

Design and establishment of a biobank in a multicenter prospective cohort study of elderly patients with venous thromboembolism (SWITCO65+)

Marie Méan · Drahomir Aujesky ·
Bernhard Lämmle · Christiane Gerschheimer ·
Sven Trelle · Anne Angelillo-Scherrer

Published online: 14 April 2013
© Springer Science+Business Media New York 2013

Abstract In the field of thrombosis and haemostasis, many preanalytical variables influence the results of coagulation assays and measures to limit potential results variations should be taken. To our knowledge, no paper describing the development and maintenance of a haemostasis biobank has been previously published. Our description of the biobank of the Swiss cohort of elderly patients with venous thromboembolism (SWITCO65+) is intended to facilitate the set-up of other biobanks in the field of thrombosis and haemostasis. SWITCO65+ is a multicentre cohort that prospectively enrolled consecutive patients aged ≥ 65 years with venous thromboembolism at nine Swiss hospitals from 09/2009 to 03/2012. Patients will be followed up until December 2013. The cohort includes a biobank with biological material from each participant taken at baseline and after 12 months of follow-up. Whole blood from all participants is assayed with a

standard haematology panel, for which fresh samples are required. Two buffy coat vials, one PAXgene Blood RNA System tube and one EDTA-whole blood sample are also collected at baseline for RNA/DNA extraction. Blood samples are processed and vialled within 1 h of collection and transported in batches to a central laboratory where they are stored in ultra-low temperature archives. All analyses of the same type are performed in the same laboratory in batches. Using multiple core laboratories increased the speed of sample analyses and reduced storage time. After recruiting, processing and analyzing the blood of more than 1,000 patients, we determined that the adopted methods and technologies were fit-for-purpose and robust.

Keywords Biobanking · Haemostasis · Cohort of elderly patients · Venous thromboembolism · DNA depository

Electronic supplementary material The online version of this article (doi:10.1007/s11239-013-0922-z) contains supplementary material, which is available to authorized users.

M. Méan (✉) · D. Aujesky
Division of General Internal Medicine, Bern University Hospital
and University of Bern, 3010 Bern, Switzerland
e-mail: marie.mean@insel.ch

B. Lämmle
Division of Hematology and Central Hematology Laboratory,
Bern University Hospital, 3010 Bern, Switzerland

C. Gerschheimer · A. Angelillo-Scherrer
Service and Central Laboratory of Hematology, Lausanne
University Hospital, 1011 Lausanne, Switzerland

S. Trelle
CTU Bern, Department of Clinical Research, Institute of Social
and Preventive Medicine (ISPM), University of Bern,
3010 Bern, Switzerland

Introduction

Biobanking is increasingly common because researchers recognize that large samples are often required to identify the molecular or genetic basis of complex diseases. Establishing a biobank is a multidisciplinary task that requires extensive knowledge of preanalytical, analytical and storage procedures, as well as expertise in data management, and related ethical concerns [1, 2].

Study-based biobanks are developed in relation to specific research questions; thus biobanks take various approaches and use different designs. An examination of the designs and methodology of biobanks across the globe may provide information regarding best practices in design and methodology [3–6].

In the field of haemostasis, many preanalytical variables influence the results of coagulation assays, such as factors

V and VIII, and may affect the quality of the subsequent results. Therefore, methods to limit these variations of results should be foreseen when building a biobank in the field of haemostasis. To our knowledge, no study describing the management of a biobank in the field of haemostasis and thrombosis has yet been published.

Moreover, a biobank of biological material from venous thromboembolism (VTE) patients is essential for translational research aiming to better characterize VTE at several levels ranging from diagnostic and prognostic classification to prediction of response to therapy.

Our detailed description of the biobank of the Swiss cohort of elderly patients with venous thromboembolism (SWITCO65+) is thus intended to facilitate set-up of other biobanks in the field of haemostasis and thrombosis and to contribute substantially to the discussion of good practice and standard operating procedure (SOP).

Materials and methods

SWITCO65+ is a prospective multicenter cohort study of patients aged 65 years or older with acute VTE. The goal of SWITCO65+ is to determine the clinical and biological factors and processes of care that drive long-term medical outcomes, health-related quality of life, and medical resource utilization for elderly patients with acute VTE. Establishment of a biobank to collect and store serum, plasma, DNA, and RNA samples was part of this project, enabling future exploration of currently unknown biological prognostic markers for VTE and treatment responses. The methods of SWITCO65+ cohort study have already been described in detail elsewhere [7].

