Received: 25 June 2023 | Revised: 8 October 2023 | Accepted: 11 October 2023

DOI: 10.1111/bcpt.13953

ORIGINAL ARTICLE

BCPT Basic & Clinical Pharmacology & Toxicology

Direct oral anticoagulant concentrations and adherence in stroke patients

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Abstract

No therapeutic ranges linking drug concentrations of apixaban and rivaroxaban to clinical outcomes have been defined. We investigated whether direct oral anticoagulant (DOAC) concentrations among patients admitted to hospital with symptoms of stroke differed between those later verified to suffer an ischaemic cerebrovascular event (stroke or transient ischaemic attack) and those having other diagnoses (control group).

Serum concentrations in 102 patients on DOAC for atrial fibrillation (84%) and thromboembolic disease (16%) were measured within 24 h of the acute event, employing ultra-high performance liquid chromatography with tandem mass spectrometry. We converted all concentrations to standardized trough levels.

DOAC concentrations were lower in the 64 patients with verified ischaemic cerebrovascular event than in the 30 controls, 255 ± 155 *versus* 329 ± 144 nmol/L ($p = 0.029$), despite no statistically significant difference in self-reported adherence and daily dosages. Calculated concentrations were 5.4–596 nmol/L (median = 229 nmol/L) in the ischaemic stroke group and 41–602 nmol/L (median = 316 nmol/L) in controls. CHA_2DS_2-VASc score was significantly higher in the ischaemic stroke group than in controls (4.9 ± 1.6 *versus* 4.1 ± 1.7; $p = 0.007$). These results may suggest that patients with high cerebrovascular risk might benefit from higher DOAC levels than those with a lower risk.

KEYWORDS
apixaban, atrial fibrillation, ischaemic stroke, patient adherence, rivaroxaban

Funding information
The Department of Laboratory Medicine, St Olav University Hospital, has supported Rachel Aakerøy with PhD funding. The authors did not receive support from any organization for the submitted work.

1 | INTRODUCTION

Phase III clinical trials have shown that the direct oral anticoagulants (DOACs), including apixaban and rivaroxaban, are at least as safe and effective as vitamin K antagonists in preventing ischaemic stroke in patients with non-valvular atrial fibrillation (AF).[1,2] For vitamin K antagonists such as warfarin, quality and safety of therapy, including the assessment of adherence, can be monitored by means of the international normalized ratio (INR). Overall, the time in the therapeutic INR range for warfarin varies, but it is about 70% in Norway.[3]

In contrast, DOACs were marketed without the intention of routine monitoring.[4] Consequently, therapeutic ranges linking drug concentrations of apixaban and rivaroxaban to clinical outcomes have not been established. Nevertheless, it has been shown that intake of standard doses of DOACs results in a wide range of plasma concentrations of the drugs.[5–7] For dabigatran and edoxaban, it has been established that too high plasma concentrations result in increased bleeding risk while too low plasma concentrations result in an increased risk of thromboembolism.[8,9] More recently, a similar trend for apixaban and rivaroxaban has been shown.[10,11]

One study observed that patients with very low DOAC plasma concentrations were more likely to suffer a thromboembolic event[10] when concentrations were measured 6–12 months before the event. More recently, another study involving 45 patients reported lower DOAC concentrations at the time of stroke than in controls.[12] Finally, low DOAC concentrations have been shown to be associated with increased stroke severity.[13] In contrast to the studies mentioned above,[10–13] we employed ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHLPC–MS–MS) for the direct measurement of apixaban or rivaroxaban concentrations, which is considered the gold standard for drug analysis.[4]

The aim of the present study was to investigate whether DOAC concentrations in patients suffering from ischaemic stroke or transient ischaemic attack (IS/TIA) differed from the concentrations in those admitted to hospital with symptoms of stroke but without having IS/TIA. In addition, we aimed to study factors that might explain lower DOAC levels, including self-reported patient adherence at the time of admission and 3 months later. Finally, the correlation between DOAC serum concentrations and anti-factor Xa activity was studied.

2 | MATERIALS AND METHOD

2.1 | Study population

Patients admitted to the Stroke Department at St. Olav University Hospital, Trondheim, Norway, with symptoms of stroke while on apixaban or rivaroxaban were eligible for inclusion. The patients were excluded if a subarachnoid haemorrhage was present, or the time interval between initial symptom presentation and hospitalization was more than 24 h. The patients were included consecutively from January 2019 to September 2021. Informed consent was obtained from each patient. The study was approved by the Regional Committee for Medical Research Ethics in Mid Norway (registration number 2018/297/REK Midt) and by the Norwegian Medicines Agency (registration number 18/15867). The study is registered in the European Union Drug Regulating Authorities Clinical Trials *(EudraCT)* database (ref. number 2018-003667-63). The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology 2023 policy for experimental and clinical studies,[14] and the article is written according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

In total, 152 patients were asked to participate, of whom 102 were included. Main reasons for exclusion were situations where informed consent was not possible to obtain and patients were reluctant to participate in the study.

