



Point-of-Care Assessment of Direct Oral Anticoagulation in Acute Ischemic Stroke: Protocol for a Prospective Observational Diagnostic Accuracy Study

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Abstract

Background Treatment of ischemic stroke with recombinant tissue plasminogen activator for intravenous thrombolysis (IVT) must be delivered within a narrow time window after symptom onset. This effective hyperacute treatment can be administered after ruling out active anticoagulation with direct oral anticoagulants (DOACs). Whenever this is impractical, e.g., due to aphasia, plasmatic DOAC levels are measured with a consequent delay in the IVT decision-making process ranging from 30 to 60 minutes of time. This study will test the hypothesis that hyperacute point-of-care assessment of clotting time in the patient's whole blood has sufficient diagnostic accuracy to determine immediately whether stroke patients are pretreated with DOAC.

Keywords

- ▶ stroke prevention
- ▶ thrombolysis
- ▶ oral anticoagulants
- ▶ inhibitors

Methods and Design This will be a prospective single-center diagnostic accuracy study in 1,850 consecutive acute ischemic stroke patients at a tertiary stroke center in Saxony, Germany. Presence of active anticoagulation with DOAC will be

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determined by point-of-care quantification of clotting time via whole blood viscoelastic testing (ClotPro) using Russell venom viper and ecarin assay compared with high-performance liquid chromatography-tandem mass spectrometry as the reference standard.

Discussion Viscoelastic point-of-care assessment of clotting time in whole blood might improve swift delivery of time-sensitive hyperacute treatment with IVT in stroke patients.

Introduction

Hyperacute Assessment of Stroke Patients: Time is Brain

Intravenous thrombolysis (IVT) with tissue-type plasminogen activator (recombinant tissue plasminogen activator [rtPA]) constitutes a state-of-the-art acute, causal therapy in patients suffering from acute ischemic stroke (AIS). IVT within up to 4.5 hours of symptom onset in AIS leads to improvement of functional outcome in terms of dependency after 90 days.¹⁻³ The use of additional imaging modalities such as perfusion computed tomography (CT) or magnetic resonance imaging (MRI) can extend the time window of eligibility for IVT up to 9 hours after onset of stroke or up to 4.5 hours after detection of symptoms with unknown onset.^{4,5} However, after stroke onset 1.9 million neurons are destroyed every minute, which substantiates the paradigm “time is brain” and may explain in parts why delivery of IVT within 45 minutes of symptom onset is associated with lower 1-year mortality rates. This was shown in a recent retrospective cohort study in 61,426 patients who received rtPA within 4.5 hours in which those who were treated within 45 minutes had an all-cause mortality of 30.8% versus 35.0% respectively (adjusted hazard ratio: 1.13 [95% confidence interval [CI]: 1.09–1.18]).⁶ Consequently, hyperacute stroke care and research has focused on strategies to accelerate delivery of IVT, which can be quantified by procedural times such as the time from the arrival of stroke patient in emergency to rtPA administration, also referred to as “door-to-needle time.”⁷ A median door-to-needle time of no more than 30 minutes is associated with a better functional outcome of AIS patients and is widely considered desirable today.⁸⁻¹⁰ Consequentially, strategies to improve speedy delivery of IVT and lower door-to-needle-times were implemented in stroke care facilities worldwide. These actions include the early prenotification of appropriate hospitals by emergency medical services in cases of AIS patients who are potentially eligible for IVT and the immediate availability of CT or MRI devices for brain imaging. Another strategy is the a priori announcement of the estimated patient arrival time of an entire stroke team with a single phone call.¹¹

Upon arrival of a potential candidate for IVT, exclusion criteria for IVT must be identified. Some of these contraindications can be easily ruled out. For instance, any intracranial bleeding will be detected on cranial CT as part of standard hyperacute workup and critical arterial hypertension will be detected on routine emergency examination.

