

Original Article

Incremental predictive value of longitudinal serum biomarker trajectories over baseline levels for rapid structural progression in knee osteoarthritis: a group-based trajectory modeling and least absolute shrinkage and selection operator Cox model

Yun Yang*, Kun Li*, Jie Zhou, Liangyu Lu

Department of Orthopedics, Pudong New Area People's Hospital, No. 490, South Chuanhuan Road, Pudong New Area, Shanghai 201299, China. *Equal contributors and co-first authors.

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Abstract: Objective: To develop a predictive model for rapid structural progression in knee osteoarthritis (KOA) using dynamic biomarker trajectory subtypes derived from longitudinal serum biomarker measurements, and to quantify the incremental prognostic value of longitudinal trajectory features beyond traditional single-timepoint baseline measurements. Methods: This single-center retrospective cohort study included patients diagnosed with KOA at our institution between January 2019 and March 2023. Group-based trajectory modeling (GBTM) was used to identify longitudinal trajectories of four biomarkers (interleukin-6 [IL-6], tumor necrosis factor- α [TNF- α], cartilage oligomeric matrix protein [COMP], and hyaluronic acid [HA]). Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression with the λ_{1se} criterion was employed to select predictors from 21 candidate variables, and Cox proportional hazards models were subsequently constructed. Harrell's C-index and Akaike Information Criterion (AIC) were used to assess model performance, with internal validation performed using 10-fold cross-validation repeated 20 times. The incremental prognostic value of longitudinal trajectory information was assessed via Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI). A 24-month landmark analysis was conducted to evaluate potential time bias. Results: A total of 450 patients with KOA were included, of whom 58 (12.9%) experienced rapid structural progression during follow-up. GBTM analysis identified four trajectory classes for IL-6, three for TNF- α , and three each for COMP and HA. LASSO regression identified five independent predictors: Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score (hazard ratio [HR] = 1.12, 95% confidence interval [CI]: 1.05-1.20), baseline IL-6 (HR = 1.23, 95% CI: 1.06-1.43), TNF- α Class 3 trajectory (HR = 1.80, 95% CI: 1.00-3.23), COMP Class 4 trajectory (HR = 2.98, 95% CI: 1.57-5.65), and HA Class 2 trajectory (HR = 6.81, 95% CI: 3.47-13.38). The primary model demonstrated excellent discrimination, with a concordance index (C-index) of 0.9034 (cross-validated C-index = 0.8982), an AIC of 538.3, and no evidence of violation of the proportional hazards assumption (global P = 0.609). Compared with the clinical-only model, the main model incorporating biomarker trajectory features achieved an IDI of 0.5801 and an event NRI of 0.5000. Conclusion: Longitudinal serum biomarker trajectory patterns provide prognostic information for rapid structural progression (SP) in KOA beyond baseline features. HA Class 2 and COMP Class 4 trajectories were identified as the strongest independent prognostic predictors. The LASSO-based main model, with only five variables, demonstrated strong discriminative ability. However, due to the single-center retrospective design and the absence of independent external validation, prospective multicenter studies are required to further validate the model.

Keywords: Knee osteoarthritis, serum biomarkers, longitudinal trajectory, group-based trajectory modeling, cox proportional hazards regression

Introduction

Knee osteoarthritis (KOA) is one of the most widespread degenerative joint diseases experi-

enced worldwide and it represents a major contributor to chronic pain, functional impairment, and reduced quality of life among middle-aged and older adults. The Global Burden of Disease

Study has demonstrated a continuous increase in the global incidence from 1990 to 2020, with a substantial further rise projected by 2050 [1-3]. Previous systematic review suggests that advanced age, obesity, prior knee injury, and metabolic abnormalities are associated with the development of KOA [4, 5]. The progression of KOA shows significant inter-individual variability; while some patients exhibit stable disease courses over extended periods, others experience rapid structural deterioration, which is commonly characterized by accelerated Kellgren-Lawrence (KL) grade progression, joint space narrowing (JSN), and need for total knee arthroplasty (TKA) [6].

KOA is increasingly recognized as a complex whole-joint disorder involving articular cartilage degeneration, synovial inflammation, subchondral bone remodeling, meniscal degeneration, and osteophyte formation [1, 7]. The underlying pathogenesis is primarily driven by imbalance between anabolic and catabolic activities of articular chondrocytes, resulting from the interaction of mechanical overload, inflammatory responses, and metabolic stress. Factors such as obesity, mechanical malalignment, chronic low-grade synovial inflammation, and systemic metabolic dysregulation contribute to structural deterioration in KOA [4, 5].

Cytokines are key immunoregulatory mediators involved in inflammatory responses and tissue stress adaptation. Inflammatory monocytes release pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), which promote matrix degradation by enhancing the expression and activity of matrix metalloproteinases (MMPs) and regulating downstream inflammatory pathways. Simultaneously, degradation products of the cartilage matrix, such as cartilage oligomeric matrix protein (COMP) and hyaluronic acid (HA), are released into the synovial fluid and the systemic circulation. Their circulating concentrations may reflect ongoing extracellular matrix turnover and joint tissue catabolism [8, 9]. IL-6 and TNF- α reflect inflammatory activation and catabolic signaling; COMP reflects cartilage extracellular matrix turnover; and HA reflects synovial dysfunction and overall joint metabolic disturbance [8-11]. Dynamic changes in these biomarkers over time may capture the evolving biological processes associated with rapid

structural progression and provide mechanistic insights into disease heterogeneity.

Currently, KOA severity assessment primarily relies on clinical symptoms and radiographic evaluation. Nevertheless, conventional radiography typically detects structural changes only after substantial joint damage has occurred, limiting its ability to provide early prognostic information [7]. Serum biomarkers, including COMP, HA, IL-6, and TNF- α , have shown potential in KOA risk stratification and progression prediction [8-11]. However, most previous studies have relied on single baseline measurements and have not incorporated longitudinal biomarker dynamics during disease evolution.

The Group-based Trajectory Model (GBTM) is a statistical approach that identifies latent trajectory subgroups within longitudinal data and is well-suited for characterizing heterogeneous disease evolution. Patients with KOA exhibit unique longitudinal changes in symptoms and functional decline, and population-average trends may obscure clinically meaningful subgroups [12, 13]. By integrating repeated measurements over time, GBTM can capture complex biomarker evolution patterns beyond those reflected by single-timepoint values or simple change scores. Although significant progress has been made in predictive models for KOA progression [14, 15], few studies have linked longitudinal serum biomarker trajectories to rapid structural progression. Whether multi-marker trajectory information offers incremental prognostic value beyond conventional baseline characteristics remains unclear.

To address this gap, GBTM was employed to characterize longitudinal trajectories of four serum biomarkers (IL-6, TNF- α , COMP, HA) at baseline, 12 months, and 24 months. Subsequently, Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression was applied to develop a multivariable predictive model. This study aimed to characterize the associations between distinct biomarker trajectory classes and rapid structural progression, evaluate the incremental predictive value of trajectory-based models compared with clinical models, and ultimately, facilitate early identification and personalized monitoring of KOA patients at high-risk of rapid structural deterioration.

Materials and methods

Study design

This was a single-center retrospective cohort study. Consecutive patients with KOA who were evaluated at the orthopedic outpatient clinic or admitted to the inpatient department of Pudong New Area People's Hospital between January 2019 and March 2023 were screened for eligibility. All patients underwent serum biomarker measurements and knee radiographic examinations at three time points: baseline (M0), 12 months (M12), and 24 months (M24). Clinical and radiographic follow-up for outcome assessment was continued for up to 36 months after baseline.

The study protocol was approved by the Medical Ethics Committee of Pudong New Area People's Hospital. Given the retrospective nature of the study, the requirement for informed consent was waived. This study was conducted in accordance with the Declaration of Helsinki [16], and was reported according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [17].

Study population

Inclusion criteria: (I) Age 45-79 years; (II) Fulfillment of the American College of Rheumatology (ACR) clinical diagnostic criteria for KOA, with a baseline KL grade ranging from 0 to 3 in at least one knee; (III) Availability of complete serum biomarker measurements, including IL-6, TNF- α , COMP, and HA, at all three time points (baseline, 12 months, and 24 months); (IV) Availability of complete baseline and longitudinal radiographic follow-up data within 36 months, including KL grade and joint space narrowing (JSN) assessments; (V) Availability of complete baseline clinical data.

Exclusion criteria: (I) Baseline KL grade 4, indicating end-stage KOA; (II) Concurrent inflammatory arthritis, including rheumatoid arthritis, gouty arthritis, psoriatic arthritis; (III) Prior surgery involving the target knee, including arthroscopic debridement, osteotomy, or arthroplasty; (IV) Severe hepatic or renal insufficiency, active malignancy, or other systemic diseases that could significantly influence serum biomarker levels; (V) Missing key variables exceeding 20%.

A total of 450 KOA patients who met the eligibility criteria were included in the final analysis, including 58 patients with rapid structural progressors (RSP) (12.9%) and 392 patients without progressors (87.1%).

Clinical data collection

Clinical data were extracted from the institutional electronic medical record system and standardized case report forms (CRF). Demographic and general clinical characteristics included age, sex, body mass index (BMI), and target knee laterality. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale [18] was assessed at baseline by trained research staff to evaluate KOA-related pain severity. The subscale consists of five items: pain while walking, stair climbing, lying in bed, sitting or lying, and standing. Each item is scored on a 0-4 scale (none to extreme), yielding a total score ranging from 0-20, with a higher score indicating greater pain severity.

Baseline Kellgren-Lawrence (KL) grades were independently assessed by radiologists, and discrepancies were resolved through consensus. The Charlson Comorbidity Index (CCI) was calculated using the updated Quan-weighted scoring system (weights of 1, 2, 3, or 6) [19]. This measure encompasses 17 comorbidity categories, including cardiovascular, cerebrovascular, pulmonary, hepatic, renal, diabetic, and malignant conditions, based on a comprehensive review of medical records. A higher CCI score indicates a greater comorbidity burden. Additional clinical variables, including previous knee injury history, hypertension, and diabetes mellitus, were collected from medical records. Previous studies have reported that symptom severity measures, such as the WOMAC scores, are associated with serum cartilage metabolism biomarkers and disease severity [9]; thus, the WOMAC pain score was included as a candidate clinical predictor. Furthermore, given the reported associations between demographic characteristics, previous injury, metabolic disorders, and KOA risk [4], BMI, knee injury history, and comorbidity burden were also included as covariates.