SWITCO65+ participants

Five university and four high-volume non-university hospitals in Switzerland participate in SWITCO65+. In- and outpatients aged ≥ 65 years with an objective diagnosis of VTE were eligible for inclusion.

Ethical considerations

Potentially eligible patients were approached for informed consent to participate in the study. Trained study nurses explained the study to each potentially eligible patient in a face-to-face interview. In addition, patients were provided an information form. The information was written in French or German using plain language. At the time of consent, patients were given a copy of both the information and the signed consent forms and were asked whether they had any questions. Because elderly patients may need more

time to understand consent information, information was repeated if necessary to improve patient comprehension of consent information [8, 9]. In fact, spending more time talking one-on-one with potentially eligible patients was shown to be the most effective way of improving study patients' understanding [10]. Final decision on the validity of the patient's consent was left to the study nurse.

Because general consent that permits all future uses of blood samples was ethically unacceptable, patients were separately asked for permission for future use of biological samples for VTE-related genetic analyses only, and for long-term preservation of samples (up to 20 years, permitted by the local ethical committees), with the provision that investigators obtain specific consent for any future use unrelated to VTE (tiered consent) [11].

To guarantee donor confidentiality during sample shipment and storage, all patient blood samples were reversibly anonymized immediately after venipuncture. Patient blood samples were labelled with a unique, computer-generated serial number that did not include any personal identifiers on blood sample tubes or cryovials.

Patients who refused to provide samples for future genetic analyses could still participate in the clinical aspect of the study and give blood samples for other purposes than genetic analyses. Patients had the right to withdraw their consent at any time. If they decided to withdraw, they were asked whether they wanted their blood samples to be destroyed or if they permitted continued use of their data and blood samples.

The study protocol and the biobank project were both approved by ethics committees at all participating hospitals and followed the recommendations of the Swiss Academy of Medical Sciences (<http://www.samw.ch/en/Ethics/Guidelines/Currently-valid-guidelines.html>) and those published in the literature (<http://www.isber.org/Pubs/BestPractices2008.pdf>) [2, 6].

Baseline sample and data collection

All consenting patients underwent blood sampling on the day of enrolment. A total of 85 ml of blood were collected from each patient, in 12 tubes (11 Sarstedt, Numbrecht, Germany and 1 Paxgene Blood RNA System tube, Pre-AnalytiX, Qiagen, Hilden, Germany), additional data are provided in an online supplement. Because we used a winged collection system, we took citrated plasma tubes in second position to avoid contamination of citrated plasma used for coagulations assays by tissue thromboplastin [12]. To measure prognostic biomarkers (ultra sensitive troponin T, N-terminal proBrain Natriuretic Peptide, ultrasensitive CRP), we used only blood samples collected within 24 h of the index VTE event (Table 1).

Table 1 Planned laboratory analyses within the SWITCO65+ cohort study

Baseline
D-dimer ELISA (Vidas)
Fibrinogen
Factor V Leiden mutation
Prothrombin G20210A mutation
N-terminal proBrain natriuretic peptide
Genotyping cytochrome P450 2C9 (CYP2C9)
Genotyping vitamin K epoxide reductase complex subunit 1 (VKORC1)
Ultrasensitive troponin T
Growth arrest-specific gene 6 product (Gas6)
Thrombin generation
Prekallikrein
Cystatin C levels
Ultrasensitive C-reactive protein
Factor IX propeptide mutation
After 12 months follow-up
D-dimer ELISA (Vidas)
Fibrinogen
Antithrombin
Protein C
Protein S
Lupus anticoagulant
Anti-beta 2-glycoprotein I antibodies
Anticardiolipin antibodies
Homocystein
Factor VIII
Factor IX
Factor XI
Growth arrest-specific gene 6 product (Gas6)
Thrombin generation
Ultrasensitive C-reactive protein

Follow-up sample and data collection

Since D-dimer, fibrinogen, antithrombin, protein C, protein S, antiphospholipid antibodies, and factor VIII, IX, and XI levels may be influenced by the presence of acute thrombosis or ongoing anticoagulation, these parameters were determined in a second fasting blood sample of 20 ml at 12 months after the index VTE. Vitamin K dependent coagulation factors (protein C, protein S, and factor IX) were not measured in patients who were still receiving vitamin K antagonists.