However, effective treatment with a direct oral anticoagulant (DOAC), another main contraindication for IVT, might not be ruled out without a considerable time delay if a current medication plan is not available and the patient is not capable of communicating accurate specifications on medication type, dosage, and last time of intake.^{12,13}

The Role of Pretreatment with Direct Oral Anticoagulants in Decision Making on Intravenous Thrombolysis for Acute Stroke

DOACs encompass dabigatran (factor IIa inhibitor) as well as rivaroxaban, apixaban, and edoxaban (factor Xa inhibitors). Direct oral anticoagulation is the standard therapy for stroke prevention in atrial fibrillation, a condition that goes along with an up to fivefold increased risk of AIS and makes up 20 to 40% of causes of AIS increasing with age.^{14,15} The percentage of patients worldwide who are treated with DOACs is exponentially increasing.¹⁶⁻¹⁸ This increment also leads to an increase in DOAC-pretreated AIS patients. A recently published nationwide cohort study conducted in Switzerland reported approximately 20% of 8,179 AIS patients pretreated with DOAC at the time of stroke onset.¹⁹ Furthermore, patients treated with DOAC due to atrial fibrillation are at higher risk for recurrent AIS compared with patients suffering from atrial fibrillation who had no prior anticoagulation.²⁰ Notably, the IVT rate was lower among patients on direct oral anticoagulation in the Swiss cohort.¹⁹ This may partially be explained by two specific challenges opposed by DOAC pretreatment.

First, there is no global consensus on when IVT in AIS patients on DOAC is considered safe. Delivery of IVT after at least 48 hours from last DOAC intake is widely accepted as safe based on the pharmacodynamic and pharmacokinetic profiles of these substances.²¹⁻²³ However, this may lead to the exclusion of AIS patients who would still benefit from IVT without compromising safety of treatment as indicated by a recent meta-analysis that synthesized data from 52,823 AIS patients.²⁴ Furthermore, that information is not readily available in the acute setting. It is noteworthy that recommendations vary between countries. While the American Heart Association recommends IVT only if screening global coagulation assays or direct factor Xa activity assays are normal without further quantification,¹² the European Stroke Organization tolerates an uncalibrated anti-Xa activity of the patient’s plasma <0.5 U/mL,¹³ which remains highly controversial,²⁵ or a thrombin time <60 seconds for direct

thrombin inhibitors. The French Society of Vascular Neurology²⁶ permits any DOAC level < 50 ng/mL if specific test results are available within 30 minutes. A threshold of < 30 ng/mL has been adopted for thrombolysis in some expert opinions.^{23,27–29}

Second, when DOAC treatment status is unknown, the presence of sub-therapeutic levels of DOAC needs to be presumed from laboratory results. There are several ways to detect the presence of DOAC in the patient's blood by routine laboratory assays, and rapid diagnostic tests for detection of Xa and IIa inhibitors in urine are available as well. However, the impact of these urine tests is limited for patients potentially eligible for IVT due to the fact that no quantitative evaluation is possible.³⁰ Routine blood analyses involve unspecific global coagulation tests and quantitative measurement for the concentration of specific anticoagulant agent in plasma. Standard laboratory tests (SLTs) such as activated partial thromboplastin time, prothrombin time (PT), and thrombin time (TT) are frequently used as global assays to screen for underlying coagulation factor imbalances and for anticoagulant drug adjustment. SLTs have some well-known advantages such as standardization, accurate quality control, and broad availability. However, SLTs show variable sensitivity and specificity for DOAC depending on the type of DOAC (which may be unknown in the hyperacute evaluation of stroke patients), because their *in vitro* effect on SLT does not correlate to the *in vivo* anticoagulant activity. Therefore, screening coagulation assays are insufficient to quantify the degree of DOAC or may fail to detect lower therapeutic DOAC levels.^{23,31} Quantitative assays include diluted TT (dTT), ecarin clotting time (ECT), ecarin chromogenic assay (ECA), as well as specific chromogenic anti-IIa and anti-Xa assays. Dilution of the patient's plasma allows for a more accurate detection of direct IIa inhibitors in the lower concentration ranges via dTT compared with TT.^{23,31} Anti-Xa and anti-IIa assays reliably detect therapeutic levels of DOAC in the patient's plasma and show a linear correlation and agreement with the actual drug concentration. Diagnostic accuracy of these methods declines at low drug concentrations < 30 ng/mL.^{23,27,31} High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) constitutes the gold standard for direct measurement of DOAC in the patient's plasma. Since HPLC-MS/MS is expensive, not routinely available, not suitable for rapid testing, and not easily operable, laboratory results of dTT and anti-Xa assays using substrate-specific calibrators and controls serve as the current standard of DOAC detection method in emergency conditions. Turnaround times (i.e., time between registration of the blood sample in the laboratory and the communication of the result) for specific anti-Xa testing usually vary between 30 and 90 minutes.^{31–34} Considering a target door-to-needle time of < 30 minutes and the narrow time window for IVT, waiting for laboratory test results imposes a highly relevant delay in IVT treatment, which compromises functional outcome and, in some cases, even leads to exceeding the eligibility criteria for IVT.²¹

