1. Title: Yeast colonization and infection in patients with inflammatory skin diseases

2. Background and aim

The pathogeneses of many inflammatory diseases are not completely understood, yet, dysregulation of the human microbiota is increasingly being investigated as a possible contributing factor[1–3]. The human microbiota includes bacteria, archaea, viruses and fungi. In general, little is known about the fungal colonization in inflammatory skin diseases although *Candida* species in oral mucosa, skin and feces seem to be increased in patients with psoriasis and atopic dermatitis[3–5]. Inflammation is also an important part of the pathogenesis in other skin diseases such as hidradenitis suppurativa, acne, vasculitis and inflammatory ulcers. Possibly, increased fungal colonization may be present in many inflammatory skin diseases, yet, this has not previously been investigated. Studies of inflammatory diseases involving other body areas such as systemic lupus erythematosus[6], Sjögren's disease[7], multiple sclerosis[1], and spondyloarthritis[2] have also demonstrated increased colonization with *Candida* species. However, it is unclear, whether colonization with *Candida* is a cause or a result of inflammatory disease. Treatment of inflammatory diseases with systemic immune-modulating drugs or recurrent antibiotic therapy may also affect yeast colonization. Emergence of biological therapies that directly or indirectly modulates the Interleukin (IL) 17-pathway emphasizes the need for further knowledge, since the IL-17 pathway is involved in the defense against yeast infections in healthy individuals[8]. Until now, investigations of yeast colonization have primarily focused on *Candida* and *Malassezia*. Whether other species of yeasts may be of interest is still uncertain[9].

This study aims to examine the prevalence of yeast colonization and yeast infection in skin and oral mucosa of a variety of patients and healthy volunteers visiting the Dermatological outpatient clinic.

3. Methods

Study execution

The project is carried out as a collaboration between Dermatological Department, Roskilde University Hospital and the Department of Microbiology, Slagelse University Hospital under the leadership of primary investigator, Senior Doctor in Dermatology, PhD, Ditte Marie Lindhardt Saunte. The project is supported by Medical Doctor in Dermatology, PhD Elisabeth Hjardem Taudorf, Dermatological study nurse Helle Anette Jensen, and the established research group at the Dermatological Department.

The samples will be analyzed at the Department of Microbiology, Slagelse University Hospital under the surveillance of specialist registrar doctor Dennis Back Holmgaard and Professor Jens Jørgen Elmer Christensen. Dept. of Mycology, Statens Serum Institute, which is a national reference laboratory for fungal infections, may be involved in cases where special tests are needed. Prof. Maiken Cavling Arendrup is the main contact person.

Study design

A case-control study comparing the incidence of yeast colonization or infection in skin and oral mucosa of patients with different skin diseases and healthy volunteers. Patients with selected skin diseases, staff at Zealand University Hospital in Roskilde, relatives to staff and students with relation to the Dermatologic Department will be asked to fill out a short questionnaire and have swaps taken from oral mucosa, as well as skin scrapings and tape strips from lesional skin (only patients) and non-lesional skin (all).

Swaps will be examined at the Department of Microbiology, Slagelse University Hospital. Samples will be cultured and cultures positive for yeast will be identified using MALDI-TOF ms (Matrix-assisted laser desorption-ionization Time of flight mass spectrometry). Skin scrapings and tape strips will be investigated for the presence of yeasts, i.e. *Candida* and *Malassezia*, and when possible investigated for subtypes. The subgroup of patients initiating systemic therapy for their skin disease at the first examination will be asked to repeat the questionnaire, swaps, skin scrapings and tape strips after approximately three months.

Clinical photos of lesional skin or mucosa will be taken and stored at a secure IT site (Sharepoint and in SP clinical report) after written consent.