2.2 | Clinical evaluation and variables

The IS or TIA diagnosis was assessed by experienced stroke physicians based on clinical signs and symptoms as well as information from MRI and CT scans, previous medical history and laboratory tests. Continuous electrocardiographic monitoring, carotid ultrasound and echocardiography investigations were also performed where

relevant to determine the underlying aetiology of the ischaemic event.[15,16] Stroke was defined as rapidly developing clinical symptoms and/or signs of focal loss of brain function, with symptoms lasting more than 24 h, with no apparent cause other than that of vascular origin.[17] TIA was distinguished from stroke on the basis of conventional 24-h cut-off for resolution of symptoms. The most common diagnoses in the control group ($n = 30$) were non-specific dysphasia/aphasia ($n = 4$), orthostatic hypotension ($n = 3$), dizziness ($n = 3$), migraine with aura ($n = 2$) and unspecified epilepsy ($n = 2$). For a complete list of diagnoses, see supporting information Table S1.

Clinical variables were retrieved from medical records. Variables included those needed to calculate the CHA_2DS_2-VASc score, which is developed for risk prediction of stroke in patients with AF but was applied irrespective of the indication for DOAC.[18] Relative estimated glomerular filtration rate (eGFR; mL/min per 1.73 m^2) was obtained automatically from the laboratory, based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[19] Absolute GFR (mL/min) was calculated from the values above, taking into consideration the patients' body weight and height, and is the GFR variable used in this article. Potentially interacting concomitant drugs were classified as CYP3A4/P-glycoprotein (P-gp) inducers or inhibitors, or platelet inhibitors, based on the overview of such drugs listed in a comprehensive guideline.[4]

2.3 | Blood sampling and analysis of DOAC concentrations

Serum or plasma samples for the analysis of rivaroxaban or apixaban were drawn at the Emergency Department and/or at the Department of Stroke. Samples were collected either in 3.5 mL Greiner Vacuette® citrate tubes with 3.8% sodium citrate, 6 mL Greiner Vacuette® tubes with CAT serum clot activator or 4 mL Greiner Vacuette® tubes with ethylenediaminetetraacetic acid (EDTA). All tubes were centrifuged within 2 h at 2200*g* for 10 min.

All samples were analysed with an UHLPC–MS–MS method with a standard range of 5–800 nmol/L, which has been described in detail previously.[20] If no serum sample was available but we had access to a citrate or EDTA plasma sample, the plasma sample was used to analyse the DOAC concentration. In a previous study from our group, the mean relative concentration difference in serum *versus* citrate plasma and serum *versus* EDTA-plasma was +17% and +4.5% for apixaban and +37% and +13% for rivaroxaban, respectively.[21] These numbers are in line with those shown in other studies.[22,23] Since the majority of retrieved samples were serum samples, we chose serum as the reference matrix. All plasma concentrations were therefore converted to serum concentrations using the percentages above.

Samples obtained less than 4 h after last dose, that is, close to the assumed concentration maximum,[5,24] were excluded. Time of sampling after last intake of medication varied from 5 to 24 h for both apixaban and rivaroxaban. The observed DOAC concentration at t hours after intake was converted to a standardized trough level concentration: 12-h concentration for apixaban and 24-h concentration for rivaroxaban, according to the following equation:

$$Ct = C \times e^{-\frac{\ln 2 \times \Delta t}{12}}$$

where Ct is the calculated trough concentration level, C is the actual measured concentration, and Δt is the time interval between the sampling time and the time of expected trough concentration (12 and 24 h for apixaban and rivaroxaban, respectively). For both apixaban and rivaroxaban, an elimination half-life of 12 h was used, as indicated in the denominator of the exponent.[5,24]

Rivaroxaban daily dosages were converted to the apixaban-equivalent daily dosages in order to merge dose information from patients using the different anticoagulants into one group. As the defined daily doses (DDDs) for apixaban and rivaroxaban when used for treatment of patients with non-valvular AF are 10 mg and 20 mg, respectively,[25] rivaroxaban doses were divided by two to achieve apixaban-equivalent doses. As published data indicate that a give concentration of apixaban and a given concentration of rivaroxaban result in the same anticoagulant effect, the two groups were considered as one when evaluating serum concentrations.[26] The concentration/dose ratio (CDR) for both drugs was subsequently calculated by dividing the measured serum concentrations by the apixaban-equivalent daily doses in milligrams.