Point-of-Care Anticoagulation Testing

Prolonged turnaround times motivated the investigation of point-of-care (POC) testing alternatives in emergency settings. Specific quantitative anti-IIa or anti-Xa assays do not exist in the POC setting. Examples for POC devices for SLT parameters in within seconds are CoaguChek POC or the Hemochron Signature Elite POC system, but DOACs cannot be reliably detected. Sensitivity, specificity, and diagnostic agreement with plasma drug concentrations measured by the gold standard show high variability depending on the type of DOAC and results were convincing for edoxaban only.^{28,35} Viscoelastic testing (VET) of the whole blood is an alternative way to access functional testing in a POC setting considering all components of coagulation, based on the cellular model. The (ClotPro) device employs a rotating cup using an elastic element with whole blood while the pin is stationary. Clotting decelerates the cup rotation due to its increasing elasticity.³⁶ In most devices the rotation deceleration is quantified and plotted as a viscoelastometry amplitude over 40 to 60 minutes. Devices vary in the way they quantify deceleration. Based on viscoelastometry amplitude characteristics, VET can evaluate clot formation, firmness, and lysis to estimate different coagulation pathways under approximated *in vivo* conditions.

VET devices are frequently used in emergency care medicine where they are predominantly used in the management of bleeding in trauma or surgical patients.^{37–39} Efforts have been made to investigate the capability of thromboelastography and rotational thromboelastometry devices to identify DOACs. Both devices detect the presence of oral anticoagulation including vitamin K antagonists, yet discrimination capacity between different types of oral anticoagulants is not warranted in a nonexperimental setting and diagnostic accuracy at lower DOAC concentrations in a defined target population remains to be further investigated.^{40–43} ClotPro (Haemonetics, Boston, Massachusetts, United States) is a recently developed VET analyzer that is designed to allow drug monitoring and discriminate between different classes of DOACs by providing additional specific assays (Russell viper venom [RVV] test and ECA test) that can be run simultaneously with standard assays.³⁶ A previous exploratory prospective observational study evaluated the ability of ClotPro to detect DOAC in the patient's plasma at drug concentrations ≥ 50 and ≥ 100 ng/mL in an inhomogeneous population including healthy controls and with low sample size.⁴⁴ Another prospective observational study investigated the ability of ClotPro to detect and quantify DOAC in trauma patients, where timely knowledge about specific DOAC pre-treatment is indispensable for adequate reversal.⁴⁵

Recent efforts have been made to detect DOAC in urine samples using DOAC dipsticks.⁴⁶ However, potential correlations with the time of last bladder emptying and the patient's renal or liver function remain to be investigated. Furthermore, study design limitations encompass a low sample size of patients with known DOAC status limiting the application of study results as a screening tool in a real-life scenario where a urine sample is not readily available as well.⁴⁶ Prospectively collected data on the diagnostic

accuracy of ClotPro in detecting DOAC at concentration cut-offs relevant for IVT in a homogeneous study population with unknown anticoagulation status who are potentially eligible for IVT are missing.