Preanalytical procedures

Control of preanalytical variables is critical for coagulation testing and to ensure clinical reliability of the results [13,

14]. All participating centers were provided with the necessary material for blood collection and storage: ready-to-use kits in plastic bags (one ready-to-use kit per patient). Each kit contained two multi-sampling needles with a pre-assembled holder (Multifly-Set, Sarstedt, Nümbrecht); adaptators for PAXgene Blood RNA System tubes (Adaptator Luer, Becton–Dickinson AG, Basel); pre-labeled blood collection tubes and storage cryovials with polyester cryoresistant labels (EPI, West Sussex, UK); and pre-labeled plastic bags for storage. Blood samples were drawn after minimal venostasis with a 21-gauge needle which is appropriate for use on elderly patients. Because we used blood collection tubes from Sarstedt and Qiagen, multi-sampling needles were required to permit a single venipuncture for drawing blood through a winged collection system (a second needle was provided as rescue).

Data and sample management

We used an interactive, password-secured, web-based database for data and sample management of the SWITCO65+ cohort study and biobank, ensuring the server was secure for storage of epidemiological and clinical data (Fig. 1). The web-based data management system automatically generated a unique patient screening number for each enrolled patient. This screening number could be linked to a unique biobank number printed on the label of the plastic bag of the ready-to-use kit. Specimens could be traced through a protected reversible link to the database. To avoid typographical errors and to allow rapid and secure entry of the biobank number in the web database, numbers were transcribed into barcodes and scanned into the database with a commercial barcode scanner. The material for baseline and 12-month blood samples was provided in blue and red-labeled plastic bags, respectively. By this method, pre-labelled bags containing prelabelled material for blood collections were prepared centrally and in advance. Thus, we could match the screening numbers of consecutive patients recruited in a single hospital to their biobank numbers.

To avoid errors in the preanalytical and analytical phases, we chose to prelabel the blood collection tubes and cryovials with cryoresistant labels and used a colour code for labels and cryovial caps. Because barcoding is effective for reducing identification errors and has been recommended as an evidence-based “best practice” [15], we decided to print an individual 8-digit European Article Number (EAN8) barcode on all labels (Fig. 2). The first digit referred to time point one or two; i.e., baseline or 12-months follow-up. Digits two through five referenced the individual patient biobank number. The sixth digit referred to the cryovial type code, and the seventh digit referred to the number of cryovials processed. The eighth digit was a standard check number to verify whether the previous