Serum biomarker measurement

Fasting peripheral venous blood samples (5 mL) were collected from all participants at

baseline (M0), month 12 (M12), and month 24 (M24). Samples were centrifuged at 3,000 r/min (centrifugation radius: 10 cm) for 10 minutes within 2 hours of collection to isolate serum. The separated serum samples were aliquoted and stored at -80°C until analysis.

Four serum biomarkers were measured using enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme-linked Biotechnology Co., Ltd., China). The corresponding kits included human IL-6 ELISA (lot no. EL-IL6-2021), TNF- α ELISA (lot no. EL-TNF α -2021), COMP ELISA (lot no. EL-COMP-2021), and HA ELISA (lot no. EL-HA-2021). All assays were conducted following the manufacturer's instructions. The intra-assay and inter-assay coefficients of variation (CV) for all biomarker were maintained below 15%. Measurements obtained at the three time points were then used to identify longitudinal biomarker trajectory classes using GBTM. These trajectory classes were used as candidate predictors in Cox regression models for predicting rapid structural progression.

Outcome measures

The primary outcome was rapid structural progression of KOA within three years. RSP was defined as the occurrence of either of the following events: an increase of ≥ 2 grades in the KL grade of the target knee within 36 months compared to baseline, or undergoing total TKA of the target knee within 36 months. Participants who met either criterion were classified into the RSP group (Progressor), whereas those who did not meet any criterion were classified as the non-progression group (Non-progressor). Progression-free survival (PFS) time was defined as the interval (in months) from enrollment until the occurrence of the first qualifying progression event or until the last follow-up for participants without progression.

The secondary outcome was the occurrence of TKA within three years, with the event time defined as the interval from enrollment to TKA. Given the relatively small number of TKA events ($n = 22$), analyses of this secondary outcome was considered exploratory.

A threshold of ≥ 2 KL grade increase was adopted to distinguish between rapid structural deterioration and slow or physiological progression. A one-grade increase over 36 months may rep-

resent the expected natural course of KOA rather than accelerated progression. According to prior studies using data from the Osteoarthritis Initiative (OAI), this definition aligns with those where radiographic progression is defined as a KL grade change of ≥ 1 or ≥ 2 grades, with the latter representing a more stringent criterion for rapid/accelerated progression [6].

The inclusion of TKA as an additional component of the composite endpoint captured patients who progressed to clinically meaningful end-stage structural failure requiring joint replacement within the 36-month window. Previous studies have used KL grade progression, JSN, and arthroplasty as indicators of radiographic progression or structural deterioration in KOA. Accordingly, this study employed a composite RSP endpoint consisting of KL progression and TKA to improve the clinical relevance of the outcome definition.

Statistical analysis

All statistical analyses were performed using R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria) and SPSS software (version 27.0; IBM Corp., Armonk, NY, USA). Continuous variables were first assessed for normality using the Shapiro-Wilk test. Variables following a normal distribution were expressed as mean $\pm$ standard deviation (SD) and compared using the independent-samples t-test. Non-normally distributed variables were expressed as median (interquartile range [IQR]) and compared using the Mann-Whitney U test. Categorical variables were expressed as frequencies (percentages) and compared using the χ^2 test or Fisher's exact test, as appropriate. A P value < 0.05 was considered statistically significant.

Longitudinal changes in the four serum biomarkers across the three time points were visualized using line plots, with values presented as mean $\pm$ standard error (SE). Wilcoxon rank-sum tests were employed for comparisons between groups at each time point. GBTM was performed using the flexmix package in R, with time points (0, 12, and 24 months) as a fixed effect and individual identifier as a random effect. For each biomarker, models containing 1-4 trajectory classes were fitted to the longitudinal data. The optimal number of trajectory classes was determined based on the Bayesian

information criterion (BIC), together with practical criteria including an average posterior probability ≥ 0.70 and a minimum class proportion $\geq 5\%$. Each participant was assigned to the trajectory class with the highest posterior probability.

Using the lowest-risk trajectory class as the reference category, univariable Cox proportional hazards regression models were constructed to evaluate relationships between each biomarker trajectory classes and RSP, with hazard ratios (HR) and 95% confidence intervals (CI) estimated. Candidate predictors included seven clinical variables (age, BMI, WOMAC pain score, Charlson CCI, KL grade, sex, knee injury history), four baseline biomarker levels, and ten trajectory class indicator variables (21 candidate variables in total). LASSO Cox regression was performed using the glmnet package. Ten-fold cross-validation was used to identify the optimal regularization parameter (λ). The final selection of predictors was made using the λ_{1se} criterion to obtain a simpler yet stable model.

Subsequently, four candidate Cox proportional hazards models were constructed: Model 1 (clinical variables only); Model 2 (clinical variables + baseline biomarker levels); Model 3 (LASSO λ_{1se} main model); Model 4 (clinical variables + all class trajectory variables). Model discrimination was evaluated using Harrell's C-index, and model fit was assessed using the Akaike Information Criterion (AIC). Schoenfeld residual tests (cox.zph function) were used to test the proportional hazards assumption of each model. Ten-fold cross-validation with 20 repeats was conducted to assess internal validation stability and compute the cross-validated C-index and its optimism (Optimism = apparent C-index - cross-validated C-index).

Using the clinical-only model as a reference, the incremental prognostic value of trajectory information was evaluated by calculating the Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI) for the LASSO λ_{1se} model. The robustness of the main model's discrimination was evaluated in BMI-stratified subgroups (dichotomized according to the median BMI). In addition, the predictive performance of a simple change-score model (from baseline to 12 months) was compared with that of the trajectory model. A land-

mark sensitivity analysis was conducted to assess potential time-related bias. Participants with a progression event before 24 months ($n = 19$) were excluded from the analysis. The main LASSO λ_{1se} model was then refitted in the remaining 431 participants using a new time origin at 24 months (Time_lm = Time - 24) to estimate the association biomarker trajectories and progression risk during the interval from 24 to 36 months. Results of the landmark analysis are presented in [Table S1](#).

Results

Baseline patient characteristics

A total of 450 patients with KOA were included in the final analysis. Among them, 392 (87.1%) were in the non-progression group (Non-progressor, Group P) and 58 (12.9%) were in the rapid structural progression group (Progressor, Group C). Twenty-two patients underwent total TKA within 3 years. Baseline characteristics of the two groups are summarized and compared in **Table 1**.

Compared with Group C, patients in Group P were significantly older [(64.90 $\pm$ 5.77) years vs. (61.60 $\pm$ 7.62) years, $Z = -3.16$, $P = 0.002$], had a significantly higher BMI [(30.96 $\pm$ 4.02) kg/m² vs. (27.95 $\pm$ 3.91) kg/m², $t = -5.34$, $P < 0.001$], and reported significantly higher WOMAC pain scores [(11.03 $\pm$ 3.44) vs. (7.19 $\pm$ 3.02), $Z = -7.39$, $P < 0.001$].

Compared with Group C, Group P showed significantly higher proportions of patients with baseline KL grade 3 (43.1% vs. 19.6%) and a history of knee injury (51.7% vs. 30.6%, $\chi^2 = 9.21$, $P = 0.002$).

Regarding the follow-up duration, Group P had a significantly shorter follow-up than Group C [(26.16 $\pm$ 4.99) months vs. (34.41 $\pm$ 1.23) months, $Z = -11.20$, $P < 0.001$], which was consistent with their earlier occurrence of progression endpoints.

No significant differences were observed between the two groups in terms of sex, knee laterality, hypertension, or diabetes (all $P > 0.05$).

Longitudinal trends of serum biomarkers

Figure 1 illustrates the longitudinal changes in IL-6, TNF- α , COMP, and HA at baseline, 12

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Table 1. Comparison of baseline data between the progressors and non-progressors

Characteristics	Non-progressor (N = 392)	Progressor (N = 58)	Statistic	P value
Age (years)	61.60 ± 7.62	64.90 ± 5.77	Z = -3.16	0.002
BMI (kg/m ²)	27.95 ± 3.91	30.96 ± 4.02	t = -5.34	< 0.001
WOMAC Pain Score	7.19 ± 3.02	11.03 ± 3.44	Z = -7.39	< 0.001
Charlson CCI	1.42 ± 0.59	1.19 ± 0.63	Z = -2.60	0.009
Follow-up (months)	34.41 ± 1.23	26.16 ± 4.99	Z = -11.20	< 0.001
Sex, n (%)			χ ² = 1.21	0.272
Male	155 (39.5%)	18 (31.0%)		
Female	237 (60.5%)	40 (69.0%)		
Knee Side, n (%)			χ ² = 0.06	0.810
Left	193 (49.2%)	27 (46.6%)		
Right	199 (50.8%)	31 (53.4%)		
KL Grade, n (%)			Fisher	< 0.001
KL Grade 0	70 (17.9%)	4 (6.9%)		
KL Grade 1	107 (27.3%)	9 (15.5%)		
KL Grade 2	138 (35.2%)	20 (34.5%)		
KL Grade 3	77 (19.6%)	25 (43.1%)		
Injury History, n (%)			χ ² = 9.21	0.002
No	272 (69.4%)	28 (48.3%)		
Yes	120 (30.6%)	30 (51.7%)		
Hypertension, n (%)			χ ² = 3.13	0.077
No	228 (58.2%)	26 (44.8%)		
Yes	164 (41.8%)	32 (55.2%)		
Diabetes, n (%)			χ ² = 2.55	0.110
No	328 (83.7%)	43 (74.1%)		
Yes	64 (16.3%)	15 (25.9%)		

Notes: BMI, Body Mass Index; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; CCI, Charlson Comorbidity Index; KL, Kellgren-Lawrence; TKA, Total Knee Arthroplasty.

months, and 24 months. Generally, serum levels of IL-6, TNF-α, and COMP were significantly higher in Group P than Group C at all three time points, with the most pronounced differences observed at 24 months. Differences in HA levels between the two groups became more apparent at 12 and 24 months. Notably, COMP levels in Group P showed a continuous upward trend, but remained relatively stable in Group C.

GBTM results

GBTM was employed to identify longitudinal trajectories of the four serum biomarkers. Using BIC minimization as the criterion for determining the optimal number of classes, 3-4 clinically interpretable trajectory classes were identified for each biomarker.

