SUPPLEMENTARY FILE

Laboratory methods

DNA extraction and analysis

Stool samples and rectal and appendiceal swabs

Bacterial DNA from rectal and appendiceal swabs and faecal samples are extracted with semiautomated DNA extraction kit together with NorDiag Arrow extraction instrument (Diasorin). The following sample preparation is undertaken before the extraction: 500 µl of the sample is added to 700 µl of stool stabilizer in a 1.4 mm Ceramic Powerbead Tube (Qiagen, Hilden, Germany) and homogenized with MO BIO PowerLyzer 24 Bench Top Bead-Based Homogenizer (MO BIO Laboratories, Inc., USA) at 1000 rpm for 3 minutes followed by centrifugation at 5000 x g for 5 minutes and 600 µl of the supernatant is transferred into a new tube, the centrifugation is repeated and 500 µl of the supernatant is transferred into anew tube. This pretreatment is followed by the DNA extraction, which is performed according to the manufacturer´s instructions.

Appendiceal biopsy: Microbiological DNA is extracted from appendix biopsy stored in RNAlater with semiautomated DNA extraction kit using NorDiag Arrow extraction instrument. At first RNA later is removed by centrifugation and the pellet is resuspended into ultrapure water. Subsequently, the resuspension containing also the biopsy is treated with proteinase K in thermomixer. From the resulting solution DNA extraction is carried out according to manufacturer's instructions.

The concentration of eluted DNA from all sample types is measured with Qubit dsDNA HS or BR assay kit (Thermo Fisher Scientific) using Qubit fluorometer (Life Technologies, Carlsbad, California, USA). DNA is divided into two aliquots and stored at -75◦C for later analysis. Microbial

profile of samples will be analysed with NGS approach using appropriate methods and Illumina Miseq system.

Analyses of metagenome, transcriptome and proteome from appendiceal biopsy

Total RNA and proteins from the appendiceal biopsy are extracted with appropriate methods. Transcriptome and metagenome are analysed with Illumina Hiseq system. In addition, the expression of specific genes is quantified with quantitative real-time PCR. Proteome is characterized using mass spectrometry-based methods with qualitative and quantitative approach.

Culture methods

Appendiceal swabs are cultured with both aerobic and anaerobic culture methods. For aerobic culture the following growth media are used: CHROMagar Orientation (Becton Dickinson, Franklin Lakes, New Jersey, USA), Trypticase soy agar with 5% sheep blood (Becton Dickinson), Yersinia selective agar and Streptococcus selective agar (in house production). If the appendectomy is performed during the office hours, an additional anaerobic culture is made in connection with the sample collection. Samples for anaerobic culturing are collected with a sterile cotton swab from the appendix and cultured for fastidious anaerobe agar with blood (FAA) and kanamycin-vancomycin laked blood agar (KVLB) (both in house production), which are immediately transferred into anaerobic jar with anaerobic gas generating sachet (Anaerogen 3,5 l, Thermo Fisher Scientific).

Cultures are incubated at +35 °C for 18-24 hours. Morphologically distinct colonies are subcultured until a pure culture is obtained. Isolated species are frozen in a mixture of skim milk and glycerol at −75 °C.

MALDI-TOF mass spectrometry

The identification of isolated bacteria is done with Bruker matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Isolates are inoculated and cultured for 18-48 hours at +35 °C before the analysis. For the analysis, colony is applied either directly or through ethanol/formic acid extraction on a steel plate and HCCA matrix (α-cyano-4-hydroxycinnamic acid in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonics, Bremen, Germany)) is added according to the manufacturer´s instructions. The identification is done using Microflex LT instrument and MALDI Biotyper software (Bruker Daltonics). If isolates cannot be identified due to the absence of reference peaks in the database, the isolate is identified with sequencing the 16S rRNA gene.

Antimicrobial susceptibility testing

Phenotypic antimicrobial susceptibility testing for the isolated aerobic and anaerobic bacteria is performed by disk diffusion and MIC methods, according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. For certain bacteria, the Clinical and Laboratory Standards Institute (CLSI) guidelines can be used.

Further molecular analysis for antimicrobial resistance or virulence genes using e.g., PCR, Sanger sequencing and whole genome sequencing (WGS) are also performed for selected isolates.

Appendicolith analysis

Appendicoliths are assessed both on the surface and cross-sectional morphology. Based on the morphological characteristics and a degree of hardness, all appendicoliths will be classified. The composition of selected appendicoliths are analysed with physical and chemical methods.

Immunological analysis

The appendiceal biopsies are analysed immunohistochemically by determining the presence of different cell types and blood vessel surface markers. Neutrophils, leukocytes and monocytes will be analysed and special interest will be focused on lymphocyte subtypes (i.e., CD4/CD8 and more detailed subgroup analyses such as regulatory T cells) and monocyte markers (i.e., macrophage M1/M2 / receptor MHCII). Moreover, certain inflammation induced markers on endothelium such as VAP-1, E-selectin and P-selectin will be evaluated. In order to compare possible differences between patients with successful antibiotic therapy to patients with failed antibiotic therapy or complicated acute appendicitis the serum samples will be screened to identify possible inflammatory or immunological markers for identifying the different forms of the disease. Cytokines, chemokines and growth factors will be tested using Bio-Plex Pro Human Cytokine 48-Plex Screening Panel (BIO-RAD) containing the following analytes: Basic FGF, CTACK, eotaxin, G-CSF, GM-CSF, GRO-a, HGF, ICAM-1, IFN-a2, IFN-g, IL-1a, IL-1b, IL-1ra, IL-2, IL-2Ra, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-16, IL-17A, IL-18, IP-10, LIF, MCP-1 (MCAF), MCP-3, M-CSF, MIF, MIG, MIP-1a, MIP-1b, b-NGF, PDGF-BB, RANTES, SCF, SCGF-b, SDF-1a, TNF-a, TNF-b, TRAIL, VCAM-1 and VEGF-A. Moreover, we will use in house analyses to measure soluble VAP-1 (inflammatory) and CD73 (anti-inflammatory). In addition, metabolomics approach using nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LC-MS) metabolomics platforms will be used for biomarker analysis. The results obtained from immunological analyses will be correlated to the clinical parameters and to the microbiological findings.