Aggravated airway inflammation: research on genomics and optimal operative treatment (AirGOs operative)

Hengitysteiden vaikea tulehdus: perintötekijät ja leikkaushoidon optimoiminen

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Study plan

1. Introduction and our previous findings

Chronic rhinosinusitis (CRS) and asthma have the prevalence of around 10 % and are a major public health problem (1-4, 16). They are characterized by chronic inflammation with mucus hypersecretion, oedema, variable obstruction, and fatigue. Asthma and CRS can roughly be divided into T helper cell 2 (Th2) -low (neutrophilic) and Th2-high (eosinophilic) phenotypes (16-17). Th2-high phenotypes include allergic and /or eosinophilic disorders varying from mild to severe progressive forms. Moreover, CRS can be subdivided into two major clinical phenotypes based on nasal endoscopy: CRS with (CRSwNP) and CRS without (CRSsNP) nasal polyps, the former being usually a severe Th2-high phenotype (17-18). Acetylsalicylic acid (ASA-) exacerbated respiratory disease (AERD) is another example of severe Th2-high phenotype (12). AERD is characterized by a tetrad of CRS with nasal polyposis, chronic otitis, asthma, and sensitivity to all COX-1–inhibiting non-steroidal anti-inflammatory drugs (14-15). Its prevalence is 1 % in general population, but it might raise up to 50 % in tertiary care patients who have co-existing asthma and CRS. NSAID ingestion may lead to congestion, periorbital swelling, bronchoconstriction and laryngospasm. AERD is characterized by overproduction of cysteinyl leukotriene, underproduction of prostaglandin E2, and eosinophilic and mast-cell rich inflammation (14-15). COX-1 inhibition releases the 5-lipooxygenase-brake by inhibiting prostaglandin E2. AERD lacks safe diagnostics. It is diagnosed by a provocative ASA-challenge (14-15). 10 % decrease of nasal nitric oxide might be diagnostic for AERD in ASA-challenge (19.) Functional Eicosanoid Testing and Typing (FET) is an example of a low-risk diagnostic method under development (19). Despite advances in treatment, asthmatics bear higher all-cause mortality-risk compared to controls (HR=1.3, 95% CI 1.1-1.5, p=0.001) in our Adult Asthma in Finland -study (AAF) of 3,800 subjects (20). We showed that severe asthma is associated with higher all-cause morbidity, AERD, strong exposure to infections since birth, or tobacco smoke or molds, and low education.

The development of chronic airway inflammation has a strong genetic predisposition (22-24). Since 2007, over 46 Genome-wide association studies (GWAS) and over 29 GWAS reviews have been published of these traits (Table 1) (22-23). They show about 100 asthma and/or allergy -associating single nucleotide polymorphisms (SNPs). The interactions between host genome and environmental infections and irritants are crucial, yet mostly unknown, in pathogenesis of persistent eosinophilic inflammation in the airways.

The treatment of CRS and asthma consists of prolonged medical anti-inflammatory treatment (1-2). Conservative treatment of CRS is supplemented by functional endoscopic sinus surgery (ESS) in case of medical treatment failure. Mean revision rate of ESS in Finland is 10%, but it is higher in CRSwNP and in severe disease (13). Half of patients operated for CRSwNP have been operated earlier (9). ESS has been shown to improve hrQol and productivity, and to decrease cost of treatment in CRS (5-11, 25). Asthma incidence is high in uncontrolled CRS and seems to decrease after surgical treatment (25). Although ESS for CRS has been shown to decrease symptoms, emergency visits and need of medication in asthma, adequate evidence in the subgroup of CRSwNP and sustainability of benefit has not been shown

