BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-019325
Article Type:	Protocol
Date Submitted by the Author:	26-Aug-2017
Complete List of Authors:	Kringelbach, Tina; Copenhagen University Hospital Herlev, Bio- and Genome Bank Denmark and the Danish Rheumatologic Biobank, The Molecular Unit, Department of Pathology Glintborg, B.; Rigshospitalet Glostrup, Copenhagen Centre for Arthritis Research, Center for Rheumatology and Spine Diseases, the Danish DANBIO Registry, and the Danish Rheumatologic Biobank Hoegdall, Estrid; Copenhagen University Hospital Herlev, Bio- and Genome Bank Denmark and the Danish Rheumatologic Biobank, The Molecular Unit, Department of Pathology; University of Copenhagen, Department of Clinical Medicine, Faculty of Health and Medical Sciences Johansen, Julia; Copenhagen University Hospital Herlev, Departments of Medicine and Oncology, and the Danish Rheumatologic Biobank; University of Copenhagen, Department of Clinical Medicine, Faculty of Health and Medical Sciences Lund Hetland, Merete; Rigshospitalet Glostrup, Copenhagen Centre for Arthritis Research, Center for Rheumatology and Spine Diseases, the Danish DANBIO Registry, and the Danish Rheumatologic Biobank; University of Copenhagen, Department of Clinical Medicine, Faculty of Health and Medical Sciences
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Epidemiology
Keywords:	Inflammatory rheumatic disease, Personalised treatment, Biomarkers, Danish Rheumatologic Biobank, DANBIO, RHEUMATOLOGY

Identification of new biomarkers to promote personalized treatment of patients with inflammatory rheumatic disease, an open cohort study

Running title: A Rheumatologic Biomarker Protocol

T M Kringelbach[1,2], B Glintborg[1,3,4], E V Høgdall[1,2,5], J Johansen[1,5,6], M L Hetland[1,3,4,5], and The Biomarker Protocol Study Group[7]

T M Kringelbach and B Glintborg contributed equally to this paper and share first co-authorship

[1]The Danish Rheumatologic Biobank
[2]Bio- and Genome Bank Denmark, The Molecular Unit, Department of Pathology, Copenhagen University Hospital Herlev, Herlev, Denmark
[3]Copenhagen Centre for Arthritis Research, Center for Rheumatology and Spine Diseases, Rigshospitalet, Glostrup, Denmark
[4]The Danish DANBIO registry, Rigshospitalet, Glostrup, Denmark
[5]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
[6]Departments of Medicine and Oncology, Copenhagen University Hospital Herlev, Herlev, Denmark
[7]The Biomarker Protocol Study Group is defined under acknowledgements

ClinicalTrials.gov ID: NCT03214263

Corresponding author: Merete L. Hetland, Department of Rheumatology, Rigshospitalet Glostrup, Glostrup, Denmark, phone: 0045-38633330, e-mail: merete.hetland@dadlnet.dk

Author disclosures:
B Glintborg: Biogen, AbbVie
M L Hetland: Orion, BMS, AbbVie, Biogen, Pfizer, MSD, Roche, Celltrion, Crescendo, UCB
The remaining authors: none declared

Word count:
Abstract: 288 words
Text: 3,638 words

ABSTRACT

Introduction: The medical treatment of inflammatory rheumatic diseases has improved dramatically during the last decades primarily due to the introduction of biological disease modifying anti-rheumatic drugs (bDMARDs). However, bDMARD treatment failure occurs in 30-40% of patients due to lack of effect or adverse events, and the tools to predict treatment outcomes in individual patients are currently limited. The objective of the present study is to identify diagnostic, prognostic and predictive biomarkers, which can be used to 1) diagnose inflammatory rheumatic diseases early in the disease course with high sensitivity and specificity, 2) improve prognostication, or 3) predict and monitor treatment effectiveness and tolerability for the individual patient.

Methods and analysis: Observational and translational open cohort study with prospective collection of clinical data and biological materials (primarily blood) in patients with inflammatory rheumatic diseases treated in routine care. Patients contribute with one cross-sectional blood sample (i.e. EDTA whole blood, plasma and buffy coat, serum, and blood in PAXgene RNA tubes), and/or are enrolled for longitudinal follow-up upon initiation of a new DMARD (blood sampling after 0, 3, 6, 12, 24, 36, 48, 60 months of treatment). Other biological materials will be collected when accessible and relevant. Demographics, disease characteristics, comorbidities, and lifestyle factors are registered at inclusion; DMARD treatment and outcomes are collected repeatedly during follow-up. Currently (July 2017), >5,000 samples from ≈3,000 patients have been collected. Data will be analysed using appropriate statistical analyses.

Ethics and dissemination: The protocol is approved by the Danish Ethics Committee and The Danish Data Protection Agency. Participants give written and oral informed consent. Biomarkers will be evaluated and published according to REMARK, STROBE, and STARD guidelines. Results will be published in peer-reviewed scientific journals and presented at international conferences.

ClinicalTrials.gov ID: NCT03214263

Strengths and limitations of this study

- Nation-wide collection of biological materials and corresponding extensive clinical data provides the opportunity to discover and/or validate a wide range of diagnostic, prognostic, and predictive biomarkers in patients with inflammatory rheumatic disease
- Recruitment of patients treated in routine care is expected to provide valuable data on "real life patients" (e.g. elderly patients with comorbidities), which are different from the more homogeneous patient population in randomised controlled trials
- Standardised collection of samples and quality control ensures comparability between samples from different departments, and enables research in less common rheumatic diseases
- Patient recruitment and follow-up in routine care and across several rheumatic diagnoses and treatments will be associated with some limitations in clinical and biological data
- The non-randomised study design inherits a risk of confounding and thorough statistical analysis and confounder adjustment is therefore important

INTRODUCTION

Rheumatoid arthritis (RA), psoriatic arthritis (PsA), and axial spondyloarthritis (AxSpA) are examples of chronic inflammatory rheumatic diseases characterised by pain, disability, and progressive decrease in workability, and are associated with comorbidity and risk of early death [1,2]. The impact of these chronic diseases on the patients should be minimized through early diagnosis followed by targeted therapy with minimal side effects. If and when remission is achieved, the patient has the potential to maintain a life with few restrictions – a desirable outcome both for the patient and for society.

The medical treatment of inflammatory rheumatic diseases has improved dramatically during the last decades. This is mainly due to an increased acknowledgement of the treat-to-target concept with conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) in RA, which implies strict monitoring and aggressive treatment strategies with add-on or switching of therapy according to clinical response or side effects [3–5]. Furthermore, the biological DMARDs (bDMARDs), e.g. tumour necrosis factor alpha inhibitors, or other specific modulators of inflammatory signal transduction, have improved outcomes for patients otherwise refractory to treatment with csDMARDs [4]. New treatment modalities including targeted synthetic DMARDs (tsDMARDS, e.g. Janus Kinase (JAK) inhibitors) are being introduced, and the first biosimilar bDMARDs have been marketed. Biological DMARDs and JAK inhibitors are expensive, and treatment failure, defined as lack of effect or serious adverse events, occurs in 30-40% of patients treated with bDMARDs [6,7]. Tools to predict treatment outcomes and side effects in the individual patient are currently limited [8].

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [9,10]. Biomarkers are divided in three categories: 1) diagnostic biomarkers, which may be used for early diagnosis of a given disease [11,12]. The ideal diagnostic biomarker should establish the correct diagnosis with high sensitivity and specificity; 2) prognostic biomarkers, which correlate with specific clinical outcomes, and thus progression of disease, regardless of any treatment; and 3) predictive biomarkers, which may be used to predict whether a given patient may benefit from a given treatment [13,14]. Hence, biomarkers may be

promising tools to personalise the treatment of patients with inflammatory rheumatic disease. Biomarkers in blood and tissue include a wide range of molecules with different characteristics such as DNA, single nucleotide polymorphisms (SNPs), RNA, microRNA (miRNA), proteins, and metabolites. Genetic variation can be caused by SNPs and Copy Number Variations (CNVs), among which the SNP is the most common type of genetic variation [15–19]. MicroRNAs are small single-stranded, endogenous, non-coding RNAs (18-25 nucleotides) and play essential roles in regulating gene expression, cell development, differentiation, and proliferation [19–21]. The human proteome constitute all expressed human proteins and reflects the biological activity of the patient, and proteomics is increasingly used to investigate treatment response [22,23] or to stratify responding versus non-responding patients [24,25]. The metabolome is defined as the complete set of metabolites <1500 Da found in a given biological sample. It is a dynamic entity, which reflects the interaction between the individual genetic background and factors such as pathophysiological conditions, diet, and pharmacologic treatment [26]. In patients with RA, PsA, and AxSpA these biomarkers may be related to the disease itself, the associated inflammation or treatment-related pharmacokinetics. Biomarkers can be detected in peripheral blood, synovial fluid, circulating cells or cell-free DNA in plasma, or in tissue (e.g. cartilage, bone, and synovial membrane).

Currently, some biomarkers are used as part of the classification of arthritis patients, e.g., IgM rheumatoid factor (IgM-RF), anti–citrullinated protein antibodies (anti-CCP), C-reactive protein (CRP) and human leukocyte antigen B27 (HLA-B27) [27–29]. However, for individual patients, these few biomarkers cannot differentiate a patient from a healthy subject with high specificity or predict mild versus severe disease. Radiographic imaging is used routinely to assess cumulated joint damage, however, biomarkers have the potential of being a more feasible, specific, and reproducible tool for both diagnostic and prognostic purposes and for the monitoring of treatment and disease progression.

