fit R^2=0.322), only sex remained significant in the final model (**Table 2**).

Using average patient characteristics observed in our cohort for the clinical covariates currently recommended for apixaban dosing (77.2 years, 85.5 kg, 103.1 μmol/L serum creatinine) (**Table 2**) [19], the regression model predicts that concomitant high-dose amiodarone (400 mg) therapy would result in 1.61-fold higher peak and trough concentrations at a 5 mg dose (**Figure 2a**). For *ABCG2* c.421C>A genotype, heterozygous and homozygous carriers would be predicted to have 1.10-fold and 1.33-fold greater apixaban peak and trough concentrations, respectively, than wildtype carriers (**Figure 2b**). Lastly, women would be predicted to have a 1.2-fold higher peak and trough concentration compared to men (**Figure 2c**).

Discussion

Considerable interpatient variability has been previously reported in apixaban plasma concentration in AF patients in routine care [5, 24, 33]. The present study determined that concomitant amiodarone therapy and *ABCG2* c.421C>A genotype significantly contributed to the observed variation in apixaban plasma concentration in addition to known demographic and clinical factors in 358 AF patients of Caucasian descent.

Hepatic CYP3A4/5 metabolism and direct intestinal secretion are major routes of apixaban elimination, followed by urinary excretion via glomerular filtration [11]. Apixaban is a known substrate of ABCG2 and ABCB1, two ABC efflux carriers recognized as key factors in drug disposition, mediating active drug removal into the intestinal lumen, bile and urine [34, 35]. ABCG2 may play a more important role than ABCB1 for apixaban absorption and renal elimination as suggested by studies in drug-transporter knockout rats [13]. *ABCG2* c.421C>A is an impaired-function polymorphism previously reported as a predictor of apixaban trough

concentration in a small cohort of 44 Japanese AF patients, a result later confirmed through population pharmacokinetics in a slightly larger cohort (n=81) [29, 30]. In this study, *ABCG2* c.421C>A but not c.34G>A, another common SNP, was found to be independently associated with apixaban concentration, and predicted moderately increased concentrations in carriers of the *ABCG2* c.421A allele. This finding is in concordance with previous reports showing enhanced exposure of other drug substrates for *ABCG2* c.421C>A, while *ABCG2* c.34G>A had no effect [27, 28]. Both SNPs occur most frequently in East Asians (30-60%), but have relatively low frequencies in Caucasians and African-Americans (5-10%), while other *ABCG2* polymorphisms are very rare (MAF≤1%) [35, 36]. We observed a MAF of 11% for *ABCG2* c.421C>A in this Caucasian population compared to 33% in the Japanese cohort [29]. Our study did not observe an effect for other pharmacogenetic markers assessed. Specifically, *CYP3A5**3, a splice variant resulting in partial or lack of CYP3A5 expression in heterozygous and homozygous carriers, respectively, had been previously shown to determine apixaban concentration [29]. The lack of effect may be in part due to the relatively low prevalence of the *CYP3A5**1 wildtype allele in Caucasian compared to Japanese patients (MAF 7.3% vs. 22%, respectively). Furthermore, our data do not support an association with *ABCB1* rs1045642, a synonymous variant known to cause impaired P-glycoprotein activity [37, 38], while a link between an intronic *ABCB1* variant rs4148738 and apixaban peak concentration was previously suggested in AF patients [39].

Our study further indicates that amiodarone, co-prescribed in 8% of patients, increased apixaban concentration, particularly at the 400 mg daily dose. Amiodarone, an antiarrhythmic agent frequently prescribed to treat AF, is a known inhibitor of ABCB1 and various CYP enzymes involved in apixaban metabolism, including CYP3A4, CYP1A2, and CYP2C9 [40-42].