Three months after admission, an invitation to answer a questionnaire on drug adherence and provide a new blood sample was sent per mail to the 102 participants. We were able to collect serum or plasma samples from 45 of these. Plasma concentrations were once again converted to equivalent serum concentrations, and trough concentrations, doses and CDRs were calculated as described above.

2.4 | Analysis of anti-factor Xa activity

At the Stroke Department, a citrate plasma sample was drawn to be analysed for anti-factor Xa activity. Plasma

samples were frozen until analysis. Anti-factor Xa activity was determined using the HemosIL Liquid Anti-Xa chromogenic assay (Instrumentation Laboratory, Bedford, MA, USA). The citrated plasma samples were analysed on the ACL Top 750 Laboratory Automation System (Instrumentation Laboratory) device, and the results were reported according to the standard HemosIL Heparin Calibrators that are used for heparin and low-molecular-weight heparins. The reportable range was 0.10 to 2.00 IU/mL, and values below and above that range were reported as <0.10 IU/mL and >2.00 IU/mL, respectively.

2.5 | Medication adherence

We assessed medication adherence to DOACs during admission by the validated 8-item Morisky Medication Adherence Scale (MMAS-8).[27,28] The MMAS-8 Scale, content, name, and trademarks are protected by the U.S. copyright and trademark laws. Permission for use of the scale and its coding is required. A licence agreement is available from MMAR, LLC., www.moriskyscale.com and was made between St. Olav University Hospital and MMAR, LLC.

Seven out of eight items in the MMAS-8 have a dichotomous response option while the last item has a 5-point Likert response. A score of 8 points is defined as high adherence, 6 to <8 points as medium adherence and <6 as low adherence. Since most of our patients scored 8, we categorized patients into two groups, high or medium/low adherence. At follow-up 3 months after admission, MMAS-8 was assessed again.

2.6 | Statistical analyses

As this is an observational study, no formal power analysis was performed. Comparisons were undertaken between the IS/TIA group and the control group. As the patients with haemorrhagic stroke were only eight in total, we excluded this group from the comparisons. We have, however, included the patients with haemorrhagic stroke for descriptive and imputation purposes as well as for assessing the correlation between serum DOAC concentration and anti-factor Xa activity.

Missing serum concentrations, ($n = 17$), MMAS-8 scores ($n = 15$) and anti-factor Xa activity levels ($n = 5$) were imputed by means of single imputation, using predictive mean matching, including all variables used in the analyses. Twelve, four and one serum concentrations were missing in the IS/TIA, control and haemorrhagic stroke groups, respectively. Variables analysed as predictors were diagnosis, serum concentration of the drug, MMAS-8 score, sex, type of drug, dose, concomitant use of pharmacokinetic inhibitors or inducers, use of drugs causing pharmacodynamic interactions, patient age, serum creatinine concentrations and anti-factor Xa activity.

Continuous variables are presented as means with standard deviations and categorical variables as frequencies and percentages. Comparisons between the IS/TIA group and the control group were carried out using the independent *t*-test for continuous variables, linear-by-linear association for ordinal variables, and as recommended by Lydersen et al.,[29] the exact unconditional Pearson chi squared test for dichotomous variables. The Pearson correlation was used to assess the association between DOAC concentration and anti-factor Xa activity. Serum drug concentrations and CDRs 3 months after admission were compared to the corresponding values at inclusion by means of paired *t*-tests. McNemar's test was used to compare dichotomized MMAS-8 scores at inclusion and 3 months later.

We used linear regression analyses to explore factors potentially influencing CDR, with CDR as dependent variable and potential explanatory factors, one at a time, unadjusted and adjusted for age and sex.

For all analyses, two-sided *p*-values <0.05 were considered to represent statistical significance. The exact unconditional test was computed using the R package "contingencytables", linear regression was performed with Stata Version 17 (Stata Statistical Software: Stata Corp LP, College Station, TX, USA), and the other statistical analyses were performed with SPSS version 27 (IBM, Armonk, NY, USA).

3 | RESULTS

The clinical and demographic characteristics of the study population at inclusion are shown in Table 1. From a total of 102 patients, 64 had a confirmed IS/TIA diagnosis, 8 had suffered a haemorrhagic stroke, while 30 were diagnosed with other conditions unrelated to cerebrovascular disease and served as a control group. Out of the 64 patients in the IS/TIA group, 14 had suffered a TIA.

There were no statistically significant differences between the IS/TIA group and the control group for age, sex, body mass index (BMI), GFR or type of DOAC used. The CHA_2DS_2-VASc score was, however, significantly higher in the IS/TIA group than in the control group (4.9 ± 1.6 *versus* 4.1 ± 1.7; $p = 0.007$). The CHA_2DS_2-VASc score remained significantly higher also when comparing the subgroup of patients prescribed DOAC for AF in the IS/TIA group and the control group ($p = 0.044$).