Study Objective

This study aims to determine the diagnostic accuracy of the ClotPro POC device in detecting whether hyperacute AIS patients have DOAC plasma concentrations that prevent or allow IVT in a real-life setting based on the consideration of a cut-off at 30 ng/mL as a clinically relevant concentration of oral anticoagulation in a patient's blood.

Trial Design

This study is a single-center prospective observational diagnostic accuracy study. Consecutive AIS patients will be screened for the presence of DOAC by quantitative laboratory assays as well as with HPLC-MS/MS (reference test) and by a ClotPro POC device with RVV assay or ECA assay (index test) in parallel and once at the time of presentation at the emergency center, regardless of any documented DOAC treatment status. In addition, urine samples will be tested with a DOAC dipstick (DOASENSE GmbH, Heidelberg, Germany) without interfering with the clinical routine.

Study results will be reported in accordance with the STARD 2015 (<https://www.equator-network.org/reporting-guidelines/stard/>) guidelines.

Methods

Study Setting

This is an investigator-initiated trial that will be conducted at a tertiary stroke center of the University Hospital Carl Gustav Carus Dresden, Germany. Patients with AIS who present at the University Emergency Department will be enrolled consecutively.

Eligibility Criteria

Adult patients above 18 years of age who present with imaging or clinically confirmed AIS will be eligible. Patients with hereditary coagulopathies (von Willebrand disease, hemophilia A, B, and C, factor VII deficiency, congenital thrombocytopenia) will be excluded. To avoid prolonged CT due to non-DOAC-related mechanisms of action, we will also exclude those on therapeutic-dose heparin. Since heparin must be injected intravenously, subcutaneously, or intranasally, pretreatment with these substances can usually be ruled out by medical history and does not require POC assessment. Furthermore, trained technicians of the in-house departments of Anaesthesiology, Forensic Medicine, Clinical Chemistry, and Laboratory Medicine will perform reference and index tests.

Index Test

ClotPro is a newly developed VET system that uses a cup and a pin to measure clot formation, with the cup rotating via an elastic element and the pin functioning as a stationary counterpart.³⁶ The mechanical deceleration of the cup rota-

tion is detected and translated into a viscoelastometric amplitude. ClotPro is a bedside-available POC device, mainly used in the intensive care unit, operation room, or emergency department. Currently there are nine different test kits available for various measurements depending on the added reagent, encompassing seven standard assays and two newly developed assays that allow drug monitoring. All test assays are provided in a ready-to-use application. Dynamics of clotting can be described by different variables including coagulation time (clotting time, seconds), clot formation time (seconds), maximum clot firmness (mm), maximum lysis (%), and lysis time (s). Unlike conventional thromboelastography devices, ClotPro developed an "active tip technology" where the dry reagent is contained in a sponge in the tip of the pipette with which the sample is drawn, enabling faster handling and better reproducibility of test results. Depending on the clinical question, a particular combination of assays and a particular parameter of clotting need to be investigated. The recent addition of snake venom RVV-V (RVV factor V activator) or ECA assays provides a possibility for rapid detection of Xa inhibitors as well as low-molecular-weight heparin (LMWH; RVV-V) and IIa inhibitors (ECA).^{44,47} Coagulation time is defined as the time from start of the test until a clot amplitude of 2 mm is reached. The RVV assay contains a factor X activator derived from the snake venom "Russell's viper." The presence of factor Xa inhibitors prolongs the coagulation time. The reference range for non-accelerated coagulation time in RVV assay is <80 seconds. The RVV test does not discriminate between LMWH and factor Xa inhibitors. The ecarin assay contains ecarin, which activates prothrombin to the thrombin intermediate like meizothrombin, which is inhibited by direct IIa inhibitors again leading to a prolongation of the coagulation time. The reference range for coagulation time in the ECA assay is <100 seconds.⁴⁸