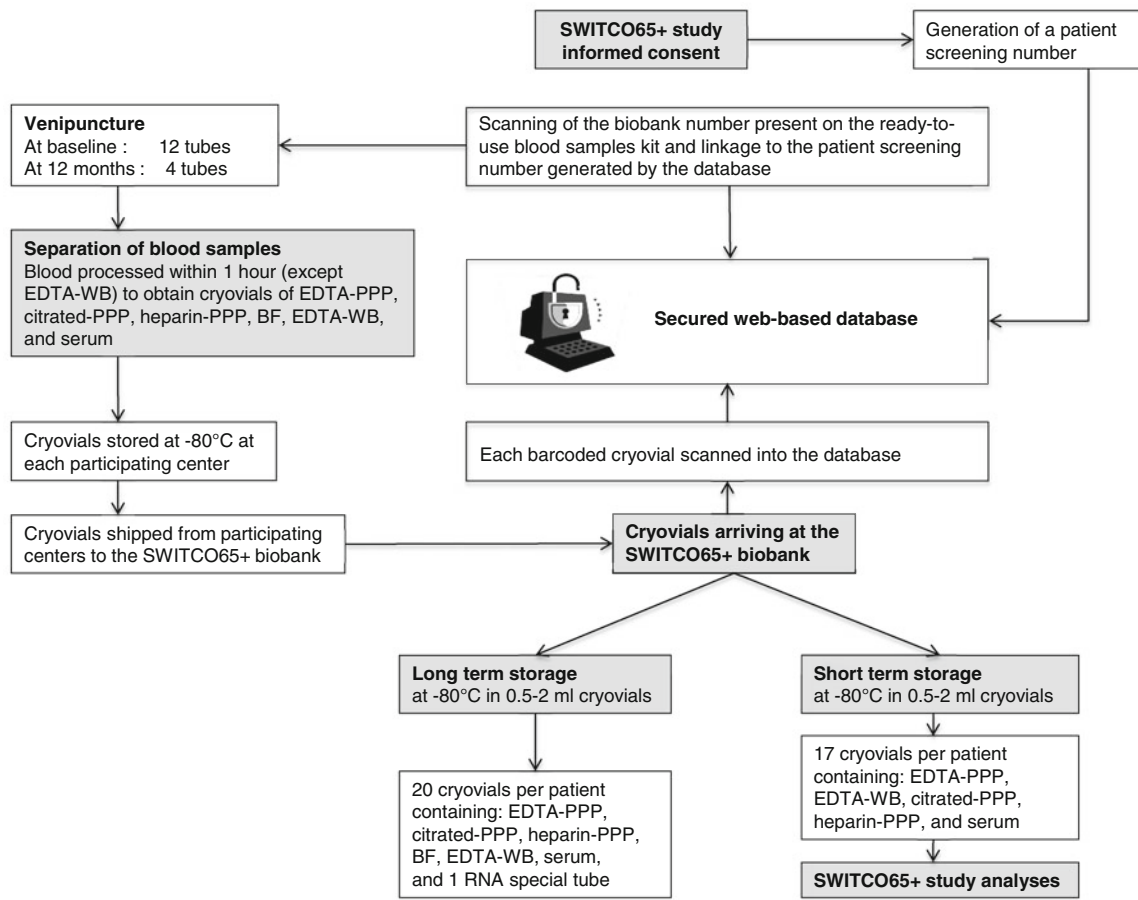


Fig. 1 Processing and storage of blood samples. For each enrolled patient, the web-based data management system automatically generates a unique patient screening number. The patient screening number is then linked to a biobank number. After collection, blood samples are processed and stored at each participating center, at -80°C

Celsius within 1 h. Cryovials are shipped to the central SWITCO65+ biobank in batches. Upon arrival at the SWITCO65+ biobank, cryovials are separated in two groups: long-term and short-term storage. *BF* buffy coat, *WB* whole blood, *PPP* platelet poor plasma

seven numbers were correct, according to a check digit algorithm. SOPs pertaining to each aspect of the SWITCO65+ cohort study and biobank were developed and are available on the study website (www.switco65.ch).

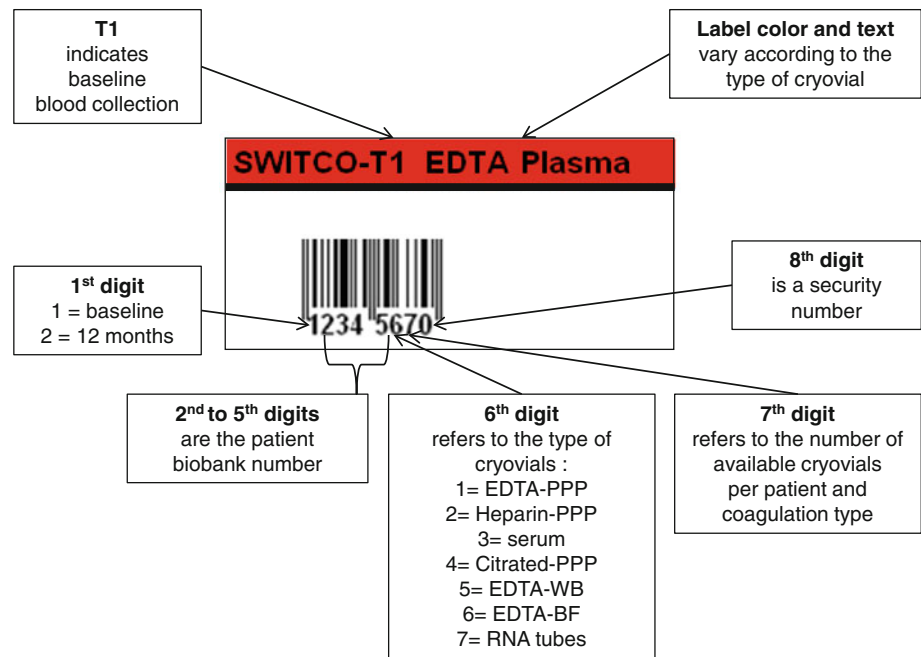
Quality control checks of sample handling, storing, and laboratory procedures are performed regularly. The electronic biobank database has automatic checks for patient identification, data completeness, and plausibility, and allows accurate tracking of any individual blood sample.