Amiodarone was not found to alter oral clearance of apixaban in 81 Japanese patients [30]. Although amiodarone dosage was not reported, this finding may be due to the small sample size. Another small retrospective study did not show an association of apixaban concentration above the expected range with concomitant administration of moderate CYP3A4 or ABCB1 inhibitors (including amiodarone), the latter prescribed to 34.4% of the 87 patients on apixaban, however amiodarone dose was not assessed [43]. At present, the effect of concurrent amiodarone use on major bleeding risk remains inconclusive. Spontaneous adverse event reporting suggests 3.5% to 9.5% of all reported bleeding events (n=1215 total in Australia, Canada and USA) in patients prescribed apixaban likely occurred due to the use of concurrent amiodarone [44]. A retrospective cohort study (n=12,886) reported an increased risk for major bleeding among amiodarone users [45] while recent analyses of the ARISTOTLE trial showed amiodarone users had similar or reduced rates of bleeding compared with non-users [46, 47], indicating further research is required to elucidate the clinical relevance of increased apixaban exposure in routine care patients prescribed amiodarone, particularly at a maintenance dose of 400 mg daily or higher. Furthermore, implications of high amiodarone loading doses (up to 1600 mg daily) on apixaban pharmacokinetic interactions are currently not known. We did not observe an effect for concomitant diltiazem, a calcium-channel blocker and moderate CYP3A4/5 and weak ABCB1 inhibitor. Previously, co-administration of 360 mg daily diltiazem was shown to moderately increase apixaban exposure by 40% [16], thus our observation may be due to the limited number of individuals prescribed a 360 mg dose (**Supplemental Table S1**). Relative to amiodarone, diltiazem had a greater effect on the exposure of simvastatin, a sensitive CYP3A substrate [48, 49]. Our results suggests that CYP3A-mediated metabolism may play a more limited role in apixaban pharmacokinetics, also supported

by reports of an only 2-fold increase in apixaban exposure after concomitant ketoconazole, a strong CYP3A/ABCB1 inhibitor [16].

The association of higher apixaban concentration with increased age, female sex, and impaired renal function observed here was consistent with previous studies involving healthy and AF subjects [6, 20, 21, 23]. An effect of bodyweight on apixaban pharmacokinetics has been previously observed by others and us [6, 22]. Interestingly, findings of this study support a more pronounced role of sex than body weight. Overall, 34% of the interpatient variability in apixaban concentration in Caucasian patients has been explained with the herein assessed covariates, compared to 35.8% in Japanese patients [29].

Our results indicate that interpatient variability is considerably high among routine care patients with a coefficient of variation of up to 55% and 71% for apixaban peak and trough concentration, respectively, with similar results reported (CV 42% for C_{max}; 68% for C_{min}) [33]. In addition, our data suggest that the predicted apixaban peak concentration for the average patient in terms of weight, age and renal function at the recommended 5 mg dose can exceed the 95th percentile observed in clinical trials [19] if patients receive concomitant high-dose amiodarone, harbor 2 impaired function *ABCG2* 421A alleles, or if they are female (**Figure 2**). Though a link to clinical outcomes is currently unclear, an increasing number of studies indicate that a concentration-effect relationship is likely [50, 51]. Concerning apixaban, two recent observational studies in 565 AF patients of the Italian START-2 register (including 208 patients on apixaban) suggested more frequent bleeding events overall in patients with higher peak levels (199-658 ng/mL for apixaban) compared to those within the lowest quartile [10], while patients with lower trough levels (22-145

ng/mL for apixaban) showed more frequently thromboembolic events compared to those within the three higher quartiles [52]. Such association is further supported by findings of the *post hoc* analysis of the AVERROES trial [3], where anti-Xa activity correlated with minor bleeding [9].

Due to a wider therapeutic index, a therapeutic range remains undefined for direct oral anticoagulants such as apixaban, and efficacy and general safety for a fixed dosing regimen has been demonstrated in clinical trial and real-world settings [50]. However, uncertainty remains concerning patient groups not or under-represented in clinical trials, for example, patients taking interacting drugs, patients at the extremes of body weight and the very elderly, where additional guidance of therapy may be achieved by measuring drug concentration [50, 51, 53]. Successful dose adjustments have been reported in patients requiring antiepileptic medication such as carbamazepine and phenobarbital, both strong CYP3A/ABCB1 inducers, to augment apixaban concentration [54, 55], as well as in a case of decreased absorption due to short bowel syndrome [56]. Importantly, studies that establish a therapeutic window for direct oral anticoagulants such as apixaban are urgently required to aid in the clinical interpretation of a patient's drug concentration.

There are several limitations of this study: i) results are based on single time point measurements rather than a complete pharmacokinetic profile, ii) plasma samples were not drawn at fixed times after dosing (i.e. trough level), iii) findings represent results from a single clinical center, iv) anticoagulation outcomes were not assessed, and v) results are only representative for a Caucasian population.

Conclusion

In conclusion, we demonstrate that co-prescribed amiodarone, a moderate ABCB1/CYP3A4 inhibitor, is a significant predictor of apixaban concentration in AF patients of Caucasian descent, with variability further explained by *ABCG2* c.421C>A genotype. These findings provide new insights concerning the role of interacting medication and genetic markers modulating ABCG2 and ABCB1 transport that explain interpatient variability in apixaban pharmacokinetics beyond known clinical factors, while their relevance for anticoagulant response requires further investigation.

