BMJ Open

BIOSKIN: A Protocol for the Copenhagen Translational Skin Immunology Biobank and Research Programme

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To cite: Løvendorf MB, Johansen JD, Skov L. BIOSKIN: A Protocol for the Copenhagen Translational Skin Immunology Biobank and Research Programme. *BMJ Open* 2024;**14**:e077207. doi:10.1136/bmjopen-2023-077207

► Prepublication history for this paper is available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2023-077207).

Received 28 June 2023
Accepted 28 January 2024

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ABSTRACT

Introduction Psoriasis, atopic dermatitis and contact dermatitis are common chronic inflammatory skin diseases that have a significant impact on individuals and society.

Methods and analysis The Copenhagen Translational Skin Immunology Biobank and Research Programme (BIOSKIN) is a translational biobank and research study that aims to prospectively collect high-quality biological samples and clinical data from 3000 patients with psoriasis, atopic dermatitis and contact dermatitis over a minimum period of 5 years. The longitudinal open design allows participants to enter and leave the study at different time points depending on their disease and treatment course. At every visit, the investigator collects biological samples, conducts interviews and assembles self-reported questionnaires on disease-specific and general health-related information. Clinical examination and biological sampling will be conducted at enrolment, during and after disease flare, before and after initiation of new treatment and at least once per year. The clinical examination includes dermatological verification of diagnosis, evaluation of disease severity and detailed information on phenotype. The biological samples include blood and when accessible and relevant, skin biopsies, tape strips and skin swabs. The data collected will undergo rigorous statistical analysis using appropriate analytical methods. As of December 2023, 825 patients have been enrolled in the study.

Ethics and dissemination The study is approved by the Scientific Ethical Committee of the Capital Region (H-21032986) and the Danish Data Protection Agency. Results will be published in peer-reviewed scientific journals and presented at national and international conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ BIOSKIN is a Danish prospective cohort study of 3000 patients with either psoriasis, atopic dermatitis, or contact dermatitis where accurate and repeated monitoring and collection of biological samples will provide a compelling data set and biobank.
⇒ At each study visit, a comprehensive set of data is gathered, encompassing clinical characteristics, as well as biological samples such as blood, skin biopsies, tape strips and skin swabs. Additionally, participants complete questionnaires covering both disease-specific details and general health-related information.
⇒ The comprehensive data from the BIOSKIN cohort can be combined with the unique Danish registers and electronic medical patient records enabling unique large-scale data mining to determine associations between a variety of health outcomes.
⇒ The cohort is limited by the potential population bias due to recruitment primarily from the capital area of Denmark and the cohort consists mainly of referred patients with moderate to severe skin disease.

INTRODUCTION

Psoriasis, atopic dermatitis and contact dermatitis are examples of chronic inflammatory skin diseases that are common and represent a major health burden for society and the affected individuals. In Northern Europe, 3%–4% of the population has psoriasis,[1] 3%–5% of adults and 15% of children have atopic dermatitis,[2] and 15% of adults have contact dermatitis.[3] These skin diseases cause significant morbidity and strongly impact the quality of life. In addition, some but not all patients develop comorbidity, for example, arthritis, cardiovascular disease, or airway disease.

Recent developments in immunology, molecular biology and genetics have greatly improved our knowledge of skin disease mechanisms which in turn has improved treatment modalities.[4–8] In recent years, several effective treatments have been introduced, especially for psoriasis and atopic dermatitis. However, treatments for patients with severe skin disease are often expensive, and many patients may only experience short-term beneficial effects from biological therapies, with the added risks of adverse drug reactions.[9] Additionally, molecular

biomarkers that can predict skin disease characteristics, prognosis, risk of comorbidity and overall disease trajectories are currently missing, which in turn leads to insufficient and delayed treatment.[10–12] Furthermore, a major clinical obstacle to personalised treatment and prevention of these diseases is our inability to reliably distinguish between different inflammatory skin diseases and to optimise treatment by stratifying subgroups within the same disease spectrum.

Today, most knowledge about common inflammatory skin diseases is based on cross-sectional studies. Existing biobanks typically contain biological material with limited access to clinical data and samples from the same patients over time are missing. The UK Biobank, which is a large-scale longitudinal study that follows the health of 500 000 volunteers,[13] is an example of a biomedical database and research resource that aims to circumvent these limitations. The Newcastle Dermatology Biobank,[14] the Skin Lesion Biobank,[15] the UNMC Dermatology Tissue and Blood Bank[16] and BIOMAP[17] are initiatives that in contrast to the population-based UK Biobank have a focus on different areas within dermatology.