For IL-6, four trajectory classes were identified (BIC = 5,023.7; average posterior probability =

0.850). Class 1 (n = 46, 10.2%) represented a consistently high-level trajectory; Class 2 (n = 48, 10.7%) represented the low-level stable trajectory; Class 3 (n = 197, 43.8%) represented the low-moderate trajectory; and Class 4 (n = 159, 35.3%) represented the moderate-level trajectory.

For TNF-α, three trajectory classes were identified, with a BIC of 5,805.6 and an average posterior probability of 0.854. Four trajectory classes were identified for COMP (BIC = 18,795.8) with an average posterior probability of 0.842. For HA, three trajectory classes were identified, with a BIC of 12,424.9 and an average posterior probability of 0.913 (**Table 2**).

Table 3 presents the distribution of patients across trajectory classes and the corresponding proportions of progressors and non-progressors. Significant differences in progression status were observed among trajectory class-

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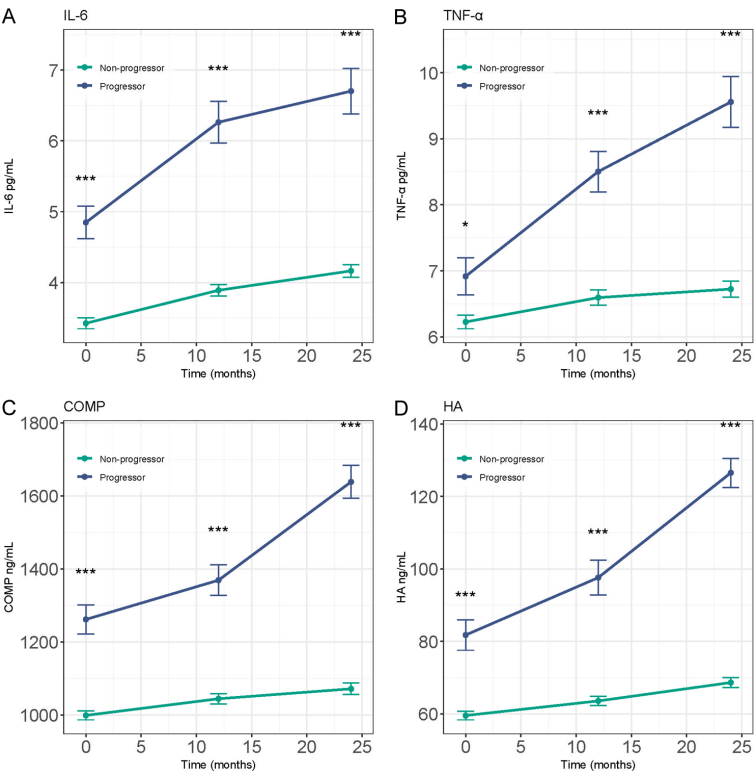


Figure 1. Longitudinal trends of four serum biomarkers over 24 months. A. IL-6 (pg/mL); B. TNF- α (pg/mL); C. COMP (ng/mL); D. HA (ng/mL). Data are presented as mean $\pm$ SE. *P < 0.05, **P < 0.01, ***P < 0.001 (Wilcoxon test, Non-progressor vs. Progressor at each time point). Note: IL-6, Interleukin-6; TNF- α , Tumor Necrosis Factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; SE, Standard Error.

Table 2. Model fit statistics for group-based trajectory models of four serum biomarkers

Biomarker	Classes	BIC	Avg_Posterior_Prob
IL-6	4	5023.7	0.85
TNF- α	3	5805.6	0.854
COMP	4	18795.8	0.842
HA	3	12424.9	0.913

Note: IL-6, Interleukin-6; TNF- α , tumor necrosis factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; BIC, Bayesian Information Criterion.

es. Those with higher COMP trajectory classes demonstrated a greater likelihood of RSP events. Specifically, in COMP Class 4 (the high-level, continuously increasing trajectory), 62% (31/50) experienced progression, whereas 38% (19/50) did not. Similarly, in HA Class 2 (the moderate-to-high trajectory), 47.3% (44/93) experienced RSP. In contrast, IL-6 Class 2 (low-level stable trajectory) and COMP Class 3 (low-level trajectory) consisted exclusively of non-progressors (**Figure 2**).

Univariable associations between trajectory classes and structural progression

Using Class 1 trajectory of each biomarker as the reference, univariable Cox proportional hazards regression analyses were performed to examine the associations between trajectory classes and RSP, with HR and 95% CI estimated (**Table 4**). IL-6 Class 1 corresponding to a persistently high-level trajectory, demonstrated a progression rate of 60.9%; TNF- α Class 1 corresponding to a low-level trajectory, demonstrated a progression rate of 4.0%; COMP Class 1 corresponding to a moderate-level trajectory, demonstrated a 5.2% progression rate; HA Class 1 corresponding to a low-level trajectory, demonstrated a progression rate of 2.4%. In classes where no events occurred, such as IL-6 Class 2 and COMP Class 3, complete separation precluded the estimation of HRs;

therefore, Firth penalized likelihood Cox regression was applied.

The differences in PFS among trajectory classes were visualized using the Kaplan-Meier curves. Log-rank tests revealed significant differences in PFS across trajectory classes for all four biomarkers (all P < 0.001). For IL-6, Class 1 (the persistently high-level trajectory) exhibited the lowest PFS rate, whereas IL-6 Class 2 (the low-level stable trajectory) maintained 100% PFS rate throughout follow-up. For COMP and HA, the high-risk trajectory classes (COMP Class 4 and HA Class 2) showed early separation of survival curves (approximately 12-18 months), indicating that these high-risk trajectories may have early warning value (**Figure 3**).

LASSO variable selection and model construction

All 21 candidate variables, comprising 7 clinical variables, 4 baseline biomarker measure-

Table 3. Distribution of trajectory classes by progression status

Biomarker	Class	Non-progressor (P)	Progressor (C)
IL-6	Class 1	18 (39.1%)	28 (60.9%)
IL-6	Class 2	48 (100.0%)	0 (0.0%)
IL-6	Class 3	187 (94.9%)	10 (5.1%)
IL-6	Class 4	139 (87.4%)	20 (12.6%)
TNF- α	Class 1	119 (96.0%)	5 (4.0%)
TNF- α	Class 2	196 (90.7%)	20 (9.3%)
TNF- α	Class 3	77 (70.0%)	33 (30.0%)
COMP	Class 1	181 (94.8%)	10 (5.2%)
COMP	Class 2	107 (86.3%)	17 (13.7%)
COMP	Class 3	85 (100.0%)	0 (0.0%)
COMP	Class 4	19 (38.0%)	31 (62.0%)
HA	Class 1	122 (97.6%)	3 (2.4%)
HA	Class 2	49 (52.7%)	44 (47.3%)
HA	Class 3	221 (95.3%)	11 (4.7%)

Note: IL-6, Interleukin-6; TNF- α , tumor necrosis factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; P, Non-progressor; C, Progressor.

ments, and 10 trajectory class indicator variables, were incorporated into the LASSO Cox regression model for predictor selection. Spearman correlation analysis demonstrated no strong correlations among candidate predictors (absolute correlation coefficient $|\rho| > 0.7$), and all variance inflation factors (VIF) were below 6, suggesting no substantial multicollinearity (Figure S1).

LASSO regression with 10-fold cross-validation and the $\lambda.1se$ (one standard error) criterion identified five predictors for the final model: WOMAC pain score (WOMAC_Pain), baseline IL-6 level (IL6_0), TNF- α Class 3 (TNFa_cls3), COMP Class 4 (COMP_cls4), and HA Class 2 (HA_cls2) (Figure 4). The $\lambda.min$ criterion retained 14 variables, yet the $\lambda.1se$ criterion was chosen to obtain a more parsimonious and clinically interpretable model.

Based on the LASSO selection results, four candidate Cox proportional hazards regression models were constructed and compared (Table 5): Model 1 included clinical variables only (7 variables), Model 2 incorporated clinical variables and baseline biomarker levels (11 variables in total), Model 3 represented the LASSO $\lambda.1se$ model (5 variables), and Model 4 included clinical variables and all trajectory class indi-

cators (17 variables). Model 1 had a C-index of 0.8668 (AIC = 589.7). Model 2 improved discrimination, with a C-index of 0.9034 (AIC = 555.4). Model 3, despite containing only 5 variables, achieved comparable discrimination to Model 2 (C-index = 0.9034) and further reduced the AIC to 538.3. Model 4 showed the highest C-index (0.9427); however, it failed the proportional hazards assumption (Global P = 0.030). In contrast, Model 3 satisfied the PH assumption (Global P = 0.609) (Figure S2), suggesting greater statistical robustness.

Considering model simplicity, discrimination performance, and compliance with model assumptions, the LASSO $\lambda.1se$ model (Model 3) was chosen as the primary prediction model. The final model incorporated five predictors: WOMAC_Pain, baseline IL-6 level, TNF- α Class 3 trajectory, COMP Class 4 trajectory, and HA Class 2 trajectory.

Model performance evaluation and validation

Ten-fold cross-validation (with 20 repeats) demonstrated that the Model 3 maintained stable predictive performance, with a cross-validated (CV) C-index of 0.8975, an apparent C-index of 0.9034, and an optimism of only 0.0058, outperforming the other models (Model 1: CV C-index = 0.8540, optimism = 0.0128; Model 2: CV C-index = 0.8844, optimism = 0.0190), indicating good internal stability of the LASSO $\lambda.1se$ model (Table 5).

Time-dependent receiver operating characteristic (ROC) analysis demonstrated that the area under the curve (AUC) of Model 3 consistently exceeded that of Model 1 throughout 6-36-month follow-up period. The AUC of Model 3 reached its peak at 24 months (Figure 5). Calibration plots at 24 months (Figure 6) showed good agreement between predicted probabilities and observed event rates across the evaluated models.