Reference Standard

HPLC-MS/MS directly quantifies DOAC concentrations as well as the concentration of active metabolites in the patient's plasma. HPLC-MS/MS constitutes a gold standard in the measurement of DOAC concentrations, yet the high costs, low throughput, and the need for specifically trained technicians limit its routine use. Hence, the use of the specified quantitative laboratory assays (dTT and anti-Xa assays) represents a generally accepted compromise.²³ These assays are comparable to the gold standard when the plasma concentration of the DOAC is above 30 to 50 ng/mL. However, in lower DOAC levels these tests display lower accuracy and cannot be used interchangeably with HPLC-MS/MS.^{23,27,31,49,50}

Blood samples will be collected (one tube per patient) in citrate tubes (S-Monovette, SARSTEDT AG & Co. KG, Nümbrecht, Germany) and processed at our central laboratory by trained staff and in accordance with manufacturers' instructions. All analysis will be performed on STA R Max devices (Stago, Asnières-sur-Seine, France). As part of clinical routine in every AIS patient with either known negative or unknown DOAC status dTT will be determined and a LMWH-calibrated

anti-Xa assay⁵¹ will be performed as an initial screening tool to identify an anti-Xa activity below 0.35 U/mL, which for every DOAC translates into a concentration <30 ng/mL. In patients with known DOAC status, specific anti-IIa measurement via dTT or specific anti-Xa measurements will be run and the quantity of DOAC in the patient's plasma will be reported as concentration (ng/mL). Additional confirmatory HPLC-MS/MS will be performed for all samples as reference test to attain exact concentration values.

Anti-IIa Measurement

The concentration of dabigatran will be inferred from the dTT. The dTT resembles the coagulation time of the patient's diluted plasma. As clotting assay, HEMOCLOT Thrombin Inhibitor Kit (HYPEN BioMed, CoaChrom Diagnostica GmbH, Maria Enzersdorf, Austria) is used together with a specific calibrator with dabigatran concentrations of 500, 250, and as low as 50 ng/mL (BIOPHEN Dabigatran Calibrator Low, HYPEN BioMed, CoaChrom Diagnostica GmbH, Maria Enzersdorf, Austria) and a specific control (BIOPHEN Dabigatran Control Low, HYPEN BioMed, CoaChrom Diagnostica GmbH, Maria Enzersdorf, Austria) with known titration for dabigatran. After plotting of the calibration curve with coagulation time on the y-axis and dabigatran concentration (ng/mL) on the x-axis, the concentration of dabigatran in the patient's plasma can be inferred from the calibration curve. The lower detection limit is 10 ng/mL.

Anti-Xa Measurement

The concentration of direct anti-Xa inhibitors in the patient's plasma will be inferred from the one-stage chromogenic assay STA-Liquid Anti-Xa assay (Stago Deutschland GmbH, Düsseldorf, Germany), which is used together with a drug-specific calibrator and control (STA-Apixaban Calibrator and STA-Apixaban Control, STA-Rivaroxaban Calibrator and STA-Rivaroxaban Control, STA-Edoxaban Calibrator and STA-Edoxaban Control, Stago, Asnières-sur-Seine, France). Chromogenic tests measure the capacity of residual factor Xa of the patient's plasma to hydrolyze a chromogenic substrate by measuring the change in optical density per minute, which is inversely correlated with the concentration of direct Xa inhibitor in the patient's plasma. After plotting a drug-specific calibration curve with anti-Xa activity (IU/mL) on the y-axis and the concentration of the respective direct Xa inhibitor (ng/mL) on the x-axis, the concentration of the respective direct Xa inhibitor in the patient's plasma can be inferred from the calibration curve. The lower detection limit is 25 ng/mL for rivaroxaban, 20 ng/mL for apixaban, and 10 ng/mL for edoxaban.