The present protocol is an observational, prospective, translational research study of rheumatologic patients followed in the nationwide Danish DANBIO registry [30] and the Danish Rheumatologic Biobank [31,32]. The objective is to identify new diagnostic, prognostic and predictive biomarkers, which can be used to 1) diagnose inflammatory rheumatic diseases early in the disease course with high sensitivity and specificity, 2) predict patient prognosis regardless of treatment, or 3) predict and monitor the effective

treatment for the individual patient with minimised risk of side effects. This protocol has been prepared and presented according to the REMARK and STROBE guidelines [13,33].

MATERIALS AND METHODS

Study design and setting

Biological samples and clinical data are collected prospectively in rheumatic patients treated in routine care. Clinical data and outcomes are registered in the Danish nationwide quality registry DANBIO [30,34] and biological samples are collected via the Danish Rheumatologic Biobank [31,32]. Patient inclusion started in May 2015 and will continue until 2025 with follow-up until 2030. If needed, the inclusion period can be expanded.

DANBIO was established in year 2000 and data collection occurs prospectively by a web-based system used in routine care at Danish hospital Departments of Rheumatology and in primary care (private practising specialists of rheumatology). It is mandatory to monitor patients with inflammatory rheumatic diseases treated with bDMARDs and patients with newly diagnosed RA irrespective of treatment [34]. Data registered in DANBIO are listed in the "Clinical data" section below. DANBIO represents an excellent tool for monitoring patients in routine care and for research purposes.

The Danish Rheumatologic Biobank was established in 2015 through nationwide collaboration between Departments of Rheumatology and Departments of Clinical Biochemistry in Denmark. The Danish Rheumatologic Biobank is organised according to the infrastructure of the well-established Danish CancerBiobank [31], and both biobanks are part of the Bio- and Genome Bank Denmark funded by Danish Regions (the governmental organisation who runs the public hospitals in DK). The foundation of the Danish Rheumatologic Biobank was funded by the Danish Rheumatism Association and Danish Regions. By June 1st, 2017, 12 hospitals from all parts of Denmark (Rigshospitalet; North Denmark Regional Hospital; King Christian 10th Hospital for Rheumatic Diseases, Graasten; Aarhus University Hospital; Copenhagen University Hospital, Gentofte; Zealand University Hospital, Køge; OUH Svendborg Hospital; Odense University Hospital; Aalborg University Hospital; Hospital Lillebaelt, Vejle; Randers Regional Hospital; and University Hospital Bispebjerg and Frederiksberg) participated in the Danish Rheumatologic Biobank, and

additional hospitals are continuously joining. Different types of biological material (e.g. blood, tissue, synovial fluid, and urine) are collected, handled and stored according to nationally approved Standard Operating Procedures (SOPs) [31] (see section "Biological Samples").

Patients contribute primarily with blood samples, but also other types of biological materials (synovial fluid and surgical tissue), when these are accessible and relevant. Patients contribute with one or more of the following samples: 1) cross-sectional blood samples: patients provide one cross-sectional sample when they meet for a scheduled routine clinical visit (Figure 1A); 2) longitudinal blood samples: patients may be enrolled for longitudinal follow-up when they start treatment with a new DMARD. Switching from csDMARD to bDMARD, or from one bDMARD to another bDMARD, indicates a new baseline sample (Figure 1B); and 3) other biological materials: Patients may contribute with representative samples of biologic material if they are scheduled for joint puncture with aspiration (synovial fluid), surgery, or biopsies (synovia, cartilage, bone, bone-marrow or other tissues). Cross-sectional sampling may be done at any given disease stage and at any time point during treatment. Longitudinal blood samples are collected at baseline and after 3, 6, 12, 24, 36, 48 and 60 months of treatment. In case of serious adverse events or treatment withdrawal, additional blood sampling is performed (Figure 1B). Approximately half of the material will be used for the present study. The other half can be made available, according to guidelines in the Bio- and Genome Bank Denmark, to other researchers who wish to cooperate.

The present protocol is designed to investigate a broad range of biomarkers in patients with inflammatory rheumatic diseases. One of the first longitudinal cohorts in the study included patients who switched from originator Infliximab (IFX, Remicade) to biosimilar IFX (CT-P13, Remsima). According to national guidelines issued in 2015, all Danish patients diagnosed with inflammatory rheumatic diseases (RA, PsA, or AxSpA), and treated with originator IFX, were switched to CT-P13 [35]. In association with this non-medical switch, the aim was to investigate the following biomarkers and clinical outcome: 1) effects of the switch on serum IFX (sIFX) and presence of anti-drug antibodies (ADA), and 2) association between sIFX and ADA at the time of switch on adherence to CT-P13 treatment [36]. Clinical data and longitudinal blood samples were collected as described in the present protocol.

Participants

The Biomarker Protocol is an open cohort study, i.e., participants may enter and leave the population at different time points during monitoring. Patients are eligible for inclusion if they are followed in routine care and monitored in DANBIO with one of the following diagnoses: RA, AxSpA, PsA, other inflammatory rheumatic diseases or tissue disorders, or are suspected for one of the above. Patients must be able to give written and oral informed consent and be aged ≥18 years. There are no exclusion criteria. Patient inclusion and follow-up will be performed by nurses and physicians when the patients meet for scheduled routine clinical visits. The number of potentially eligible patients for the study is shown in Figure 1.

Clinical data

At the time of inclusion the following clinical data are collected in DANBIO [30,34,37]:

1) Patient demographics: e.g. age, gender, body weight, diagnosis, and disease duration
2) Exposures: i.e. previous and current treatment with DMARDs including dosing schedule, start and stop date, and reason for treatment withdrawal
3) Outcomes: patient reported outcomes (e.g. visual analogue scales (VAS) for pain, fatigue, patient's global, Health Assessment Questionnaire (HAQ), quality of life), Disease Activity Score 28-joints (DAS28), serum CRP concentration, radiographic status (for RA: erosions on X-rays of hand or feet), and bone mineral density (BMD). In axial disease: Bath Ankylosing Spondylitis (BAS)-scores for disease activity (BASDAI), function (BASFI), and metrology index (BASMI) are registered
4) Comorbidities and lifestyle factors: serum cholesterol, diabetes, blood pressure, cardiovascular disease or other comorbidities, smoking status, and exercise habits

Upon every new collection of biological material, exposure and outcome data are re-evaluated and registered within 30 days before/after the collection of biological material. Any prescription of medical treatment and the monitoring of disease status (radiographic status, BMD, etc.) are done as part of routine care and do not follow a specific study protocol. Data registration in the DANBIO registry follows DANBIO guidelines [34,37].

Biological samples

The collected biological material is primarily blood. Synovial fluid, tissue, cartilage, bone and bone marrow may also be collected, when accessible and relevant. Peripheral blood is collected in one EDTA tube (9 ml), two serum tubes (2x9 ml), and one PAXgene blood RNA tube (2.5 ml, Becton & Dickinson, Lyngby, Denmark). Blood samples are processed according to the nationally approved SOP for blood (Figure 2) [31]. In brief, EDTA whole blood (1.5 ml) is isolated followed by the centrifugation of EDTA and serum tubes at 2000xg and 4˚C for 10 min. After centrifugation 2x2 ml EDTA plasma, 1x EDTA buffy coat and 4x2 ml serum are isolated. PAXgene Blood RNA tubes are kept at room temperature for 2-72 hours, hereafter frozen at -20˚C for 24-72 hours, and finally stored long term at -80˚C. Whole blood and buffy coat are stored at ≤-20˚C; plasma and serum are stored at -80˚C.

Synovial fluid is collected in EDTA tubes (9 ml) and centrifuged at 2000xg and 4˚C for 10 min. The cell-free supernatant is transferred to 5 ml cryotubes and each cell-pellet is resuspended in 1 ml supernatant and pooled in 5 ml cryotubes. Sample processing results in: ≤20x5 ml cell-free synovial fluid and ≤2x5 ml cell-pellet, which are stored long term at -80˚C.

Pre-analytical factors such as date and time of sampling, handling and storage, temperature during transportation, and the exact handling procedure are registered in the nationwide Bio- and Genome Bank Denmark registry. All samples are pseudonymised before storage.

Assay methods

The protocol aims to investigate the following biomarkers in blood, synovial fluid, or tissue:

1) Genetic variation using Next Generation Sequencing (NGS) and Whole Genome Sequencing (WGS), and RNA and miRNA expression profiles
2) Protein biomarker profiles of inflammation, and bone- and cartilage-metabolism, using, e.g., the Multi Biomarker Disease Activity (MBDA) score (a panel of 12 proteins) [38] (Cresendo Bioscience Inc., South San Francisco, CA, USA), Proseek Multiplex protein arrays (panels of 92 proteins) (Olink Proteomics, Uppsala, Sweden, www.olink.com), or proteomics platforms, such as mass spectrometry, protein-arrays, or multiplexed-ELISA

3) Metabolites using Nuclear Magnetic Resonance (NMR)-spectroscopy

4) ADA against IFX and IFX-drug concentrations using a target-based assay fully automated on the AutoDELFIA® (PerkinElmer, Waltham, MA, USA) immunoassay platform (Oslo University Hospital, Radiumhospitalet, Oslo, Norway)

All samples will be analysed in pseudonymised form to ensure blinded testing by the laboratory personnel. The list of specific diagnostic, prognostic, and predictive biomarkers will be updated continuously according to new discoveries. The methods for biomarker analysis are rapidly expanding and improving, and the best available method will be used at time of analysis.

