Lectin Microarray-Based Glycosylation Profiling and Explainable Machine Learning Reveal Shared Synovial Fluid Osteoarthritis Biomarkers in Humans and Companion Animals

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DISCLOSURES: HLR (3C,4; Calyxis Biosciences, Inc.), AGP (N), PR (N), CC (N), SR (3B; Novartis Pharmaceuticals, Advance Medical, Enovis; 4; Jannu Therapeutics, Inc., Overture Medical; 5; Virginia Toulmin Foundation, OREF, Arthritis Foundation, Angiocrine Biosciences, Inc.; 8; Associate Editor, American J. Sports Medicine), KH (N), LKM (3B; Vector Laboratories, Inc.)

INTRODUCTION: Post-traumatic osteoarthritis (PTOA) is a leading cause of morbidity in humans and companion animals. Despite its prevalence, early diagnostic biomarkers remain limited, and imaging modalities such as radiography lack sensitivity for detecting early disease. Synovial fluid (SF) represents a localized biofluid that reflects joint pathology, yet systematic glycomic analyses across species are lacking. We hypothesized that lectin microarray-based profiling of SF, combined with explainable machine learning, would reveal conserved glycosylation signatures capable of distinguishing OA from healthy joints in humans, dogs, and horses.

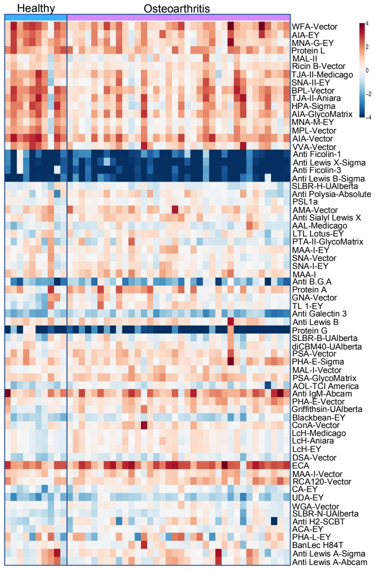
METHODS: SF was collected from canine (n=60; control = 9, OA = 51), equine (n=118; control = 41, OA = 77), and human (n=52; control =16, OA =36) subjects of both sexes, classified by joint as 'healthy' control or osteoarthritis (OA). SF was collected from dogs diagnosed with naturally occurring cranial cruciate ligament rupture, horses diagnosed with naturally occurring osteochondral fragmentation, and humans diagnosed with anterior cruciate ligament rupture or moderate-to-severe knee OA. Dual-color lectin microarray profiling was performed using 62 probes to assess glycosylation patterns **(Figure 1)**. Student's t-tests with Benjamini-Hochberg FDR corrections identified differentially bound lectins, and CatBoost machine learning models were applied to classify OA versus healthy samples. Model performance was assessed using stratified 10-fold cross-validation and a holdout test set. SHAP analysis was used to determine lectin contributions to classification. This study was performed with both IACUC and IRB approval.

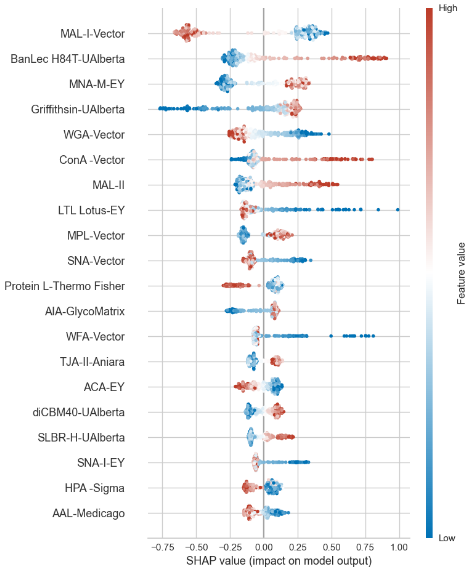
RESULTS: Distinct glycomic alterations were observed across species. Canine OA joints exhibited reduced α2,6- and α2,3-sialylation, while equine OA joints showed elevated Core 1/3 *O*-glycans. Human OA joints displayed elevated core fucosylation and lower abundance of *O*-glycan structures. A conserved enrichment in high mannose glycans was detected in all three species. The CatBoost model achieved strong classification performance (mean accuracy 0.91, AUC 0.98; holdout accuracy 87%, recall 100%). SHAP analysis identified high-mannose–binding lectins (Griffithsin, BanLec) and sialic acid–binding lectins (SNA, MAL-I) as top predictors of OA across all 3 species **(Figure 2)**. In addition to species-specific differences, cross-species comparisons revealed that canine and equine OA glycan profiles were more similar to each other than to those of humans, likely reflecting the study design in which canine and equine samples were obtained from independent OA and control subjects, whereas human synovial fluid was collected from the contralateral knees of OA patients.

DISCUSSION: This cross-species study demonstrates that synovial glycan alterations, particularly conserved increases in high-mannose structures and shifts in sialylation and core fucosylation, are key features of OA **(Figure 3)**. Machine learning revealed that these glycomic signatures robustly discriminate OA from healthy joints. Species-specific differences in glycosylation patterns may reflect variation in experimental design (paired sampling in humans but not in veterinary species) or underlying biology. Limitations include small control cohorts, contralateral 'healthy' controls in human samples, and lack of external validation. These findings highlight the value of a One Health approach, demonstrating that companion animal models can provide insights into shared and divergent pathways of OA pathogenesis, ultimately strengthening translational relevance for biomarker discovery.

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes synovial glycomic profiling as a minimally invasive approach for OA biomarker discovery. The identification of conserved glycan signatures across humans, dogs, and horses highlights translational potential and supports the development of glycan-based tools for early OA detection and patient stratification. By integrating glycomics with explainable machine learning, this study demonstrates a scalable framework for cross-species biomarker discovery that may accelerate both veterinary and human OA diagnostic and therapeutic development.

Weill Cornell Medical College/Center for Translational Science NIH Pilot Award (UL1 TR002384); Harry M. Zweig Memorial Fund for Equine Research; Cornell Veterinary Biobank (NIH R24 GM082910.).





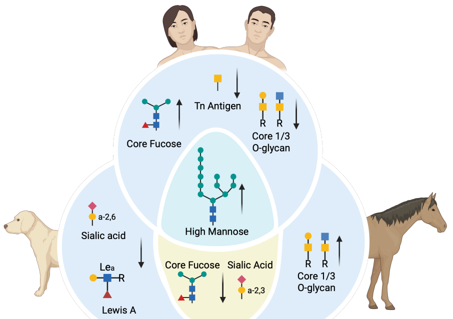


Figure 1. Heatmap of human synovial fluid lectin microarray results. **Figure 2.** SHAP Analysis of Lectin Microarray. SHAP summary plot displaying the impact of individual lectin-epitopes on the classification of OA versus control synovial fluid samples. **Figure 3.** Comparative glycomic analysis across human, canine, and equine OA samples reveals both conserved and species-specific glycan alterations. High-mannose N-glycans (center), are consistently enriched in all three species, representing a robust, cross-species OA signature.