PROTOCOL
MAS STUDY
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Title of the Study	*BASELINE DOAC MEASUREMENT IN NVAF PATIENTS AND INCIDENCE OF BLEEDING OR THROMBOEMBOLIC COMPLICATIONS DURING FOLLOW-UP: A PROSPECTIVE, MULTICENTER, OBSERVATIONAL STUDY* *The MAS (Measure And See) Study*
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List of abbreviations and acronyms

VKA	Vitamin K Antagonists
eCRF	Electronic Case Report Form
DOAC	Direct Oral Anticoagulants
SD	Standard Deviation
dTT	Diluted Thrombin Time
ECA	Ecarin Clotting Assay
FCSA	Italian Federation of Thrombosis Centres (Federazione Centri per la diagnosi della trombosi e la Sorveglianza delle terapie Antitrombotiche)
FDA	Food and Drug Administration
GCP	Good Clinical Practice
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
NVAF	Non Valvular Atrial Fibrillation

1. Background Information and rationale

Oral anticoagulant therapy is recommended in many clinical conditions to treat and prevent venous and arterial thromboembolism. Until a few years ago, vitamin K antagonists (VKA) were the only available drugs that, owing to their pharmacological characteristics, require frequent laboratory monitoring and expert dose adjustment (1). The direct oral anticoagulants (DOAC) have been introduced in clinical practice for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (NVAF) and for the prevention and treatment of venous thromboembolism (2-5). Contrary to VKA, DOACs have been proposed without the need for laboratory testing and dose-adjustment because phase III clinical trials showed efficacy and safety at fixed dose based only on clinical criteria (2-6). Nevertheless, DOAC inter-individual variability has been evaluated in previous studies, and a high inter-individual variability has been demonstrated with all DOAC at different dosages. Furthermore, post-hoc analysis and FDA reports of RE-LY, Rocket and Hokusai trials (7-13) showed that plasma concentrations of dabigatran, rivaroxaban, and edoxaban were associated with the relative risk of bleeding and thromboembolic complications, suggesting that DOAC measurements could be clinically relevant for increasing safety and efficacy of these treatments. As a consequence, an open clinical question is if, in real world patient population, measurements of DOAC levels are associated with subsequent bleeding and/or thromboembolic events.

The guidelines of the Italian Federation of Thrombosis Centres (Federazione Centri per la diagnosi della trombosi e la Sorveglianza delle terapie Antitrombotiche, FCSA) (14) suggest to do follow-up visits after 1 month from the first therapy prescription, and later every 3-6 months, to assess the patient's compliance with treatment (since a low adherence rate could diminish the benefit of treatment), to investigate regarding the occurrence of thromboembolic and bleeding complications potentially related to the prescribed DOAC, and any new co-medications with possible drug–drug interactions, and to perform a blood sampling for the necessary exams (15, 16). The execution of specific laboratory tests after 15-30 days gives a DOAC level at the steady- state.

2. Study objectives

The MAS Study is an observational, multicentre, prospective cohort study in NVAF patients treated with one the DOACs available in Italy for NVAF patients.

The general aim is to deepen the knowledge of DOAC treatment in NVAF patients, by measuring the plasma concentration of anticoagulant drugs and their correlation with any adverse events that may occur during treatment.

The study has an exclusively observational purpose in order to be the starting point for collaborative clinical studies, enabling their planning and execution; it is not aimed to influence the treatment of the individual patients included in the study.

The primary study objective is to evaluate the possible relationship between DOAC anticoagulant levels at the trough, measured at steady state (within the first 2-4 weeks of treatment) and occurrence of bleeding and thromboembolic events during the subsequent one year follow up.

3.Investigational plan

3.1 Study design

The MAS Study is an observational, prospective cohort study, double blind, multicentre, international and no Profit.

3.2 Setting

Anticoagulation clinics, affiliated or not to the Italian FCSA, will be asked to take an active part in the study, provided they have the facilities for blood sampling and processing.

3.3 Study population

4000 consecutive NVAF outpatients, 1000 for each single drug, aged >18 years and starting anticoagulation with one of the four DOAC (apixaban, dabigatran, edoxaban, rivaroxaban) will be enrolled at the moment of the first prescription. Patients will receive the type and dosage of DOAC on the base of clinical characteristics at the discretion of the attending physician, as the normal clinical practice, and the study will not influence the decision of the type and dosage of DOAC.