Statistical methods

For the longitudinal samples it is expected that the numbers collected during a 10-year period will provide sufficient statistical power to identify prognostic and predictive biomarkers if these are present among >10% of the patients. Knowledge within the field is still insufficient, thus, it is not possible to perform a comprehensive power calculation; this will, however, be performed before any biomarker analysis is done.

In general, statistical analyses will be done according to available data; the following statistical tests may be used (the list is not complete): comparison of group demographics will be done with Student's t-test, Pearson's chi-square test or Mann-Whitney U-test according to the distribution of data. Due to the large size of the dataset the probability for type II error in testing the hypothesis will be low. Treatment duration and time to event can be explored with Kaplan-Meier curves, log-rank statistics and Cox regression analyses. Treatment outcomes across groups or according to specific biomarkers will be analysed with logistic regression analyses. Multivariable analyses will be performed in order to study the impact of potential confounders. These confounders may be identified in the DANBIO registry (gender, age, smoking status, or other baseline characteristics). All included patients are recruited and treated in routine care across Denmark and this will inevitably lead to some missing data (missing sampling of biological material, missing registration of corresponding clinical data, whenever biological material is collected, patient lost to follow-up, etc.). For sensitivity, various statistical methods may be applied in order to test the robustness of the results. This may be done as last observation carried forward in case of lacking data on clinical outcomes,

non-responder imputation, or statistical multiple imputation of missing data. Statistical expertise will be included when necessary.

ETHICS AND DISSEMINATION

The protocol is approved by the Danish Ethics Committee (H-2-2014-086, supplementary protocol 49419) and The Danish Data Protection Agency (RH-2015-297, I-Suite 04318). The Danish Rheumatologic Biobank is approved by The Danish Data Protection Agency (GLO-2015-6, I-Suite 03490). All patients receive verbal and written information before enrolment, and give oral and written consent at baseline according to the guidelines from the Danish Ethics Committee. All patients are informed that they can withdraw from the study at any time without it having consequences for their treatment. In case of withdrawal, samples are discarded and all patient-related registrations deleted from the Bio- and Genome Bank Denmark registry.

The sampled volume of blood for the study is 26.5 ml per patient-visit and maximum 240 ml/year. The sampling of blood for the study is performed simultaneously with scheduled routine blood sampling, thus minimizing the discomfort for the patient. Synovial fluid, surgical tissue, or bone marrow will only be collected if relevant interventions occur as part of routine care and surplus material, not used for diagnostic or therapeutic purposes, is available. The patients will be contacted and informed regarding the overall study results if they indicate interest in this in the patient study consent form. Direct feedback to the patient may be relevant in case of the discovery of mutations in known disease-linked genes, or as random discoveries, and will occur according to the guidelines directed by the Danish Ethics Committee (document number 1293688, October 2013). The physician in charge of the project at the individual department is responsible for conducting the study in accordance with the Helsinki declaration. Study participation does not affect the treatment course of individual patients and the patients will be treated according to clinical practise.

Due to the large number of included patients, it will be possible to perform exploratory as well as validation biomarker studies. We plan to evaluate and publish study results according to the REMARK [13], STROBE [33], and STARD [39] guidelines. Results will be published in international and peer-reviewed scientific journals and presented at international conferences. Negative, positive as well as inconclusive results will be published. If relevant, collaborations with international researchers will be established to

facilitate the right expertise for biomarker analyses. The first results (measurements of s-IFX and ADA drug levels up to one year after switch from originator to biosimilar IFX) have been presented [36,40].

STUDY STATUS

Patient recruitment started in May 2015 and is expected to continue until January 1st, 2025, with follow-up until January 1st, 2030. Currently, ≈3,000 patients have been enrolled in the study and >5,000 blood samples have been collected.

DISCUSSION AND POTENTIAL LIMITATIONS

In this observational, prospective, and translational biomarker study of patients with inflammatory rheumatic diseases, blood samples are collected in routine care and closely linked to extensive clinical data regarding rheumatic disease status and activity, medical treatment, treatment efficacy and adverse events, and comorbidities. The study protocol allows for a large-scale collection of blood and other biological materials with the aim to identify new biomarkers that can be used for improved personalised treatment of patients with inflammatory rheumatic diseases. Additionally, the nationwide collection of biological materials and clinical data is intended to further promote research collaboration within inflammatory rheumatic diseases, both nationally and internationally, in order to ensure research of the highest quality for the benefit of the patients.

Positivity for IgM-RF and anti-CCP are established risk factors for development of RA, and they are currently used as part of classification criteria and as prognostic markers [3]. In AxSpA, HLA-B27 is part of the disease classification [41]. Apart from the erythrocyte sedimentation rate and serum-CRP level, no biomarkers are used in routine care, and they cannot predict treatment responses or side effects. The wide range of currently available and future bDMARDs with different modes of action for the treatment of inflammatory arthritis, and the recent introduction of biosimilars and tsDMARDS, stresses the importance of improved ability to select the most effective treatment in the individual patient. Development of diagnostic, prognostic and predictive biomarkers will benefit the treatment of future patients and facilitate personalised medicine.

Patient recruitment and follow-up in routine care will lead to some limitations in clinical and biological data. Since patients are recruited across several rheumatic diagnoses and treatments, patient inclusion may take some time in order to obtain enough samples for a specific research question. However, since it is mandatory to register patients receiving biological treatment in DANBIO, coverage is high (≈96%) [42] and the risk of selection bias low. The risks for the patient are minimal and are out-weighted by the benefits for future patients. The non-randomised study design inherits the risks of confounding, and thorough statistical analysis and confounder adjustment is therefore important. On the other hand, the wide recruitment of patients treated in routine care may provide valuable data on, e.g., elderly patients with comorbidities. This may be a valuable supplement to data generated in randomised trials.

Hopefully, the results of the present study will provide us with new biomarkers that will improve our ability to a) diagnose rheumatic diseases more accurately and at an earlier stage, b) prognosticate the development of rheumatic diseases, and c) predict and monitor treatment effectiveness in the individual patient (personalised treatment).

Researchers, who are interested in collaboration regarding samples and/or clinical data from DANBIO should contact the Danish Rheumatologic Biobank [31] and DANBIO [30], respectively.

Funding

The Danish Rheumatism Association and Danish Regions funded the establishment of The Danish Rheumatologic Biobank. Danish Regions are funding the continued collection of biological samples and clinical data. The funders will have no influence on study design, data analysis, interpretation of results, or publications. Biomarker analyses will be funded by research grants.

Contributors

MLH and JJ wrote the protocol for the Research Ethics Committee. MLH, JJ, EH, TK and BG contributed to study concept. TK and BG drafted and revised the manuscript after feedback from all authors. All authors contributed to review of the present manuscript and approved the final version of the manuscript.

Competing interests

None

Provenance and peer review

Not commissioned; externally peer review.

Acknowledgements

The Danish Rheumatologic Biobank is acknowledged for handling and storage of biological material. Lab technician Teresa Rozenfeld and IT specialist Niels Steen Krogh are acknowledged for their assistance. The Biomarker Protocol Study Group, listed below, is acknowledged for their contribution to data registration or sample collection, and for review of the present manuscript: Asta Linauskas, North Denmark Regional Hospital; Gitte Meyer Larsen, North Denmark Regional Hospital; Anne Gitte Loft, Aarhus University Hospital; Charlotte Modin, Aarhus University Hospital; Karin Skovgaard, Aarhus University Hospital; Inge Juul Sørensen, Rigshospitalet; Britt Corfixen, Rigshospitalet; Oliver Hendricks, King Christian 10th Hospital for Rheumatic Diseases, Graasten; Charlotte Drachmann, King Christian 10th Hospital for Rheumatic Diseases, Graasten; Hanne Lindegaard, Odense University Hospital; Marina Bjørling-Poulsen, Odense University Hospital; Lene Dreyer, Copenhagen University Hospital, Gentofte; Johnny Raun, Hospital Lillebaelt, Kolding; Mikkel Østergaard, Rigshospitalet; Kim Hørslev-Petersen, King Christian 10th Hospital for Rheumatic Diseases, Graasten; Henning Bliddal, Frederiksberg Hospital; Claus Rasmussen, North Denmark Regional Hospital; Torkell Ellingsen, Odense University Hospital; Søren Jacobsen, Rigshospitalet; Tove Lorenzen, Silkeborg Regional Hospital; Inger Marie Jensen Hansen, OUH Svendborg Hospital; Annette Schlemmer, Aalborg University Hospital; Salome Kristensen, Aalborg University Hospital; Kristian Stengaard-Pedersen, Aarhus University Hospital; Michael Stoltenberg, Zealand University Hospital, Køge; Henrik Nordin, Zealand University Hospital, Køge; Peter Mosborg, Randers Regional Hospital; Susanne Juhl Pedersen, Rigshospitalet; Christoffer Tandrup Nielsen, Rigshospitalet; Ellen-Margrethe Hauge, Aarhus University Hospital; Bent Deleuran, Aarhus University; Cecilie Brahe, Rigshospitalet; Christian Gytz Ammitzbøll, Aarhus University Hospital; Tue Wenzel Kragstrup, Aarhus University; Karen Carlsen, North

Zealand University Hospital, Hillerød; Niklas Rye Jørgensen, Rigshospitalet; Dorte Vendelboe Jensen, Copenhagen University Hospital, Gentofte; Heidi Lausten Munk, Odense University Hospital; Michael Kruse Meyer, North Denmark Regional Hospital; and Tine Lottenburger, Hospital Lillebaelt, Vejle.