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Compliance with ethical standards

Conflict of interest disclosure Authors have no conflict of interest to disclose.

Ethics approval and consent to participate This study involving adult patients has been approved by the Research Ethics Board of Western University, London, Canada (REB15586). All subjects provided written informed consent.

Figure legend

Figure 1. Single time point apixaban plasma concentrations between 3 and 12 hours after dose (a) and peak and trough concentration (b) at 2.5 mg and 5 mg twice a day (BID, bis in die). Concentrations 3-4 hours after dosing were defined as peak and 8-12 hours as trough. Data are presented as median, mean (+), quartiles (box), 10-90th percentiles (whiskers) and outliers (●).

Figure 2. Predicted apixaban peak (*left panels*) and trough (*right panels*) plasma concentration at steady state by concomitant amiodarone therapy (a), *ABCG2* c.421C>A genotype (b), and sex (c). Data are presented as predicted means (95% CI) and were calculated using the β coefficients derived from multivariate regression of apixaban concentration in our cohort (n=358, **Table 2**). Observed mean values were employed for clinical covariates including weight (85.5 kg), age (77.2 years), and serum creatinine (103.1 μmol/L); apixaban concentrations were predicted for a 5 mg dose at 3 (peak) and 12 hours (trough) after dosing. Predicted *on therapy* range of apixaban concentration in clinical trials depicted as horizontal lines (Median [5th, 95th percentiles]: 171 [91, 321] for C_{max}; 103 [41, 230] for C_{min}). *Eliquis*® (apixaban) 2.5 mg and 5 mg product monograph. Bristol-Myers Squibb.

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Table 1. Characteristics of the study population including 358 patients with atrial fibrillation prescribed apixaban.

Characteristics	
Ethnicity, Caucasian, *n* (%)	358 (100%)
Sex, Female, *n* (%)	166 (46.4%)
Age at enrolment	
Mean ± SD, years	77.2 ± 10.5
Median (min-max, 5-95th percentile), years	79 (41−97, 58−92)
≤65 years, *n* (%)	48 (13%)
>65 to <80 years, *n* (%)	146 (41%)
≥80 years, *n* (%)	164 (46%)
Body weight	
Mean ± SD, kg	85.5 ± 23.2
Median (min-max, 5-95th percentile), kg	82.1 (43.0−221.1, 55.0-132.0)
≤60 kg, *n* (%)	34 (10%)
>60 to <120 kg, *n* (%)	294 (82%)
≥120 kg, *n* (%)	30 (8%)
Level of renal impairment [a]	
Mean ± SD, mL/min	69.8 ± 38.2
Median (min-max, 5-95th percentile), mL/min	63 (15−287, 23−142)
Normal (eCrCl >80 mL/min) , *n* (%)	108 (30.2%)
Mild (50 ≤ eCrCl ≤ 80 mL/min) , *n* (%)	123 (34.3%)
Moderate (30 ≤ eCrCl < 50 mL/min), *n* (%)	97 (27.1%)

Severe (15 ≤ eCrCl < 30 mL/min), *n* (%)	30 (8.4%)
Serum Creatinine	
Mean ± SD, µmol/L	103.1 ± 43.6
Median (min-max, 5-95th percentile), µmol/L	91 (41−316, 58−188)
< 133 µmol/L, *n* (%)	287 (80%)
≥ 133 µmol/L, *n* (%)	71 (20%)
Apixaban Dose	
2.5 mg twice daily, *n* (%)	171 (48%)
5 mg twice daily, *n* (%)	187 (52%)
Time after dose, median (min-max), hours	5.8 (3-12)
Apixaban concentration, median (min-max), ng/ml	208.3 (32.3-1329.0)
Concomitant ABCB1 and CYP3A4 Inhibitor	
Amiodarone, *n* (%)	28 (8%)
Diltiazem, *n* (%)	53 (15%)
Minor allelic frequency, variant alleles/ total alleles (%)	
ABCB1 c.3435C>T	377/716 (52.5%)
ABCG2 c.34G>A	25/716 (3.5%)
ABCG2 c.421C>A	79/716 (11.0%)
CYP3A4 *22	36/716 (5.0%)
CYP3A5 *3	666/716 (92.7%)

Abbreviations: eCrCl, estimated creatinine clearance. [a] Creatinine clearance was calculated using the Cockcroft-Gault equation.

