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# Clinical evaluation of laboratory methods to monitor apixaban treatment in patients with atrial fibrillation

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ABSTRACT

*Introduction:* The direct factor-Xa inhibitor apixaban is approved e.g. for the prevention of stroke in patients with atrial fibrillation (AF). Although routine monitoring of apixaban therapy is currently not recommended, selective monitoring could be useful to optimize efficacy and safety in certain clinical situations. We studied the exposure and effect of apixaban using different laboratory methods in a clinical setting with a well-defined cohort of AF patients.
*Material and methods:* Seventy AF patients (72 ± 7.4 years, 64 % men, mean $CHADS_2$ score 1.7) treated with apixaban 2.5 (n = 10) or 5 mg BID (n = 60). Trough plasma apixaban concentrations determined by liquid chromatography-tandem mass-spectrometry (LC-MS/MS) were compared to the coagulation assays Anti-factor Xa, PT-INR and aPTT.
*Results:* The apixaban plasma concentration determined by LC-MS/MS varied more than 10-fold overall. The range was between 15-83 and 29-186 ng/mL for the 2.5 mg BID and 5 mg BID respectively, with patients receiving 5 mg BID having significantly higher apixaban concentrations ($p < 0.001$). A strong correlation between LC-MS/MS and anti-FXa-assay was found ($p < 0.001$), while aPTT and PT-INR were not sensitive enough. There were no significant correlations between gender, creatinine clearance, body weight or age and apixaban exposure.
*Conclusions:* Anti-FXa-assay performed well upon apixaban concentrations in a normal exposure range. Still LC-MS/MS remains the "gold standard" method, covering also low concentrations. Compared to clinical trials, we observed relatively lower apixaban exposure and a more pronounced difference between high and low dose. Additional information regarding apixaban exposure and benefit-risk profile is needed in order to individualize treatment.



## Introduction

Atrial fibrillation (AF) is the most common arrhythmia and the prevalence increase with age. Patients with AF have a major risk of suffering thromboembolic events such as ischemic stroke. Treatment with anticoagulants reduces that risk but increase the risk for bleeding. Therefore only patients with a predominant risk for thromboembolism due to co-existing risk factors are recommended life-long treatment with anticoagulants. The $CHADS_2$ score, an acronym including congestive hear failure, hypertension, ages > 75 years or previous stroke (doubled), has often been used for this risk evaluation [1]. Today, warfarin is still the most commonly used anticoagulant in AF and reduces the risk for stroke by approximately 60 % [2]. This treatment requires frequent monitoring of the international normalized ratio of prothrombin time (PT-INR) with target values of 2.0-3.0 to yield the most optimal balance between efficacy and safety [3]. In ARISTOTLE, the pivotal study on the direct factor-Xa inhibitor apixaban in AF, patients were randomized to receive apixaban 5 mg BID or warfarin titrated to a target INR of 2.0-3.0 [4,5]. A lower dose of apixaban (2.5 mg BID) was administered to a subpopulation expected to be exposed to higher drug concentration in plasma, i.e. to elderly patients with low weight and/or reduced renal function, see below. Overall, the ARISTOTLE study showed that apixaban reduced the risk of stroke and caused less bleeding compared with warfarin [5] and the drug is approved for the prevention of stroke and systemic embolism in patients with non-valvular AF.

*Abbreviations:* (AF), Atrial fibrillation; (NOAC), New oral anticoagulants; (SPC), Summary of product characteristics; (LC-MS/MS), Liquid chromatography-tandem mass spectrometry; (CrCl), Creatinine clearance.

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Factor Xa has a central role in the clot formation and the direct factor-Xa inhibitor apixaban inhibits free and clot-bound factor Xa and prothrombinase activity, thus preventing thrombin generation and thrombus development. Apixaban has a mainly non-renal (75%) clearance and a half-life around 12 hours. Unlike warfarin, apixaban and other direct new oral anticoagulants (NOAC) are claimed not to require routine monitoring [6,7]. However, exposure to apixaban is subject to variability and is affected by e.g. renal function, weight, age and drug interactions. A dose reduction from 5 mg BID to 2.5 mg BID is currently recommended in patients with at least two of the following characteristics; age ≥ 80 years, body weight ≤ 60 kg or serum creatinine ≥ 133 μmol/l [8], but selective monitoring of apixaban therapy to optimize its efficacy and safety in certain groups of patients could eventually prove useful. Although the variability in concentration maybe less pronounced for apixaban than for dabigatran, it is reasonable to suppose that there is a relationship between apixaban exposure and efficacy/safety that is overall in line with what has been suggested and much debated for dabigatran [9,10].

Moreover, there are also several important clinical situations that may arise where monitoring would aid clinical decision making; i.e. preparation for elective and emergency surgery, when suspecting overdose or interaction with other drugs and when bleeding or thromboembolism occurs during treatment [11]. In all, the ability to monitor patient treatment would most probably be helpful in order to achieve the best health benefit in relation to risk profile, but little is known about the performance of different laboratory methods to monitor apixaban in real life AF patients. According to the apixaban summary of product characteristics (SPC), there is a strong correlation between apixaban plasma concentration and anti-factor Xa (anti-FXa) activity and weak correlation with PT INR and activated partial thromboplastin time (aPTT) [8]. The monitoring of apixaban anticoagulant activity has also been published and have in general shown to correlate well with apixaban plasma concentrations, however different anti-FXa assays show variable results [12 - 19]. Importantly, these studies were conducted in vitro, using spiked samples [16,17,19], in healthy volunteers [15,18] or in patients with acute coronary syndrome [13,14] or venous thromboembolism [12].