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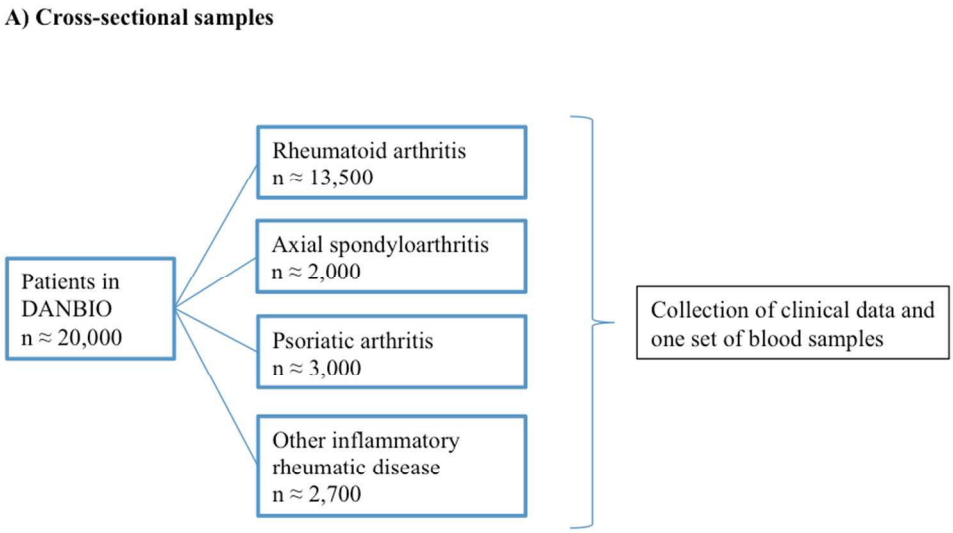

Figure 1 Schematic presentation of study design and sampling strategies. Any patient diagnosed with RA, AxSpA, PsA, other inflammatory rheumatic disease or tissue disorder, or suspected for one of these, may participate when they meet for a scheduled routine clinical visit. These patients can provide one cross-sectional blood sample (A), or may be included for longitudinal follow-up (B) when they start treatment with a new DMARD (see text). Numbers (n) indicate patients potentially eligible for inclusion in one or more of the study arms.

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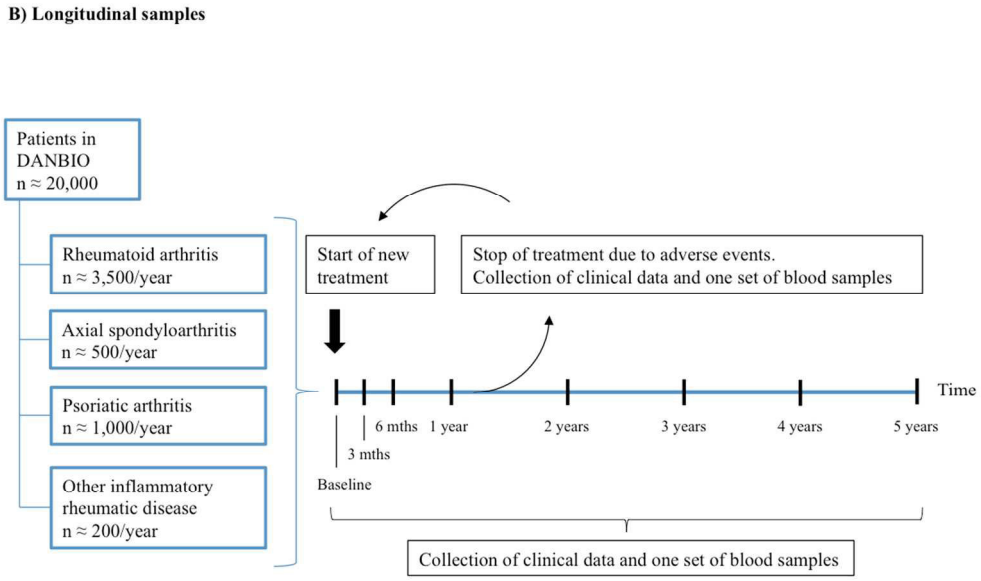

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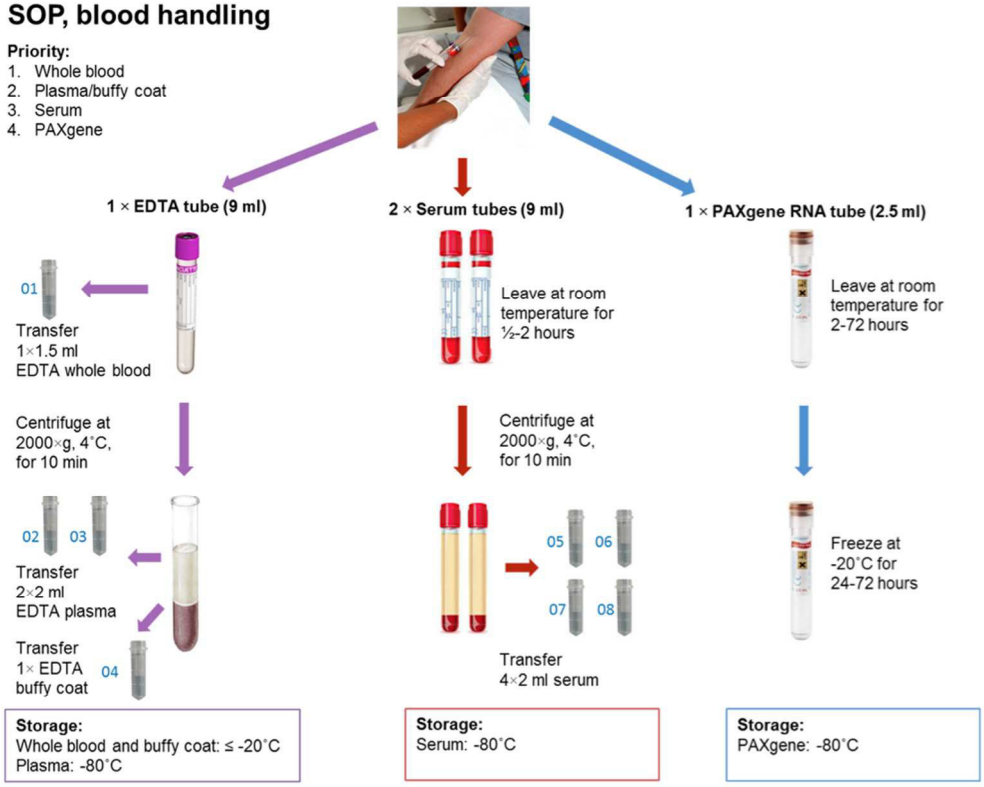

Figure 2 Standard Operating Procedure (SOP) for blood handling in the Danish Rheumatologic Biobank [31]. Peripheral blood is collected in one EDTA tube, two serum tubes, and one PAXgene blood RNA tube. Serum tubes coagulate at room temperature for 30 min to 2 hours. From the EDTA tube, 1.5 ml whole blood is isolated. EDTA and serum tubes are centrifuged at 2000xg and 4˚C for 10 min. EDTA plasma (2x2ml), EDTA buffy coat and serum (4x2ml) are isolated. Processed blood samples are stored at ≤-20˚C. PAXgene RNA tubes are kept at room temperature for 2-72 hours, then frozen at -20˚C for 24-72 hours and stored at -80˚C.

355x283mm (72 x 72 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1 and 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not relevant
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8 and 10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	8 + 10 and Fig 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	(not relevant yet, the study is still recruiting)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	10-11
		(*d*) If applicable, explain how loss to follow-up was addressed	10-11
		(*e*) Describe any sensitivity analyses	10-11

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	(not relevant yet, the study is still recruiting)
		(b) Give reasons for non-participation at each stage	(not relevant yet, the study is still recruiting)
		(c) Consider use of a flow diagram	(not relevant yet, the study is still recruiting)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	(not relevant yet, the study is still recruiting)
		(b) Indicate number of participants with missing data for each variable of interest	(not relevant yet, the study is still recruiting)
		(c) Summarise follow-up time (eg, average and total amount)	(not relevant yet, the study is still recruiting)
Outcome data	15*	Report numbers of outcome events or summary measures over time	(not relevant yet, the study is still recruiting)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	(not relevant yet, the study is still recruiting)
		(*b*) Report category boundaries when continuous variables were categorized	(not relevant yet, the study is still recruiting)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	(not relevant yet, the study is still recruiting)