Table 2. Multiple linear regression model for apixaban plasma concentration (log-transformed) in 358 Caucasian patients with atrial fibrillation.

Predictor variable	**β[a]**	**95% CI[b]**	***P*-value**
Dose, 5mg twice daily	0.100	0.081 0.119	**<0.001**
Time after dose, hours	-0.033	-0.042 -0.023	**<0.001**
Sex, female	0.080	0.035 0.124	**<0.001**
Age, year	0.006	0.004 0.008	**<0.001**
Weight, kg	-0.001	-0.002 0.0002	0.113
Serum creatinine, µmol/L	0.002	0.001 0.002	**<0.001**
Amiodarone [c]	0.068	0.005 0.131	**0.035**
Diltiazem [c]	-0.012	-0.042 0.018	0.442
ABCB1 c.3435C>T, per allele	-0.006	-0.035 0.023	0.682
ABCG2 c.34G>A, 1 or 2 alleles	0.003	-0.076 0.083	0.934
ABCG2 c.421C>A, per allele	0.048	0.002 0.093	**0.040**
CYP3A4 *22, 1 or 2 alleles	0.013	-0.057 0.082	0.721
CYP3A5 *3, 2 alleles	-0.014	-0.074 0.046	0.648
Adjusted R^2 for model, 0.344			

[a] β, beta coefficient; [b] CI, confidence interval; [c] ordinal variable according to daily dose (Supplemental Table S1)

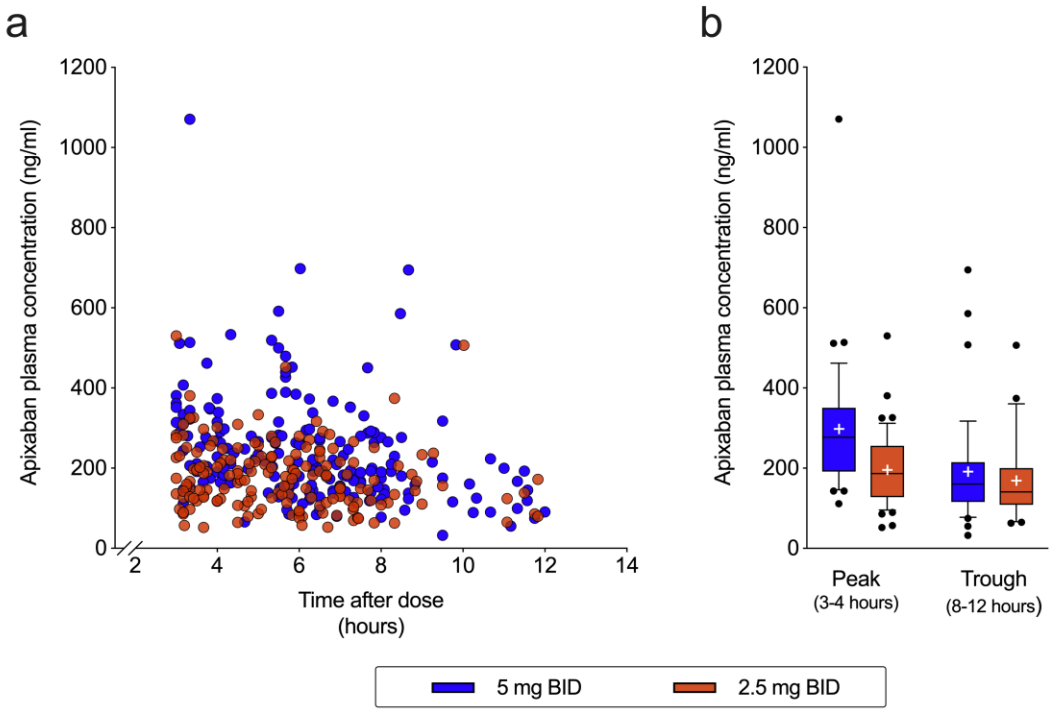

a
b
Apixaban plasma concentration (ng/ml)
1200
1000
800
600
400
200
0
2
4
6
8
10
12
14
Time after dose
(hours)
Peak
(3-4 hours)
Trough
(8-12 hours)
5 mg BID
2.5 mg BID