3.3.1 Inclusion criteria

- NVAF patients starting DOAC anticoagulation
- age > 18 years
- ability to give written informed consent
- availability, as part of the normal withdrawals, to the blood sampling for the study purpose
- availability for 12-months follow-up

3.3.2 Exclusion criteria

- age < 18 years
- indication for electrical cardioversion at the moment of drug prescription
- participation in Phase II or III clinical trials
- indication for treatment different from NVAF
- not suitable to give or not giving informed consent
- not available for blood collection or follow-up

4. Study procedures

4.1 Enrolment procedure

The active participants will propose to participate in the study to the NVAF subjects treated with DOAC that satisfy the inclusion and exclusion criteria
4000 consecutive NVAF patients, 1000 for each single drug, aged>18years and starting anticoagulation with one of the four DOAC (apixaban, dabigatran, edoxaban, rivaroxaban) will be enrolled at the moment of the first prescription. The enrolment will end at the achievement of 4000 patients, 1000 for each drug.
The study requires a mandatory plasma collection for the measurement of the anticoagulant activity of the drug at the steady state, that is within the first 2-4 weeks of treatment, obtained at the time of trough, immediately before the subsequent intake of the drug. It is up to the participating centres, depending on their availability and organization, to perform an additional plasma collection on the same day 2 hours after the last intake, or to make further blood sampling after 3, 6 or 9 months from the time of the first prescription.
The Italian FCSA guidelines recommend a follow-up visit approximately one month after the first prescription to confirm the necessary patient's adherence to the treatment and to complete the routine exams not available at the time of prescription. On that occasion, and together with the routine blood sampling, it will be collected the plasma needed to measure the DOAC concentration for the study, taking 10 ml more of blood. Samples will be treated and identified locally in order to

maintain patient's anonymity, later they will be centralized to the Biobank of Fondazione Arianna Anticoagulazione in Bologna (Italy). Afterwards, samples will be distributed to laboratories identified by the Promoter for the measurement of DOAC plasma concentration. Test results will be centrally stored and communicated to the patient's recruiting centre only at the end of the study.
Each patient will have a 12-month follow-up period, that will start at the time of the plasma collection, established within 15-30 days from the beginning of DOAC treatment.
The end of the study is at the conclusion of the 12-month follow-up of the last enrolled patient.
In the 12 months following the end of the follow-up period of the last enrolled patient, all the data will be collected and the statistical analysis and the drafting of the final report will be carried out.
All patients with NVAF who start DOAC treatment for the first time and meet the inclusion criteria are eligible for the study.
The investigator, after collection of the patient's informed consent, will collect and record in a specific electronic database (electronic CRF), the essential information collected during the routine checks performed within the previous 60 days.
The following data will be registered in the electronic CRF: patient identification number, date of birth, gender, blood cell count, creatinine, liver enzymes, diabetes, previous stroke/TIA, other comorbidities, concomitant medications (with a special attention to antiplatelet drugs), and past clinical history. The CHADS2VASC and the HASBLED score will be calculated for all patients.
A unique anonymous identifying code will be given to each patient to ensure anonymity, which will be used both to collect clinical information and to identify biological samples. The investigator of the participating centre has to keep the correspondence between the patient's personal details and the related identification code (Appendix 1).
All events occurring during the 12-month follow-up will be recorded.
Data entered in the eCRF will be stored in the database located at a server of the S. Orsola-Malpighi University Hospital in Bologna, which, thanks to an agreement between Fondazione Arianna Anticoagulazione and the S. Orsola-Malpighi Hospital, guarantees the storage, backup and maintenance of the database.
An Adjudication Committee will evaluate the adverse events occurring during the year of follow-up, examining a possible correlation with the anticoagulant activity of the drug, measured in the sample taken at the entry of the study.

4.2 Follow-up procedure

The follow-up of patients will last 12 months after blood sampling, also in patients that will stop anticoagulant treatment. In that case, a telephone follow-up is scheduled, to assess the patient's health status. A structured follow-up, as defined by FCSA guidelines, including clinical evaluation at the moment of blood sampling and each 3 to 6 months thereafter is required, in order to assess the patient’s adherence to the treatment and compliance. During the follow-up, bleeding (major and clinically relevant non-major) and thromboembolic complications (Appendix 2) will be registered. Patients not referring at established dates are promptly looked for, to avoid as much as possible, lost at follow-up.
Clinical follow up will be performed each 3 to 6 months, and the following data will be registered: weight, blood pressure, change in clinical and health status and co-medications, creatinine clearance, blood cell count, liver enzymes. The need for an adequate adherence to the treatment will be stressed to each patient on every contact occasion; on these occasions, adherence to the treatment will be evaluated by a questionnaire according to the "Morisky medication adherence scale" (25, 26), given to the patient and to be completed by the patient himself. The negative