Figure 7 presents the predictor effect sizes and overall performance of the main model. Multivariable Cox regression analysis revealed that each 1-point increase in the WOMAC pain score was associated with a 12% increase in the progression risk (HR = 1.12, 95% CI: 1.05-1.20, P < 0.001). A 1-unit increase in baseline IL-6 concentration was associated with a 23% increase in RSP risk (HR = 1.23, 95% CI: 1.06-

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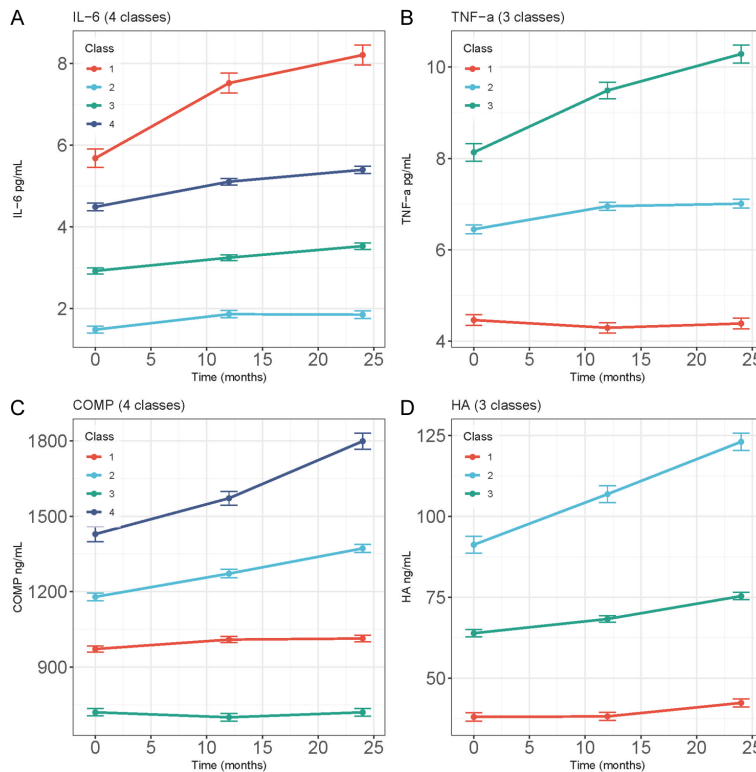


Figure 2. GBTM-derived longitudinal trajectory classes for four serum biomarkers. A. IL-6 (4 classes); B. TNF- α (3 classes); C. COMP (4 classes); D. HA (3 classes). Note: IL-6, Interleukin-6; TNF- α , Tumor Necrosis Factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; SE, Standard Error.

Table 4. Univariable associations between biomarker trajectory classes and rapid structural progression

Biomarker	Comparison	HR	95% CI	P value
IL-6	Class 2 vs. Class 1 (Ref)	0.00	(0.00-Inf)	0.997
IL-6	Class 3 vs. Class 1 (Ref)	0.06	(0.03-0.12)	< 0.001
IL-6	Class 4 vs. Class 1 (Ref)	0.16	(0.09-0.28)	< 0.001
TNF- α	Class 2 vs. Class 1 (Ref)	2.30	(0.86-6.13)	0.095
TNF- α	Class 3 vs. Class 1 (Ref)	8.31	(3.24-21.29)	< 0.001
COMP	Class 2 vs. Class 1 (Ref)	2.70	(1.24-5.90)	0.013
COMP	Class 3 vs. Class 1 (Ref)	0.00	(0.00-Inf)	0.998
COMP	Class 4 vs. Class 1 (Ref)	16.91	(8.26-34.61)	< 0.001
HA	Class 2 vs. Class 1 (Ref)	25.94	(8.05-83.62)	< 0.001
HA	Class 3 vs. Class 1 (Ref)	1.98	(0.55-7.10)	0.295

Note: HR, Hazard Ratio; CI, Confidence Interval; IL-6, Interleukin-6; TNF- α , tumor necrosis factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; Ref, Reference.

1.43, $P = 0.007$). Compared with the reference trajectory class, TNF- α Class 3 trajectory was associated with an 80% increase in risk of progression (HR = 1.80, 95% CI: 1.00-3.23, $P = 0.049$). COMP Class 4 trajectory was associat-

ed with a 198% increase in the risk (HR = 2.98, 95% CI: 1.57-5.65, $P < 0.001$). The HA Class 2 trajectory demonstrated the strongest association among the selected predictor, with an HR of 6.81 (95% CI: 3.47-13.38, $P < 0.001$).

Incremental value of biomarker trajectory information

The incremental prognostic value of incorporating biomarker trajectory information into the prediction model was evaluated by comparing the LASSO λ_{1se} model (Model 3) with the clinical-only model (Model 1) using NRI and IDI analyses (Table 6). Compared with Model 1, Model 3 achieved an IDI of 0.5801, suggesting improved overall risk discrimination. In the event group, the NRI (event) was 0.5000, indicating that the main model accurately reclassified 50% of progressors into a higher-risk category. However, the non-event NRI value was -0.1633, indicating certain degree of over-reclassification of non-progressors. Overall, the total NRI was 0.3367, representing a net improvement in risk classification with the addition of biomarker trajectory information.

Sensitivity analyses

Several sensitivity analyses were conducted to assess the robustness of the main model, with results summarized in Table S1. In BMI-stratified analyses, the C-index of the model was 0.9315 in

the lower-BMI group ($<$ median; $n = 225$, 14 events) and 0.8857 in the higher-BMI group ($\geq$ median; $n = 225$, 44 events), indicating consistent discrimination across different BMI strata. The full trajectory model with all 17 tra-

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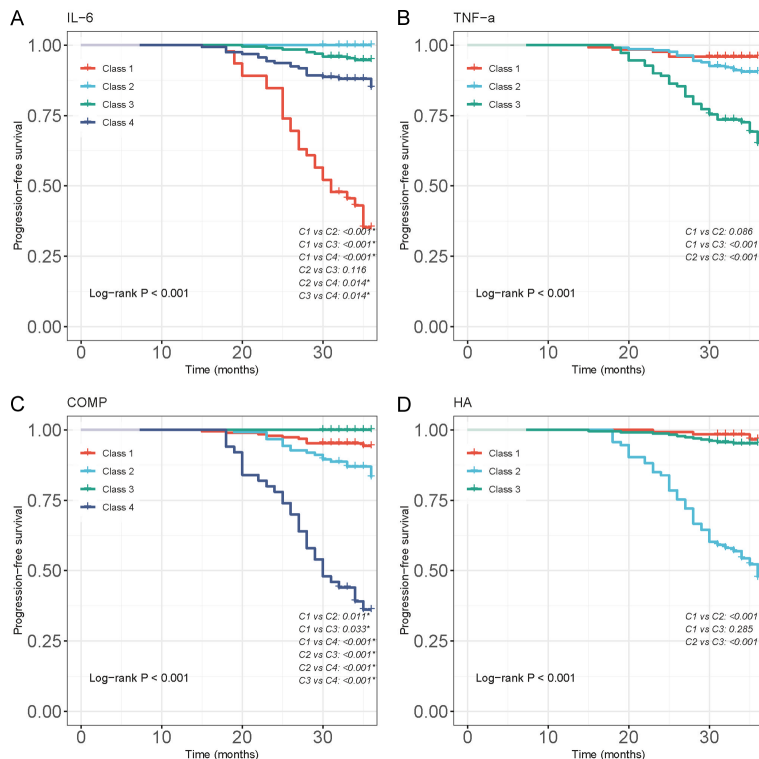


Figure 3. Kaplan-Meier curves of progression-free survival stratified by trajectory classes. A. IL-6; B. TNF- α ; C. COMP; D. HA. *P* values are from the log-rank test. Pairwise comparisons use BH-adjusted *P* values. Note: IL-6, Interleukin-6; TNF- α , Tumor Necrosis Factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; BH, Benjamini-Hochberg.

jectory classes (Model 4) achieved a C-index of 0.9427, higher than the main model. However, as previously mentioned, this model violated the PH assumption, limiting its reliability for Cox regression-based prediction. A simplified model using only baseline-to-12-month change scores demonstrated a lower C-index of 0.7891 compared with the trajectory-based model. This finding further validates the superiority of GBTM over simple change scores in longitudinal analysis. The 24-month landmark sensitivity analysis (excluding participants who experienced a progression event before 24 months; $n = 431$, 39 events) resulted in a main model C-index of 0.9176, indicating that the association between biomarker trajectories and subsequent progression risk remained robust after accounting for time-related bias.

Secondary outcome: prediction of total TKA

An exploratory analysis was conducted using TKA within 3 years as the second outcome. The overall incidence of TKA was 4.9% (22 out of

450). The incidence in Group P was 22.4% (13/58), compared to 2.3% (9/392) in Group C ($P < 0.001$). During data quality assessment, an outlier (> 4 standard deviations from the mean) was identified at the 12-month assessment points for both IL-6 and HA; however, exclusion or retention of these values did not materially affect the study findings.

In the progression group, a subset of patients exhibited lower biomarker levels at 24 months compared with baseline (IL-6: 19.0%; TNF- α : 17.2%, COMP: 6.9%, HA: 8.6%), indicating that longitudinal biomarker patterns may not always follow a monotonic increasing trend during KOA progression.

Discussion

This study used single-center retrospective cohort data from 450 patients with KOA to characterize longitudinal trajectories of four serum biomarkers (IL-6, TNF- α , COMP, and HA) at baseline, 12 months, and 24 months, and integrated trajectory-derived features into Cox proportional hazards models for predicting RSP. The results indicated that each biomarker exhibited heterogeneous longitudinal patterns, with 3-4 clinically distinct trajectory classes. Among these trajectories, HA Class 2 (HR = 6.81), COMP Class 4 (HR = 2.98), and TNF- α Class 3 (HR = 1.80) were independent risk factors for RSP in KOA. The LASSO λ_{1se} -selected model incorporating five variables achieved good discriminatory ability (C-index = 0.9034, CV C-index = 0.8975) and demonstrated predictive performance compared to the clinical-only model (IDI = 0.58, NRI = 0.34). The serum biomarker trajectories over time provided additional prognostic information beyond conventional baseline measurements. However, given the single-center retrospective design and the absence of external validation, the conclusions should be regarded as exploratory. Castagno et al. [14] demonstrated that interpretable automated machine