(11, 25) ESS usually includes uncinectomy, middle meatal anthrostomy and opening of the bulla ethmoidalis. In polyposis, recurrent disease may be treated with progressively extended procedures. So far, there is inadequate evidence that total ethmoidectomy and removal of ethmoid cells below the skull base would be better than less extensive approaches. This is due to heterogenous patient samples and other methodological flaws of previous studies (11). Total ethmoidectomy with opening of other sinuses may be more efficient in reducing symptoms and revision rates in the severest form of CRSwNP. Preliminary results of our retrospective study indicate that patient history of oral corticosteroids and antibiotics for CRSwNP before surgery was related to uncontrolled disease in the follow-up. Furthermore, extended surgery seemed to decrease revisions rates. These factors in patient history and the extension of surgery related to uncontrolled disease need to be evaluated in a prospective randomized study design (31, 34). However, total ethmoidectomy combined with opening of other sinuses is potentially more susceptible to skull base, orbital and vascular complications. It is therefore usually only performed by experienced surgeons using navigation. Availability of experience, navigation and longer operation time are required when compared with limited approaches such as partial ethmoidectomy reaching posterior ethmoids. Recently, timing of surgery has been found to be important in the course of airway disease in CRS and asthma (11, 26). Prolonged uncontrolled disease may thus compromise treatment results. Therefore, early adequate surgery in the uncontrolled severe CRSwNP may prove cost effective.

A limited number of biomarkers are currently available the endotyping of CRS patients in clinical practise. Several potential biomarkers might be used: eosinophils in blood, total IgE, specific IgE (e.g. Aspergillus fumigatus, Staphylococcus enterotoxin), eosinophil cationic protein (ECP), eosinophil mucin or total IgE in nasal secretions, and periostin in serum (28-29).

Taken together, clinical trials as well as search for new genome-based markers and therapeutics are needed in attempt to hit early and to prevent permanent modulations of airway structure and function in patients suffering from severe CRSwNP, asthma and AERD.

2. Aims and significance of the study

This is a randomized controlled multicenter study that focuses on severe CRSwNP ± asthma ±AERD. Our primary objectives of this study are to find out:

(1) Does extended surgery (total ethmoidectomy with opening of large sinuses) reduce HRQoL more effectively than partial ethmoidectomy? The primary end-point is the disease-spesific questionnaire SNOT-22. The secondary objectives are to:
   1) Evaluate the outcome of surgery on polyp size, symptoms, general HRQoL, asthma specific HRQoL, olfaction, nasal patency, revision-rate, exacerbation-rate, lung function, costs and safety
   2) Develop the care pathway of CRS+/-asthma+/-AERD patients to improve outcomes and cost effectiveness of treatment
   3) Develop safe, specific and sensitive genome-based biomarkers for detection and management models for of severe airway inflammation and to further develop markers for progressive disease forms.
   4) To see if patients with certain genetic and/or clinical predispositions will benefit from extended surgery.

Significance: The clinical significance of this study is to develop cost-effective treatment models that will facilitate risk-estimation and prevention of the onset and the progression of severe CRSwNP, asthma and AERD. The potential societal impact is that it reduces the burden of inflammatory airway diseases. It will increase co-work between

clinicians, standardize treatment protocols and increase equality in health care in Finland. The academic impact is to train PhD students and clinicians. We will publish in peer reviewed international open access medical journals.

3. Methods

A. Operative study

The study is performed as a randomized prospective controlled multicenter trial. A total of 174 adult severe CRSwNP patients ± asthma ± AERD participate in the study. The patients are recruited at the Departments of Otorhinolaryngology and Head and Neck Surgery, Helsinki University Hospital (HUH) (n=60-80), Tampere University Hospital (TUH) (n=20), Kuopio University Hospital (KUH) (n=15), Oulu University Hospital (OUH) (n=20) and Turku University Hospital (Tyks) (n=15). Otorhinolaryngological department of the Academic Medical Center, AMC, Amsterdam will recruit 60-80 patients for the study. Patients refusing to participate in the study are asked to contribute with HRQoL data, and age and gender are recorded. The study will be monitored by a professional monitor. Electronic CRF and paper/electronic patient questionnaires provided by HUS will be used (eCRF and patient questionnaires, Granitics).