TABLE 1 Demographic and clinical characteristics at index hospitalization.

	Ischaemic stroke/TIA $N = 64$	Haemorrhagic stroke $N = 8$	Other diagnoses $N = 30$	Total $N = 102$
Female	28 (44%)	4 (50%)	13 (43%)	45 (44%)
Age (years)	79.4 ± 8.6	84.2 ± 5.3	78.7 ± 7.5	79.6 ± 8.1
BMI (kg/m^2)	26.8 ± 5.9	25.2 ± 3.7	26.8 ± 5.0	26.6 ± 5.5
HbA1c (mmol/mol)	44.4 ± 10.9	40.5 ± 4.7	43.2 ± 9.6	43.8 ± 10.2
LDL cholesterol concentration (mmol/L)	2.7 ± 1.0	2.8 ± 0.8	2.5 ± 0.9	2.7 ± 1.0
Total cholesterol concentration (mmol/L)	4.1 ± 1.0	4.2 ± 1.1	3.9 ± 0.9	4.1 ± 1.0
Serum creatinine concentration (μmol/L)	82.1 ± 35.9	80.9 ± 22.3	87.1 ± 29.2	83.5 ± 33.1
Glomerular filtration rate (mL/min)	81.6 ± 25.0	73.4 ± 21.9	77.5 ± 25.3	79.7 ± 24.8
Diabetes mellitus	17 (27%)	None	5 (17%)	22 (22%)
Previous TIA	14 (22%)	1 (13%)	4 (13%)	19 (19%)
Previous ischaemic stroke	25 (40%)	2 (25%)	7 (23%)	34 (34%)
Previous intracranial bleeding	None	None	None	None
Current smoker	5 (8%)	None	4 (13%)	9 (9%)
Previous smoker	29 (46%)	3 (43%)	10 (33%)	42 (42%)
Hypertension	46 (73%)	4 (50%)	22 (73%)	72 (71%)
Liver disease[a]	2 (3%)	None	2 (7%)	4 (4%)
Previous bleeding[b]	None	1 (13%)	3 (10%)	4 (4%)
High alcohol consumption[c]	3 (5%)	None	2 (7%)	5 (5%)
Coronary artery disease	19 (30%)	2 (25%)	11 (37%)	32 (31%)
Peripheral artery disease	10 (16%)	2 (25%)	4 (13%)	16 (16%)
Heart failure	20 (32%)	None	4 (13%)	24 (24%)
CHA_2DS_2-VASc score	4.9 ± 1.6	4.0 ± 0.7	4.1 ± 1.7	4.6 ± 1.6

Note: Numbers are *n* (%) or mean ± standard deviation (SD) unless otherwise specified.

Abbreviations: BMI = body mass index; LDL = low density lipoprotein; HbA1c = glycated haemoglobin; TIA = transient ischaemic attack.

[a]Cirrhosis or steatosis.

[b]Bleeding peptic ulcer or other significant bleeding causing anaemia.

[c]High alcohol consumption noted in hospital medical records.

Variables related to the treatment with DOACs, including its indication and other drugs at the index hospitalization according to diagnosis, are presented in Table 2. There were no differences in the DOAC dose between groups, but the IS/TIA group had significantly lower DOAC concentrations, CDRs and anti-factor Xa activities than the control group (Table 2 and Figure S1). DOAC serum concentrations ranged from 5.4 to 596 nmol/L (median = 229 nmol/L) in the IS/TIA group and from 41 to 602 nmol/L (median = 316 nmol/L) in the control group. Four patients, all in the IS/TIA group, were outliers with very low DOAC concentrations, ranging from 5.4 to 12 nmol/L. These four all had concentrations below 10% of the overall mean. They also had CDRs below 10% of the overall mean, ranging from 0.5 to 2.4 (nmol/L)/(mg/d). The lowest concentration in the control group was 41 nmol/L.

In the linear regression analysis exploring variables potentially influencing CDR, we found statistically significant, positive coefficients for increased age, female sex, lower GFR and lower BMI (Table 3). In contrast, concomitant use of enzyme/P-gp inhibitors or inducers or the MMAS-8 score had no statistically significant influence on CDR (Table 3).

Correlations between anti-factor Xa activity and DOAC serum concentrations were generally high (Figure 1), varying from $r = 0.85$ ($p < 0.001$) in the control group to $r = 0.94$ ($p = 0.002$) in the haemorrhagic stroke group and $r = 0.96$ ($p < 0.001$) in the IS/TIA group (Figure 1).

Variables related to DOAC treatment at inclusion and 3 months after admission for the 45 patients from whom information was available at both time points are presented in Table 4. Notably, 3 months after admission, the

TABLE 2 Variables related to treatment with direct oral anticoagulants (DOACs) and other drugs at the index hospitalization, according to diagnosis ($N = 102$).