High-Performance Liquid Chromatography-Tandem Mass Spectrometry

A 500 µL of every blood sample taken in the acute setting will be frozen and multiple samples will be analyzed in by HPLC-MS/MS using a 1260 Infinity Quaternary LC System (Agilent Technologies, Santa Clara, California, United States), equipped with a Phenomenex Luna Pentafluorophenyl Column (length: 150 mm, internal diameter: 2 mm, particle

size: 5 µm), and a 3200 Q Trap (AB Sciex Germany GmbH, Darmstadt, Germany) mass spectrometer.⁵² Calibration reagents encompass apixaban, edoxaban, and rivaroxaban supplied by Cayman Chemical Company (Ann Arbor, United States), dabigatran etexilate supplied by Sigma Aldrich (St. Louis, United States), and dabigatran supplied by Biosynth/Carbosynth (Thal, Switzerland). Apixaban-13C-d3 (Cayman Chemical Company, Ann Arbor, United States) is used as the internal standard. The HPLC-MS/MS method for the simultaneous determination of DOACs that will be used in this study has recently been developed and validated by members of our group.⁵²

Outcomes

The primary outcomes of this study are sensitivity and specificity of the ClotPro POC device in detecting DOAC plasma concentrations above a threshold of 30 ng/mL in consecutive AIS patients presenting at the emergency department. HPLC-MS will serve as the reference standard for any DOAC type. ClotPro RVV and ECA assays (index test) will be run once for every AIS patient by time of presentation and in parallel with a measurement of the DOAC concentration by the respective reference standard. A dichotomized test will serve to calculate the diagnostic accuracy of ClotPro to correctly classify DOAC concentrations in the whole blood as contraindicating or permitting IVT at a cut-off concentration of 30 ng/mL with respect to the reference standard test. Secondary study outcomes of diagnostic accuracy comprise positive predictive value (PPV) and negative predictive value (NPV). Moreover, positive and negative likelihood ratios will be calculated in terms of proportions. PPV and NPV will be investigated in the subgroup of patients with assumed DOAC intake and higher pretest probability. In addition, sensitivity and specificity of the ClotPro device will be investigated for every DOAC subtype as well as applying a 50 ng/mL threshold as a relevant DOAC plasma concentration. The correlation and diagnostic agreement between DOAC concentrations as inferred by standard testing, DOAC dipstick results, and coagulation times of ClotPro assays will be investigated. Furthermore, turnaround times of reference and index tests as well as door-to-needle times in case of IVT will be documented as an explorative indicator for potential time saving. The association of ClotPro data with the risk of bleeding and clinical outcome in AIS patients will be investigated.

Sample Size

As primary outcome sensitivity and specificity of the ClotPro device to correctly classify DOAC concentrations in the patient's blood will be investigated, an estimated overall number of $N=1,850$ AIS patients will be consecutively enrolled. Accounting for a drop-out rate of 15%, a total of $n=1,610$ AIS patients need to be analyzed. We performed a sample size calculation that is sensitive to the prevalence of individual DOACs in our target population of patients presenting with AIS at the emergency center. The study will therefore be able to estimate the diagnostic accuracy of POC test results in a real-life setting.⁵³

The prevalence of DOAC pretreatment in AIS patients at our local tertiary stroke center was estimated as follows: in 2020 during an observation period of 200 days, 126 of 580 patients (22%) presenting with AIS were pretreated with oral anticoagulants including phenprocoumon. Of these, 4.5% were pretreated with edoxaban, 4.0% were pretreated with rivaroxaban, 6.2% were pretreated with apixaban, and 1.1% were pretreated with dabigatran. Overall, 15.8% were pretreated with DOAC. These numbers are in line with external observations on DOAC pretreatment in AIS patients.^{17–19}

To determine the diagnostic accuracy of ClotPro test results for the detection of DOACs in the patient's blood, the clinically acceptable width of a 95% CI is set to be 10% ($w=0.10$). This translates to an acceptance to assume that the true value of the respective sensitivity or specificity lies with a probability of 95% within a $\pm 10\%$ range of the assumed value.