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	(not relevant yet, the study is still recruiting)
Discussion			
Key results	18	Summarise key results with reference to study objectives	(not relevant yet, the study is still recruiting)
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	(not relevant yet, the study is still recruiting)
Generalisability	21	Discuss the generalisability (external validity) of the study results	(not relevant yet, the study is still recruiting)
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Identification of new biomarkers to promote personalized treatment of patients with inflammatory rheumatic disease: protocol for an open cohort study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-019325.R1
Article Type:	Protocol
Date Submitted by the Author:	13-Nov-2017
Complete List of Authors:	Kringelbach, Tina; Copenhagen University Hospital Herlev, Bio- and Genome Bank Denmark and the Danish Rheumatologic Biobank, The Molecular Unit, Department of Pathology Glintborg, B.; Rigshospitalet Glostrup, Copenhagen Centre for Arthritis Research, Center for Rheumatology and Spine Diseases, the Danish DANBIO Registry, and the Danish Rheumatologic Biobank Hogdall, Estrid; Copenhagen University Hospital Herlev, Bio- and Genome Bank Denmark and the Danish Rheumatologic Biobank, The Molecular Unit, Department of Pathology; University of Copenhagen, Department of Clinical Medicine, Faculty of Health and Medical Sciences Johansen, Julia; Copenhagen University Hospital Herlev, Departments of Medicine and Oncology, and the Danish Rheumatologic Biobank; University of Copenhagen, Department of Clinical Medicine, Faculty of Health and Medical Sciences Lund Hetland, Merete; Rigshospitalet Glostrup, Copenhagen Centre for Arthritis Research, Center for Rheumatology and Spine Diseases, the Danish DANBIO Registry, and the Danish Rheumatologic Biobank; University of Copenhagen, Department of Clinical Medicine, Faculty of Health and Medical Sciences
Primary Subject Heading:	Rheumatology
Secondary Subject Heading:	Epidemiology
Keywords:	Inflammatory rheumatic disease, Personalised treatment, Biomarkers, Danish Rheumatologic Biobank, RHEUMATOLOGY

Identification of new biomarkers to promote personalized treatment of patients with inflammatory rheumatic disease: protocol for an open cohort study.

Running title: A Rheumatologic Biomarker Protocol

T M Kringelbach[1,2,#], B Glintborg[1,3,4,#], E V Hogdall[1,2,5], J Johansen[1,5,6], M L Hetland[1,3,4,5], and The Biomarker Protocol Study Group[7]

[#] T M Kringelbach and B Glintborg contributed equally to this paper and share first co-authorship

[1]The Danish Rheumatologic Biobank

[2]Bio- and Genome Bank Denmark, The Molecular Unit, Department of Pathology, Copenhagen University Hospital Herlev, Herlev, Denmark

[3]Copenhagen Centre for Arthritis Research, Center for Rheumatology and Spine Diseases, Rigshospitalet, Glostrup, Denmark

[4]The Danish DANBIO registry, Rigshospitalet, Glostrup, Denmark

[5]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

[6]Departments of Medicine and Oncology, Copenhagen University Hospital Herlev, Herlev, Denmark

[7]The Biomarker Protocol Study Group is defined under acknowledgements

ClinicalTrials.gov ID: NCT03214263

Corresponding author: Merete L. Hetland, Department of Rheumatology, Rigshospitalet Glostrup, Glostrup, Denmark, phone: 0045-38633330, e-mail: merete.hetland@dadlnet.dk

Author disclosures:

B Glintborg: Biogen, AbbVie

M L Hetland: Orion, BMS, AbbVie, Biogen, Pfizer, MSD, Roche, Celltrion, Crescendo, UCB

The remaining authors: none declared

Word count:

Abstract: 293 words

Text: 3,708 words

ABSTRACT

Introduction: The introduction of biological disease modifying anti-rheumatic drugs (bDMARDs) has improved the treatment of inflammatory rheumatic diseases dramatically. However, bDMARD treatment failure occurs in 30-40% of patients due to lack of effect or adverse events, and the tools to predict treatment outcomes in individual patients are currently limited. The objective of the present study is to identify diagnostic, prognostic and predictive biomarkers, which can be used to 1) diagnose inflammatory rheumatic diseases early in the disease course with high sensitivity and specificity, 2) improve prognostication, or 3) predict and monitor treatment effectiveness and tolerability for the individual patient.

Methods and analysis: The present study is an observational and translational open cohort study with prospective collection of clinical data and biological materials (primarily blood) in patients with inflammatory rheumatic diseases treated in routine care. Patients contribute with one cross-sectional blood sample and/or are enrolled for longitudinal follow-up upon initiation of a new DMARD (blood sampling after 0, 3, 6, 12, 24, 36, 48, 60 months of treatment). Other biological materials will be collected when accessible and relevant. Demographics, disease characteristics, comorbidities, and lifestyle factors are registered at inclusion; DMARD treatment and outcomes are collected repeatedly during follow-up. Currently (July 2017), >5,000 samples from ≈3,000 patients have been collected. Data will be analysed using appropriate statistical analyses.

Ethics and dissemination: The protocol is approved by the Danish Ethics Committee and The Danish Data Protection Agency. Participants give written and oral informed consent. Biomarkers will be evaluated and published according to the REporting recommendations for tumour MARKer prognostic studies (REMARK), STrengthening the Reporting of OBservational studies in Epidemiology (STROBE), and the Standards for Reporting of Diagnostic Accuracy (STARD) guidelines. Results will be published in peer-reviewed scientific journals and presented at international conferences.

ClinicalTrials.gov ID: NCT03214263

Strengths and limitations of this study

- Nation-wide collection of biological materials and corresponding extensive clinical data provides the opportunity to discover and/or validate a wide range of diagnostic, prognostic, and predictive biomarkers in patients with inflammatory rheumatic disease
- Recruitment of patients treated in routine care is expected to provide valuable data on "real life patients" (e.g. elderly patients with comorbidities), which are different from the more homogeneous patient population in randomised controlled trials
- Standardised collection of samples and quality control ensures comparability between samples from different departments, and enables research in less common rheumatic diseases
- Patient recruitment and follow-up in routine care and across several rheumatic diagnoses and treatments will be associated with some limitations in clinical and biological data
- The non-randomised study design inherits a risk of confounding and thorough statistical analysis and confounder adjustment is therefore important

INTRODUCTION

Rheumatoid arthritis (RA), psoriatic arthritis (PsA), and axial spondyloarthritis (AxSpA) are examples of chronic inflammatory rheumatic diseases characterised by pain, disability, and progressive decrease in workability, and are associated with comorbidity and risk of early death [1,2]. The impact of these chronic diseases on the patients should be minimized through early diagnosis followed by targeted therapy with minimal side effects. If and when remission is achieved, the patient has the potential to maintain a life with few restrictions – a desirable outcome both for the patient and for society.

The medical treatment of inflammatory rheumatic diseases has improved dramatically during the last decades. This is mainly due to an increased acknowledgement of the treat-to-target concept with conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) in RA, which implies strict monitoring and aggressive treatment strategies with add-on or switching of therapy according to clinical response or side effects [3–5]. Furthermore, the biological DMARDs (bDMARDs), e.g. tumour necrosis factor alpha inhibitors, or other specific modulators of inflammatory signal transduction, have improved outcomes for patients otherwise refractory to treatment with csDMARDs [4]. New treatment modalities including targeted synthetic DMARDs (tsDMARDS, e.g. Janus Kinase (JAK) inhibitors) are being introduced, and the first biosimilar bDMARDs have been marketed. Biological DMARDs and JAK inhibitors are expensive, and treatment failure, defined as lack of effect or serious adverse events, occurs in 30-40% of patients treated with bDMARDs [6,7]. Tools to predict treatment outcomes and side effects in the individual patient are currently limited [8].

In 1998, the National Institutes of Health Biomarkers Definitions Working Group defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” [9,10]. Biomarkers are divided in three categories: 1) diagnostic biomarkers, which may be used for early diagnosis of a given disease [11,12]. The ideal diagnostic biomarker should establish the correct diagnosis with high sensitivity and specificity; 2) prognostic biomarkers, which correlate with specific clinical outcomes, and thus progression of disease, regardless of any treatment; and 3) predictive biomarkers, which may be used to predict whether a given patient may benefit from a given treatment [13,14]. Hence, biomarkers may be

promising tools to personalise the treatment of patients with inflammatory rheumatic disease. Biomarkers in blood and tissue include a wide range of molecules with different characteristics such as DNA, RNA, microRNA (miRNA), proteins, and metabolites. Genetic variation can be caused by single nucleotide polymorphisms (SNPs), nucleotide insertions/deletions, and Copy Number Variations (CNVs), among which the SNP and insertions/deletions are the most common types of genetic variation [15–19]. MicroRNAs are small single-stranded, endogenous, non-coding RNAs (18-25 nucleotides) and play essential roles in regulating gene expression, cell development, differentiation, and proliferation [19–21]. The human proteome constitute all expressed human proteins and reflects the biological activity of the patient, and proteomics is increasingly used to investigate treatment response [22,23] or to stratify responding versus non-responding patients [24,25]. The metabolome is defined as the complete set of metabolites <1500 daltons found in a given biological sample. It is a dynamic entity, which reflects the interaction between the individual genetic background and factors such as pathophysiological conditions, diet, and pharmacologic treatment [26]. In patients with RA, PsA, and AxSpA these biomarkers may be related to the disease itself, the associated inflammation or treatment-related pharmacokinetics. Biomarkers can be detected in peripheral blood, synovial fluid, circulating cells or cell-free DNA in plasma, or in tissue (e.g. cartilage, bone, and synovial membrane).

Currently, some biomarkers are used as part of the classification of arthritis patients, e.g., IgM rheumatoid factor (IgM-RF), anti–citrullinated protein antibodies (anti-CCP), C-reactive protein (CRP) and human leukocyte antigen B27 (HLA-B27) [27–29]. However, for individual patients, these few biomarkers cannot differentiate a patient from a healthy subject with high specificity or predict mild versus severe disease. Radiographic imaging is used routinely to assess cumulated joint damage, however, biomarkers have the potential of being a more feasible, specific, and reproducible tool for both diagnostic and prognostic purposes and for the monitoring of treatment and disease progression.