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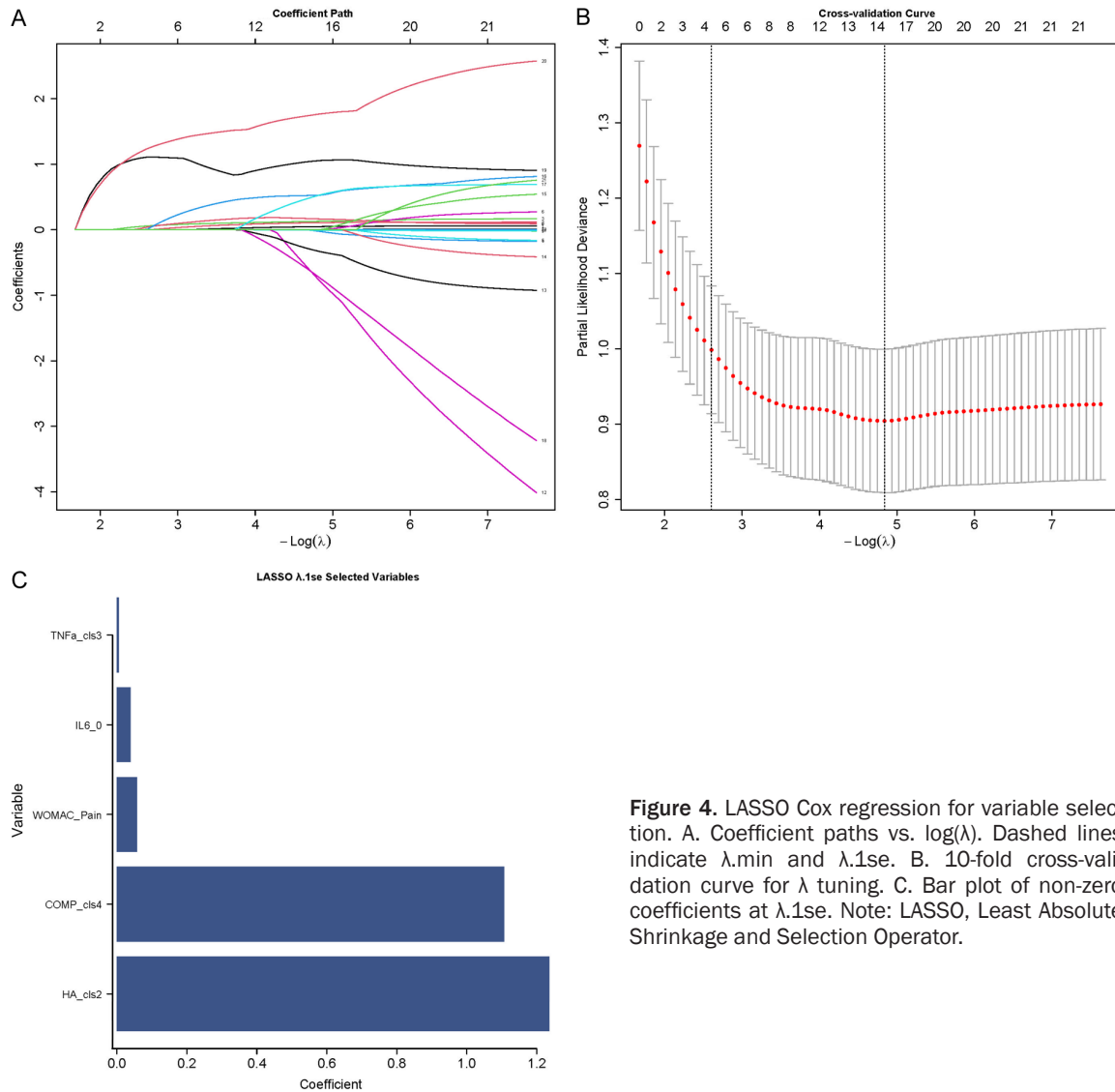


Figure 4. LASSO Cox regression for variable selection. A. Coefficient paths vs. $\log(\lambda)$. Dashed lines indicate $\lambda_{\min}$ and λ_{1se} . B. 10-fold cross-validation curve for λ tuning. C. Bar plot of non-zero coefficients at λ_{1se} . Note: LASSO, Least Absolute Shrinkage and Selection Operator.

learning algorithms could identify individuals at increased risk of rapid KOA progression. Similarly, previous research [15] suggested that peripheral blood molecular signatures combined with machine learning may improve prediction of KOA progression. These findings are consistent with present study, which further extends this concept by incorporating dynamic serum biomarker patterns rather than relying solely on baseline measurements.

GBTM analysis revealed that COMP and HA trajectories showed the strongest association with RSP. Patients assigned to COMP Class 4, had the highest observed progression rate (62.0%). This pattern may reflect sustained alterations in cartilage matrix turnover and

increased extracellular matrix degradation activity during disease progression. Akinmade et al. [8] reported that serum COMP could be used to predict the onset and progression of KOA. Kraus et al. [11] later confirmed that a serum proteomic panel could provide predictive information for KOA progression. In contrast, the low-level stable COMP trajectory was not associated with observed progression events in this cohort, suggesting that relatively stable cartilage matrix metabolism may reflect a lower-risk disease phenotype.

The strong association between HA Class 2 and RSP may reflect the combined impact of synovial inflammation, abnormal cartilage matrix metabolism, and disturbed joint homeostasis

Table 5. Comparison of predictive model performance

Model	Variables	C_index	AIC	PH_Global_p
Clinical only	7	0.866780749	589.7125569	0.318606119
Clinical + Baseline biomarkers	11	0.90340107	555.4294449	0.366352157
LASSO λ_{1se} (MAIN)	5	0.903358289	538.2516285	0.609257175
Clinical + All trajectory classes	17	0.942673797	522.6537935	0.030260668

Note: AIC, Akaike Information Criterion; PH, Proportional Hazards; LASSO, Least Absolute Shrinkage and Selection Operator.

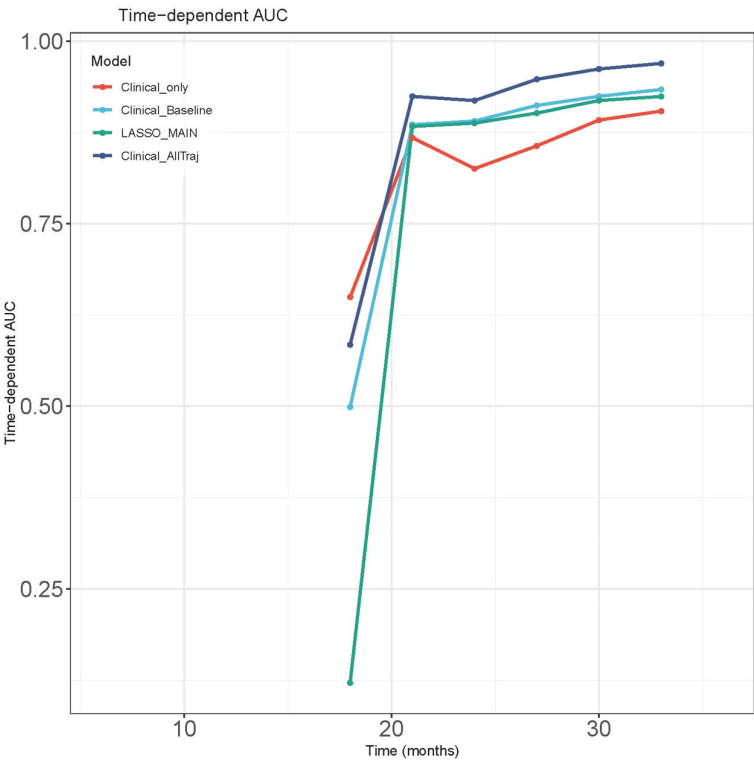


Figure 5. Time-dependent AUC curves for candidate models (6-36 months). Curves represent AUC values at each time point for the four candidate models. Note: AUC, Area Under the Curve.

on KOA progression. Papanephytous et al. [9] reported that serum HA and cartilage metabolism-related markers were associated with KOA risk factors, pain severity, and disease status. Kraus et al. [20] also proposed that molecular biomarkers may capture different stages along the continuum of osteoarthritis pathophysiology.

Inflammatory trajectories also contributed to risk stratification. Patients with persistently high IL-6 levels had a relatively higher progression rate. The high-level TNF- α trajectory was also retained in the final model, indicating that the inflammatory pathway might be involved in the structural deterioration of KOA. Rankothgedera et al. [21] reported that serum

immune-inflammatory markers may facilitate identification of distinct KOA phenotypes. These findings support the incorporation of inflammatory cytokines such as IL-6 and TNF- α into multidimensional prediction models for KOA progression.

From a biological perspective, the finding that HA Class 2 showed the strongest predictive association (hazard ratio = 6.81) is noteworthy and may reflect the unique role of HA in multiple pathological processes involved in KOA. Unlike COMP, which primarily reflects cartilage matrix degradation, serum HA levels may be influenced by synovial inflammation, altered joint tissue metabolism, and impaired clearance from the joint compartment. Thus, HA reflects a broader spectrum of pathological alterations related to joint homeostasis.

COMP Class 4 trajectory, characterized by persistently increasing COMP levels rather than just elevated levels, showed a substantially higher progression risk compared with more stable trajectories. These findings suggest that the dynamic pattern of biomarker change, rather than the absolute biomarker concentration alone, may provide additional prognostic information. This reinforces the central hypothesis of this study that longitudinal biomarker trajectories may better characterize disease evolution than its level at any single time point.

The trajectory-informed LASSO main model, which included only five variables, achieved discrimination comparable to the clinical-plus-baseline-biomarker model, while demonstrat-

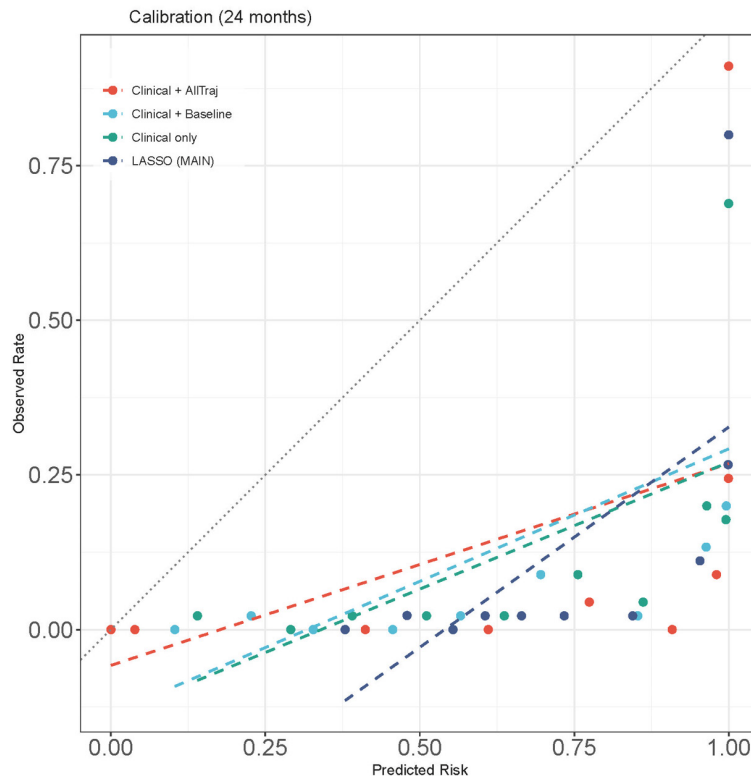


Figure 6. Calibration plots of predicted vs. observed progression risk at 24 months. Dashed lines: loess-smoothed calibration for each model. Dotted diagonal: ideal calibration (predicted = observed). Note: LOESS, Locally Estimated Scatterplot Smoothing.

ing a lower AIC. Incorporating trajectory information can enhance prediction capability. The substantially lower C-index of the simplified change-score model based on baseline-to-12-month difference compared with the trajectory model highlights the potential limitations of using simple two-timepoint changes to represent complex biomarker evolution. Chang et al. [13] reported that pain experiences in KOA are characterized by different natural history trajectories. Individuals with KOA or those at risk of developing KOA often experience altered pain sensitization (i.e., hyperalgesia and allodynia), which can evolve along different long-term trajectories [22]. These observations further support the concept that KOA represents a heterogeneous disease entity rather than a uniformly progressive condition.