Sensitivity and specificity of ClotPro assays for the detection of anti-Xa inhibitors determined by plasma-based chromogenic assays have been previously reported in trauma patients and a mixed group of patients and controls.^{44,48} In one of these studies,⁴⁵ a 50 ng/mL cut-off level for dabigatran plasma concentration was defined clinically relevant. By contrast, we defined a 30 ng/mL cut-off level relevant to decide whether IVT can be applied safely in AIS patients. A more conservative guess of the respective DOAC has been adopted for this study: for edoxaban a sensitivity of 100% and a specificity of 38% have been reported, for rivaroxaban a sensitivity of 100% and a specificity of 24% have been reported, for apixaban a sensitivity of 95% and a specificity of 45% have been reported, and for dabigatran a sensitivity and specificity of 100% have been reported. In case values reached 100%, 98% was used for calculation for direct anti-Xa inhibitors and 95% for dabigatran.

We proceeded as described in detail by Buderer⁵³ and received the following results for the number of AIS patients who need to be consecutively enrolled: for edoxaban $n_e = 167$, for rivaroxaban $n_r = 188$, for apixaban $n_a = 294$, for dabigatran $n_d = 960$ patients. Because DOAC is mutually exclusive, the total number of consecutive AIS patients who need to be enrolled adds up to $n = 1,610$. This number likely represents a slight overestimation of the actual number needed to analyze because patients taking no DOAC serve as negatives for all DOAC types. Conversely, the fraction of patients that is positive for one DOAC may not be accounted for as negative control for a different DOAC.

Recruitment

Patients who are admitted to the emergency department of the University Hospital Carl Gustav Carus with clinically or imaging confirmed AIS will be consecutively enrolled in the study. All trial investigators have real-time electronic access to incoming patient data and will be able to detect AIS candidates by time of presentation. With an average of 870 patients admitted to our emergency department every year with main diagnosis of AIS, an overall recruitment period of 26 months is anticipated.

Data Collection

Screening or specific quantitative laboratory assays will be obtained by trained technical staff at our central laboratory and provided via the clinical information system as part of the routine diagnostics. Any accessible information on DOAC type and last time of intake will be made available to the central laboratory to allow for optimal evaluation of specific dTT and anti-Xa assays as part of clinical routine. ClotPro assays specific for anti-IIa and anti-Xa activity will run simultaneously to laboratory assays for all participants. A 500 μL of every sample will be frozen and analyzed in batches by HPLC-MS/MS. Data will be primarily collected offline in an Excel sheet (Microsoft Corporation, Albuquerque, New Mexico, United States).

Blinding

Staff assessing the HPLC-MS/MS reference standard will receive number-coded anonymous probes and thereby be unaware of laboratory as well as ClotPro test results. Results of the reference standard will not be available to ClotPro assessors due to a delayed processing of the samples by HPLC-MS/MS. Thereby, a blinding of outcome assessors will be assured at any time.

Data Management

All clinical data will be transferred to a secure password-protected online database. Backup copies will be stored offline at the trial center.

Statistical Methods

Descriptive analysis will be used to describe categorical and continuous data. For continuous data, normal distribution will be checked by means of the Shapiro–Wilk test. Non-normally distributed data will be presented using median and interquartile range and normally distributed continuous data using mean and standard deviation. Categorical data will be specified using frequency and percentages. Diagnostic accuracy of the coagulation time in ClotPro RVV and ECA assays in classifying DOAC concentration levels as obtained by the reference/standard test method at a threshold of 30 ng/mL in eligible versus noneligible for IVT will be analyzed. Diagnostic accuracy parameters comprising sensitivity, specificity, PPV, and NPV as well as positive and negative likelihood ratio for ClotPro test results with respect to the standard test will be calculated by commonly known means as described elsewhere.⁵⁴ Receiver operating characteristic curves will be plotted for coagulation time in ClotPro RVV and ECA assays and Youden's index will be derived.