answer to all four questions in the questionnaire indicates a good adherence to the treatment, while the presence of even one positive answer suggests criticality in the adherence to therapy (26).
What is required to do during follow up should be in line with the indications for local and usual care management. In patients discontinuing anticoagulant treatment for whatever reason before the end of the follow-up period, a form detailing reason for discontinuation should be completed in the eCRF. Any surgical interventions or invasive procedures resulting in temporary therapy interruption (for at least 2 days) with no DOAC administration must be reported in the eCRF.

4.3 Definition of the conclusion of the study

The study will be concluded for each patient at the end of the 12-months follow-up and, globally, at the completion of the last follow-up of the last patient enrolled

5. Study evaluations and measurements

5.1 Laboratory assessment

Plasma samples will be collected in NVAF patients, after collection of written informed consent, within the first 15-30 days of treatment. During routine periodic checks, clinical information (weight, blood pressure, changes in clinical status and co-medications, creatinine clearance, blood count), and adverse events defined by the protocol (Appendix 2) will be recorded. The drug dose intake is recommended in the morning and evening for dabigatran and apixaban, and in the morning for rivaroxaban (during breakfast) and for edoxaban.
Blood sampling should be performed as follows:

1. at trough level for each anticoagulant drug used, in fasting subject: 12 hours or 24 hours after the last dose intake of dabigatran and apixaban, or of rivaroxaban and edoxaban, respectively. This blood sample is mandatory for the study.

It is up to the participating centres, depending on their availability and organization, to perform a blood sample at peak, after 2 hours of dose intake, or further blood samples at 3rd, 6th and 9th month of treatment.

See Appendix 3 for technical aspects of blood sampling and processing (19).
The frozen samples in anonymous form will be initially stored at the participating centre, then centralized and stored c/o the Biobank of Fondazione Arianna Anticoagulazione in Bologna for 5 years (compatibly with their quantitative availability). The following specific tests for DOAC levels measurement will be performed: dTT (diluted thrombin time) and ECA (Ecarin chromogenic assay) calibrated for dabigatran; chromogenic anti-Xa assays, calibrated for apixaban, rivaroxaban, or edoxaban. These assays are indirect methods that do not measure quantitatively DOAC concentrations (as the gold standard liquid chromatography-tandem mass spectrometry LC-MS/MS), but the biological activity of the drug. Nevertheless, many published studies have shown that the DOAC activity measured by coagulative and chromogenic methods is significantly correlated with the DOAC levels measured by LC-MS/MS (20-23), and their use is recommended by Italian (24) and international (8) guidelines.
All the dedicated methods available (from all the companies) will be assessed in laboratories identified by the Promoter to measure the anticoagulation level of the four direct oral anticoagulant drugs. Each assay, for all collected samples, will be carried-out in the same laboratory for that assay (to avoid inter-laboratory variability). The tests will be carried out when

appropriate in relation to the optimal number of samples to be tested. Results of the tests will be kept blind both to attending clinicians and patients; they will be open to the participating centre only at the end of follow-up period.

5.2 Outcomes during the follow-up

5.2.1 Primary outcomes

1. Bleeding complications (Appendix 2): a composite of:
    - Major bleeding (ISTH definition) (17)
    - Clinically relevant non-major bleeding (18)
2. Thromboembolic complications (Appendix 2):
    - objectively documented cerebral vascular event
    - systemic embolism with angiographic demonstration of abrupt arterial occlusion
3. Mortality (cardiovascular and total)

5.2.2 Secondary outcomes

- Drug discontinuation
- Adverse drug reaction (determining change of treatment)
- Thromboembolic complications (venous and/or arterial)
- A composite of thromboembolic and total mortality
- A composite of bleeding complications and total mortality
- Incidence of thromboembolic/haemorrhagic complications by CHADS-Vasc subgroups

5.3 Safety control

Every 6 months data regarding the events occurred in the interval period and the possible relationship with the individual results of drug levels measured at inclusion will be communicated to the Safety Committee. The Safety Committee, nominated by the Promoter, may propose the interruption of the study in case of excessive outcomes in relation to the drug levels results.

