Will immunologic and inflammatory variables associated with persistent post-treatment fatigue in breast cancer survivors be reduced by acupuncture treatments?

Protocol

Introduction

This sub-study is an offshoot from the main study AcuBreast (2017/2197REK sør-øst A). Subjects in the present study will be asked to participate when they are approached as participants in the main study. Participants in the present study will be drawn from both the treatment group and the control group i.e., 40 participants from each group.

The AcuBreast study is a pragmatic, randomized controlled trial and follows the methodological criteria for this type of study. Adding an explanatory aspect as an sub-study to a pragmatic study will increase the power of the main study and will be noticed in international research, since it will be one of the first large studies to link acupuncture, CRF and biomarkers together.

Title

"Will immunologic and inflammatory variables associated with persistent post-treatment fatigue in breast cancer survivors be reduced by acupuncture treatments?"

Responsible researchers

Professor Terje Alræk is the principal investigator for AcuBreast, while for the present sub-study associate professor Martin Frank Strand will be the principal investigator.

Collaborating partners

The research team at Kristiania University College will be responsible for taking the blood samples, storing them according to established procedures and analysing the samples. Our collaborating partners, as described in the AcuBreast protocol, will assist in writing articles from this data.

Research question

Will there be any changes in immunologic and inflammatory variables associated with cancer related fatigue in breast cancer survivors after acupuncture treatments?

Background

In our REK-approved AcuBreast protocol we thoroughly described the background, together with the status of present research on acupuncture and cancer related fatigue. Since the present study is an offspring from AcuBreast we think it is unnecessary to repeat the same information in the present application.

Purpose

This project is connected to the main study, AcuBreast, which was funded by the Pink Ribbon with NOK4.7 million in 2019. The present study is funded by Kristiania University College (NOK146,000).

Low-grade inflammation has been associated with cancer related fatigue (CRF). It affects up to 99% of patients during treatment but, perhaps more importantly, continues to be a burden after completion of treatment in one quarter to one third of survivors, limiting day-to-day activities and the resumption of a pre-cancer lifestyle. CRF is a complex and serious condition. Findings from a review of the literature suggest that CRF is associated with immunological, inflammatory, metabolic, neuroendocrine and genetic biomarkers. Several genes and signalling molecules have been found to be associated with fatigue. Several studies show a change in IL1, IL6, TNF-α, CRP and aPL in addition to the current genotype genes TNF-308 and IL-6-174. It is these biomarkers we want to measure in the present project.

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Data for both the treatment and control groups will be obtained from questionnaires from the main study and kept against blood samples from the subproject. Including biomarkers will increase the validity of the main study and will be noticed in international research, as the study will be one of the first to link acupuncture, CRF and biomarkers together in such a large study.

Inclusion

The sub-study will use participants who were included in the AcuBreast study. It will use the same randomized participants for the treatment and control groups. We will start with patient number 100 from the main study until we have 80 participants, 40 in each group. We estimate that 10–15% will drop out. After being informed about the AcuBreast study and completing all questionnaires, each participant will receive information about the sub-study and be invited to participate in the study. Those who agree to participate will receive information about the sub-study and an additional information sheet and consent form to sign. The participant will be given a copy of the signed consent form. The blood sample will then be taken.

The first blood sample will be taken during their first appointment for both groups. Next blood sample will be taken after the end of treatments for the treatment group and twelve weeks after the first appointment for the control group. For both group a blood sample will be taken 12 weeks after the last one.

Procedure

Blood samples will be taken in the morning, preferably at the same time for all candidates. (There is no absolute requirement for this, but it minimizes the chance of diurnal variation.) Candidates will be requested not to perform intense physical activity prior to sampling (although such activity is unlikely for patients experiencing post-treatment fatigue).

Following the guidelines in *Prosedyrebok for NoPSC biobank*, all samples will be taken as close together as possible, since pre-analytical variations can affect downstream analysis.

The guidelines are as follows:

- As few samplers as possible
- It is desirable not to stress the person during sampling
- The patient should be sitting or lying down during sampling
- The sampling tubes should always be taken in the same order
- Carefully invert the sampling tubes 6–8 times
- All deviations are registered on a separate form.

As a rule, we want to strive for only one venipuncture per patient. Where this is not possible, the rest of the tubes, including a new BD Vacutainer CAT, will be drained by a new venipuncture.

The following equipment will be used for sampling:

- Tourniquet
- 70% alcohol swabs for skin
- Vacuette Safety VPS 23G, 19mm (needle). Also called a 'butterfly', this is a sterile disposable cannula with a tube that facilitates sampling. It prevents backflow of liquid from the CTAD and PAX generator tubes.
- Cotton-wool ball
- Medical tape

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- Sampling tubes (NB: Always check the durability of extra tubes in the basket.)
- a puncture-resistant sharps container
- Study number.

Blood samples will be collected into an EDTA whole blood sample tube and a serum sample tube (pyrogen/endotoxin free tubes). Whole blood samples will immediately be transferred to aliquots (1 mL), be labelled with a study number and frozen for storage at -80 °C (up to one year). For serum, the blood sample will be left to coagulate for 30-40 minutes at room temperature. Coagulated blood and serum will be separated at 4000RPM for 15 minutes in a refrigerated centrifuge. Centrifuged tubes will be kept at an angle of 2–8 degrees during pipetting after centrifugation. The supernatant (serum samples, each labelled with study number) will be transferred to aliquots (1 mL) for storage at -80 °C (up to one year).

Serum will be thawed and used in the Elisa assay according to the associated protocol for analysis of IL-1ra, TNF alfa, IL-6 and CRP.

The whole blood samples will be thawed and used for genotype analysis according to protocol.

Patient information will be given both in writing and orally before the blood sample is to be taken. The information will be provided in such a way that the subject can make an informed decision.

Side effects

There is a risk to both patients and health workers if the phlebotomist is unaware of the patient's health status. Risk can be reduced by following best practices in infection prevention and control, after obtaining informed consent from the participants.

Data handling and storage

All blood samples are to be made anonymous and labelled with the same trial number that the participants received in the main AcuBreast study. All personal information and the index that links trial numbers with individual participants are to be kept in a locked room in a filing cabinet locked with a key, in the possession of our coordinator. Only the trial number can be used to identify individual computerized data from the blood samples.

The biobank

Blood and serum samples will be marked with the participant's trial number and stored in a research-specific biobank at -80 °C for up to one year. The biobank freezer is in a locked room to which lab technicians and project leader (Martin Strand) have access. All samples will be destroyed after analysis of the analytes described in the project, or no later than one year after sample collection.