The present protocol is an observational, prospective, translational research study of rheumatologic patients followed in the nationwide Danish DANBIO registry [30] and the Danish Rheumatologic Biobank [31,32]. The objective is to identify new diagnostic, prognostic and predictive biomarkers, which can be used to 1) diagnose inflammatory rheumatic diseases early in the disease course with high sensitivity and

specificity, 2) predict patient prognosis regardless of treatment, or 3) predict and monitor the effective treatment for the individual patient with minimised risk of side effects. This protocol has been prepared and presented according to the REporting recommendations for tumour MARKer prognostic studies (REMARK) and STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines [13,33].

MATERIALS AND METHODS

Study design and setting

Biological samples and clinical data are collected prospectively in rheumatic patients treated in routine care. Clinical data and outcomes are registered in the Danish nationwide quality registry DANBIO [30,34] and biological samples are collected via the Danish Rheumatologic Biobank [31,32]. Patient inclusion started in May 2015 and will continue until 2025 with follow-up until 2030. If needed, the inclusion period can be expanded.

DANBIO is a nationwide, Danish register which serves as a clinical database for monitoring of clinical quality of treatment and which may be used for research purposes. DANBIO was established in year 2000 and data collection occurs prospectively by a web-based system used in routine care at Danish hospital Departments of Rheumatology and in primary care (private practising specialists of rheumatology). It is mandatory to monitor patients with inflammatory rheumatic diseases treated with bDMARDs and patients with newly diagnosed RA irrespective of treatment [34]. Data registered in DANBIO are listed in the "Clinical data" section below. DANBIO represents an excellent tool for monitoring patients in routine care and for research purposes.

The Danish Rheumatologic Biobank was established in 2015 through nationwide collaboration between Departments of Rheumatology and Departments of Clinical Biochemistry in Denmark. The Danish Rheumatologic Biobank is organised according to the infrastructure of the well-established Danish CancerBiobank [31], and both biobanks are part of the Bio- and Genome Bank Denmark funded by Danish Regions (the governmental organisation who runs the public hospitals in Denmark). The foundation of the Danish Rheumatologic Biobank was funded by the Danish Rheumatism Association and Danish Regions. By June 1st, 2017, 12 hospitals from all parts of Denmark (Rigshospitalet; North Denmark Regional Hospital;

King Christian 10th Hospital for Rheumatic Diseases, Graasten; Aarhus University Hospital; Copenhagen University Hospital, Gentofte; Zealand University Hospital, Køge; OUH Svendborg Hospital; Odense University Hospital; Aalborg University Hospital; Hospital Lillebaelt, Vejle; Randers Regional Hospital; and University Hospital Bispebjerg and Frederiksberg) participated in the Danish Rheumatologic Biobank, and additional hospitals are continuously joining. Different types of biological material (e.g. blood, tissue, synovial fluid, and urine) are collected, handled and stored according to nationally approved Standard Operating Procedures (SOPs) [31] (see section "Biological Samples").

Patients contribute primarily with blood samples, but also other types of biological materials (synovial fluid and surgical tissue), when these are accessible and relevant. Patients contribute with one or more of the following samples: 1) cross-sectional blood samples: patients provide one cross-sectional sample when they meet for a scheduled routine clinical visit (Figure 1A); 2) longitudinal blood samples: patients may be enrolled for longitudinal follow-up when they start treatment with a new DMARD. Switching from csDMARD to bDMARD, or from one bDMARD to another bDMARD, indicates a new baseline sample (Figure 1B); and 3) other biological materials: Patients may contribute with representative samples of biologic material if they are scheduled for joint puncture with aspiration (synovial fluid), surgery, or biopsies (synovia, cartilage, bone, bone-marrow or other tissues). Cross-sectional sampling may be done at any given disease stage and at any time point during treatment. Longitudinal blood samples are collected at baseline and after 3, 6, 12, 24, 36, 48 and 60 months of treatment. In case of serious adverse events or treatment withdrawal, additional blood sampling is performed (Figure 1B). Approximately half of the material will be used for the present study. The other half can be made available to other researchers, who wish to cooperate, according to guidelines in the Bio- and Genome Bank Denmark.

The present protocol is designed to investigate a broad range of biomarkers in patients with inflammatory rheumatic diseases. One of the first longitudinal cohorts in the study included patients who switched from originator Infliximab (IFX, Remicade) to biosimilar IFX (CT-P13, Remsima). According to national guidelines issued in 2015, all Danish patients diagnosed with inflammatory rheumatic diseases (RA, PsA, or AxSpA), and treated with originator IFX, were switched to CT-P13 [35]. In association with this non-medical switch, the aim was to investigate the following biomarkers and clinical outcome: 1) effects of

the switch on serum IFX (sIFX) and presence of anti-drug antibodies (ADAb), and 2) association between sIFX and ADAb at the time of switch on adherence to CT-P13 treatment [36]. Clinical data and longitudinal blood samples were collected as described in the present protocol.

Participants

The Biomarker Protocol is an open cohort study, i.e., participants may enter and leave the population at different time points during monitoring. Patients are eligible for inclusion if they are followed in routine care and monitored in DANBIO with one of the following diagnoses: RA, AxSpA, PsA, other inflammatory rheumatic diseases or tissue disorders, or are suspected for one of the above. Patients must be able to give written and oral informed consent and be aged ≥18 years. There are no exclusion criteria. Patient inclusion and follow-up will be performed by nurses and physicians when the patients meet for scheduled routine clinical visits. The number of potentially eligible patients for the study is shown in Figure 1.

Clinical data

At the time of inclusion the following clinical data are collected in DANBIO [30,34,37]:

1) Patient demographics: e.g. age, gender, body weight, diagnosis, and disease duration
2) Exposures: i.e. previous and current treatment with corticosteroids, non-steroid anti-inflammatory drugs (NSAIDs) and DMARDs including dosing schedule, start and stop date, and reason for treatment withdrawal
3) Outcomes: patient reported outcomes (e.g. visual analogue scales (VAS) for pain, fatigue, patient's global, Health Assessment Questionnaire (HAQ), quality of life), Disease Activity Score 28-joints (DAS28), serum CRP concentration, radiographic status (for RA: erosions on X-rays of hand or feet), and bone mineral density (BMD). In axial disease: Bath Ankylosing Spondylitis (BAS)-scores for disease activity (BASDAI), function (BASFI), and metrology index (BASMI) are registered
4) Comorbidities and lifestyle factors: serum cholesterol, diabetes, blood pressure, cardiovascular disease or other comorbidities, smoking status, and exercise habits

Upon every new collection of biological material, exposure and outcome data are re-evaluated and registered within 30 days before/after the collection of biological material. Any prescription of medical treatment and the monitoring of disease status (radiographic status, BMD, etc.) are done as part of routine care and do not follow a specific study protocol. Data registration in the DANBIO registry follows DANBIO guidelines [34,37].

Biological samples

The collected biological material is primarily blood. Synovial fluid, tissue, cartilage, bone and bone marrow may also be collected, when accessible and relevant. Peripheral blood is collected in one EDTA tube (9 ml), two serum tubes (2x9 ml), and one PAXgene blood RNA tube (2.5 ml, Becton & Dickinson, Lyngby, Denmark). Blood samples are processed according to the nationally approved SOP for blood (Figure 2) [31]. In brief, EDTA whole blood (1.5 ml) is isolated followed by the centrifugation of EDTA and serum tubes at 2000xg and 4˚C for 10 min. After centrifugation 2x2 ml EDTA plasma, 1x EDTA buffy coat and 4x2 ml serum are isolated. PAXgene blood RNA tubes are kept at room temperature for 2-72 hours, hereafter frozen at -20˚C for 24-72 hours, and finally stored long term at -80˚C. Whole blood and buffy coat are stored at ≤-20˚C; plasma and serum are stored at -80˚C.

Synovial fluid is collected in EDTA tubes (9 ml) and centrifuged at 2000xg and 4˚C for 10 min. The cell-free supernatant is transferred to 5 ml cryotubes and each cell-pellet is resuspended in 1 ml supernatant and pooled in 5 ml cryotubes. Sample processing results in: ≤20x5 ml cell-free synovial fluid and ≤2x5 ml cell-pellet, which are stored long term at -80˚C.

Pre-analytical factors such as date and time of sampling, handling and storage, temperature during transportation, and the exact handling procedure are registered in the nationwide Bio- and Genome Bank Denmark registry. All samples are pseudonymised before storage.

Assay methods

The protocol aims to investigate the following biomarkers in blood, synovial fluid, or tissue:

1) Genetic variation using Next Generation Sequencing (NGS) and Whole Genome Sequencing (WGS), and RNA and miRNA expression profiles
2) Protein biomarker profiles of inflammation, and bone- and cartilage-metabolism, using, e.g., the Multi Biomarker Disease Activity (MBDA) score (a panel of 12 proteins) [38] (Cresendo Bioscience Inc., South San Francisco, CA, USA), Proseek Multiplex protein arrays (panels of 92 proteins) (Olink Proteomics, Uppsala, Sweden, www.olink.com), or proteomics platforms, such as mass spectrometry, protein-arrays, or multiplexed-ELISA
3) Metabolites using Nuclear Magnetic Resonance (NMR)-spectroscopy
4) ADAb against bDMARD and drug concentrations (e.g. IFX) using a target-based assay fully automated on the AutoDELFIA® (PerkinElmer, Waltham, MA, USA) immunoassay platform (Oslo University Hospital, Radiumhospitalet, Oslo, Norway)

All samples will be analysed in pseudonymised form to ensure blinded testing by the laboratory personnel. The list of specific diagnostic, prognostic, and predictive biomarkers will be updated continuously according to new discoveries. The methods for biomarker analysis are rapidly expanding and improving, and the best available method will be used at time of analysis.

