Supplementary file 2: Investigational parameters

History

The history will include questions regarding previous and current OA-related medical and surgical events and treatments or treatments and/or events that might affect any of the biomarkers. Comorbidities will be recorded according to the Charlson index.[1] Smoking habits and menopausal status will also be registered. Menopause is defined by last menstrual bleeding ≥ 12 months ago, without using oral anticonceptives etc.

Physical and joint examination

Physical examination consists of a general examination and examination of knees, hips and hands. Knee examination will include warmth of the knee, effusion (positive patellar tap), passive ranges of flexion and extension, presence of a flexum, presence of varus or valgus and pain and grinding in the patellofemoral joint (grinding test). Physical examination of hips will include passive range of motions and presence of a flexum. On all hand joints, number and location of osteophytes and inflamed joints, defined by soft tissue swelling, will be evaluated. Deformity of Carpometacarpal joint 1 (CMC-1), Distal Inter Phalangeal (DIP) joints 2 and 3 and Proximal Inter Phalangeal (PIP) joints 2 and 3 and subluxation of the first metacarpal joint (MCP-1) will be reported. The articular index of Doyle will be used to determine severity of hand OA by grading pain (0-3) in 24 joints and joint groups by pressure or passive movement.[2]

Imaging index knee

Posteroanterior weight-bearing semiflexed (metatarsophalangeal) radiographs will be obtained according to the protocol of Buckland-Wright.[3]

Conventional and novel MRI techniques (e.g. T2 mapping) of the index knee will be performed to evaluate OA progression. These techniques visualize different tissue characteristics of the knee joint, possibly different involved in the OA process.

High resolution CT of the index knee will be performed for evaluation of bone architecture and shape analysis.

Imaging of other joints

Conventional standardized radiographs of hands will be obtained. WBLDCT will be conducted to evaluate concomitant hip, shoulder and spine OA. The overall radiation exposure during two years will be approximately 6.5 mSv (table 1). A standard clinical CT of the pelvis is 10 mSv.

Table 1: Effective dose for radiographs and CT (mSv per visit)

	Screening	BL	6M	12M	24M
Index knee					
- Radiograph	0.0002	-	0.0002	0.0002	0.0002
- CT	-	0.25	-	-	0.25
Other imaging					
- Radiographs (other knee + hands)	-	0.0004	-	-	0.0004
- WBLDCT	-	3	-	-	3
Total effective dose (mSv)	0.0002	3.2504	0.0002	0.0002	3.2506

BL: Baseline. **WBLDCT:** Whole Body Low Dose CT

Central reading

In order to minimize variability, the reading and analyses of images will be done at central facilities. Knee radiographs will be graded for structural OA features according to KIDA[4], Kellgren and Lawrence (KL) grade[5] and Osteoarthritis Research Society International (OARSI) grade[6] and hand radiographs will be evaluated according to KL grade, OARSI grade and Verbruggen-Veys score.[7] Bone shape will be analyzed by the Active Shape and Appearance

Models which provide quantitative, spatial and temporal understanding of all component tissues within a given joint. Subchondral bone analysis will be performed by fractal models.

Standard MRI techniques will be used to evaluate OA features in a quantitative[8] and semi-quantitative way using MRI Osteoarthritis Knee Score (MOAKS) analysis.[9] T2-mapping will give a qualitative measurement for assessing collagen distribution and is used to evaluate cartilage morphology and condition, anterior and posterior meniscal horns, cruciate ligaments, anterior and posterior femoral and tibial osteophytes, superior and inferior patellar osteophytes, subchondral bone cysts and attrition.[10] The novel exploratory method of Active Shape and Appearance Models will also be used on MRI images since bone shape is highly specific for the presence of OA and a sensitive measurement of progression, being more responsive than radiographic joint space width and MRI cartilage volume.[11]

Bone architecture on high resolution knee CT includes bone mineral density, anisotropy, inhomogeneity, variogram slope and entropy. For shape analyses, statistical Shape Model (SSM) applications in OA using predefined landmarks on 2D or 3D images produces a mean shape and modes of shape variation of the joint. In WBLDCT it is possible to reconstruct high quality 3D data providing more detail than standard radiographs.[12] Semi-quantitative grades on a 0-3 scale will be used to score joint space narrowing and osteophytes, disc degeneration, hip OA, glenohumeral and acromioclavicular OA and OA of facet joints.[13]