Depending on the distribution of anti-Xa and anti-IIa concentrations as well as RVV and ECA clotting times, Pearson's correlation or Spearman's correlation will be applied to investigate the correlation between both test results. Regression analysis will be applied to identify potential risk factors for over- and underestimation by ClotPro test results with reference to the standard test results. Complete case analysis will be performed, and missing data will not be imputed. Statistical analysis will be performed using Stata software (StataCorp 2021, Stata Statistical Software Release

17; StataCorp LLC, College Station, Texas, United States) and R version 4.0.3 (R Core Team 2021; R: A language and environment for statistical computing; R Foundation for Statistical Computing, Vienna, Austria).

Data Monitoring

This is a noninterventional observational study. Diagnostic accuracy of a POC testing device will be compared with laboratory reference standards. Measurements of the patient's blood will be performed *in vitro*, and results will not be incorporated in treatment decisions. Therefore, data monitoring is not needed.

Harms

Given the nature of the study, no harms are expected.

Auditing

No auditing will be necessary.

Discussion

Long turnaround times of quantitative laboratory tests of DOAC concentrations in plasma prolong the decision-making process on IVT in patients with AIS, worsening functional outcome. If this study confirms sufficient diagnostic accuracy of ClotPro for the detection of significant concentrations of DOAC in AIS patients, the use of the ClotPro POC device might enable substantial acceleration of door-to-needle times in patients with unknown treatment status of DOACs and allow otherwise eligible patients with unknown treatment status of DOACs to also benefit from IVT. Moreover, measurement results are valid for hemolytic probes with a high bilirubin concentration, where chromogenic assays are invalid. Viscoelastic POC quantification of coagulation time in the whole blood might pose a shift toward personalized medicine in the hyperacute decision-making on IVT in stroke patients. While it is commonly accepted to perform IVT in patients who have not taken any DOAC over the past 48 hours,^{21–23} exceptions to this rule of thumb might apply where pharmacokinetic characteristics are altered due to hepatic or renal conditions, advanced age, or drug interactions with cytochrome P450 isoenzymes or P-glycoprotein.⁵⁵

Moreover, the results of this study will have implications to the management of patients suffering from acute intracerebral hemorrhage as well, where timely knowledge on the presence of clinically significant levels of anticoagulation can allow targeted antagonization.

Author Contributions

A.S., L.H., O.T., J.P., J.B.-W., V.P., P.S., and T.S. conceptualized the study. A.K. and A.S. were involved in sample size calculation and statistical analysis. A.S. wrote the first draft of the manuscript. O.T., J.P., J.B.-W., P.S., and T.S. provided resources. L.H., A.K., O.T., J.P., M.M., K.B., T.M., J. B.-W., V.P., P.S., and T.S. reviewed the manuscript for intellectual content. All authors agreed to be accountable for the content of the work.

Ethical Approval

The study has been conducted according to the current revised form of the Helsinki Declaration (2000, Edinburgh, Scotland). The study protocol has been approved by the institutional review board (IRB) of Technische Universität Dresden (Study reference: BO-EK 515102021). This study is registered with the German Clinical Trials Register DRKS00028597.

Informed Consent

No written informed consent is necessary as all screening or specific quantitative laboratory assays are performed as part of clinical routine. Thereby, a prospective database is generated. The index test as well as the reference test is intended to measure the same parameters but with a different method and diagnostic accuracy.

Confidentiality

Physical copies of informed consent forms will be stored onsite and converted to electronic files. Case report forms will not contain patients' initials or birth dates but only a study number. All the data will be treated confidentially and only trial investigators will be authorized to access to the hard drives.

Access to Data

The final trial dataset will be available to all investigators of the study.

Dissemination Policy

This protocol paper was written in accordance with the SPIRIT 2013 Statement on the composition of protocol papers (<https://www.spirit-statement.org/protocol-version/>). The complete dataset and statistical code will be available upon reasonable request.

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Conflict of Interest

T.S. received grants from the German Federal Ministry of Health and Kurt Goldstein Institut, royalties from AstraZeneca for consulting and from Dresden International University for serving as a program director and a lecturer of the Master's Program in Clinical Research. None of these activities were related to the current study. The other authors report no conflict of interest.

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