Statistical methods

For the longitudinal samples it is expected that the numbers collected during a 10-year period will provide sufficient statistical power to identify prognostic and predictive biomarkers if these are present among >10% of the patients. Knowledge within the field is still insufficient, thus, it is not possible to perform a comprehensive power calculation; this will, however, be performed before any biomarker analysis is done.

In general, statistical analyses will be done according to available data; the following statistical tests may be used (the list is not complete): comparison of group demographics will be done with Student's t-test, Pearson's chi-square test or Mann-Whitney U-test according to the distribution of data. Due to the large size of the dataset the probability for type II error in testing the hypothesis will be low. Treatment duration and time to event can be explored with Kaplan-Meier curves, log-rank statistics and Cox regression analyses. Treatment outcomes across groups or according to specific biomarkers will be analysed with logistic

regression analyses. Multivariable analyses will be performed in order to study the impact of potential confounders. These confounders may be identified in the DANBIO registry (gender, age, smoking status, or other baseline characteristics). All included patients are recruited and treated in routine care across Denmark and this will inevitably lead to some missing data (missing sampling of biological material, missing registration of corresponding clinical data, whenever biological material is collected, patient lost to follow-up, etc.). For sensitivity, various statistical methods may be applied in order to test the robustness of the results. This may be done as last observation carried forward in case of lacking data on clinical outcomes, non-responder imputation, or statistical multiple imputation of missing data. Statistical expertise will be included when necessary.

ETHICS AND DISSEMINATION

The protocol is approved by the Danish Ethics Committee (H-2-2014-086, supplementary protocol 49419) and The Danish Data Protection Agency (RH-2015-297, I-Suite 04318). The Danish Rheumatologic Biobank is approved by The Danish Data Protection Agency (GLO-2015-6, I-Suite 03490). All patients receive verbal and written information before enrolment, and give oral and written consent at baseline according to the guidelines from the Danish Ethics Committee. All patients are informed that they can withdraw from the study at any time without it having consequences for their treatment. In case of withdrawal, samples are discarded and all patient-related registrations deleted from the Bio- and Genome Bank Denmark registry.

The sampled volume of blood for the study is 26.5 ml per patient-visit and maximum 240 ml/year. The sampling of blood for the study is performed simultaneously with scheduled routine blood sampling, thus minimizing the discomfort for the patient. Synovial fluid, surgical tissue, or bone marrow will only be collected if relevant interventions occur as part of routine care and surplus material, not used for diagnostic or therapeutic purposes, is available. The patients will be contacted and informed regarding the overall study results if they indicate interest in this in the patient study consent form. Direct feedback to the patient may be relevant in case of the discovery of mutations in known disease-linked genes, or as random discoveries, and will occur according to the guidelines directed by the Danish Ethics Committee (document number 1293688, October 2013). The physician in charge of the project at the individual department is responsible for

conducting the study in accordance with the Helsinki declaration. Study participation does not affect the treatment course of individual patients and the patients will be treated according to clinical practise.

Due to the large number of included patients, it will be possible to perform exploratory as well as validation biomarker studies. We plan to evaluate and publish study results according to the REMARK [13], STROBE [33], and the Standards for Reporting of Diagnostic Accuracy (STARD) [39] guidelines. Results will be published in international and peer-reviewed scientific journals and presented at international conferences. Negative, positive as well as inconclusive results will be published. If relevant, collaborations with international researchers will be established to facilitate the right expertise for biomarker analyses. The first results (measurements of s-IFX and ADAb drug levels up to one year after switch from originator to biosimilar IFX) have been presented [36,40].

STUDY STATUS

Patient recruitment started in May 2015 and is expected to continue until January 1st, 2025, with follow-up until January 1st, 2030. Currently, ≈3,000 patients have been enrolled in the study and >5,000 blood samples have been collected.

DISCUSSION AND POTENTIAL LIMITATIONS

In this observational, prospective, and translational biomarker study of patients with inflammatory rheumatic diseases, blood samples are collected in routine care and closely linked to extensive clinical data regarding rheumatic disease status and activity, medical treatment, treatment efficacy and adverse events, and comorbidities. The study protocol allows for a large-scale collection of blood and other biological materials with the aim to identify new biomarkers that can be used for improved personalized treatment of patients with inflammatory rheumatic diseases. Additionally, the nationwide collection of biological materials and clinical data is intended to further promote research collaboration within inflammatory rheumatic diseases, both nationally and internationally, in order to ensure research of the highest quality for the benefit of the patients.

Positivity for IgM-RF and anti-CCP are established risk factors for development of RA, and they are currently used as part of classification criteria and as prognostic markers [3]. In AxSpA, HLA-B27 is part of the disease classification [41]. Apart from the erythrocyte sedimentation rate and serum-CRP level, no biomarkers are used in routine care, and they cannot predict treatment responses or side effects. The wide range of currently available and future bDMARDs with different modes of action for the treatment of inflammatory arthritis, and the recent introduction of biosimilars and tsDMARDS, stresses the importance of improved ability to select the most effective treatment in the individual patient. Development of diagnostic, prognostic and predictive biomarkers will benefit the treatment of future patients and facilitate personalized medicine.

Patient recruitment and follow-up in routine care will lead to some limitations in clinical and biological data. Since patients are recruited across several rheumatic diagnoses and treatments, patient inclusion may take some time in order to obtain enough samples for a specific research question. However, since it is mandatory to register patients receiving biological treatment in DANBIO, coverage is high (≈96%) [42] and the risk of selection bias low. The risks for the patient are minimal and are out-weighted by the benefits for future patients. The non-randomised study design inherits the risks of confounding, and thorough statistical analysis and confounder adjustment is therefore important. On the other hand, the wide recruitment of patients treated in routine care may provide valuable data on, e.g., elderly patients with comorbidities. This may be a valuable supplement to data generated in randomised trials.

Hopefully, the results of the present study will provide us with new biomarkers that will improve our ability to a) diagnose rheumatic diseases more accurately and at an earlier stage, b) prognosticate the development of rheumatic diseases, and c) predict and monitor treatment effectiveness in the individual patient (personalized treatment).

The Danish Rheumatologic Biobank provides an infrastructure for national and international research collaboration. Thus, researchers, who are interested in collaboration regarding samples and/or clinical data from DANBIO should contact the Danish Rheumatologic Biobank [31] and DANBIO [30], respectively.

Funding

The Danish Rheumatism Association and Danish Regions funded the establishment of The Danish Rheumatologic Biobank. Danish Regions are funding the continued collection of biological samples and clinical data. The funders will have no influence on study design, data analysis, interpretation of results, or publications. Biomarker analyses will be funded by research grants.

Contributors

MLH and JJ wrote the protocol for the Research Ethics Committee. MLH, JJ, EH, TK and BG contributed to study concept. TK and BG drafted and revised the manuscript after feedback from all authors. All authors contributed to review of the present manuscript and approved the final version of the manuscript.

Competing interests

None

Provenance and peer review

Not commissioned; externally peer review.

Acknowledgements

The Danish Rheumatologic Biobank is acknowledged for handling and storage of biological material. Lab technician Teresa Rozenfeld and IT specialist Niels Steen Krogh are acknowledged for their assistance. The Biomarker Protocol Study Group, listed below, is acknowledged for their contribution to data registration or sample collection, and for review of the present manuscript: Asta Linauskas, North Denmark Regional Hospital; Gitte Meyer Larsen, North Denmark Regional Hospital; Anne Gitte Loft, Aarhus University Hospital; Charlotte Modin, Aarhus University Hospital; Karin Skovgaard, Aarhus University Hospital; Inge Juul Sørensen, Rigshospitalet; Britt Corfixen, Rigshospitalet; Oliver Hendricks, King Christian 10th Hospital for Rheumatic Diseases, Graasten; Charlotte Drachmann, King Christian 10th Hospital for Rheumatic Diseases, Graasten; Hanne Lindegaard, Odense University Hospital; Marina Bjørling-Poulsen, Odense University Hospital; Lene Dreyer, Copenhagen University Hospital, Gentofte; Johnny Raun, Hospital

Lillebaelt, Kolding; Mikkel Østergaard, Rigshospitalet; Kim Hørslev-Petersen, King Christian 10th Hospital for Rheumatic Diseases, Graasten; Henning Bliddal, Frederiksberg Hospital; Claus Rasmussen, North Denmark Regional Hospital; Torkell Ellingsen, Odense University Hospital; Søren Jacobsen, Rigshospitalet; Tove Lorenzen, Silkeborg Regional Hospital; Inger Marie Jensen Hansen, OUH Svendborg Hospital; Annette Schlemmer, Aalborg University Hospital; Salome Kristensen, Aalborg University Hospital; Kristian Stengaard-Pedersen, Aarhus University Hospital; Michael Stoltenberg, Zealand University Hospital, Køge; Henrik Nordin, Zealand University Hospital, Køge; Peter Mosborg, Randers Regional Hospital; Susanne Juhl Pedersen, Rigshospitalet; Christoffer Tandrup Nielsen, Rigshospitalet; Ellen-Margrethe Hauge, Aarhus University Hospital; Bent Deleuran, Aarhus University; Cecilie Brahe, Rigshospitalet; Christian Gytz Ammitzbøll, Aarhus University Hospital; Tue Wenzel Kragstrup, Aarhus University; Karen Carlsen, North Zealand University Hospital, Hillerød; Niklas Rye Jørgensen, Rigshospitalet; Dorte Vendelboe Jensen, Copenhagen University Hospital, Gentofte; Heidi Lausten Munk, Odense University Hospital; Michael Kruse Meyer, North Denmark Regional Hospital; and Tine Lottenburger, Hospital Lillebaelt, Vejle.