Power calculation: The mean postoperative difference in the Sinonasal Outcome test (SNOT-22) was assumed to be at least 9 between the treatment arms. In a previous study the response within each subject group was normally distributed with standard deviation 20. If the true difference in the experimental and control means is 9, we will need to study 79 experimental subjects and 79 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0,8. The Type I error probability associated with this test of this null hypothesis is 0,05. Taking into account the patients lost to follow-up, 10%, the number of patients needed to recruit is 174.

The diagnosis of CRS with nasal polyps (NP) is based on positive history, nasal endoscopy and computed tomography scans (EPOS 2012). EPOS-criteria for CRS include: Inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip): ± facial pain/pressure, ± reduction or loss of smell.

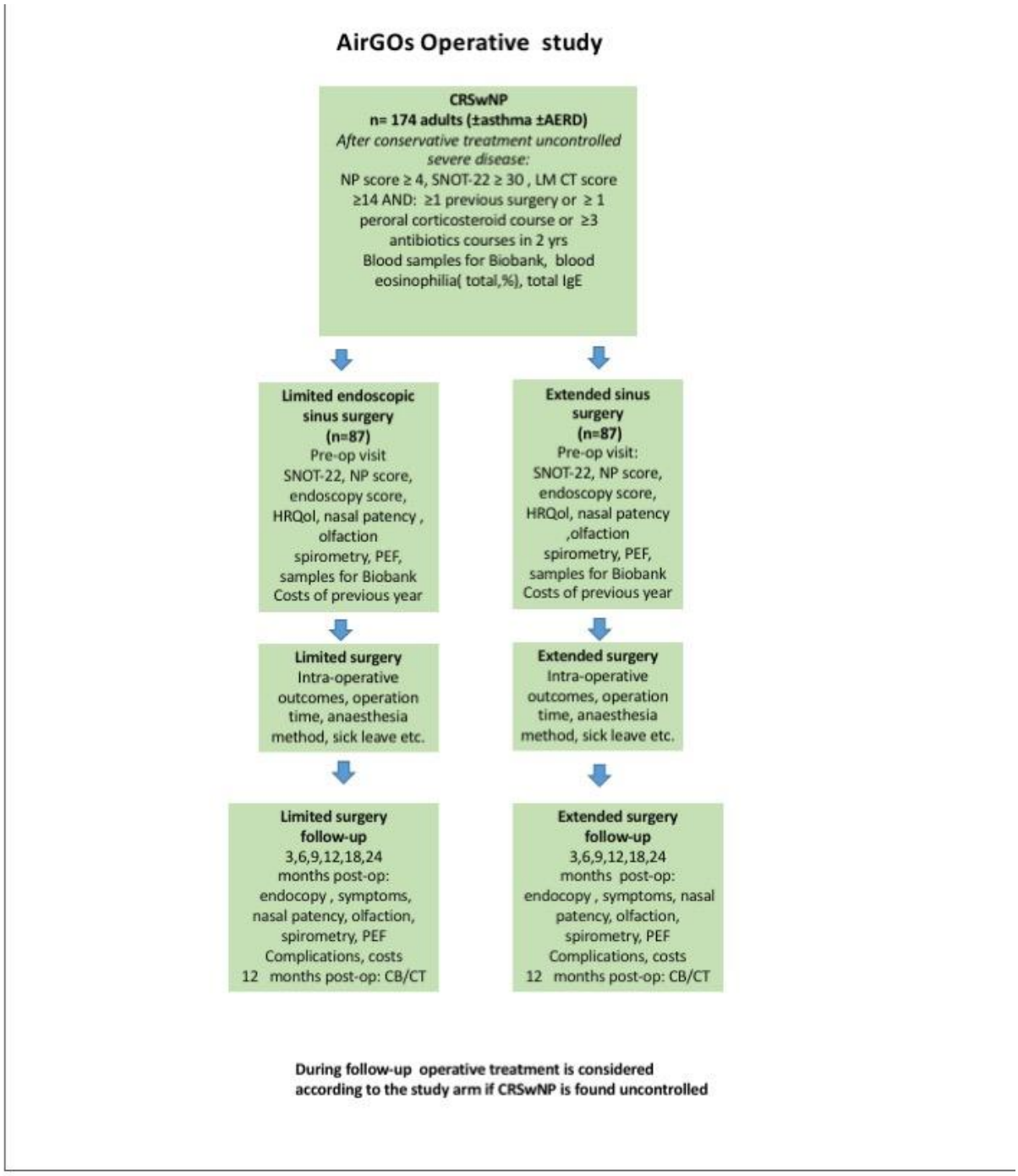
The diagnosis of asthma is based on spirometry, methacholine or histamine challenge and peak expiratory flow (PEF). Allergy is verified with positive history and skin prick test or specific igE. AERD is verified by typical history.