Processing of blood samples

Trained study nurses processed blood samples at each center according to the SWITCO65+ SOPs. Aliquots of samples were cryovialled in 3 ml polypropylene tubes with screw caps (Sarstedt). Screw cap colors were assigned to designate the content of the cryovials: black for EDTA whole blood, blue for buffy coat, red for EDTA platelet

poor plasma (PPP), green for citrated PPP, yellow for heparin PPP and white for serum. Blood collection tubes selected for plasma or serum isolation were centrifuged for 10 min at $2,700\times g$. To simplify blood processing, we chose one speed: $2,700\times g$ was selected because of its possible application to the centrifugation equipment of all participating centers. Evidence suggests that increasing the relative centrifugation forces and reducing the centrifugation time is possible without affecting the reliability of coagulation testing [16–18]. The supernatant plasma from citrated blood samples was re-centrifuged for 10 min at $2,700\times g$ to remove remaining platelets [19]. The resulting PPP was cryovialled within 1 h of blood collection and immediately stored at -80°C in the local analytical laboratory. We completed the blood processing within 1 h of blood collection to avoid significant measurement variation [20]. In fact a recent study observed clinically significant changes, defined as a percentage change of more than 10 %

Fig. 2 Cryovial label and barcoding. An example of a pre-labeled cryovial with a cryoresistant label. On the label is printed an individual 8-digit European Article Number (EAN) 8-digit barcode. *BF* buffy coat, *WB* whole blood, *PPP* platelet poor plasma



from the initial value, for factor VIII clothing activity level when the plasma was frozen more than 1 h after blood sampling [21].

For one of the citrated tube, we used a recent method to collect platelet and leukocyte-free plasma for thrombin generation measurement. Following the first centrifugation, approximately half the citrated plasma volume was collected. We made sure not to aspirate the top surface of the plasma, where some light platelets may be found, and were careful not to disturb the buffy coat by aspirating too close to the platelet layer [22]. This approach prevented plasma contamination by platelets and leukocytes that could alter thrombin generation measurement results.

SWITCO65+ biobank

After blood collection and processing, we temporarily stored cryovials at -80° at each participating center (Fig. 1). Cryovials and PAXgene Blood RNA System tubes were left in an upright position in their original plastic bag. A commercial shipping company periodically transported the samples, packed in dry ice, to the SWITCO65+ biobank. While frozen samples were centrally stored at the SWITCO65+ biobank at the Central Laboratory of Hematology of Lausanne University hospital, SWITCO65+ study laboratory analyses were performed at four different university hospital laboratories.

The biobank was divided into two different sections, for short-term and long-term storage (Fig. 1). Samples were stored in the short-term racks according to plans for analysis and their destinations. Samples were shipped and transit was

tracked securely from one laboratory to another through the password-secured, web-based database. Because storage time was limited to 24 months for D-dimers, fibrinogen, factor IX, antithrombin and protein C, and 18 months for factor VIII, factor XI and protein S (allowing for 10 % variation) [23], analyses were performed within 6 months of collection, in order to reduce variation to <5 % over baseline for factor VIII, factor XI and protein S [23, 24].

In long-term storage racks, cryovials from each patient containing 0.5–2.5 ml of different plasma, serum or whole blood and one RNA blood system tube containing 2.5 ml were stored at baseline. Additional cryovials taken at the patient's 12 months follow-up appointment were subjected to long-term storage (Fig. 1).

Storing of blood samples

The blood samples from the SWITCO65+ study are stored in a single -80°C freezer at the Central Laboratory of Hematology of Lausanne University hospital. There will be approximately 35,000 cryovials, and 1000 PAXgene Blood RNA System tubes stored in the biobank. The freezer has a capacity of 793 l (Thermo Fisher, Fisher Scientific, Wohlen, Switzerland) and is connected to the institution's generator system, in case of power failure. To avoid internal temperature variations that may influence the quality of the stored blood samples, freezer temperature is continuously controlled through a thermal sensor placed inside the freezer [24].