	Ischaemic stroke/TIA $N = 64$	Other diagnoses $N = 30$	P value[a]	Haemorrhagic stroke $N = 8$
Type of DOAC				
Apixaban	53 (83%)	26 (87%)	0.67	7 (88%)
Rivaroxaban	11 (17%)	4 (13%)		1 (12%)
Indication for DOAC				
Atrial fibrillation	51 (80%)	26 (87%)	0.62	7 (88%)
Venous thromboembolism[b]	11 (17%)	4 (13%)		1 (12%)
Unknown	2 (3%)	None		None
Apixaban-equivalent dose[c] (mg/d)				
5 mg	23 (36%)	9 (30%)	0.39	2 (25%)
7.5 mg	4 (6%)	0 (0%)		0 (0%)
10 mg	37 (58%)	21 (70%)		6 (75%)
Serum DOAC concentration (nmol/L)	255 ± 155	329 ± 144	**0.029**	241 ± 136
Concentration/dose ratio (CDR) [(nmol/L)/(mg/d)]	32.4 ± 17.9	42.8 ± 26.8	**0.028**	26.9 ± 11.7
Anti-factor Xa activity (IU/mL)[d]	0.57 ± 0.53	0.85 ± 0.50	**0.017**	0.43 ± 0.27
MMAS-8 score[e]				
High	54 (84%)	22 (73%)	0.22	8 (100%)
Medium or low	10 (16%)	8 (27%)		None
Concomitant use of enzyme/P-gp inducer[f]	5 (8%)	1 (3%)	0.45	None
Concomitant use of enzyme/P-gp inhibitor[g]	1 (2%)	3 (10%)	0.085	None
Concomitant use of platelet inhibitor[h]	15 (36%)	8 (27%)	0.77	None

Note: Numbers are *n* (%) or mean ± standard deviation (SD) unless otherwise specified. Bold values represent statistically significant *p*-values.
Abbreviations: DOAC = direct oral anticoagulant; MMAS-8 = 8-item Morisky Medication Adherence Scale; P-gp = P-glycoprotein; SD = standard deviation; TIA = transient ischaemic attack.
[a]*P* values for comparisons between the groups ischaemic stroke/TIA and the control group.
[b]Deep venous thrombosis and/or pulmonary embolism.
[c]Rivaroxaban doses were divided by 2 according to the differences in potency between the two drugs.
[d]In the calculations of means and SDs, concentrations below the lower limit of quantification of the analytical method (<0.1 IU/mL) were set to 0.05 IU/mL, whereas concentrations above the upper limit of quantification (>2.0 IU/mL) were set to 2.1 IU/mL.
[e]Morisky Medication Adherence Scale is a validated scale of self-reported medication adherence, range from 0 to 8, with 8 representing high adherence, 6 to <8 medium adherence and <6 low adherence. The MMAS-8 scale, content, name and trademarks are protected by the U.S. copyright and trademark laws. Permission for use of the scale and its coding is required. A licence agreement is available from MMAR, LLC., www.moriskyscale.com.
[f]Enzyme/P-gp inducer in all cases was prednisolone.
[g]Enzyme/P-gp inhibitors were verapamil and amiodarone.
[h]Platelet inhibitors were acetylsalicylic acid, clopidogrel, escitalopram, citalopram, sertraline and venlafaxine.

MMAS-8 scores were significantly lower than at inclusion ($p = 0.039$). There were otherwise no statistically significant differences. There was no substantial change in concomitant use of enzyme/P-gp inducers and inhibitors during the 3-month period.

4 | DISCUSSION

Our main finding is that among patients using DOACs admitted to hospital with symptoms of stroke, the calculated DOAC concentrations were significantly lower in those with a verified IS/TIA compared to those who turned out to have other diagnoses. At the same time, the IS/TIA group had a more unfavourable cerebrovascular risk profile, as measured by the CHA_2DS_2-VASc score. Although CHA_2DS_2-VASc score has conventionally been used to assess stroke risk in patients with AF, recent studies have shown that the score may be used to assess the risk of stroke also in patients without AF.[30] We found no clear-cut evidence for generally reduced self-reported adherence in the IS/TIA group compared to the control

TABLE 3 Linear regression with dose-adjusted serum concentration (concentration/dose ratio) of direct oral anticoagulants as dependent variable ($N = 102$).