4. Statistics

Prevalence and Odds Ratios (OR) will be determined. With a two-sided significance level of 95%, a power of 80 %, an expected ratio of healthy controls vs. patients with skin disease of 0.5 and an expected OR of 2.7[3] a minimum sample size of 127 patients per skin disease and 64 healthy controls are needed. We aim to include approximately of 130 patients per skin disease. In total, a maximum of 700 individuals will be included, of which 70 will be healthy controls. Inclusion of patients with rare skin diseases may necessitate fewer patients in some groups.

5. Test subjects

Inclusion criteria

Legally competent women and men aged 18 years or older can be included.

Exclusion criteria

Patients in actual systemic or topical antifungal therapy cannot participate. Pregnant or lactating patients will likewise not be included.

6. Risks, side effects and drawbacks

All samples consist of non-invasive mucosal swaps, skin scrapings or superficial tape strips which holds no risk of known adverse effects. It may be associated with minimal discomfort during sample collection and participants may use an additional 15 minutes of their time in order to receive information regarding the project. Apart from that, this project causes no known risks, side effects or drawbacks.

7. Biologic material

Swaps from mucosal membranes, skin scrapings and superficial tape strips from the skin surface will be collected and analyzed for the presence of yeasts whereupon samples will be destroyed. There is no biobank involved in this project.

8. Information from patient files

Participation will be registered in paper case report forms (CRFs), which are kept behind locked doors in the Research Department. Consent forms will be signed, and personal identification numbers replaced by specific research identification numbers (rID). The identification key linking rID to identity will be kept behind double locked doors. Primary Investigator, research workers appointed to take care of this project and regulatory authorities will gain access to patient health files including the electronic files. Access to health information will include disease severity, duration and medication at initiation of the project and if relevant at a scheduled follow-up. This procedure is necessary in order to execute the research project, will ensure precise research data for the publication and allow required controls, including self-monitoring, quality control and monitoring of the project. There are no sponsors involved in this project.

The patient data including clinical photos, the sample information and epidemiological data will be entered in a database on a secure SharePoint teamsite. Data will be kept for ten years in accordance with and after approval from the Danish Dataprotection Agency. Data will be saved with unique rIDs.

9. Handling of personal information

The project will be notified to the Danish Data Protection Agency via the Notification for Region Zealand, and the General Data Protection Regulation as well as the the Data Protection Act will be respected. Personal data is not shared nationally or internationally.

10. Economy

The Dermatologic Department, Roskilde University Hospital provides sample materials and Department of Microbiology, Slagelse University Hospital analyzes the samples. Both departments cover their own expenses. The project is completely independent and does not receive financial support from third parties. None of the involved research workers receive payment for participation or has conflicts of interest related to the project.

11. Remuneration for trial participants

Participants will not receive any remuneration or compensation related to study participation.

12. Recruitment of trial participants and informed consent

Patients, relatives and staff associated to the Dermatologic Department in Roskilde will be asked to participate. Patients and relatives will be recruited during a routine visit to the clinic, while staff will be offered participation during a regular staff meeting. A private consultation will take place, where each participant will receive individual oral and written information about the project in a quiet atmosphere. The participant can bring a bystander and will be offered time for consideration. If requested, an extra

interview with repeated possibility to bring a bystander will be provided. If a person wants to participate in the project and meet criteria for inclusion, informed consent will be signed, a short questionnaire filled out and samples will be collected during their regular outpatient visit or by individual appointment.

13. Publication of results

Positive as well as negative results will be published in relevant international Scientific Journals and presented at Scientific meetings and congresses.

14. Research ethics

Many patients, staffs and relatives will be asked to participate in this case-control study, spend a short amount of extra time during an appointment at the clinic and may experience minimal discomfort during collection of samples. In contrast, information about yeast colonization in different skin diseases may provide a completely new insight and form the basis for further studies to detect the possible causality between yeast colonization and inflammatory skin diseases. Thus, the potential small disadvantages for project participants can by far be justified by the large amount of new knowledge obtained.

15. Information on compensation

Participants in this project are covered by the general rules for patient compensation in the Danish healthcare system.

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