Adult patients with severe CRSwNP ± asthma ± AERD are recruited. Clinical baseline information of the patients is collected including smoking, allergy, asthma, medication, previous operations, co-morbidities, duration of symptoms. During preoperative visit/s in the ENT clinic nasal medication including treatment of exacerbations during the previous year is recorded. Medication for asthma during the previous year is counted and emergency care visits for airway symptoms/asthma attacks, hospitalizations, and other visits for asthma are monitored**.**

Preoperatively the patients undergo endoscopy (35) and (CB)CT. Polyps are scored on a 0-4 scale on each side and a Lund-Mackay computer tomography score (LM CT score, range, 0-24) is obtained (30, 31). Quality of life data and data for cost analysis are collected by validated questionnaires: Sinonasal outcome test (SNOT-22), generic HRQoL questionnaires (15D, EQ-5D-5L). Work Productivity and Activity Impairment Questionnaire: General Health (WPAI:GH), Productivity Costs Questionnaire (iPCQ), Medical Comsumption Questionnaire (iMCQ) and asthma control tests (ACT,

miniAQLQ). The patients undergo spirometry with bronchodilatation and PEF (2 weeks as a diagnostic test, 1 week in follow-up with patients own medication).

Figure 1.



Inclusion criteria: Patients having uncontrolled CRSwNP and not responding to medical treatment are asked to participate in the study. Conservative treatment consists of corticosteroid drops and nasal lavage lasting at least 3 months before surgery is considered. Uncontrolled CRSwNP is defined as high polyp grade (≥4, bilateral), and SNOT-22 ≥30, and high CT Lund-Mackay score (LM score ≥14). In addition, patient should have a history of at least one of

the following: ≥1 previous surgeries for CRSwNP; ≥1 oral corticosteroids during the past two years; ≥3 antibiotic courses during the past two years. In patients with contraindication/adverse effects in using oral steroids additional criteria are not required.

Exclusion criteria: Age <18 years, age > 65 years, complication of CRS (f.e. mucocele, invasive fungal rhinosinusitis), other diagnosis than CRSwNP (inverted papilloma, antrochoanal polyp etc), previous external sinus surgery or Draf 3 procedure (or indication for external approaches or Draf 3, bleeding diathesis, pregnancy/ breastfeeding, cystic fibrosis, primary ciliary dyskinesia (PCD), sarcoidosis, granulomatosis with polyangitis (GPA), eosinophilic granulomatosis with polyangitis (EGPA), immunosuppression (diagnosed Spesific Antibody Deficiency (SAD), CVI, HIV or use of biologicals/immunosuppressive medication), immunotherapy, daily use of systemic corticosteroids, communication problems (f.e. neurological/psychiatric disease, language skills), unlikely to comply, other severe disease, inability to be operated.

Randomization and surgery. The randomisation is performed by a professional statistician in blocks and for each center. The randomisation group of consecutive patients is sealed in numbered envelops and kept in a locked room; the envelop is opened during the visit after informed consent. The patients are randomized for 1. total ethmoidectomy +sphenotomy +frontal opening (Draf2a) and 2. removal of polyps with uncinectomy, +/-opening of the natural ostium +/- ground lamella but not opening sphenoid or frontal sinus unless already opened in a previous surgery (Figure 1). Sinus operations are performed under general or local anesthesia on both sides with standard techniques and instrumentation by ENT specialists. Videoendoscopy is recorded at completion of extended surgery. Extended sinus surgery is usually performed with navigation. If not contraindicated, the patients may receive peroral corticosteroids 10-14 days perioperatively starting a few days before surgery.

