Assessment of Serum Biomarkers for Determining Severity of Knee Osteoarthritis based on Two Different Radiographic Scoring Systems

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Introduction: Osteoarthritis (OA) is a significant cause of pain and disability in patients worldwide. Knee OA is most commonly diagnosed based on patient-reported symptoms, physical examination findings, and radiographic assessments, which are only currently effective in irreversible stages of disease. Numerous studies have assessed serum biomarkers for their potential for diagnosing and staging knee OA. However, there is significant variability among studies such that no serum biomarkers have been validated for clinical use in determining OA severity in patients. Factors that may contribute to this lack of clinical application include the limits of the often-used Kellgren-Lawrence (K-L) grading system and failure to account for contralateral knee OA in the analyses. To address the limitations of K-L grading, our group developed the Composite OA grading system, which includes a much broader range of OA severity scores than the K-L system based on a greater number of features of OA assessed to determine scores for each compartment of the knee. As such, the Composite OA grading system allows for more distinct assignment of patients into knee OA phenotype cohorts based on involvement and severity. Therefore, this study was designed to compare differences in serum biomarkers based on OA severity as defined by K-L and Composite OA systems for operated, contralateral (non-operated), and both knees of patients undergoing total knee arthroplasty (TKA) for treatment of symptomatic OA. It was hypothesized that serum biomarkers could effectively delineate knee OA severity for both the K-L and Composite scoring systems, and that severity of OA in the non-operated knee would significantly affect the analyses.

Methods: With IRB approval (IRB#1208392) and informed patient consent, serum samples were collected from patients (n=93, mean age: 63.5y, mean BMI: 33.82, sex: 63F) undergoing TKA for treatment of symptomatic OA. Pre-surgical VAS pain scores, bilateral anteroposterior (AP) standing knee radiographs and skyline radiographs from the operated and non-operated knees of these patients were obtained. ***Radiographic Grading*:** Radiographs of the operated (OP) and non-operated (NON) knees were graded by an experienced musculoskeletal research radiologist, blinded to patient history, using the K-L (0-4) and Composite (0-29) radiographic grading systems. The Composite system grades instability of the tibiofemoral joint (grades 0-4); the medial and lateral tibiofemoral joint spaces separately for: joint space (0-4), osteophytes (0-3), sclerosis (0-1), cysts (0-1), and chondrocalcinosis (0-1); and the patellofemoral joint for: alignment (0-3) and joint space (0-2). The scores for each region of the Composite system can be summed to provide a score for the entire knee (CTS, 0-29). ***Biomarker testing*:** Serum was tested for COMP, GRO-α, TNF-α, IL-6, IL-8, MCP-1, MCP-3, MIP-1α, MIP1-β, MMP-2, MMP-3, DKK-1, OC, OPG, OPN, PTH, SOST, TIMP-1, TIMP-2, TIMP-3, TIMP-4, and VEGF. ***Statistical analysis:*** The serum data was log transformed for analysis, and patients were separated into cohorts based on the K-L and CTS grades of their OP knee, NON knee, and OP+NON knee. Significant ($p<0.05$) differences in serum biomarker concentrations between patient cohorts were determined using a univariate mixed linear model with patient age, sex, BMI, and VAS pain as covariates, and a LSD post-hoc analysis.

Results: ***Significant changes in Serum biomarker concentrations based on patient K-L grades (Fig. 1)*:** For the OP K-L grade cohorts, serum MCP-3, IL-8, TNF-α, and SOST were significantly higher in patients with a grade of 2 compared to a grade of 4. For the NON K-L grade cohorts, serum MMP-2 and TNF-α were significantly higher, and OPG and PTH was significantly lower, in patients with a knee replacement than patients with a K-L grade of 3 or 4. For the OP+NON K-L grade cohort, patients with grades of 3 or higher in both knees had significantly lower serum MMP-2, MMP-3, and TNF-α compared to patients with lower K-L grades in both knees. ***Significant changes in Serum biomarker concentrations based on patient Composite grades (Fig. 2)*:** For the OP CTS grade cohorts, patients with a CTS grade <5 had significantly higher serum OPG compared to patients with a CTS grade of 10. For the NON-CTS grade cohorts, serum DKK-1 was significantly higher in patients with a CTS grade of 8-11 compared to patients with a previous TKA and CTS grade <8, that serum MIP-1β was significantly higher in patients with a CTS grade of 8-9 compared to patients with a previous TKA, and patients with a previous TKA had significantly lower serum PTH. For the OP+NON-CTS grade cohort, serum DKK-1 was significantly lower in patients with a combined grade of 15 compared to other grades, serum TIMP-3 was significantly lower in patients with a combined grade of 17 compared to other grades, and serum SOST was significantly higher in patients with a combined grade of 14 compared to other grades.

Discussion: The data from this study elucidated significant differences in serum biomarkers among OA severity cohorts based on K-L grade or Composite sum scores of the operated knee, the non-operated knee, and both knees combined in patients undergoing TKA for treatment of symptomatic OA in one knee. Interestingly, biomarkers that distinguished OA severity based on K-L grades were significantly different from those that distinguished OA severity based on Composite scoring. Further, biomarkers that distinguished OA severity in the operated knee were significantly different from those that distinguished OA severity in the non-operated knee and in both knees combined. Taken together, these results suggest that the method for defining OA severity significantly impacts serum biomarker analyses and is critically important for determining the clinical relevance of biomarkers used for knee OA diagnosis and staging.

Significance: The results of this study support the use of the Composite OA Scoring System for distinguishing severity of knee OA for development and validation of serum biomarkers for knee OA diagnosis and staging. Further research is needed in order to optimize relative weighting of OA severity in each knee with respect to determining the clinical relevance of serum biomarkers. Ongoing studies in our lab are aimed at analyses of larger data sets in order to identify valid biomarkers for clinical application.