	Unadjusted analysis			Age- and sex-adjusted analysis		
	Coefficient	**95% CI**	***P* value**	**Coefficient**	**95% CI**	***P* value**
Age, years	0.625	0.127 to 1.123	**0.015**	-	-	-
Female sex	10.5	2.37 to 18.6	**0.012**	-	-	-
GFR (mL/min)	−0.36	−0.52 to −0.22	**<0.001**	−0.36	−0.55 to −0.17	**<0.001**
Self-reported medication adherence[a]	−0.80	−5.44 to 3.84	0.73	−3.10	−7.78 to 1.64	0.20
Body mass index (kg/m^2)	−1.09	−1.85 to −0.33	**0.005**	−1.04	−1.85 to −0.24	**0.012**
Use of enzyme/P-gp inducer[b]	9.21	−8.36 to 26.8	0.30	7.08	−9.93 to 24.1	0.41
Use of enzyme/P-gp inhibitor[c]	0.92	−20.5 to 22.3	0.93	5.57	−15.2 to 26.3	0.60
Type of DOAC, rivaroxaban[d]	−17.1	−28.0 to −6.13	**0.003**	−17.2	−27.7 to −6.73	**0.002**

Abbreviations: CI = confidence interval; DOAC = direct oral anticoagulant; GFR = glomerular filtration rate; P-gp = P-glycoprotein. Bold values represent statistically significant *p*-values.

[a]Measured by Morisky Medication Adherence Scale-8, with range 0–8, and 8 as reference corresponding to high adherence, 0 corresponding to low adherence.

[b]Enzyme/P-gp inducer in all cases was prednisolone.

[c]Enzyme/P-gp inhibitors were verapamil and amiodarone.

[d]Apixaban as reference.

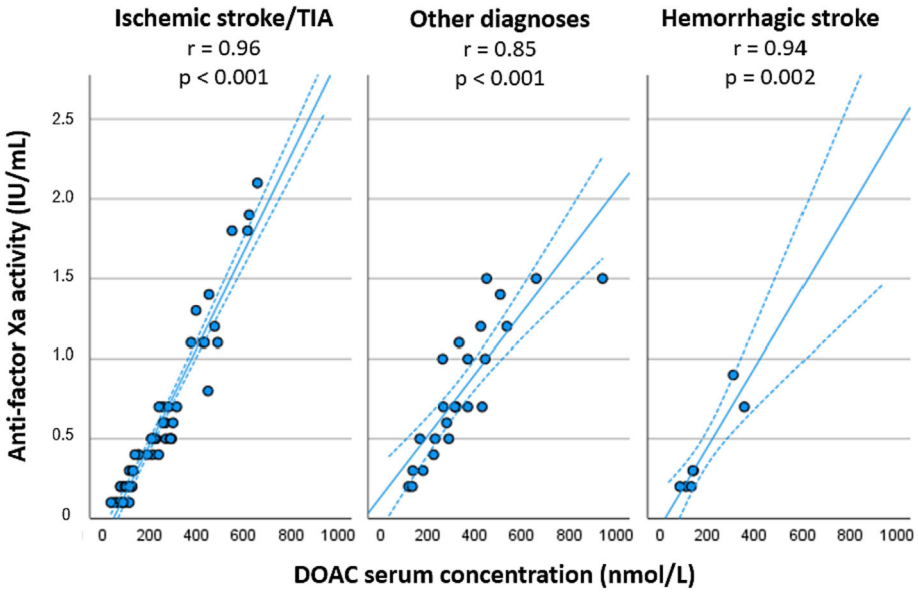

FIGURE 1 DOAC concentration *versus* anti-factor Xa activity in the 86 patients from whom the samples for analysis of the DOAC concentrations and the anti-factor Xa activity were drawn at the same time ($n = 54$ in the ischaemic stroke/TIA group, $n = 25$ in the other diagnoses group, $n = 7$ in the haemorrhagic stroke group). DOAC, direct oral anticoagulant.

group. These findings largely concur with a previous study in that most ischaemic strokes in patients using DOACs occur despite patients having measurable antithrombotic activity on admission.[13] Our study also corroborates other studies in finding that DOAC concentrations are lower in stroke patients.[10,12,13] Thus, using UHLPC–MS–MS technology and calculations to normalize the concentrations, our data support results from previous studies which have used indirect measurements for quantifying DOAC concentrations.

Self-reported adherence at admission was high for most patients in our study, irrespective of the diagnosis. This finding differs from earlier studies, where only about 30 to 60% of patients in primary care and outpatient clinics were classified as being highly adherent to DOACs.[31,32] In the first of these,[31] MMAS-8 was used to evaluate adherence with a similar cut-off for high adherence as we used, whereas high adherence was defined based on pill counts with a cut-off of 80% in the latter.[32] We found no significant differences in self-reported adherence between the IS/TIA group and the control group. Self-reported adherence alone does therefore not seem to explain the differences in DOAC serum concentrations and CDRs between these two groups, even though four patients in the IS/TIA group had so low CDRs that it might be less likely that it could be caused solely by pharmacokinetic factors. Surprisingly, self-reported adherence had decreased 3 months after

TABLE 4 Variables related to direct oral anticoagulant (DOAC) treatment at inclusion and 3 months after admission in the 45 patients from whom information was available at both time points ($n = 28$ in the ischaemic stroke/TIA group, $n = 17$ in the other diagnoses group).