Follow-ups. Patients are monitored before and during the follow-ups. The follow-ups are 3, 6, 9, 12,18 and 24 months postoperatively. During each visit the validated questionnaires and nasal endoscopy are performed. Nasal patency and olfaction as well as spirometry and PEF are monitored. Blood and tissue samples and low-dose sinus CT scans (1 year postop) are performed (Figure 1).

We also monitor intra-operative factors (such as anesthesia method, use of local e.g. cocaine, terracortril and systemic medication, e.g. antibiotics and dexametasone, operation time and bleeding, use of tampons, complications, mucopurulent excretions, early postoperative factors such as duration of hospitalization, timing of tampon removal, duration of sick leave, medication, complications, visits and hospitalizations; as well as exacerbations, need of medication (po. corticosteroids; antibiotics). Time of surgery and equipment needed is recorded.

The primary end point is the change in SNOT-22 1 and 2 years postoperatively. Secondary end points include changes in polyp score, LM CT score, hrQOL, loss af productivity, nasal patency (peak nasal inspiratory flow (PNIF) +/- acoustic rhinometry (ARM), olfaction test (Sniffin' Sticks, identification), lung function (spirometry and PEF) and pathologic findings. Safety (complications, adverse effects) costs and loss of productivity between study arms will be compared. The study will compare costs of CRSwNP and asthma treatment one year before and two years after surgery. In addition to data from medical records and patients, data may be collected from national health records.

During and after follow-up, patients can undergo revision operation or other treatment, if needed and patients will still be followed for the duration of the study. Patient´s disease control is monitored postoperatively by ENT-doctor as described earlier. Patients who have uncontrolled disease not responding to medication undergo CT scans and are evaluated for the need of removal of polyps and re-ethmoidectomy according to their study arm. In addition, AERD-patients are evaluated for their willingness in ASA desensitization and to participate in the AirGOs clinical trial.

B. Genome-scale search for biological key-factors

Peripheral blood, intranasal mucus and cell brushing samples, nasal polyp and/or nasal mucosal biopsies are taken from clinical trial participants and volunteer control subjects. The samples will be stored at Helsinki Biobank. These samples are collected in a time-series manner (pre-intervention, 24w post start of intervention) and biopsies are taken under local anesthesia Nasal brushings and mucus swabs are collected from nasal mucosa, under middle turbinate, without local anesthesia. If possible, subjects are asked to be for 7-14 days without CRS medication before taking nasal specimens.
We aim at performing during the years 2-7 exome sequencing, mRNA sequencing and metatranscriptomics, immunohistochemistry, western-blot and ELISA in order to search for biological key-factors relevant for aggravated CRS and asthma and/or AERD.

For exome sequencing DNA is isolated from peripheral blood leucocytes, PCR enriched, and libraries are processed and sequenced on an Illumina HiSeq. For DNA methylation profiling, raw bisulphite treatment is performed on DNA. Oligonucleotide primers are synthesized. Libraries are processed and sequenced. SNPs and indels are called and annotation is performed by ANNOVAR. For metatranscriptomics RNA-Seq reads are trimmed and high-quality reads from each dataset are mapped to the non-redundant and truncated version of the ribosomal RNA SILVA reference. Reads are mapped to rRNA reference sequences and outputs are summarized for each phylotype. For transcriptomics total RNA from nasal cell brushings is extracted, enriched RNA-seq library is prepared and Nextera Primers are used. miRNA is PCR enriched and smallRNA libraries sequenced. Reads are corrected and aligned to the reference human genome (GRCh38/hg38). For metatranscriptomics RNA-Seq reads are trimmed and high-quality reads from each dataset are mapped to the non-redundant and truncated version of the ribosomal RNA SILVA reference. Reads are mapped to rRNA reference sequences and outputs are summarized for each phylotype. For quality control original cDNAs and RT-qPCR are used in the validation. cDNAs are amplified using TaqMan Universal PCR Master Mix. The expression levels are normalized with TBP or GAPDH. Small interfering RNAs targeting candidate genes are used. For exploring key-proteins we will perform immunohistochemistry, western-blot and ELISA to nasal specimens.