A specifically trained biobank nurse is in charge of the reception, management, and storage of the biosamples. The

blood samples received from participating centers were immediately stored in original plastic bags in the freezer. The biobank nurse then opened the plastic bag containing the cryovials, scanned each barcoded cryovial into the database, and stored the material in boxes (able to hold up to 81 cryovials per box) and racks according to type of cryovial (Fig. 1).

SWITCO65+ blood analyses

Laboratory analyses were performed in batches at the University hospitals of Lausanne, Bern, Geneva and Zürich. All analyses of the same type were performed in the same laboratory.

We distributed the analysis tasks based on the specializations of each of the university hospital laboratories. Using multiple core laboratories increased the speed of sample analyses and reduced storage time. Quick turnover ensured the availability of space in the biobank freezer for samples arriving from the various study sites. Because cycles of freezing and thawing may degrade DNA and have a negative impact on the quality of biomarker measurements, laboratory analyses were performed in batches to avoid freeze–thaw cycles [24, 25]. Unused sample material was either returned to the study biobank or destroyed according to existing disposal standards.

SWITCO65+ DNA/RNA depository

VTE is a complex disease with genetic components. Heritability estimates indicate that ~60 % of disease risk is genetic [26, 27]. The genome-wide association (GWA) strategy has recently been applied to thromboembolic phenotypes, but sample sizes were relatively small [28, 29]. The SWITCO65+ study is suited for such an experiment due to its sample size of 1,003 patients.

Two buffy coat cryovials, one PAXgene Blood RNA System tube and one EDTA-whole blood sample were collected at baseline and will be used for RNA/DNA extraction. The SWITCO65+ DNA and RNA depository was designed to permit both GWA-replication studies and GWA studies, with the single limitation that 99.8 % of the study patients were Caucasians. Thus, the results may not be generalizable to other racial or ethnic groups.

Preliminary results

Between September 8, 2009, and March 31, 2012, a total of 1,863 patients with VTE were screened. We excluded 462 patients (24.8 %) who had at least one exclusion criteria and 398 patients (21.4 %) who refused to provide informed

consent, our initial study sample thus comprised 1,003 patients (53.8 % of initially screened patients).

Six patients withdrew their consent and did not allow us to use their data or blood sample, which reduced the analysable cohort to 997. Nine-hundred-and-five patients (90.8 %) had blood collected at baseline. The proportion of patients who agreed to participate in the cohort study but refused genetic analyses was 6.5 % (65 out of 997).

By October 16, 2012, after an average follow-up time of 512 days, 799 (80.1 %) patients were still actively participating, 150 (15.0 %) had died, and 1 (0.1 %) was lost to follow-up. Forty-seven out of 1,003 (4.7 %) patients had withdrawn their consent, but only 6 (0.6 %) did not allow us the use of their data and blood samples for analysis.

By November 2012, 20,802 cryovials had been stored in the central SWITCO65+ biobank and 7,852 vials had already been sent for subsequent analyses in one of the four participating laboratories (Lausanne, Zürich, Bern, Geneva) (Table 1).

Discussion

In this prospective multicenter cohort study of 1,003 elderly patients with acute symptomatic VTE, we observed that 21.4 % of the screened patients refused to provide informed consent, whereas very few patients willing to participate refused genetic testing (6.5 %). Therefore, we believe that the primary reason for nonparticipation to this cohort study is not due to biobanking or genetic analyses but most probably to the high number and complexity of baseline and follow-up assessments, a known obstacle to study participation in the elderly [30].

A classical issue concerning biobanks is the establishment of standard procedures for blood sample collection, processing and storage. While the design and the development of biobanking procedures has been described for cardiovascular disease, such as coronary artery disease, [5] and for cancer, [3] design and development of biobanking procedures in the field of haemostasis and thrombosis is currently lacking.

Therefore, we have described the entire process of biobanking blood samples from a cohort of elderly patients with acute VTE, beginning with the decision to collect blood, and ending with the safe storage of samples. We designed and developed this process to guarantee the highest quality of stored material and the best conditions for subsequent analyses.