5.4 Results of the study

The study will evaluate DOAC plasma concentrations. In particular, dabigatran levels will be measured by dTT (diluted thrombin time) or ECA (chromogenic test for anti-IIa activity), while apixaban, edoxaban and rivaroxaban levels will be measured by a chromogenic method for anti-Xa activity. The possible relationship between DOAC plasma concentrations and the occurrence of haemorrhagic or thrombotic events during follow-up will be examined.

6. Statistical Considerations

6.1 Bias

To avoid patient's selection bias, the investigators are asked to consecutively include the observed patients or, if it is not possible, to declare the criterion adopted for enrolment (e.g. one day a week, one week a month, etc.).

6.2 Sample size

We estimate that the global incidence of thromboembolic events and bleeding complications to be around 2% and 10% in NVAF patients per year. The range of drug levels at trough or peak, from the quantification level to the highest measured level, will be divided into classes. Comparisons will be made by contrasting the first vs the fourth class (for efficacy: increased incidence of thromboembolism in the first vs last class; for safety: increased incidence of bleeding in the last vs first class). We assume that the incidence of thromboembolism is 6% in the first class, and 1% in the fourth class, thus requiring 211 patients per class (total 844) to demonstrate differences in the efficacy end point, with an alpha and beta error equal to 0.05 and 0.80, respectively. We assume that the incidence of bleeding is 8% in the first class, and 16% in the fourth class, thus requiring 252 patients per class (total 1004) to demonstrate differences in the safety end point, with an alpha and beta error equal to 0.05 and 0.80, respectively.
We therefore aim at evaluating 1000 NVAF patients per drug subgroup (total 4000 patients).
For the statistical analysis, follow-up will be interrupted and patients censored when:
- a primary outcome is reached
- therapy is definitively interrupted
- end of study

6.3 Statistical analysis plan

Calculation of the mean (+ -SD) and median (interquartile distribution) of the drug blood levels at the trough and peak level. Investigation of the distribution of primary and secondary events in relation to drug blood levels, and study of correlations.
Patients who will not be present at the controls on the established dates will be immediately contacted, to avoid as much as possible the number of subjects lost to follow-up.
Patients with no frozen plasma aliquots to perform the tests, or for which test can not be performed for any reason, are excluded from the study.
Patients with no available clinical follow-up are excluded from the study, but the performed laboratory tests are used to evaluate the overall variability of the anticoagulation levels.
Patients with an available follow-up lower than the expected 1-year observation, are considered only for the available follow-up period.

7. Safety management

Since the study procedures are not greater than minimal risk, Serious Adverse Events (SAEs) are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB in accordance with CHOP IRB SOP 408: Unanticipated Problems Involving Risks to Subjects. Clinical adverse events (AEs) that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review. Adverse drug reactions should be reported according to the rules for spontaneous reporting (post-marketing).

8. Study administration

8.1 Data collection and management

Patient's personal data will be collected (in anonymous form), date of birth, gender, weight, height, liver and hepatic enzymes, past clinical history, thrombotic/haemorrhagic risk factors, lifestyle habits, type of treatment, clinical events during the follow-up.
The monitor of the study will check data quality and completeness by remote monitoring.
Data will be anonymously entered by the investigator in the eCRF, by assigning a patient identification code.
The adopted database is mySQL and it will be produced by Softime90 Snc company.
Data anonymously entered in the eCRF will be recorded and stored in the server of the S. Orsola-Malpighi University Hospital in Bologna.
Each access to the eCRF will be managed by personal user name and a password.
The security system requires that the password be changed at the first access; it requires a length of at least 8 characters and provides for a periodic 3-month expiry.
It is obviously possible to disable the login, if it is deemed necessary by the system administrator.
Investigators can have full access only to their patient's data.
Every night the data backup is automatically performed.
The Study Monitor will send blind to the Safety Committee the data of the occurred events and all the necessary related information for their assessment.

8.2 Ethics

The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and in compliance with the protocol and ICH-GCP.
This is an observational study and all drug prescriptions and clinical investigations are independent from the study.
Patients will receive the type and dosage of DOAC on the base of clinical characteristics at the discretion of the attending physician, as the normal clinical practice, independently from the decision to enrol the patient in the study.
The protocol and any amendments and the informed consent form (or information/non-opposition letter, as allowed by local regulations) will have to obtain Institutional Review Board or Ethics committee (IRB/EC) approval prior to initiation of the study.