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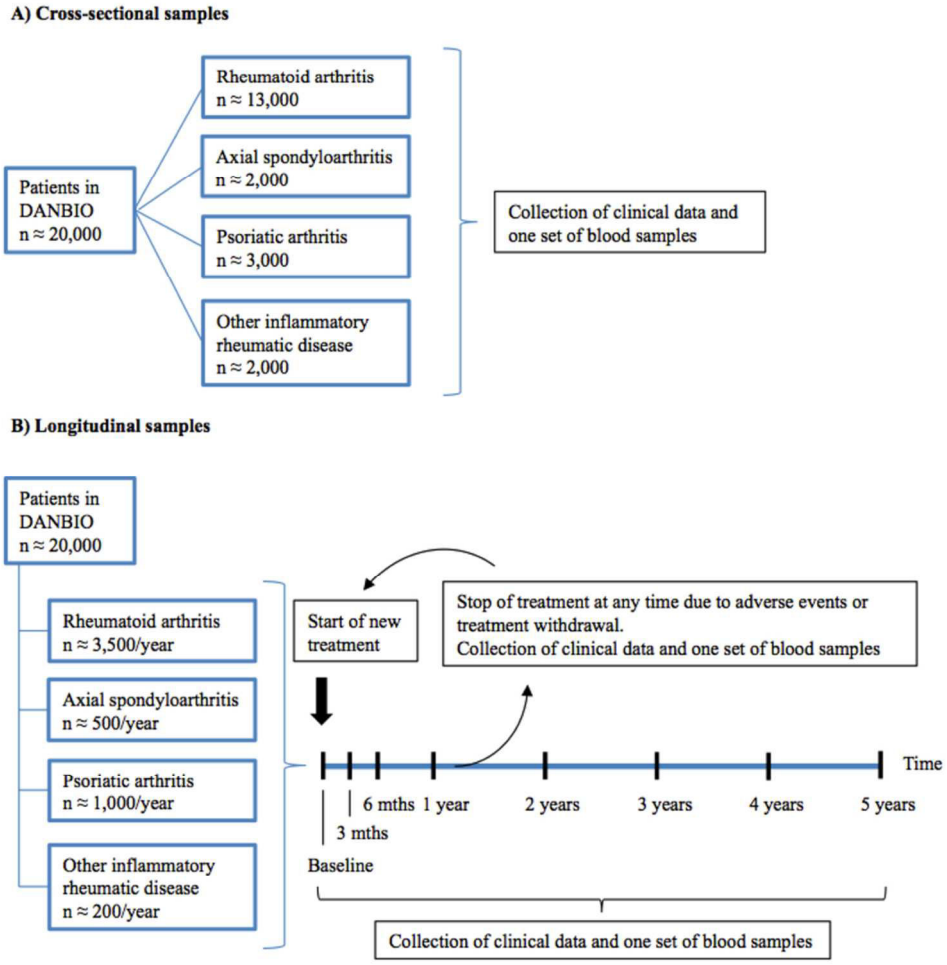
FIGURE LEGENDS

Figure 1 Schematic presentation of study design and sampling strategies. Any patient diagnosed with RA, AxSpA, PsA, other inflammatory rheumatic disease or tissue disorder, or suspected for one of these, may participate when they meet for a scheduled routine clinical visit. These patients can provide one cross-sectional blood sample (**A**), or may be included for longitudinal follow-up (**B**) when they start treatment with a new DMARD (see text). See Figure 2 for details on blood handling and storage. Numbers (n) indicate patients potentially eligible for inclusion in one or more of the study arms.

Figure 2 Standard Operating Procedure **(**SOP) for blood handling in the Danish Rheumatologic Biobank [31]. Peripheral blood is collected in one EDTA tube, two serum tubes, and one PAXgene blood RNA tube. Serum tubes coagulate at room temperature for 30 min to 2 hours. From the EDTA tube, 1.5 ml whole blood is isolated. EDTA and serum tubes are centrifuged at 2000xg and 4˚C for 10 min. EDTA plasma (2x2ml), EDTA buffy coat and serum (4x2ml) are isolated. Processed blood samples are stored at ≤-20˚C. PAXgene blood RNA tubes are kept at room temperature for 2-72 hours, then frozen at -20˚C for 24-72 hours and stored at -80˚C.



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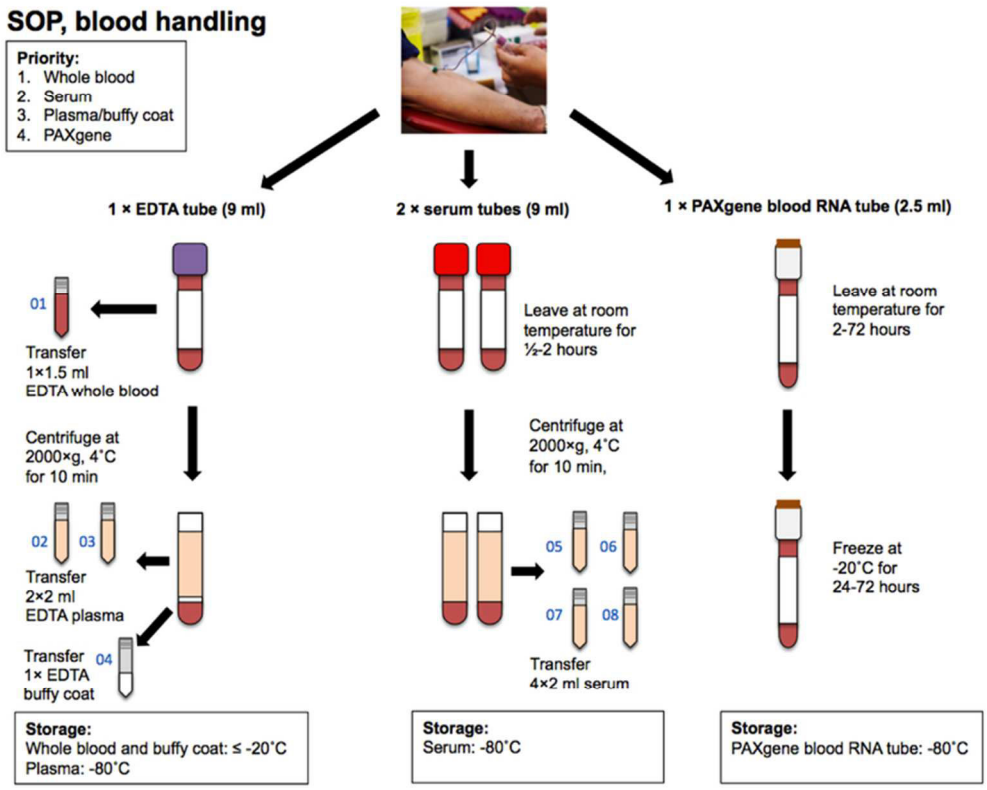

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71x56mm (300 x 300 DPI)

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1 and 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4-5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6-7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6-9
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	Not relevant
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7-8 and 10
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	9-10
Bias	9	Describe any efforts to address potential sources of bias	10
Study size	10	Explain how the study size was arrived at	8 + 10 and Fig 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	(not relevant yet, the study is still recruiting)
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	10
		(*b*) Describe any methods used to examine subgroups and interactions	10
		(*c*) Explain how missing data were addressed	10-11
		(*d*) If applicable, explain how loss to follow-up was addressed	10-11
		(*e*) Describe any sensitivity analyses	10-11

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	(not relevant yet, the study is still recruiting)
		(b) Give reasons for non-participation at each stage	(not relevant yet, the study is still recruiting)
		(c) Consider use of a flow diagram	(not relevant yet, the study is still recruiting)
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	(not relevant yet, the study is still recruiting)
		(b) Indicate number of participants with missing data for each variable of interest	(not relevant yet, the study is still recruiting)
		(c) Summarise follow-up time (eg, average and total amount)	(not relevant yet, the study is still recruiting)
Outcome data	15*	Report numbers of outcome events or summary measures over time	(not relevant yet, the study is still recruiting)
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	(not relevant yet, the study is still recruiting)
		(*b*) Report category boundaries when continuous variables were categorized	(not relevant yet, the study is still recruiting)
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	(not relevant yet, the study is still recruiting)

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	(not relevant yet, the study is still recruiting)
Discussion			
Key results	18	Summarise key results with reference to study objectives	(not relevant yet, the study is still recruiting)
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	(not relevant yet, the study is still recruiting)
Generalisability	21	Discuss the generalisability (external validity) of the study results	(not relevant yet, the study is still recruiting)
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.