We have established a biobank that includes DNA and RNA samples, which can be used to study future, yet unknown, predictors of VTE-related prognosis. We believe that association studies between genetic variants and different clinical VTE phenotypes and VTE recurrence, and between RNA expression levels and phenotypes and VTE recurrence might improve our understanding of the pathophysiology of

VTE events. The low dropout rate of 4.8 % and near-complete blood collection are other strengths.

After recruiting, processing and analyzing the blood of more than 1,000 study participants, we determined that the adopted methods and technologies were fit-for-purpose and robust. Additional information about the cohort, i.e. the study protocol, data collection forms, study progress, ongoing nested projects, and planned or published articles, can be found on the study website (www.switco65.ch) or at the study registry ClinicalTrials.gov (identifier NCT00973596). The Steering Committee of SWITCO65+ encourages national and international collaborative research projects related to the SWITCO65+ study, including sharing anonymized data and biological samples. Requests for access to SWITCO65+ data or material should be submitted to the Steering Committee, using the Subproject Proposal Form (available at www.switco65.ch or from the SWITCO65+ project manager: marie.mean@insel.ch).

What is known about this topic?

- Biobanking is increasingly common because researchers recognize that large samples are often required to identify the molecular or genetic basis of complex diseases, such as VTE.
- In the field of haemostasis, many preanalytical variables influence the results of coagulation assays and may affect the quality of the subsequent results. Therefore, methods to limit these factors affecting the quality of laboratory results should be anticipated when building a biobank in the field of haemostasis.

What does this paper add?

- We provide the first description of biobanking of blood for subsequent DNA, RNA, plasma and serum analyses in the field of haemostasis and thrombosis.
- We recommend a set of “best practices” for the development of standardized biobanking processes in the field of haemostasis and thrombosis to guarantee the best quality of the blood samples stored.

Acknowledgments This cohort study is supported by the Swiss National Science Foundation (Grant no. 33CSO-122659/139470). We thank Kali Tal, Ph.D. for her editorial work.

Conflict of interest The authors have no conflict of interest.

References

1. Salvaterra E, Lecchi L, Giovanelli S, Butti B, Bardella MT, Bertazzi PA et al (2008) Banking together. A unified model of informed consent for biobanking. *EMBO Rep* 9:307–313
2. Riegman PH, Morente MM, Betsou F, de Blasio P, Geary P (2008) Biobanking for better healthcare. *Mol Oncol* 2:213–222
3. Field JK, Liloglou T, Niaz A, Bryan J, Gosney JR, Giles T et al (2009) EUELC project: a multi-centre, multipurpose study to investigate early stage NSCLC, and to establish a biobank for ongoing collaboration. *Eur Respir J* 34:1477–1486
4. Molnar MJ, Bencsik P (2006) Establishing a neurological-psychiatric biobank: banking, informatics, ethics. *Cell Immunol* 244:101–104
5. Jayasinghe SR, Mishra A, Van Daal A, Kwan E (2009) Genetics and cardiovascular disease: design and development of a DNA biobank. *Exp Clin Cardiol* 14:33–37
6. Schroh AS, Wurtz S, Kohn E, Banks RE, Nielsen HJ, Sweep FC et al (2008) Banking of biological fluids for studies of disease-associated protein biomarkers. *Mol Cell Proteomics* 7:2061–2066
7. Méan M, Righini M, Jaeger K, Beer HJ, Frauchiger B, Osterwalder J et al (2013) The Swiss cohort of elderly patients with venous thromboembolism (SWITCO65+): rationale and methodology. *J Thromb Thrombolysis*
8. Sudore RL, Landefeld CS, Williams BA, Barnes DE, Lindquist K, Schillinger D (2006) Use of a modified informed consent process among vulnerable patients: a descriptive study. *J Gen Intern Med* 21:867–873
9. Sugarman J, McCrory DC, Hubal RC (1998) Getting meaningful informed consent from older adults: a structured literature review of empirical research. *J Am Geriatr Soc* 46:517–524
10. Flory J, Emanuel E (2004) Interventions to improve research participants’ understanding in informed consent for research: a systematic review. *JAMA* 292:1593–1601
11. Mello MM, Wolf LE (2010) The Havasupai Indian tribe case—lessons for research involving stored biologic samples. *N Engl J Med* 363:204–207
12. Adcock DM, Hoefner DM, Kottke-Marchant K, Marlar RA, Szamosi DI, Warunek DI (2008) Collection, transport and processing of blood specimens for testing plasma-based coagulation assays and molecular hemostasis assays: approved guideline, 5th edn. Clinical and Laboratory Standards Institute, Wayne, PA
13. Favaloro EJ, Lippi G, Adcock DM (2008) Preanalytical and postanalytical variables: the leading causes of diagnostic error in hemostasis? *Semin Thromb Hemost* 34:612–634
14. Lippi G, Franchini M, Montagnana M, Salvagno GL, Poli G, Guidi GC (2006) Quality and reliability of routine coagulation testing: can we trust that sample? *Blood Coagul Fibrinolysis* 17:513–519
15. Snyder SR, Favoretto AM, Derzon JH, Christenson RH, Kahn SE, Shaw CS et al (2012) Effectiveness of barcoding for reducing patient specimen and laboratory testing identification errors: a Laboratory Medicine Best Practices systematic review and meta-analysis. *Clin Biochem* 45:988–998
16. Sédille-Mostafaie N, Engler H, Lutz S, Korte W (2012) Advancing haemostasis automation—successful implementation of robotic centrifugation and sample processing in a tertiary service hospital. *Clin Chem Lab Med* 15:1–6
17. Lippi G, Salvagno GL, Montagnana M, Manzato F, Guidi GC (2007) Influence of the centrifuge time of primary plasma tubes on routine coagulation testing. *Blood Coagul Fibrinolysis* 18:525–528
18. Sultan A (2010) Five-minute preparation of platelet-poor plasma for routine coagulation testing. *East Mediterr Health J* 16:233–236
19. Favaloro EJ, Funk DM, Lippi G (2012) Pre-analytical variables in coagulation testing associated with diagnostic errors in hemostasis. *Lab Med* 43:1–10
20. Ostroff R, Foreman T, Keeney TR, Stratford S, Walker JJ, Zichi D (2010) The stability of the circulating human proteome to variations in sample collection and handling procedures measured with an aptamer-based proteomics array. *J Proteomics* 73:649–666