8.3 Informed consent forms

Participation in this study is entirely voluntary. Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP and local regulations. A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the trial. Information should be given in both oral and written form and subjects (or their legal representatives) must be given ample opportunity to inquire about details of the study. If appropriate and required by the local IRB/IEC, assent from the subject will also be obtained. If a subject is unable to sign the informed consent form (ICF) a legal representative may sign for the subject. A copy of the signed consent form will be given to the subject or legal representative of the subject and the original will be maintained with the subject's records.
Once a patient has been enrolled in the study, he/she may withdraw his/her consent to participate in the study at any time without prejudice. Patients will not be entered in the database if the informed consent has not been obtained.

8.4 Administrative issues

8.4.1 Support of the study

Participation in the MAS study is entirely voluntary and no form of remuneration or refund is given to the investigators.
The MAS study is supported by own funds of the Promoter, for the preparation, working and maintenance of all the necessary activities, i.e. informatic and monitoring activities, centralization, storage and distribution of plasma aliquots, reagents for laboratory activities, secretary needs, statistical analysis of results, production of reports and publication of scientific papers.
Fondazione Arianna Anticoagulazione will free provide to the Italian centres the material for plasma collection, buying the cryogenic storage tubes.
Becton Dickinson supports the study by the free supply of blood sampling tubes for Italian centres.
The Promoter will also support the costs for the shipment of the material to Italian centres and for the centralization of Italian plasma samples and their further distribution to the centres chosen for the execution of laboratory tests.

9. Publication

9.1 Role of Promoter and Investigators

The Promoter and the investigators may suggest specific analysis, regarding all or some data of the central database. The authorship of the article must provide who proposed the analysis, who performed the data analysis, who took part in writing the article.
The role of the Promoter and investigators in the study (study design, collection, management, data analysis and interpretation, report writing, authorship) should be described.

9.2 Data handling and record keeping

All data and results and all intellectual property rights to the data and results derived from the study will be the property of the Promoter Fondazione Arianna Anticoagulazione according to D.M. 17 Dicembre 2004. This study is a No Profit Study (D.M. 17 Dicembre 2004, Art. 1, comma 2, lettera c)

9.3 Final report and publication

Within 12 months from the completion of the trial and in accordance with ICH-GCP, the promoter provides the IRB/IEC with a summary of the trial's outcome, and the regulatory authority(ies) with any reports required. All data and results and all intellectual property rights to the data and results derived from the study will be the property of Fondazione Arianna Anticoagulazione, who may utilize the data in various ways, such as for submission to government regulatory authorities or disclosure to other investigators. All publication or communication (oral or written) will respect the international requirements: "Uniforms requirements for Manuscripts Submitted to Biomedical Journals".

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APPENDIX 1

Labelling code

1. Alphanumeric code chosen by the centre
2. Date of collection
3. V (uppercase) for trough sample and P (lowercase) for peak sample

Example of labelling: “Alphanumeric code/01/01/2018/V”, that identify the trough sample of a patient dated 01/01/2018.
That code has to be inserted in the proper field in the eCRF (tube code)

APPENDIX 2

OUTCOME DEFINITIONS

VENOUS THROMBOEMBOLISM (VTE)

Pulmonary embolism (PE)
Symptoms of PE with one of the following findings.

- A new intraluminal filling defect in (sub) segmental or more-proximal branches on spiral computed tomography (CT) of the chest.
- A new intraluminal filling defect, or an extension of an existing defect, or a new sudden cutoff of vessels more than 2.5 mm in diameter on the pulmonary angiogram.
- A new perfusion defect of at least 75% of a segment, with a local normal ventilation result (high probability) on ventilation/perfusion lung scintigraphy (VQ scan).
- Inconclusive spiral CT, pulmonary angiography, or VQ scan evidence of a new or recurrent PE, with demonstration of a new or recurrent deep vein thrombosis (DVT) in the lower extremities by compression ultrasound (CUS) or venography.

Proximal deep vein thrombosis (DVT)
Symptoms of DVT with one of the following findings.
(a) For a NEW DVT: abnormal CUS, or an intraluminal filling defect on venography.
(b) For a RECURRENT DVT:

- abnormal CUS where compression had been normal or, if non-compressible during screening, a substantial increase (4 mm or more) in diameter of the thrombus during full compression, or
- an extension of an intraluminal filling defect, or a new intraluminal filling defect, or an extension of non-visualization of veins in the presence of a sudden cut-off on venography.

DEATH

For all patients who died during the study, the cause of death was adjudicated to one of the following categories.