	At inclusion	After 3 months	*P* value
Serum DOAC concentration (nmol/L)			
Total group	266 ± 150	299 ± 195	0.17
Ischaemic stroke/TIA	226 ± 153	268 ± 207	0.23
Other diagnoses	332 ± 123	351 ± 167	0.50
Concentration/dose ratio [(nmol/L)/(mg/d)]			
Total group	32.2 ± 21.4	35.8 ± 26.9	0.26
Ischaemic stroke/TIA	26.7 ± 17.6	32.1 ± 29.9	0.25
Other diagnoses	41.3 ± 24.5	41.9 ± 20.7	0.86
MMAS-8 score			
High	38 (84%)	31 (69%)	
Medium or low	7 (16%)	14 (31%)	**0.039**
Apixaban-equivalent dose (mg/d)			
5 mg	11 (24%)	9 (20%)	
7.5 mg	1 (2%)	1 (2%)	N/A
10 mg	33 (74%)	35 (78%)	
Type of DOAC[a]			
Apixaban	37 (82%)	39 (87%)	
Rivaroxaban	8 (18%)	6 (13%)	N/A

Note: Numbers are *n* (%) or mean ± standard deviation (SD) unless otherwise specified. Bold values represent statistically significant *p*-values.
Abbreviations: DOAC = direct oral anticoagulant; MMAS-8 = 8-item Morisky Medication Adherence Scale; N/A = not applicable; TIA = transient ischaemic attack.
[a]Two of the patients who were on rivaroxaban at inclusion were later switched to apixaban. All the patients treated with apixaban on admission were on the same treatment 3 months later.

admission. In other settings, secondary prevention has been associated with improved adherence when compared to primary prevention.[33] One would therefore expect self-reported adherence to improve 3 months after the acute event. One explanation for this incongruity could be that self-reported adherence was overestimated at hospital admission when the patients were interviewed and that it would be easier to answer truthfully in a questionnaire sent by mail after 3 months. Similar conclusions have been drawn previously where a higher level of adherence in patients with AF has been reported in studies involving direct patient contact than in database analyses.[32] Future studies could benefit from combining self-reported assessments with serial plasma concentration measurements of both parent drug and metabolites, since the metabolic ratio might give an even more objective assessment of adherence than measuring the parent drug only.[34]

Since differences in self-reported adherence seemingly cannot explain the significantly lower DOAC concentrations and CDRs in the IS/TIA group than in the control group, we also examined the influence of pharmacokinetic factors. In general, there is a large intraindividual variability in serum concentrations achieved after intake of standard doses of DOAC.[7] We checked for differences between the two groups in medications that might have inhibited or induced metabolism or transport of apixaban and rivaroxaban via CYP3A4 and P-gp, respectively. We found no significant effects of such inducers and inhibitors on CDR, and no differences in the use of such drugs between groups. However, it should be noted that prednisolone was the only CYP3A4/P-gp inducer present that was listed in the source we used for categorizing interacting drugs.[4] Prednisolone has only a weak enzyme inducing effect in vivo,[35] which may explain the lack of any influencing effect. Some patients used strong inhibitors, but these might have been too few to reveal any statistically significant effects.

Age, female sex, lower BMI and lower GFR all increased serum concentrations of DOAC and therefore CDRs, similar to the findings in earlier studies.[36,37] However, there were no statistically significant differences between the IS/TIA group and the control group for any of these variables.

In our material, there were more patients on doses less than 10 mg in the IS/TIA group than in the control group. However, this difference was not statistically significant, which could perhaps be explained by too small a sample size. However, considering the statistically significant lower CDR, where the concentration is corrected for dose, in the IS/TIA group, it follows that the higher proportion of lower doses in the IS/TIA group cannot explain the difference in serum DOAC concentrations.

We found a high correlation between serum DOAC concentration and anti-factor Xa activity, which is in agreement with previous studies.[26,38] The anti-factor Xa activity in the IS/TIA group was significantly lower compared to the control group, which is in line with the lower serum DOAC concentrations. Nosal et al.[12] also assessed DOAC concentrations and anti-factor Xa activity at the time of acute embolic stroke but without taking into consideration the accurate time interval since the last dose. They found lower anti-factor Xa activity in stroke patients when compared to trough inpatient control samples. As opposed to that study,[12] where the control group was from an in-hospital setting where adherence is expected to be optimal, our control group reflected real-life adherence. We also standardized our DOAC concentrations by calculating trough levels in both groups.