The parsimonious nature and interpretability of the LASSO Cox model may facilitate clinical practice. Risk stratification based on WOMAC pain score, baseline IL-6 level, and selected biomarker trajectory classifications may pro-

vide a feasible approach for identifying patients at higher risk of rapid progression and optimizing follow-up strategies. Bonakdari et al. [23] developed a warning algorithm based on machine learning to screen patients at risk of KOA structural progression. Additionally, Joseph et al. [24] noted that machine learning models are being increasingly used for clinical and structural prediction of KOA, although the generalizability and interpretability remain significant challenges. Therefore, the LASSO Cox model, selected for its relative parsimony and interpretability in this study, may be more clinically translatable.

Compared with previous KOA prediction studies, our study provides a complementary approach by integrating longitudinal trajectories of multiple serum biomarkers into a survival prediction framework. Prior studies have primarily

focused on baseline biomarker levels, imaging features, or individual molecular markers. Almhdie-Imjabbar et al. [25] employed bone texture imaging features and Siamese neural networks using data from the Osteoarthritis Initiative (OAI) and the Multicenter Osteoarthritis Study (MOST) cohorts to predict KOA progression. Yin et al. [26] demonstrated that a deep-learning model based on bilateral knee imaging could predict radiographic OA progression. Jamshidi et al. [27] reported that machine-learning and deep-learning approaches based on microRNA signatures can aid in early prediction of KOA structural progression. Li et al. [28] also developed predictive models for radiographic and pain progression using data from the Foundation for the National Institutes of Health (FNIH) OA Biomarkers Consortium study. Collectively, these studies suggest that integrating multi-source biological information with longitudinal data may represent an important direction for improving KOA progression prediction. Although this study did not incorporate radiomic or multi-omic features, the serum bio-

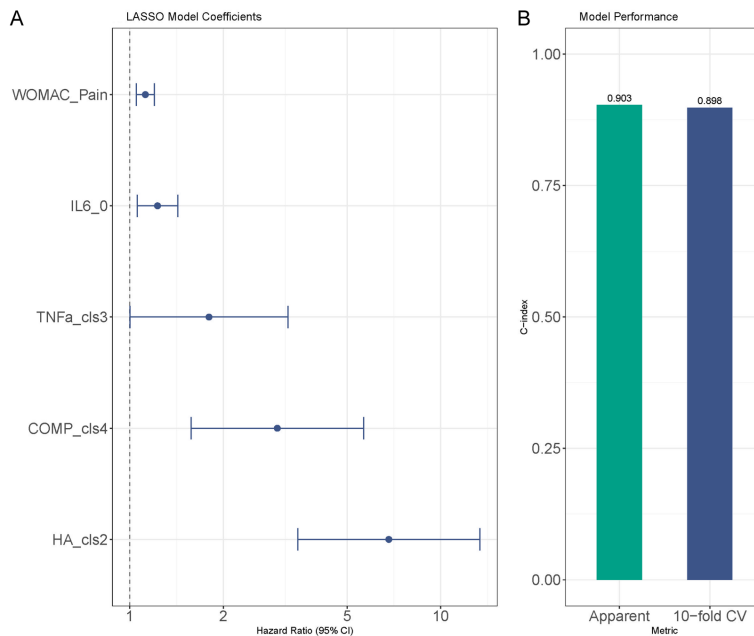


Figure 7. LASSO $\lambda.1se$ main model: forest plot of hazard ratios and model performance. A. Forest plot showing HR (95% CI) for each variable in the main model. B. Apparent C-index and 10-fold cross-validated C-index of the main model. Note: HR, Hazard Ratio; CI, Confidence Interval; LASSO, Least Absolute Shrinkage and Selection Operator.

Table 6. Incremental prognostic value of the LASSO $\lambda.1se$ main model over the clinical-only model

Metric	Value	Comparison
IDI	0.5801	LASSO MAIN vs. Clinical only
NRI (event)	0.5000	LASSO MAIN vs. Clinical only
NRI (non-event)	-0.1633	LASSO MAIN vs. Clinical only
NRI (total)	0.3367	LASSO MAIN vs. Clinical only

Note: IDI, Integrated Discrimination Improvement; NRI, Net Reclassification Improvement; LASSO, Least Absolute Shrinkage and Selection Operator. NRI (event): proportion of progressors correctly reclassified; NRI (non-event): proportion of non-progressors correctly reclassified; NRI (total): sum of NRI (event) and NRI (non-event).

markers evaluated are relatively accessible, and the trajectory classes are highly interpretable. Therefore, this framework may offer a practical strategy for identifying high-risk KOA populations in primary care and routine clinical settings.

Regarding the secondary outcome of TKA, the incidence of TKA was significantly higher among patients with RSP than those without. Although the LASSO main model achieved good discrimination for TKA prediction, this finding should be interpreted cautiously. The decision to undergo TKA is influenced by numerous factors, includ-

ing pain severity, functional limitation, patient preference, healthcare resources, and radiographic structural damage. Therefore, TKA occurrence cannot be considered a direct surrogate for biological disease progression alone. Saxer et al. [29] demonstrated that the B-score could estimate joint replacement risk across different OA phenotypes. D'Assignies et al. [30] further indicated that machine-learning models incorporating multiple MRI features and clinical phenotypes could predict joint replacement outcomes in patients with KOA. Therefore, TKA prediction requires the integration of structural, symptomatic, and clinical decision-making factors. Similarly, previous studies have suggested that patellofemoral joint radiomics features may improve prediction of knee replacement risk [31]; Sun et al. [32] reported that a nomogram based on X-ray radiomics could predict pain progression in KOA. Collectively, these findings indicate that TKA prediction requires integration of structural abnormalities, symptom burden, and clinical decision-making factors. Therefore, the TKA analysis in this study should be considered an exploratory supplementary

analysis rather than a definitive prediction model for joint replacement.

From a clinical translation perspective, a major strength of the final five-variable model is its parsimony. The model requires only the WOMAC pain score, one baseline biomarker level, and three binary trajectory indicators, without the need for advanced imaging processing or multi-omics platforms. This characteristic may facilitate routine clinical application after external validation. However, the negative NRI for non-events (-0.1633) warrants careful consideration. The model may misclassify some non-

progressors as high-risk individuals, which could cause unnecessary patient anxiety or additional clinical assessments. Therefore, whether the overall improvement in reclassification (total NRI = 0.3367) translates into meaningful clinical benefit should be evaluated within specific clinical contexts, such as population-level risk stratification or specialized orthopedic follow-up, rather than interpreted solely based on statistical performance metrics. Importantly, the proposed model is not intended to replace radiographic monitoring or clinical evaluation, but rather in enabling risk-adapted follow-up strategies. Patients with low-risk biomarker trajectories can be safely monitored at longer intervals, while patients with HA Class 2 or COMP Class 4 trajectories require intensified follow-up.

This study has several limitations. First, it was a single-center retrospective study with a relatively limited sample size and a small number of progression events. In addition, the model lacked independent external validation. Thus, the generalizability of the model requires further evaluation in multicenter prospective cohorts. Second, trajectory classes were identified using the entire study population, and although internal validation was performed using repeated cross-validation, some degree of optimism bias cannot be completely excluded. Third, ELISA results can be influenced by reagent batches, laboratory procedures, and assay platforms. Fourth, some trajectory classes contained relatively small sample sizes, and trajectory assignment was based on maximum posterior probability (hard assignment), without fully considering classification uncertainty. Fifth, this study used biomarker trajectories from baseline to 24 months to predict progression events occurring within 36 months. Consequently, some events may have occurred before completion of the trajectory assessment period, introducing potential temporal sequence bias. Although the 24-month landmark sensitivity analysis produced results consistent with the primary analysis, residual bias cannot be completely excluded. Sixth, the negative NRI for non-events indicated that the model may inaccurately classify some non-progressors as higher-risk individuals. This poses a risk of inducing undue anxiety and over-intervention in clinical translation.

Although machine-learning approaches show promise for predicting KOA progression, issues

related to model validation, population heterogeneity, and clinical interpretability remain significant barriers to translation [33]. As highlighted by Fuggle et al. [34], the management of KOA has evolved from a symptom-focused approach toward more individualized strategies incorporating clinical phenotypes, imaging features, and molecular biomarkers. Further studies should focus on prospective applications, broader external validation, and integration of prediction models into clinical decision-making pathways.

Conclusion

This study developed a prediction model for rapid structural progression in KOA by integrating longitudinal trajectory information of four KOA-related serum biomarkers (IL-6, TNF- α , COMP, and HA) identified through GBTM into a LASSO Cox regression framework. The five-variable prediction model demonstrated good discrimination and internal validation stability.

The trajectory patterns of HA Class 2, COMP Class 4, and TNF- α Class 3 were independently associated with increased risk of rapid structural progression. This model provides a potential framework for early stratification and personalized monitoring of KOA populations at high-risk of rapid structural progression. However, further validation in independent, multicenter, prospective cohorts is required to determine its clinical utility.

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Disclosure of conflict of interest

None.