- VTE-related death
  - ✓ PE (based on objective diagnostic testing, autopsy)
  - ✓ Unexplained death (and VTE cannot be ruled out)
  - ✓ Sudden death (and VTE cannot be ruled out).
- Cardiovascular (CV)-related death
  - ✓ Myocardial infarction (MI)
  - ✓ Stroke
  - ✓ Other CV event (to be specified).
- Other
  - ✓ Cancer
  - ✓ Bleeding

- ✓ Infectious disease
- ✓ Other known cause (to be specified).

BLEEDING EVENTS

Major bleeding event

A major bleeding event was defined as a bleeding event (as per International Society on Thrombosis and Haemostasis guidelines [11]), as follows.

- Acute clinically overt bleeding accompanied by one or more of the following.
  - ✓ a decrease in hemoglobin of 2 g/dl or more
  - ✓ a transfusion of 2 or more units of packed red blood cells
  - ✓ bleeding that occurs in at least one of the following critical sites: intracranial, intraspinal, intraocular, pericardial, intra-articular, intramuscular with compartment syndrome, retroperitoneal
  - ✓ fatal bleeding

Clinically relevant non-major bleeding event

The definition of clinically relevant non-major bleeding was acute clinically overt bleeding that consists of:

- any bleeding compromising hemodynamics
- any bleeding leading to hospitalization
- subcutaneous hematoma larger than 25 cm^2, or 100 cm^2 if there was a traumatic cause
- intramuscular hematoma documented by ultrasonography
- epistaxis that lasted for more than 5 minutes, was repetitive (i.e. two or more episodes of bleeding more extensive than spots on a handkerchief within 24 hours), or led to an intervention (e.g. packing or electrocoagulation)
- gingival bleeding occurring spontaneously (i.e. unrelated to eating or tooth brushing) or lasting for more than 5 minutes
- hematuria that was macroscopic and was spontaneous or lasted for more than 24 hours after instrumentation (e.g. catheter placement or surgery) of the urogenital tract macroscopic gastrointestinal hemorrhage, including at least one episode of rectal blood loss, if more than a few spots on toilet paper
- hemoptysis, if more than a few speckles in the sputum and not occurring within the context of PE
- any other bleeding type considered to have clinical consequences for a patient such as medical intervention, the need for unscheduled contact (visit or telephone call) with a physician, or temporary cessation of a study drug, or associated with pain or impairment of activities of daily life

Minor bleeding events

All acute clinically overt bleeding events not meeting the criteria for either major bleeding or clinically relevant non-major bleeding were classified as minor bleeding.

Fatal bleeding event

A fatal bleeding event was defined as a bleeding event that the adjudication committee determined was the primary cause of death or contributed directly to death.

APPENDIX 3

Sample collection protocol

1. Select the patient treated with one of the direct acting oral anticoagulants, which you want to include in the study. Besides other characteristics (see protocol for further details) the patient must express (after appropriate information) the availability to perform a trough and a peak (optional) withdrawal, to be carried out preferably in the morning, just before taking the daily dose (or one of two daily doses, for double-dose drugs). Trough withdrawal should be performed 12 hours after the last dose of dabigatran or apixaban and 24 hours after the last intake of rivaroxaban or edoxaban.
2. For each sample it is required a total blood volume of 9-10 ml (e.g. 2 tubes of 4.5 ml or 4 tubes of 2.7 ml), taken with a vacuum system, containing citrate at the concentration of 0.109 M (3.2%).
3. Centrifuge (within 2 hours from sampling) for 15 minutes at 2,000xg at controlled room temperature (20°C).
4. Separate the plasma, without disturbing the cells, and mix it in a single plastic tube. Dispense the plasma into cryogenic storage tubes in aliquots of 0.5 mL, taking care to prepare as many as possible (with that blood volume - see above - the tubes will be at least 7-8). Each tube will be labelled by a code (see Appendix 1) that identifies centre, patient, date of collection and indicates whether it is a trough or peak sample.
5. Cap the tubes and move them into a freezer -70°C as soon as possible. If it is possible, it would be recommended to quickly freeze the plasma, before transfer it to the freezer, by immersion for a few seconds in liquid nitrogen or 10 minutes in an alcohol and dry-ice bath. If a freezer -70°C is not available, samples can be stored in a freezer -30°C for up to 3 months.
6. Move the samples to the Biobank of the Promoter, according to the planning and method indicated in the study protocol.