Figure 1

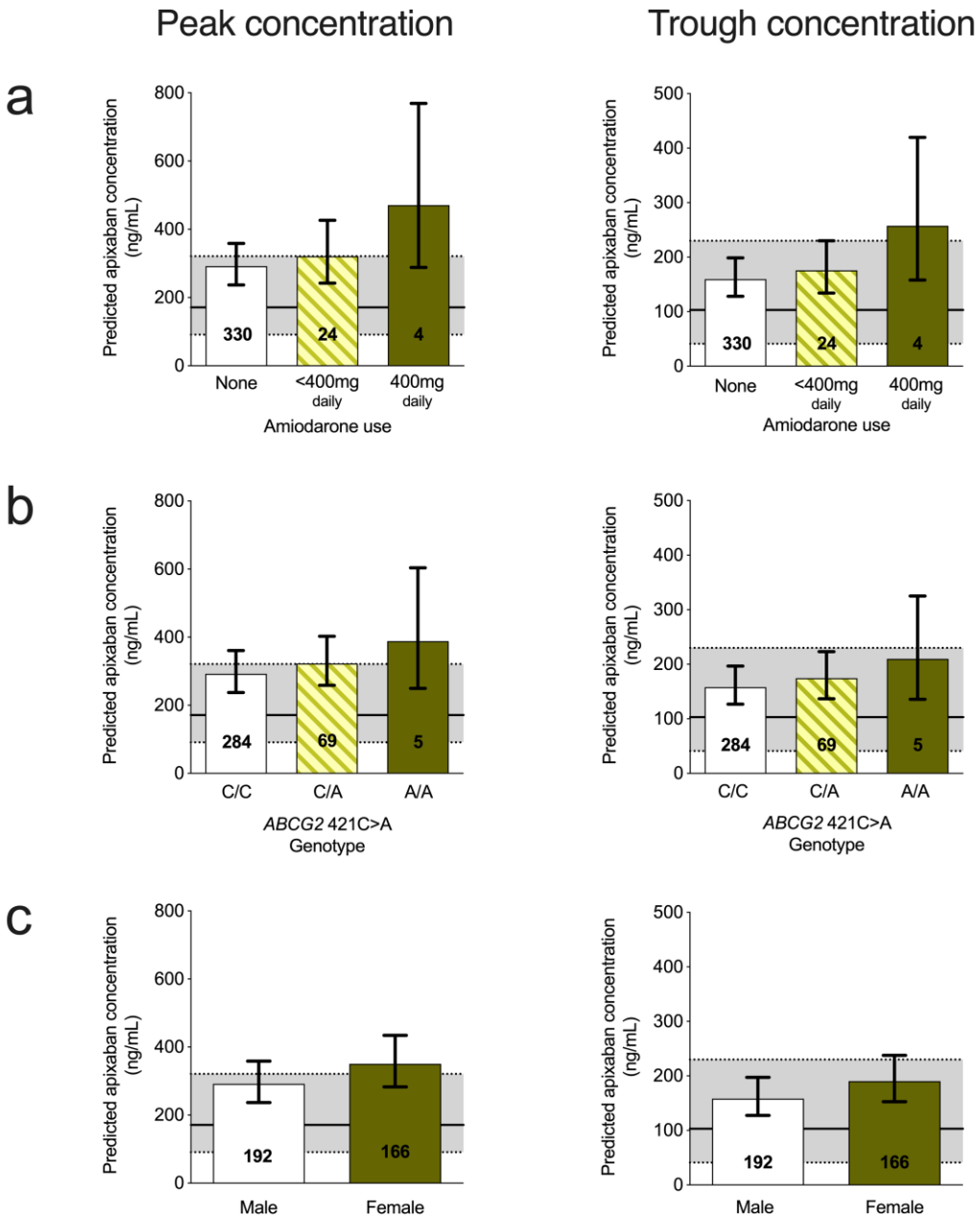
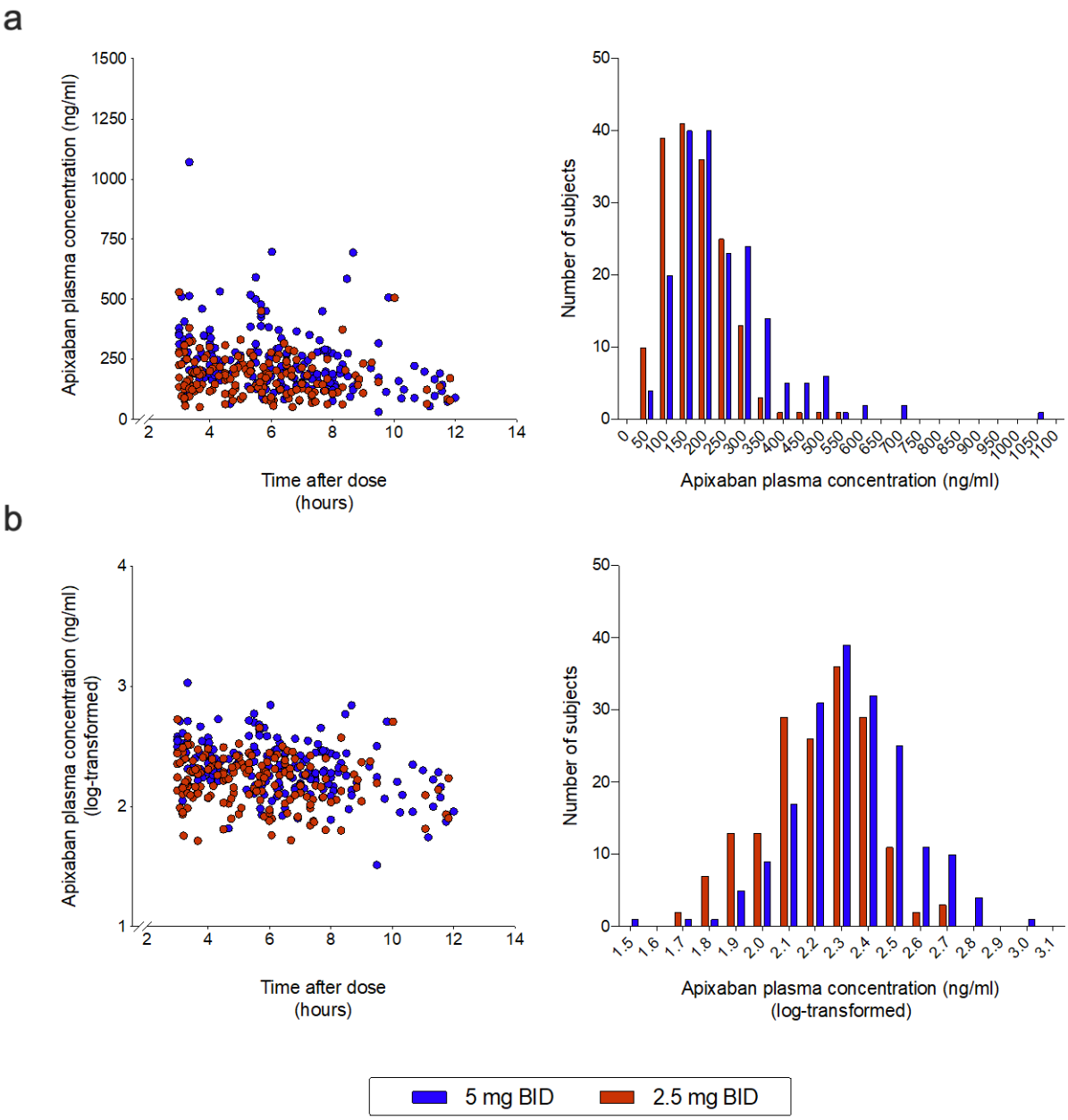