Testa et al.[10] observed a relationship between low trough DOAC concentrations and the occurrence of thrombotic events in patients with non-valvular AF. In that study, DOAC concentrations were measured by indirect methods and within 15–25 days of initiation of treatment rather than at the time of the ischaemic event using UHLPC–MS–MS. Our study showed higher CHA_2DS_2-VASc scores in the IS/TIA group than in the control group (4.9 *versus* 4.1). This is in line with Testa et al.,[10] who found mean scores of 5.3 in patients with thrombotic events *versus* 3.0 in the control group. Taken together, this indicates that the combination of high cerebrovascular risk with low anticoagulant levels may indeed expose patients to a greater risk of thrombotic complications. Although speculative, one could hypothesize that patients with a high cerebrovascular risk might benefit from higher DOAC levels than those with a lower risk.

4.1 | Limitations of the study

Our observational study cannot prove a causal relationship between low serum DOAC concentrations and thromboembolic risk. The most severely ill stroke patients may not have been included in the study due to the nature of their condition and urgency of the situation. This may have introduced a selection bias, which could have led to an underestimation of the difference between the IS/TIA group and the control group. We also did not gather data on severity or subtype of stroke.

Ideally, all blood samples should have been collected in tubes with the same type of additive to avoid having to calculate the equivalent value in serum from plasma concentrations. However, we have used conversion factors based on results from the three previous studies having investigated this topic thoroughly, and these studies were consistent regarding the size of the differences in measured concentrations between plasma and serum.[21–23] We therefore consider that the impact of this weakness is limited at the group level, particularly as the proportion of patients with concentrations measured in plasma was the same in the IS/TIA group and in the control group.

Trough levels were calculated from samples obtained from 5 to 24 h after the last dose, using a fixed elimination half-life of 12 h for all subjects. This procedure has obviously introduced a certain degree of imprecision as compared to having obtained the samples exactly at trough.

We have merged patients using apixaban and rivaroxaban into one group. We would have preferred to analyse rivaroxaban and apixaban groups separately, but the sample sizes were too small to draw robust conclusions for each of these subgroups. As can be seen from Table 3, the use of rivaroxaban had a significant impact on CDR. However, as the proportion using rivaroxaban was small and also similar in the IS/TIA group and the control group (17% *versus* 13%), the effect on the endpoints studied will be limited.

Our study enrolled a relatively small group of stroke patients, although we included more stroke patients than earlier key studies.[10,12] Our study would clearly have benefited from a larger control group, and it can also be debated whether the current control group is ideal or whether use of different groups could have been more informative. The relatively low number of subjects in total also introduced a power issue when evaluating both the impact of the degree of adherence and of pharmacokinetic drug interactions, as there were surprisingly few individuals that reported low adherence and also very few that used interacting drugs. Those data must be interpreted with this limitation in mind.

Pharmacogenetic variants of the *ABCB1* gene, which encodes P-gp, might also account for some of the inter-individual variability of DOACs.[39] We did not have available genotyping methods for *ABCB1* variants, but we do not consider this as a major limitation as the genetic contribution of specific *ABCB1* variants on drug disposition is still unclear.[40]

5 | CONCLUSION

Among the patients using DOACs admitted to hospital with symptoms of stroke, those with a verified diagnosis of IS/TIA had lower DOAC serum concentrations than those in whom a stroke diagnosis was subsequently ruled out. Our observational study cannot prove a causal relationship between low serum DOAC concentrations and the thromboembolic risk. However, it confirms and extends previous findings that the patients suffering IS/TIA have a higher cerebrovascular risk as assessed by CHA_2DS_2-VASc score and lower serum DOAC concentrations. Although speculative, one could hypothesize that patients with high cerebrovascular risk might benefit from higher DOAC levels than those with a lower risk, and further research is needed to clarify this issue. As always in patients having a low drug concentration, adherence must be thoroughly assessed before any dose increase is considered.

ACKNOWLEDGEMENTS

We would like to thank the research staff, John Bjørn Storvold and Mats Storvold Olsen at the Stroke Department, St. Olav University Hospital. The MMAS-8 scale, content, name and trademarks are protected by the U.S. copyright and trademark laws. Permission for use of the scale and its coding is required. A licence agreement is available from MMAR, LLC., www.moriskyscale.com.

CONFLICT OF INTEREST STATEMENT

We declare that the authors have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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How to cite this article: Aakerøy R, Gynnild MN, Løfblad L, et al. Direct oral anticoagulant concentrations and adherence in stroke patients. *Basic Clin Pharmacol Toxicol*. 2024;134(1):175-185. doi:10.1111/bcpt.13953