51 QUANTITATIVE PROTEOMICS OF DIFFERENT ZONES IN HUMAN ARTICULAR CARTILAGE REVEALS UNIQUE PATTERNS OF PROTEIN DISTRIBUTION


Purpose: The progress in proteomics technology development during the last decade has made tissue proteomics available also for the study of extracellular matrices such as cartilage and bone. We have used quantitative proteomics used for a detailed study of different zones in articular cartilage to increase our knowledge of structure in relation to cartilage biology.

Methods: Our gel-free proteomics approach includes tissue extraction, digestion by trypsin and tagging of peptides with ITraq for quantification followed by 2D LC-separations coupled to tandem mass spectrometry for their identification and quantification. The tissue was sliced from top to bottom in 10μm thin sections and the full thickness cartilage was divided into 9 pools including superficial, intermediate and deep zones.

Results: Previous known distributions of e.g. superficial zone protein (lubricin) were confirmed but also novel findings were observed e.g. asporin which was predominantly seen in the top layers. In total approximately 200 proteins were identified and quantified showing different patterns.

Conclusions: As an alternative to immunohistochemistry we used proteomics technology to study the protein abundance across full thickness articular cartilage. The advantage of this approach is that it allows multiple targets to be studied simultaneously and that it is independent of antibody availability. Other advantages include unambiguous identifications and improved quantifications as an unbiased detection of proteins and information on some of their structural qualities. The work has shown novel information on the differences of different layers of cartilage of value in understanding changes in early pathology.

52 VERIFICATION OF POTENTIAL DISEASE BIOMARKERS IN MESENCHYMAL STEM CELLS FROM OSTEOARTHRITIS PATIENTS BY SELECTED REACTION MONITORING ASSAYS


Purpose: To verify the potential role of the proteins which were found regulated in Bone Marrow-Mesenchymal Stem Cells (BM-MSCs) isolated from OA patients using Selected Reaction Monitoring (SRM) assays.

Methods: BM aspirates were obtained from the femoral channel of two OA patients (age: 52 and 89 years) and from three subjects without OA (mean age 73.3 years, range 47-90) at the time of orthopedic surgery. Protein extracts (150μg) of BM-MSCs were obtained by standard methods. Proteins were electrophoresed and after Coomasie blue staining three consecutive bands (Fig. 2 x 2 mm) were cut out and incubated with acetonitrile and 10 mM DTT. After several acetonitrile washes bands were trypticized with sequencing grade porcine trypsin. Protein extracts were finally analyzed by SRM assays performed on an hybrid triple-quadrupole mass spectrometers that enable the multiplexed, efficacious filtering of selected peptide sequences derived from specific proteins of interest. The statistical comparison of the OA and control groups was performed based on Student’s t-test. Values with p ≤ 0.05 were considered significant.

Results: Using SRM multiplexed analysis we have validated the differential expression of potential protein biomarkers in BM-MSCs from OA patients. These proteins were either metabolic enzymes (cathespin B chain B and dihydropyrimidinaselel 2 variant) or proteins related to cytoskeleton and cell motility (tubulin [nun-supported Character – Symbol Font ≤]6, Lcademson II and destrin isofrom B). SRM enabled the precise analysis of specific isoforms beyond commercial antibody-based assays, that usually analyze several protein isoforms simultaneously.

Conclusions: Using SRM multiplexed analysis we have validated the differential expression of potential protein biomarkers in BM-MSCs from OA patients and reinforced the hypothesized preactivation of these cells by signaling events produced by the subchondral bone. In addition we also corroborate the feasibility of using SRM for the multiplexed identification and quantitation of biomarker sets in clinically relevant samples with high sensitivity and specificity.
The mean pre-operative OKS for cemented UKR was 23.4/48 and improved to 38.9 at two years. For cementless UKR the pre-operative OKS was 24.4/48 which improved to 41.9 at two years. There was no significant difference (p=0.225, Mann-Whitney).

Conclusions: Cementless Oxford UKR has equivalent clinical outcome in the short term to the proven cemented prostheses. It has an increased amount of tibial migration, expressed as subsidence, compared to cemented fixation, but there is early stabilisation and no further migration after six months. This suggests that the particular kinematics of the Oxford UKR allow a mechanical environment under which a hydroxyapatite coated prosthesis can gain secure fixation that should provide very long term survivability.

54 CLINICAL OUTCOME AFTER TOTAL KNEE REPLACEMENT: FORGOTTEN KNEE. A SINGLE-CENTRE PROSPECTIVE PRAGMATIC STUDY

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Purpose: Total knee replacement is the radical surgical treatment of knee osteoarthritis and usually produces good or very good results in terms of pain and function. However the patient may report that the knee, although free of pain, does not feel normal. As a result, the patient’s assessment may be less favourable than the surgeon’s assessment of the outcome. A “forgotten” knee is a joint that feels completely normal to the patient and is therefore the best possible outcome from the patient’s viewpoint.