HandScan

The HandScan is a novel method for visualizing inflammation of the joints of hands and wrists. A pressure cuff around the lower arms is inflated, resulting in pooling of blood in the hands. This blood pooling is different in inflamed joints compared to healthy joints. The blood pooling is measured by diffuse optical transmission. Patient hands are illuminated with red/near-infrared light and the transmitted light is detected by a camera. Blood absorbs the light so blood pooling will give a decrease in the transmitted light. The scan takes about 2-3 minutes and provides an indirect measurement of joint inflammation.[14]

Motion analysis

Patients will be asked to walk 20 meters at their own self-selected speed, while six GaitSmart™ sensors are attached to their body (two at the hips, two at the thighs and two at the calves).[15] Data will be analyzed with Poseidon software. Obtained gait parameters include range of motion in the sagittal plane of pelvis, hips, thighs, knees and calves, medial-lateral movement of thighs and calves, knee stance flexion, joint and segment symmetry, and stride duration.

Performance-based tests

Two different performance based tests will be performed. For the 40-meter self-paced walk test patients will be asked to walk as quickly but safely as possible to a mark 10 meters away, return, and repeat for a total distance of 40 meters. For the 30-second chair stand-up test, patients are asked to stand-up from a chair and sit down again without use of their hands and arms and repeat this for 30 seconds.

Questionnaires

Index knee

Patients will be asked to fill in several questionnaires. The Knee injury and Osteoarthritis Outcome Score (KOOS)[16] and Intermittent and Constant OA Pain questionnaire (ICOAP)[17] are used to evaluate OA progression in the index knee. A pain Numeric Rating Scale (NRS)[18] is used to evaluate pain for the index knee.

Other questionnaires

The Hip disability and Osteoarthritis Outcome Score (HOOS)[19], ICOAP[17] for the most painful hip, Functional Index for Hand OsteoArthritis (FIHOA)[20] and aforementioned Pain NRS[18] will

be used to assess OA in other joints. PainDETECT[21] and Short Form 36 (SF-36)[22] are used to evaluate possible other causes of pain and quality of life, respectively. In addition, a one month pain diary including three questions (1. Do you have pain in your most affected knee? 2. Did you take painkillers or analgesics for your most affected knee? 3. Did you take painkiller of analgesics for other reasons?) will be used to evaluate pain.

Biological samples

Blood and midstream urine samples (fasted at baseline) will be collected, unless this is not possible for logistic reasons (e.g. long travel distance to clinical site). Fasting condition and time of last meal, physical activity within the last 24 hours and physiotherapy or spa activity within the last 72 hours will be recorded since these activities might influence biochemical markers, metabolomics and lipidomics. A maximum total volume of 113 mL blood per participant will be collected during two years (table 2)

Samples will be processed and stored at the clinical sites. Serum and plasma samples will be centrifuged at 2500xg and 4ºC. Then serum will be pooled. Both, serum and plasma will be stored at -70ºC to -80ºC in aliquots of 0.5mL and 1 aliquot of 5mL (only serum). DNA and RNA samples will directly be stored at -70ºC to -80ºC . Urine samples will be centrifuged at 1500xg at 4ºC and stored at -70ºC to -80ºC . Samples are shipped to central laboratories on a regular basis.

Parameters associated with joint pathology and metabolism will be measured. The biological pathways of interest are cartilage and bone metabolism (e.g. CTX-II), inflammation (e.g. C-reactive protein, pro-inflammatory cytokines), oxidative damage and antioxidant status (e.g. carotenoids, isoprostanes), and muscular anabolism (e.g. pro-collagen-3 n-terminal peptide). At the end of the study, the most state-of-the-art analytic approach will be used to examine these pathways.

Analysis of genomic markers (DNA and RNA) is optional and separate informed consent will be obtained. The aim is to investigate associations between genomic markers and knee OA, to assess whether different phenotypes have different genomic profiles at baseline or at study end.

Biological samples will eventually be stored in the central repository site, Biostorage (Germany), for duration of the consortium agreement. Thereafter samples will be relocated to original institutes and stored for a maximum of 15 years.

Table 2: Volume (mL) of blood and urine collected during the APPROACH study

	BL	6M	12M	24M
Urine for biomarkers	50mL	50mL	50mL	50mL
Blood for biomarkers				
- Plasma	21mL	21mL	21mL	21mL
- Serum	7mL			
Blood for genomic markers				
- DNA	8.5mL			8.5mL
- RNA	2.5mL			2.5mL

BL: Baseline. **mL:** milliliter. **DNA:** Deoxyribonucleic acid. **RNA:** Ribonucleic acid.

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