Here we present the Copenhagen Translational Skin Immunology Biobank and Research Programme (BIOSKIN) which is an observational, prospective, translational biobank and research study following patients with the most prevalent chronic inflammatory skin diseases: psoriasis, atopic dermatitis and contact dermatitis. The aim of the project is to intensify translational research in dermatology by collecting high-quality biological samples and clinical data from 3000 patients over a minimum period of 5 years. Together, this will enable a thorough characterisation of the patient's disease trajectories, response to treatment and risk of comorbidities. Ultimately, the results from this research programme will improve the quality of life for a large group of patients and lead us closer to finding a cure for disabling inflammatory skin diseases.

MATERIALS AND METHODS

Cohort description

The BIOSKIN cohort is an ongoing prospective and observational study recruiting patients with inflammatory skin diseases including psoriasis, atopic dermatitis and contact dermatitis. The study is a collaboration between the LEO Foundation Skin Immunology Research Centre (SIC), University of Copenhagen and the Department of Dermatology and Allergy, Herlev and Gentofte Hospital, Denmark where the cohort is anchored. Patient inclusion started in February 2022 and as of December 2023, 825 patients have been enrolled in the BIOSKIN cohort. The cohort will entail unprecedented biological sampling and collection of patient data for clinical assessments, for which Denmark is an ideal location because of its linked electronic medical records and national patient registers. On inclusion, the participants will be invited to provide blood samples for the recently established Danish Dermatological Biobank (DDeB)[18] which requires a specific declaration of interest. Samples acquired for DDeB can be made available to other researchers on request if the project has been approved by a Scientific Ethics Committee and by the Danish Data Protection Authority.

The prospective, longitudinal open cohort design with repeated sampling will allow participants to enter and leave the study at different time points during monitoring. The frequency of visits is tailored to each participant's specific disease severity and treatment trajectory in the clinic (figure 1). Hence, patients with moderate to severe disease that initiate a systemic or biological treatment are followed closely in the first year (every 3 months) in line with the close monitoring in the clinic. If the treatment is well tolerated and the patient exhibits a positive response, subsequent appointments are scheduled annually. If participants experience disease flares or change their treatment regimen at any point during their involvement in the BIOSKIN programme, we will closely follow-up with them, mirroring the monitoring practices of the clinic. Participants with mild disease and no flares who do not require systemic or biological treatment will only be seen once every year. In total, we will follow the patients and collect samples and data for a minimum of 5 years from 3000 patients with the most prevalent diseases, atopic dermatitis, psoriasis and contact dermatitis. The number of patients will ensure that different disease severities, subtypes and types of treatment are fully covered and will make the set of biological material and data in the biobank unique in terms of both quality and size.

Recruitment process

Patients (age>2 years) are eligible for inclusion in the BIOSKIN cohort if they have an inflammatory skin disease and can provide both written and oral informed consent. For children (<18 years), both parents are required to sign the consent form. On reaching the age of 18, the children will be asked to provide renewed consent during their subsequent visit. All patients, including children and adults, with psoriasis, atopic dermatitis and contact dermatitis seen at the Department of Dermatology and Allergy at Herlev and Gentofte Hospital will be offered the opportunity to participate in BIOSKIN. For comparative purposes, data and samples from a smaller group of patients with other inflammatory skin diseases such as alopecia areata, vitiligo, hidradenitis suppurativa and mycosis fungoides will also be enrolled. Patient inclusion and follow-up will be performed by physicians and nurses at the research unit when the patients meet for a scheduled routine clinical visit. Patients with mild diseases who are not eligible for a clinical course at the department will be recruited from advertising using various public platforms.