Address correspondence to: Liangyu Lu, Department of Orthopedics, Pudong New Area People's Hospital, No. 490, South Chuanhuan Road, Pudong New Area, Shanghai 201299, China. E-mail: lly198008@163.com

References

- [1] Gelber AC. Knee osteoarthritis. *Ann Intern Med* 2024; 177: ITC129-ITC144.
- [2] GBD 2021 Osteoarthritis Collaborators. Global, regional, and national burden of osteoarthritis, 1990-2020 and projections to 2050: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet Rheumatol* 2023; 5: e508-e522.
- [3] GBD 2021 Diseases and Injuries Collaborators. Global incidence, prevalence, years lived with disability (YLDs), disability-adjusted life-years (DALYs), and healthy life expectancy (HALE) for 371 diseases and injuries in 204 countries and territories and 811 subnational locations, 1990-2021: a systematic analysis for the Global Burden of Disease Study 2021. *Lancet* 2024; 403: 2133-2161.
- [4] Dong Y, Yan Y, Zhou J, Zhou Q and Wei H. Evidence on risk factors for knee osteoarthritis in middle-older aged: a systematic review and meta analysis. *J Orthop Surg Res* 2023; 18: 634.
- [5] Duong V, Abdel Shaheed C, Ferreira ML, Narayan SW, Venkatesha V, Hunter DJ, Zhu J, Atukorala I, Kobayashi S, Goh SL, Briggs AM, Cross M, Espinosa-Morales R, Fu K, Guillemin F, Keefe F, Stefan Lohmander L, March L, Milne GJ, Mei Y, Mobasher A, Namane M, Peat G, Risberg MA, Sharma S, Sit R, Telles RW, Zhang Y and Cooper C. Risk factors for the development of knee osteoarthritis across the lifespan: a systematic review and meta-analysis. *Osteoarthritis Cartilage* 2025; 33: 1162-1179.
- [6] Joo PY, Borjali A, Chen AF, Muratoglu OK and Varadarajan KM. Defining and predicting radiographic knee osteoarthritis progression: a systematic review of findings from the osteoarthritis initiative. *Knee Surg Sports Traumatol Arthrosc* 2022; 30: 4015-4028.
- [7] Almhdie-Imjabbar A, Toumi H and Lespes-sailles E. Radiographic biomarkers for knee osteoarthritis: a narrative review. *Life (Basel)* 2023; 13: 237.
- [8] Akinmade A, Oginni LM, Adegbehingbe OO, Okunlola AI, Jeje OA and Adeyeye AI. Serum cartilage oligomeric matrix protein as a biomarker for predicting development and progression of knee osteoarthritis. *Int Orthop* 2021; 45: 551-557.
- [9] Papanephytous C, Alabajos-Cea A, Viosca-Herrero E, Calvis C, Costa M, Christodoulides AE, Kroushovski A, Lapithis A, Lapithi VM, Papayannis I, Christou A, Messeguer R, Giannaki C and Felekis K. Associations between serum biomarkers of cartilage metabolism and serum hyaluronic acid, with risk factors, pain categories, and disease severity in knee osteoarthritis: a pilot study. *BMC Musculoskelet Disord* 2022; 23: 195.
- [10] Szilagyi IA, Vallergera CL, Boer CG, Schiphof D, Ikram MA, Bierma-Zeinstra SMA and van Meurs JBJ. Plasma proteomics identifies CRTAC1 as a biomarker for osteoarthritis severity and progression. *Rheumatology (Oxford)* 2023; 62: 1286-1295.
- [11] Kraus VB, Reed A, Soderblom EJ, Moseley MA, Hsueh MF, Attur MG, Samuels J, Abramson SB and Li YJ. Serum proteomic panel validated for prediction of knee osteoarthritis progression. *Osteoarthritis Cartilage* 2024; 6: 100425.
- [12] White DK, Neogi T, Nguyen US, Niu J and Zhang Y. Trajectories of functional decline in knee osteoarthritis: the osteoarthritis initiative. *Rheumatology (Oxford)* 2016; 55: 801-808.
- [13] Chang AH, Almagor O, Lee JJ, Song J, Muhammad LN, Chmiel JS, Moisiu KC and Sharma L. The natural history of knee osteoarthritis pain experience and risk profiles. *J Pain* 2023; 24: 2175-2185.
- [14] Castagno S, Birch M, van der Schaar M and McCaskie A. Predicting rapid progression in knee osteoarthritis: a novel and interpretable automated machine learning approach, with specific focus on young patients and early disease. *Ann Rheum Dis* 2025; 84: 124-135.
- [15] Dunn CM, Sturdy C, Velasco C, Schlupp L, Prinz E, Izda V, Arbeeva L, Golightly YM, Nelson AE and Jeffries MA. Peripheral blood DNA methylation-based machine learning models for prediction of knee osteoarthritis progression: biologic specimens and data from the osteoarthritis initiative and Johnston county osteoarthritis project. *Arthritis Rheumatol* 2023; 75: 28-40.
- [16] Wen B, Zhang G, Zhan C, Chen C and Yi H. The 2024 revision of the Declaration of Helsinki: a modern ethical framework for medical research. *Postgrad Med J* 2025; 101: 371-382.
- [17] Vandembroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ, Poole C, Schlesselman JJ and Egger M. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Med* 2007; 4: e297.
- [18] Namireddy SR, Gill SS, Yaqub Y and Ramkumar P. Computerized versus traditional approaches for total knee arthroplasty: a quantitative analysis of knee society score and Western Ontario and McMaster universities osteoarthritis index. *Orthop Surg* 2024; 16: 1530-1537.
- [19] Quan H, Li B, Couris CM, Fushimi K, Graham P, Hider P, Januel JM and Sundararajan V. Updating and validating the Charlson comorbidity

- index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. *Am J Epidemiol* 2011; 173: 676-682.
- [20] Kraus VB, Sun S, Reed A, Soderblom EJ, Moseley MA, Zhou K, Jain V, Arden N and Li YJ. An osteoarthritis pathophysiological continuum revealed by molecular biomarkers. *Sci Adv* 2024; 10: eadj6814.
- [21] Rankothgedera S, Atukorala I, Fernando C, Munidasa D, Wijayarathne L and Udagama P. A potential diagnostic serum immunological marker panel to differentiate between primary and secondary knee osteoarthritis. *PLoS One* 2021; 16: e0257507.
- [22] Aoyagi K, Wang N, LaValley M, Tilley S, Frey-Law L, Lewis CE, Nevitt M and Neogi T. Longitudinal trajectories of pain sensitization over nine years in people with or at risk of knee osteoarthritis. *Arthritis Care Res (Hoboken)* 2026; 78: 113-122.
- [23] Bonakdari H, Jamshidi A, Pelletier JP, Abram F, Tardif G and Martel-Pelletier J. A warning machine learning algorithm for early knee osteoarthritis structural progressor patient screening. *Ther Adv Musculoskelet Dis* 2021; 13: 1759720X21993254.
- [24] Joseph GB, McCulloch CE, Nevitt MC, Lane NE, Majumdar S and Link TM. Machine learning models for clinical and structural knee osteoarthritis prediction: recent advancements and future directions. *Osteoarthritis Cartil Open* 2025; 7: 100654.
- [25] Almhdie-Imjabbar A, Nguyen KL, Toumi H, Jennane R and Lespessailles E. Prediction of knee osteoarthritis progression using radiological descriptors obtained from bone texture analysis and Siamese neural networks: data from OAI and MOST cohorts. *Arthritis Res Ther* 2022; 24: 66.
- [26] Yin R, Chen H, Tao T, Zhang K, Yang G, Shi F, Jiang Y and Gui J. Expanding from unilateral to bilateral: a robust deep learning-based approach for predicting radiographic osteoarthritis progression. *Osteoarthritis Cartilage* 2024; 32: 338-347.
- [27] Jamshidi A, Espin-Garcia O, Wilson TG, Lovelless I, Pelletier JP, Martel-Pelletier J and Ali SA. MicroRNA signature for early prediction of knee osteoarthritis structural progression using integrated machine and deep learning approaches. *Osteoarthritis Cartilage* 2025; 33: 330-340.
- [28] Li X, Li C and Zhang P. Predictive models of radiographic progression and pain progression in patients with knee osteoarthritis: data from the FNIH OA biomarkers consortium project. *Arthritis Res Ther* 2024; 26: 112.
- [29] Saxer F, Demanse D, Brett A, Laurent D, Mindholm L, Conaghan PG and Schieker M. Prognostic value of B-score for predicting joint replacement in the context of osteoarthritis phenotypes: data from the osteoarthritis initiative. *Osteoarthritis Cartil Open* 2024; 6: 100458.
- [30] D'Assignies G, Demanse D, Saxer F, Laurent D, Zille P, Vesoul T, Cordelle P, Herpe G, Conaghan PG and Schieker M. Combining machine-learning assessment of multiple MRI pathologies and clinical phenotypes for predicting joint replacement in knee osteoarthritis: data from the osteoarthritis initiative. *Cartilage* 2025; [Epub ahead of print].
- [31] Zhang J, Jiang T, Chan LC, Lau SH, Wang W, Teng X, Chan PK, Cai J and Wen C. Radiomics analysis of patellofemoral joint improves knee replacement risk prediction: data from the Multicenter Osteoarthritis Study (MOST). *Osteoarthritis Cartil Open* 2024; 6: 100448.
- [32] Sun Y, Liu J, Deng C, Peng C, Pan S and Liu X. Nomograms based on X-ray radiomics for predicting pain progression in knee osteoarthritis using data from the foundation for the national institutes of health: development and validation study. *JMIR Med Inform* 2026; 14: e78338.
- [33] Liu Y, Xiao G, Zhang Y, Wang X, Jia J, Xie A, Zheng Z and Zhang K. Predictive value of machine learning in knee osteoarthritis progression: systematic review and meta-analysis. *J Med Internet Res* 2025; 27: e80430.
- [34] Fuggle NR, Chapurlat R, Laslop A, Al-Daghri N, Alokail M, Thiyagarajan JA, Balkowiec-Iskra E, Berenbaum F, Bemden AB, Borg JJ, Bruyere O, Burllet N, Cavalier E, Rosa MM, Conaghan PG, Cooper C, Dennison EM, Englund M, Im GI, Haugen IK, Hilgsmann M, Kurth A, Lane N, Lories R, Marlovits S, Matijevic R, Mobasheri A, Ormarsdottir S, Yerro MCP, Radermecker RP, Rannou F, Sepodes B, Silverman S, Torre C, Veronese N, Rizzoli R, Reginster JY and Harvey NC. Novel approaches to the stratified management of knee osteoarthritis. *Nat Rev Rheumatol* 2025; 21: 684-695.