To evaluate the prevalence of “forgotten knee” in a single-centre pragmatic prospective study of a uniform patient population, from January 2001 to December 2008.

Methods: The study patients were selected for TKR by a specialized surgeon based on tibiofemoral and/or patellofemoral knee osteoarthritis stage IV in the Albeck radiological classification, with a > 70-mm visual-analogue-scale (VAS) pain score and non response to appropriate medical management. All procedures were done by the same surgeon (MB). A mobile-bearing posterior cruciate ligament-substituting prosthesis was used. The following were recorded at baseline and during regular post-surgery visits: VAS pain score, Knee Society knee and function scores, and four functional scores (KOOS, Oxford, WOMAC, and Lequesne). Forgotten knee was assessed at last follow-up based on the patient’s answer to the following question: “Do you feel your knee is completely normal all the time?” Knee radiograph findings before and after TKR were recorded. The chi-square test was used for qualitative parameters and the t test and analysis of variance for quantitative parameters.

Results: We included 498 knees in 406 patients (63% of women). Mean age was 70±8.6 years, mean body mass index was 29 Kg/m², and mean follow-up was 6.4 (3–10) years. Varus deformity was noted in 63% and valgus deformity in 36% of patients. At last follow-up, 375 patients were still active. All functional scores improved significantly (p=0.001) from baseline to last follow-up: KOSS, 40±14 to 95±9; Oxford, 38±9 to 14±4; global WOMAC, 51±18 to 12±7; and Lequesne, 14±9 to 1±3. Among the patients, 80% felt satisfied with the procedure and 87% felt the results were consistent with their expectations, but only 44% met our criterion for “forgotten” knee. There were 17 complications (3.4%) including 5 cases of sepsis with favourable outcomes after reoperation.

Conclusions: Only 44% of patients had a forgotten knee after TKR, although 80% were satisfied and free of pain. A forgotten knee may be a criterion for complete recovery that might be of use in future studies of TKR outcomes.

55 ASSOCIATIONS BETWEEN PRE-OPERATIVE RADIOGRAPHIC CHANGES AND OUTCOMES AFTER TOTAL KNEE JOINT REPLACEMENT FOR OSTEOARTHRITIS

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Purpose: Knee joint replacement (KJR) for OA is a successful intervention, but about 20% of KJR patients obtain little or no improvement. The reasons for this are unclear. The purpose of this study was to investigate whether pre-operative radiographic changes predict pain and functional outcomes.

Methods: Data from a prospective single-centre cohort study of patients undergoing TKR between Jan 1st 2006 and Dec 31st 2007 were analysed (n = 525). Pre-operative data collection included demographics (age, sex, BMI, self-reported co-morbidities), the International Knee Society Score (IKSS), and the Short Form Health Survey (SF-12). Pre-operative (within 6 months of surgery) AP standing and lateral radiographs were read by a single observer using the Kellgren and Lawrence (K&L) and Altman atlases, and the scoring repeated on a random 10% sample to assess rater reliability. Post-operative data collection at 12 and 24 months included the IKSS and SF-12. Linear and logistic regression analyses were undertaken to assess the relationships between baseline radiographic features and pre and post-operative pain and function.

Results: Of the 525 cases 24 were excluded as they had undergone unicompartmental replacements, and 23 radiographs were rejected, leaving 478 TKR cases for inclusion. 5 and 24 were unable to complete the 12 or 24 month data respectively due to death, loss to follow-up or refusal. The amount of missing data was well below 5%. The mean age of the subjects was 70.8 years (+/- 8.3), 69% were female and the mean BMI was 32.2. Pain scores improved from a mean of 4.0(+/-2.3) to 34.9 at 12 months and 34.8 (+/- 15.9) at 24 months: however about 30% of patients still complained of moderate or severe pain in the operated knee at each time point. Function scores improved from a mean of 37.5 (+/- 18.1) pre-operatively to 58.8 and 55.5 (+/- 26.8) at one and two years respectively. Inter-rater reliability scores for radiographic features were satisfactory, ranging from a kappa of 0.80 (lateral joint space narrowing) to 0.65 (lateral osteophyte score). The majority had OA K&L grade 3 (57 with mild (3a) and 200 with severe (3b) JSN) or 4 (87 without (4a) and 119 with (4b) bone attrition). The commonest compartmental distributions were medial and patella-femoral (244), or medial only (104).

There were no significant associations between pre-operative radiographic scores and pre-operative pain or function, but radiographic severity was associated with outcome. Those with lower K&L grades and less compartmental involvement were less likely to have obtained improvements in pain at either 12 or 24 months (e.g. the odds ratio for ongoing pain in the operated joint at 12 months comparing those with K&L grades < 3 to those with grade 4b was 5.23 (CI 1.5 to 17.6), p=0.008). The relationships between pre-operative radiographs and function were similar but less strong. Outcome did not appear to differ for those with predominant medial, lateral or patella-femoral joint involvement.