Statistics. Data analysis will be carried out on the relationship between two operative treatments, and between ASA- and placebo desensitization. Variables tested in the statistical models include age, gender, exposures, infections, diseases, symptoms, exacerbation-rate, satisfaction, image-findings, endoscopic findings, operation, perioperative factors (such as operation time), complications, sick leave, biological factors (predisposing SNPs, alterations in transcriptome or microbiome), occupation, asthma, grade of nasal polyps, AERD, allergy, revision-rate, side-effects of AD, hospitalizations, sick leaves, and other factors at different time-points. We will use several software: R 3.0.2, SPSS 22.0 Statistical Software Package (SPSS, Chicago, IL) and STATA (Release 13.1 software, StataCorp, Texas, USA). For comparisons, the results will be analyzed by Logistic regression, Student´s t-, Chi-square, (nonparametric) Fisher´s exact and Mann Whitney U test, Cox's proportional-hazards model, cumulative hazard function H(t), hazard ratio, Kaplan–Meier method, and log rank test. Genetic calculations on Hardy–Weinberg equilibrium and linkage disequilibrium will be performed with Arlequin (version 3.5) using SPSS Statistics (version 20.0, IBM). P-values less than 0.05 are considered statistically significant. Results of logistic regression are reported as odds ratios (OR) with 95% confidence intervals. All models will be adjusted by selected covariates (potential confounding factors).

4. Implementation of the study plan

The **operative** trial will be performed during the years 1-5, starting in September 2018. The wetlab will be performed during the years 2-6. The statistical analyses, data management, search for literature, and manuscript(s) will be performed during the years 1-6.

5. The study group and collaborators

The study group:

Docent, Otorhinolaryngologist (HUH) Paula Virkkula is director and is responsible for the management of the project and conducts the AirGOs Operative study in ENT hospital of HUH.

Docent, Pulmonologist (HUH) Paula Kauppi, is vice-director and conducts pulmonological assessment in Skin and Allergy Hospital (HUH/IAS).

MD, PhD, Otorhinolaryngologist (HUH) Sari Hammarén-Malmi, is vice director and performs operative and clinical study in ENT Hospital

Docent Sanna Toppila-Salmi (HUH) performs in IAS and conducts data management, manuscripts and sample studies

MD, PhD, Otorhinolaryngologist (HUH) Anu Laulajainen-Hongisto and MD performs the trial in IAS and ENT Hospitals

MD, PhD Otorhinolaryngologist Lena Hafrén (HUH) performs genome-scale tests for samples.

All study group members perform each phase of the study. They correspond on planning, wetlab, data management and manuscripts.

Collaborators:

MD PhD Seija Vento, Markus Lilja PhD, Johanna Wikstén and Maija Hytönen (HUH) collaborate in planning, patient recruitment, and operative study in ENT hospital. Professor Antti Mäkitie collaborates in planning, samples and manuscripts.

Pekka Malmberg, docent in clinical physiology (HUH) conducts lung function measurements in IAS.

Saara Sillanpää & Jussi Karjalainen (TaUH), Johanna Sahlman & Jukka Kiviranta (KUH), Perttu Halme & Maritta Kilpeläinen (TyUH), as well as Antti Alakärppä & Leena Tiitto (OUH) perform and organize patient recruitment and examinations in the four collaborative centers in Finland. Wytske Fokkens, Professor and Sietze Reitsma, otolaryngologist, the study coordinator participate in conduction of the operative the study in Academic Medical Center, Amsterdam.

Heikki Turpeinen, MD, (Univ. Helsinki, HUH) is a doctoral student assisting in the study.

Docent Mikko Mäyränpää & Docent Jaana Hagström (HUSLAB, Univ, Helsinki) provide expertise in wetlab experiments of the samples and pathology.

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