### *Aim*

The aim of this study was to investigate the typical exposure, i.e. plasma concentrations, of apixaban in relation to the clinical characteristics of AF patients treated with approved and recommended dosages. Furthermore, we compared the actual plasma concentrations with functional coagulation assays, which monitor the effect of the drug. This is the first clinical study to evaluate different laboratory methods to monitor apixaban in a well-defined real-life cohort of AF patients.

## Material and Methods

Seventy patients with AF treated with apixaban were recruited from the coagulation centre at Danderyd´s Hospital in the Stockholm County during the period 2013-10-01 and 2014-06-03. The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethical Review Board in Stockholm, Sweden (Dnr 2012/1232-31/4). Oral and written informed consent was obtained from each participant. The patients were treated with apixaban according to clinical routine care. Patients were excluded from the study if they were in treatment with any possibly interacting drug according to the SPC. All study patients reported full compliance during the last three days before trough plasma samples were collected in median 12.3 hours (9.8 – 18.8; min-max) after last intake of the drug. Samples were analyzed using LC-MS/MS methodology and different coagulation assays; anti-FXa assay, aPTT and PT-INR. The apixaban concentrations were also evaluated in relation to the clinical characteristics (renal function, body weight, sex, age) of the treated patients and the prescribed apixaban dose. Moreover, the thromboembolic risk for each patient was calculated using $CHADS_2$ score [1] and renal function by the Cockcroft-Gault formula.

Blood samples were drawn by direct venipuncture from an antecubital vein, collected into standard vacuum tubes containing a 1/10th volume of trisodium citrate (Becton Dickenson USA, 0,109 mol/L in 4,5 mL blood, (3,2%)) and immediately centrifuged at 2000 ×*g* for 20 minutes at room temperature. Plasma was then frozen at –70 °C in aliquots of 0.5 mL.

### *Direct Measurement of Apixaban in Plasma by LC-MS/MS*

Plasma concentrations of apixaban were determined using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method using apixaban-$^{13}$C-d7 as internal standard. Apixaban was purchased from Selleckchem, Houston, TX. and internal standard was purchased from Alsachim, Strasbourg, France. Patient samples, 50 μL aliquots, were prepared by protein precipitation with methanol containing internal standard. After centrifugation, the supernatant was transferred and diluted with mobile phase A (0.1% formic acid) and then 7 μL of the final extract was injected on a LC-MS/MS. Separation of the analyte was achieved on an AcquityBEH column (C18, 1.7 μm, 2.1 × 50 mm) using a gradient run with mobile phase A and acetonitrile as mobile phase B. The analytes were detected by operating in positive electrospray ionization (ESI) mode utilizing selected reaction monitoring (SRM), with transitions 461 → 443 m/z for apixaban, 469 → 451 m/z for internal standard. Injection to injection time was 3.5 min. The calibration curve in plasma was linear over the range 2.0 – 500 ng/mL and between-run precision of low-, medium- and high quality control samples were below 10.3% (CV%) and between-run accuracy -9.05% to 1.48%. No analytical interference was observed.

### *Indirect Measurements of Apixaban in Plasma by Coagulation Assays*

STA® Liquid Anti-FXa (Diagnostica Stago, Asnieres, France) is a quantitative chromogenic assay for determination of apixaban anti-FXa activity. Citrated plasma is mixed with a substrate for factor Xa and incubated at 37 °C for 4 min. Upon addition of factor Xa, a competitive reaction is started in which factor Xa inhibition by apixaban occurs simultaneously as the hydrolysis of factor Xa substrate. The quantity of released paranitroaniline measured at 405 nm, is inversely proportional to the concentration of apixaban. The assay was performed according to the manufacturer's instructions and adopted for Sysmex® CS-2100 (Sysmex, Kobe, Japan). Calibration was performed with apixaban plasma calibrators from the manufacturer with the concentration range 0-475 ng/mL. Control samples (72 and 275 ng/mL) from the manufacturer measured on seven different occasions using 14 determinations per concentrations, showed precisions (CV%) of 5,6 % and 2,5 %, respectively.

aPTT using the Automate® reagent (Diagnostica Stago, Asnieres, France) was performed on a Sysmex® CS2100i (Sysmex, Kobe, Japan). Results are given in seconds with normal range ≤ 40 seconds.

PT-INR using the Owren reagent SPA + ® (Diagnostica Stago, Asnieres, France) was performed on a Sysmex® CS2100i (Sysmex, Kobe, Japan). Results are presented as INR with normal range < 1.2.

### *Statistics*

Pearson's correlation coefficients were used to estimate simple correlations between variables. Differences between groups of patients were evaluated by Wilcoxon´s Rank Sum test. The JMP package (version 10.0; SAS Institute, Cary, C, USA) was used for statistical calculations. A $p$-value < 0.05 was considered statistically significant.

## Results

Patient characteristics are given in Table 1. Patients on the 2.5 mg BID dose (n = 10) had a higher mean age, higher $CHADS_2$ scores,