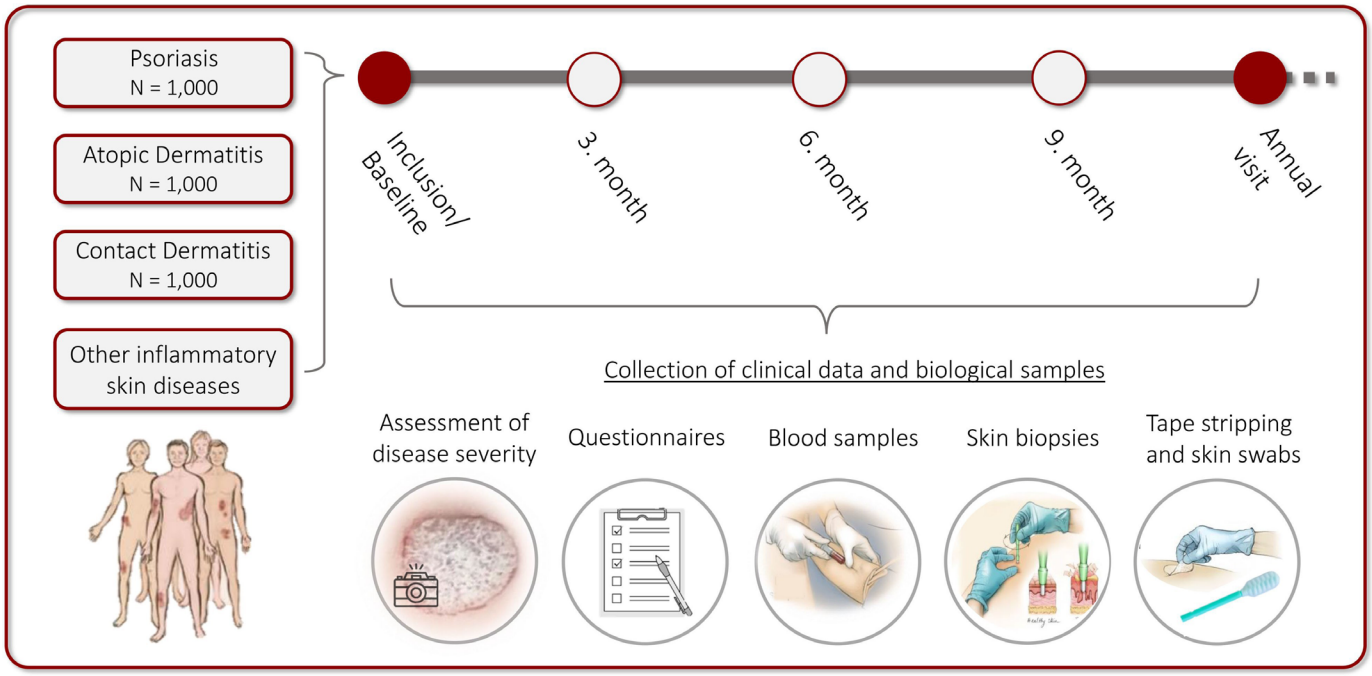

Figure 1 Schematic overview of the BIOSKIN study protocol and sampling strategies. BIOSKIN is an ongoing Danish prospective cohort study aiming to enroll patients diagnosed with inflammatory skin diseases including psoriasis, atopic dermatitis, and contact dermatitis. The BIOSKIN cohort is designed as a prospective, longitudinal open cohort, facilitating repeated sampling, and allowing participants to enter or exit the study at different time points during monitoring. The frequency of visits is tailored to each participant's specific disease and treatment trajectory. As a result, participants with moderate to severe conditions who require systemic or biological treatment undergo thorough monitoring during the initial year, with assessments scheduled every three months (grey circles), aligning with the close monitoring in the clinic. If the treatment is well tolerated and the patient has a positive response, subsequent appointments are scheduled annually (red circle). If participants experience disease flares or change their treatment regimen at any point during their participation in the BIOSKIN program, we will resume close follow-up (new baseline, red circle). Participants with mild conditions and no flares, who do not necessitate systemic or biological treatment, are scheduled for annual visits. During each study visit, the investigator conducts an interview using a predefined set of questions specific to the visit, covering disease-related inquiries and general health-related topics. A clinical examination is also performed, and biological samples are collected during every visit (Table 1). At a minimum, participants aged 18 years and above provide a blood sample, while additional samples may be collected from lesional, non-lesional, and healed skin, including punch biopsies, tape strip samples, and skin swabs, whenever accessible and relevant

Interview

During all study visits, the investigator completes an interview by asking the participant a visit-specific predefined series of questions consisting of both disease-specific questions and more general health-related questions. The disease-specific questions relate to disease history, possible symptoms, family history, potential trigger factors and if relevant, present and previous disease-specific treatments. The general part of the questions includes patients' demographics (eg, age, gender and ethnicity), lifestyle factors (eg, smoking status, alcohol consumption, diet, exercise habits and socioeconomic status) and comorbidities. In addition to the interview completed by the investigator, the participants complete several self-reported disease-specific questionnaires (table 1). The questionnaires include Dermatology Life Quality Index (DLQI), Psoriasis Symptoms and Disease Diary (PSSD), questions related to psoriatic arthritis (Early ARthritis for Psoriatic patients (EARP), Patient-Oriented Eczema Measure (POEM) and the Quality Of Life in Hand Eczema Questionnaire (QOLHEQ). All questionnaires have been either used in routine clinical care or previously validated.