21. Zürcher M, Sulzer I, Barizzi G, Lämmle B, Alberio L (2008) Stability of coagulation assays performed in plasma from citrated whole blood transported at ambient temperature. *Thromb Haemost* 99:416–426
22. Dargaud Y, Negrier C (2010) Thrombin generation testing in haemophilia comprehensive care centres. *Haemophilia* 16: 223–230
23. Woodhams B, Girardot O, Blanco MJ, Colesse G, Gourmelin Y (2001) Stability of coagulation proteins in frozen plasma. *Blood Coagul Fibrinolysis* 12:229–236
24. Rundle AG, Vineis P, Ahsan H (2005) Design options for molecular epidemiology research within cohort studies. *Cancer Epidemiol Biomarkers Prev* 14:1899–1907
25. Paltiel L, Ronningen KS, Meltzer HM, Baker SV, Hoppin JA (2008) Evaluation of freeze thaw cycles on stored plasma in the Biobank of the Norwegian mother and child cohort study. *Cell Preserv Technol* 6:223–230
26. Larsen TB, Sorensen HT, Skytthe A, Johnsen SP, Vaupel JW, Christensen K (2003) Major genetic susceptibility for venous thromboembolism in men: a study of Danish twins. *Epidemiology* 14:328–332
27. Souto JC, Almasy L, Borrell M, Blanco-Vaca F, Mateo J, Soria JM et al (2000) Genetic susceptibility to thrombosis and its relationship to physiological risk factors: the GAIT study. Genetic analysis of idiopathic thrombophilia. *Am J Hum Genet* 67:1452–1459
28. Tregouet DA, Heath S, Saut N, Biron-Andreani C, Schved JF, Pernod G et al (2009) Common susceptibility alleles are unlikely to contribute as strongly as the FV and ABO loci to VTE risk: results from a GWAS approach. *Blood* 113:5298–5303
29. Rasmussen-Torvik LJ, Cushman M, Tsai MY, Zhang Y, Heckbert SR, Rosamond WD et al (2007) The association of alpha-fibrinogen Thr312Ala polymorphism and venous thromboembolism in the LITE study. *Thromb Res* 121:1–7
30. McMurdo ME, Roberts H, Parker S, Wyatt N, May H, Goodman C et al (2011) Improving recruitment of older people to research through good practice. *Age Ageing* 40:659–665