Longitudinal biomarker trajectories predict knee osteoarthritis progression

Table S1. Sensitivity analyses of the LASSO λ_{1se} main model

Scheme	N	Events	C_index	Delta
Main (LASSO 1se)	450	58	0.903358289	0
Exclude KL grade 0	450	58	0.903358289	0
BMI < median	225	14	0.931456095	0.028097806
BMI $\geq$ median	225	44	0.885652024	-0.017706264
Baseline-12 m change	450	58	0.789090909	-0.11426738
All trajectory classes	450	58	0.942673797	0.039315508
24-month landmark	431	39	0.9176	+0.0142

Note: LASSO, Least Absolute Shrinkage and Selection Operator; KL, Kellgren-Lawrence; BMI, Body Mass Index. Δ : difference in C-index relative to the main model. The “Baseline-to-12-month change” scheme uses only the changes in variables from baseline to 12 months as predictors. “All trajectory classes” uses the full model with all trajectory class indicators.

Longitudinal biomarker trajectories predict knee osteoarthritis progression

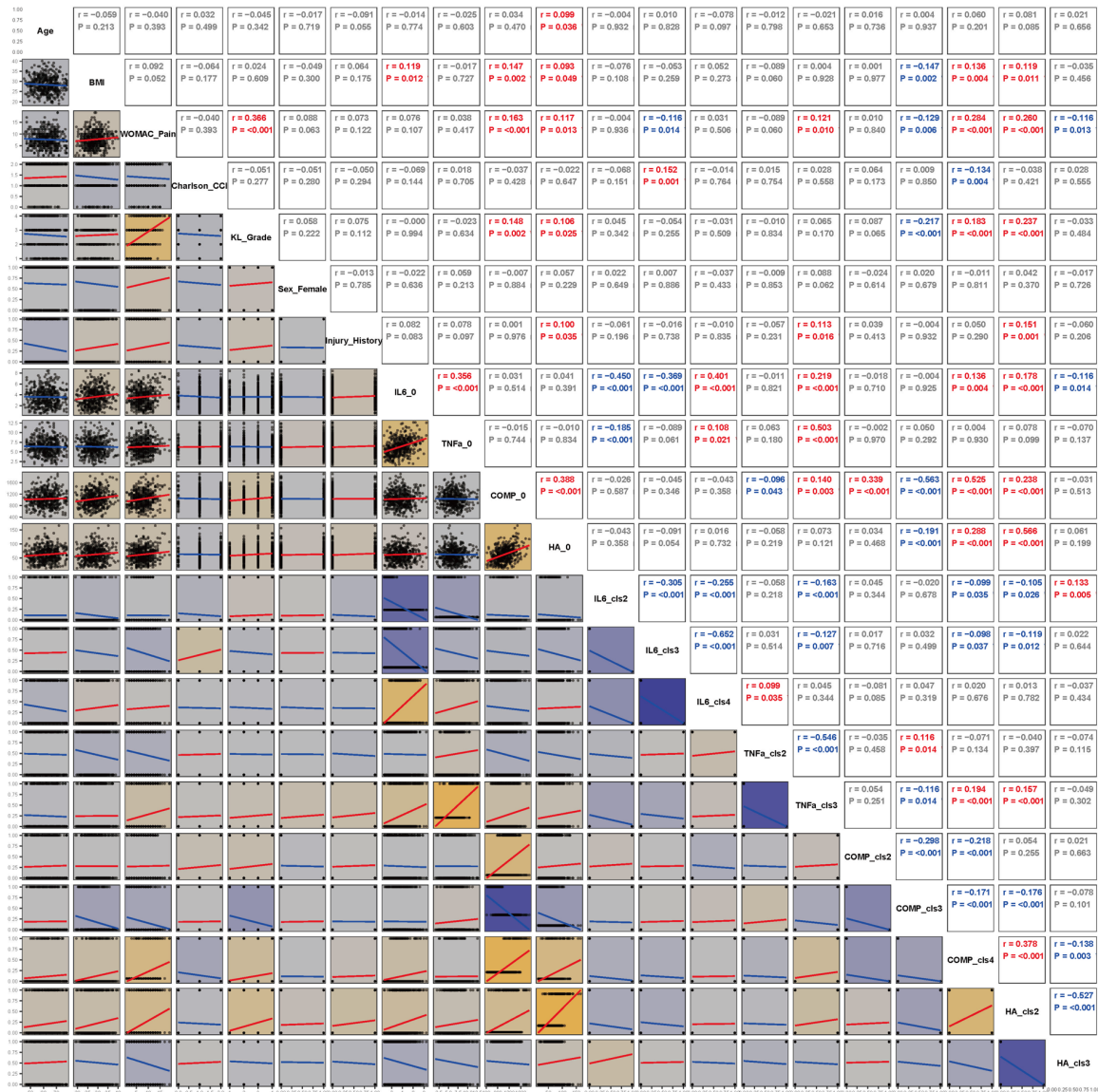


Figure S1. Spearman correlation matrix of candidate predictor variables. Upper triangle: Spearman correlation coefficient (ρ), P value, and significance level. Lower triangle: scatter plot with locally estimated scatterplot smoothing (LOESS) regression line and 95% confidence band. Diagonal: variable name and distribution density. Note: IL-6, Interleukin-6; TNF- α , tumor necrosis factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; KL, Kellgren-Lawrence; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; CCI, Charlson Comorbidity Index; BMI, Body Mass Index. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Correlation coefficients not reaching statistical significance are displayed in gray.

Longitudinal biomarker trajectories predict knee osteoarthritis progression

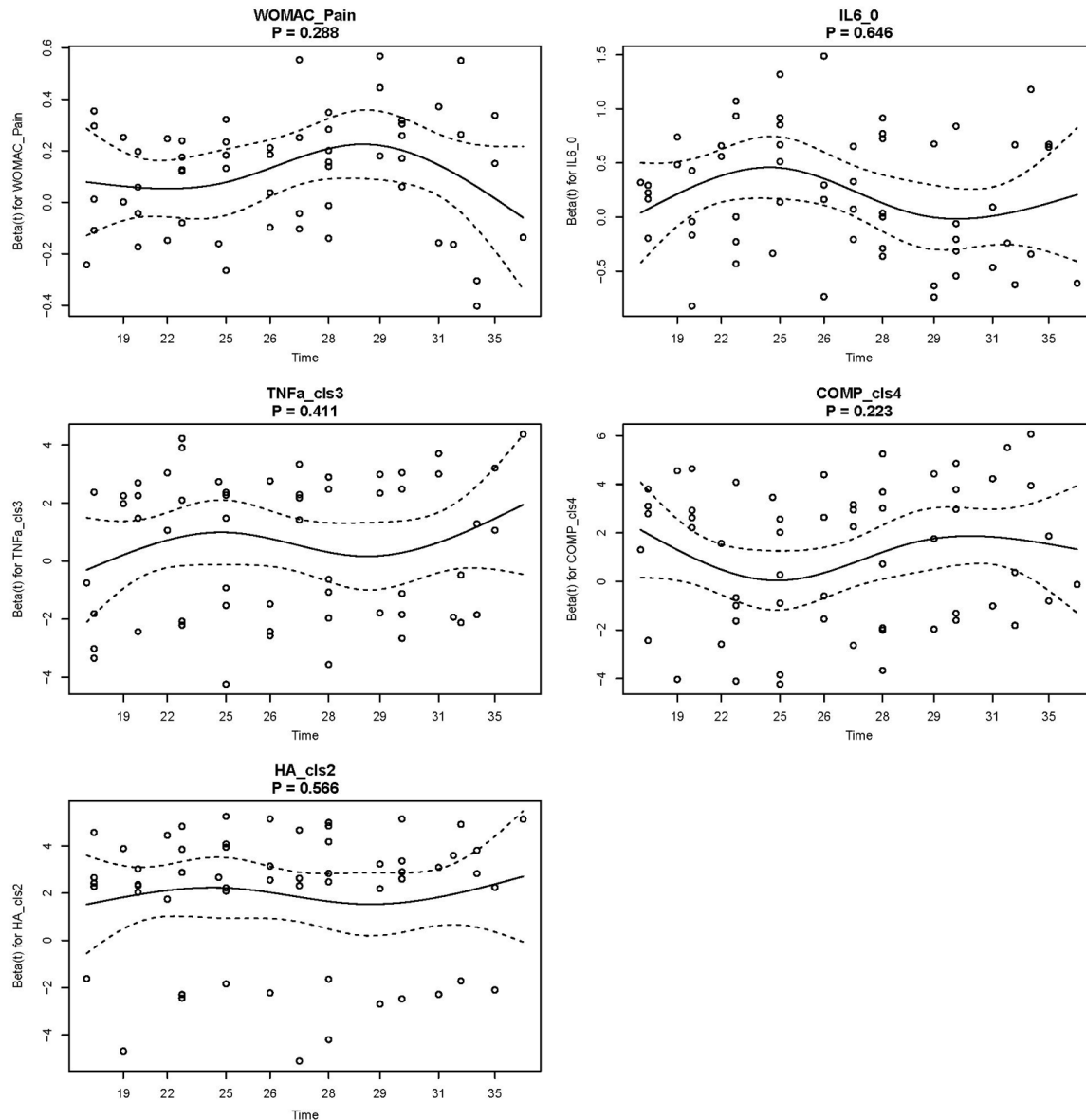


Figure S2. Schoenfeld residual plots for testing the proportional hazards assumption of the LASSO λ_{1se} main model. Each panel displays the scaled Schoenfeld residuals against time for an individual predictor variable with a LOESS smooth curve (solid line) and 95% confidence band (dashed lines). P values are from the individual variable test; Global P is from the omnibus test. A non-significant P value (> 0.05) indicates no violation of the proportional hazards assumption. Note: IL-6, Interleukin-6; TNF- α , Tumor Necrosis Factor- α ; COMP, Cartilage Oligomeric Matrix Protein; HA, Hyaluronic Acid; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index; PH, Proportional Hazards; LOESS, Locally Estimated Scatterplot Smoothing.