Clinical examination

Clinical examination will be conducted at inclusion, during and after disease flare, before and after initiation of new treatment, and at least once per year (table 1). The clinical examination includes a thorough inspection of the skin and nails for dermatological verification of diagnosis and detailed information on skin type and the present phenotype. The distribution of skin lesions will be marked on a diagram and a clinical photo including local severity score from sites of sampling will be recorded. Disease severity will be assessed using disease-specific scoring tools (Patient's and Physician's Global Assessment, Psoriasis Area and Severity Index (PASI), Nail Psoriasis Severity Index (NAPSI), Body Surface Area (BSA), Eczema Area and Severity Index (EASI), Hand Eczema Severity Index (HECSI) and SCORAD (Scoring Atopic Dermatitis)). During all study visits, vital parameters including

Table 1 Overview of study procedures

		Type of visit		
		Inclusion	Follow-up	Annual
Disease severity assessment*	**Disease**			
Psoriasis Area Severity Index (PASI)	PSO	✓	✓	✓
Body Surface Area (BSA)	PSO	✓	✓	✓
Nail Psoriasis Severity Index (NAPSI)	PSO	✓	✓	✓
Eczema Area and Severity Index (EASI)	AD/(CD)	✓	✓	✓
SCORing Atopic Dermatitis (SCORAD)	AD/(CD)	✓	✓	✓
The Hand Eczema Severity Index (HECSI)	CD/(AD)	✓	✓	✓
Disease-specific questionnaires†				
Dermatology Life Quality Index (DLQI)	PSO/AD/CD	✓	✓	✓
Diet Questionnaire	PSO/AD/CD	✓		✓
Psoriasis Symptoms and Signs Diary (PSSD)	PSO	✓	✓	✓
Early ARthritis for Psoriatic patients (EARP) questionnaire	PSO	✓		✓
The Quality of Life in Hand Eczema Questionnaire (QOLHEQ)	AD/CD	✓	✓	✓
Patient Oriented Eczema Measure (POEM)	AD/CD	✓	✓	✓
Biological samples‡				
Blood samples	PSO/AD/CD	✓	✓	✓
Skin samples (biopsi, tape strip and skin swab)	PSO/AD/CD	✓	✓	✓
Fibroscan	PSO/AD/CD	✓		✓

*Indication of the patient groups that are relevant for the different study procedures. Atopic dermatitis and contact dermatitis in parentheses are if the participant, for instance, is included as a contact dermatitis patient but has an atopic background and vice versa.
†Disease-specific questionnaires are completed by the parents together with the child (<18 years). For DLQI, participants<16 years will fill in 'The Children Dermatology Life Quality Index (CDLQI)'.
‡Blood samples are only obtained from children (<18 years), if they can be collected alongside samples samples used for disease monitoring. Skin samples from lesional, non-lesional and healed skin are obtained when accessible and pertinent. Due to the invasive nature of the skin biopsy, we will not sample skin biopsies from children nor lesions localised to the neck and the facial area.
AD, atopic dermatits; CD, contact dermatitis; PSO, psoriasis.

weight, height, waist-to-hip ratio and blood pressure will be recorded. In addition, an evaluation of the presence and extent of liver steatosis and fibrosis will be assessed using non-invasive ultrasound-based liver elastography (Fibroscan, Echosens, Paris, France). This liver evaluation aims to determine the risk of liver disease and explore potential variations in disease risks among patients with mild and moderate-to-severe conditions.

Biological samples

Biological samples will be collected at every visit (table 1). As a minimum, patients >18 years old provide a blood sample and when accessible and relevant, we also collect samples from lesional, non-lesional and healed skin including punch biopsies, tape strip samples and skin swabs (figure 1). We may also collect faeces samples and conduct skin prick (standard inhalation panel) and patch tests on a project-based initiative. We only take blood from children (<18 years) if they can be collected with blood samples used for disease monitoring. Due to the invasive nature of the skin biopsy, we will not sample skin biopsies from children nor lesions localised to the neck and the facial area.