Peak concentration
Trough concentration
a
b
c
Predicted apixaban concentration (ng/mL)
800
600
400
200
0
500
300
100
330
24
4
284
69
5
192
166
None
<400mg daily
400mg daily
Amiodarone use
C/C
C/A
A/A
ABCG2 421C>A Genotype
Male
Female

Figure 2

Supplementary Material



Supplemental Figure S1. Single time point apixaban plasma concentrations in 358 patients with atrial fibrillation at 2.5 and 5 mg twice daily doses. a) Apixaban concentration over the time after dosing segregated by dose *(left panel*) and frequency distribution of patients according to apixaban concentration and dose (*right panel*). b) Log10-transformed apixaban concentration over the time after dosing segregated by dose *(left panel*) and frequency distribution of patients according to apixaban concentration segregated by dose (*right panel*).

Supplemental Table S1. Number of patients on apixaban with concomitant use of amiodarone and diltiazem by dose and variable values used for multivariate regression model (n= 358).

Concomitant CYP3A4/ ABCB1 inhibitor	Number of patients	Variable value for regression model
Amiodarone (n= 28)		
100 mg daily	3	1
200 mg daily	20	1
300 mg daily	1	1
400 mg daily	4	2
Diltiazem (n=53)		
120 mg daily	14	1
180 mg daily	12	1.5
240 mg daily	17	2
300 mg daily	2	2.5
360 mg daily	8	3