Blood samples

Sample processing is carried out at the research laboratory facility at the Department of Dermatology and Allergy at Herlev and Gentofte Hospital by trained laboratory technicians and nurses. Peripheral blood is collected in two EDTA tubes (Hettich Labinstrument Aps, 4 mL+9 mL) and two serum tubes (Hettich Labinstrument Aps, 2×9 mL). For patients who will initiate a systemic treatment, we collect an additional 9 mL EDTA tube for isolation of peripheral blood mononuclear cells (PBMCs) and one PAXgene blood RNA tube (Becton and Dickinson). Serum tubes coagulate at room temperature for 30–120 min before centrifugation. PAXgene blood RNA tubes are kept at room temperature for 2–72 hours, then frozen at −20°C for 24–72 hours before long-term storage at −80°C. Blood samples are processed according to the nationally approved standard operating procedure for blood.[18] In brief, from the 4 mL EDTA tube, 2×2 mL whole blood is isolated. The 9 mL EDTA tube and the 2×9 mL serum

tubes are centrifuged at 2000×g (acceleration=9, deacceleration=9), 4°C for 10 min. After centrifugation, 3×1 mL EDTA plasma, 1×EDTA buffy coat and 5×1 mL serum are isolated. All blood fractions are stored at −80°C. One plasma sample and two serum samples are reserved for the DDeB. Preparation of the PBMC fraction from EDTA blood is done using density gradient centrifugation. Briefly, diluted EDTA blood (1:1, 1X Phosphate Buffered Saline (Sigma, #D8537)) is layered over a density gradient medium (Leucosep tube (VWR, #720–1840) containing 15 mL Lymphoprep (STEMCELL, #7801)) and centrifuged at 1200×g (acceleration=5, deacceleration=0), 20°C for 20 min. After centrifugation, the top plasma layer is removed, and the white cloudy mononuclear cell layer is transferred to a new 50 mL falcon tube (HOUNISEN, #62.547.254) where three subsequent washing steps are done to remove the remaining platelets (200×g (acceleration=5, deacceleration=9), 20°C, 8 min). The PBMCs are immersed in CryoStor CS5 (Sigma, #C2999) and stored in a rate-controlled container (Corning CoolCell, Merck) at −80°C before transferring to liquid nitrogen for long-term storage.

In addition to blood samples obtained for research purposes, standard blood samples will be collected from all enrolled patients as part of their disease monitoring in the clinic. These blood samples will be analysed by the Department of Clinical Biochemistry, Herlev and Gentofte Hospital and they include haematological parameters, and markers for liver function, cholesterol levels, inflammation (high-sensitivity C-reactive protein) and diabetes. In addition, an HLA-Cw06 gene variant analysis for patients with psoriasis will be conducted on inclusion. Finally, total IgE and filaggrin gene variant analysis will be evaluated in patients with atopic dermatitis and contact eczema.

Skin samples

Skin biopsies (2 mm) are collected under local anaesthesia (lidocaine, 20 mg/mL) from lesional skin, non-lesional or healed skin. After evaluating various methods for obtaining skin biopsies, we selected the most suitable approach in terms of RNA quality, yield and practical convenience (data not shown). Our chosen method involves placing the punch biopsies in 1 mL RNAlater (Thermo Fischer, #AM7020) at room temperature followed by immediate storage at −80°C to ensure the preservation of high-quality nucleic acids. For selected patients, we will acquire 4–6 mm punch biopsies for flow cytometric analysis or preservation in formalin followed by paraffin embedding. To collect material for skin microbiome analysis, skin swabs (Isohelix T-Swab, YouDoBio, #SK-S4) are rubbed 20–60 s on the lesional and non-lesional skin, respectively, and transferred to a tube containing 1 mL of DNA/RNA shield (Nordic Biosite, #R1100-250) before storage at −80°C. For tape strip samples, eight consecutive standard adhesive disks (D100 D-Squame Sampling Discs, 22 mm, Clinical & Derm) are placed on lesional, non-lesional or healed skin under a standard weight of 225 gm/cm^2 using D500 D-Square Pressure Instrument (Clinical & Derm) for 10 s. The adhesive disks are removed using forceps and placed inside a 2 mL microtube (Hounisen, #72.609) with the adhesive side facing inside and stored at −80°C.

Data management

At inclusion, all participants receive a unique participant ID, and their clinical data are recorded and managed using a Research Electronic Data Capture tool (REDCap) hosted at the Region Hovedstaden, Denmark[19 20] ensuring uniform and high-quality data collection in accordance with the FAIR principles (Findable, Accessible, Interoperable and Reusable).[21] Preanalytical factors including the date and time of sampling, handling, storage and the exact handling procedure of biological samples are recorded in the nationwide Bio- and Genome Bank Denmark registry (RBGB). All research samples will be marked with a unique fraction ID and a barcode for electronic reading. The Capital Region hospitals' freezer facilities (BIOSEK) have restricted access and temperature monitoring and will be used for long-term storage of the samples. Genetic information will be stored at the National Genome Centre according to §223a of the Danish Health Act, BEK no. 360 of 4 April 2019.

Patient and public involvement

A patient board representing patients with psoriasis, atopic dermatitis and contact dermatitis has been established to ensure the integration of the patient's perspective and the needs of future patients into the research programme. Furthermore, we have a close collaboration with patient organisations such as The Danish Psoriasis Association, The Danish Atopic Dermatitis Association and Astma Allergy Denmark. Finally, the BIOSKIN research programme appreciates international scientific counselling from SIC's Scientific Advisory Board.

Ethics and dissemination

The study is approved by the Scientific Ethical Committee of the Capital Region, Denmark (H-21032986) and the Danish Data Protection Agency (P-2021-435) and will be performed in accordance with the good clinical practice regulations and the Helsinki II declaration. Both parents of children<18 years old will give written informed consent prior to entry to the study. The Danish Dermatological Biobank is approved by the RBGB and participation will require separate consent. All relevant study results will be presented in peer-reviewed publications and presented at national and international conferences.

Perspectives and potential limitations

The BIOSKIN is unique in collecting longitudinal high-quality clinical material and data from inflammatory skin diseases including psoriasis, atopic dermatitis and contact dermatitis. The core elements are a comprehensive biobank with implemented standardised sampling and processing procedures, collection of clinical characteristics

and advanced research technologies in skin immunology which will facilitate the integration of basic and clinical science in dermatology. A key advantage of the BIOSKIN cohort lies in its unique potential for the integration of clinical and molecular data, encompassing genomic, transcriptomic and proteomic information, along with Danish registers and electronic patient records. This comprehensive approach empowers researchers to uncover associations between diverse health outcomes. However, there are limitations to this study, including potential population bias due to recruitment primarily from the capital area of Denmark, a cohort consisting mainly of referred patients with moderate to severe skin disease, and the possibility of loss to follow-up. While we attempt to mitigate these limitations by including patients from a range of different hospitals and clinics in the Greater Copenhagen area, and patients with milder forms of the disease, these limitations should be considered when interpreting the study's findings. Despite these limitations, we believe that the BIOSKIN cohort represents a valuable resource for investigating the pathogenesis of inflammatory skin diseases and identifying new biomarkers and therapeutic targets. By acknowledging these limitations and continuing to work to address them, we aim to maximise the impact of our research and ultimately improve disease management and the quality of life for a large group of patients with inflammatory skin diseases.

Acknowledgements We would like to thank Rikke Juul Wittrup and Christina Yndal Erichsen whose unwavering commitment has played an instrumental role in the commencement of this study. Also, we would like to thank the dedicated BIOSKIN team including project nurses, physicians and biotechnicians for their invaluable contributions to patient inclusion, data registration and sample collection and processing which are vital to the integrity of the data collected. Furthermore, we thank the patients for their participation. Finally, we acknowledge the support provided by the Department of Dermatology and Allergy, Herlev and Gentofte Hospital, which fostered the infrastructure, resources and clinical expertise in the realisation of this project.

Contributors MBL, LS and JDJ designed the study, created the study protocol and obtained approvals for the study design. MBL drafted the first manuscript and all authors critically revised and approved the final version of the manuscript.

Funding This work was supported by the LEO Foundation grant number (LF-ST-21-500002) and Copenhagen University Hospital, Herlev and Gentofte Hospital, Denmark.

Competing interests MBL and JDJ declare no conflict of interest. LS has been a paid speaker for AbbVie, Eli Lilly, Pfizer, Sanofi and LEO Pharma, and has been a consultant or served on Advisory Boards with AbbVie, Janssen Cilag, Novartis, Eli Lilly, LEO Pharma, UCB, Almirall, Boehringer Ingelheim, BMS and Sanofi. LS has received research and educational grant from Pfizer, Novartis, BMS, Almirall, Sanofi, Janssen Cilag and Leo Pharma.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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