Table 1
Cytokines as predictors of progression of outcomes of osteoarthritis. Odds ratios (OR) with 95% confidence intervals (CI) from logistic regression analysis with adjustments for sex, age and body mass index at first examination, and time between examinations. Statistically significant associations are indicated in bold.

		Synovial fluid conc. exam 1 (n = 132)	Synovial fluid conc. exam 2 (n = 71)	Delta conc. (2−1) (n = 71)	Increased conc. (2 > 1) (n = 71)
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Radiographic progression					
Loss of joint space	IL-6	1.02 (0.99, 1.06)	1.00 (1.00, 1.01)	1.00 (1.00, 1.01)	**5.17 (1.54, 17.32)**
	IL-8	1.04 (0.99, 1.09)	1.00 (1.00, 1.01)	1.00 (0.99, 1.01)	2.37 (0.65, 8.61)
	TNF-α	1.01 (0.80, 1.28)	**1.70 (1.15, 2.52)**	**1.34 (1.06, 1.71)**	**5.01 (1.32, 18.92)**
Osteophyte progression	IL-6	**1.05 (1.00, 1.09)**	1.00 (1.00, 1.01)	1.00 (0.99, 1.01)	1.51 (0.49, 4.66)
	IL-8	1.05 (0.99, 1.10)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.33 (0.37, 4.79)
	TNF-α	**1.35 (1.03, 1.75)**	1.05 (0.82, 1.36)	0.90 (0.73, 1.11)	0.71 (0.22, 2.35)
ROA progression	IL-6	1.05 (0.98, 1.13)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	2.70 (0.80, 9.13)
	IL-8	1.03 (0.97, 1.10)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	2.73 (0.67, 11.03)
	TNF-α	1.24 (0.90, 1.70)	1.27 (0.91, 1.76)	1.07 (0.84, 1.37)	1.41 (0.36, 5.55)
KOOS progression					
Pain	IL-6	1.01 (0.99, 1.03)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.02 (0.29, 3.52)
	IL-8	1.02 (0.97, 1.08)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.29 (0.30, 5.64)
	TNF-α	1.12 (0.87, 1.44)	1.16 (0.88, 1.54)	1.11 (0.88, 1.38)	1.72 (0.40, 7.34)
Other symptoms	IL-6	1.00 (0.98, 1.02)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.71 (0.46, 6.37)
	IL-8	1.00 (0.95, 1.06)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.63 (0.37, 7.16)
	TNF-α	1.08 (0.84, 1.40)	0.97 (0.72, 1.31)	0.99 (0.79, 1.24)	1.27 (0.32, 5.04)
Function of daily living	IL-6	1.01 (1.00, 1.03)	1.01 (1.00, 1.01)	1.01 (1.00, 1.01)	0.97 (0.24, 3.89)
	IL-8	1.04 (0.99, 1.10)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	0.99 (0.21, 4.57)
	TNF-α	1.18 (0.90, 1,55)	**1.50 (1.07, 2.09)**	1.25 (0.97, 1.62)	7.25 (0.81, 65.33)
Function in sports and recreation	IL-6	**1.08 (1.01, 1.14)**	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.29 (0.38, 4.32)
	IL-8	1.04 (0.98, 1.09)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	1.24 (0.33, 4.67)
	TNF-α	1.06 (0.83, 1.36)	1.17 (0.89, 1.53)	1.09 (0.88, 1.34)	1.65 (0.45, 6.09)
Knee-related quality of life	IL-6	1.02 (0.99, 1.04)	1.00 (1.00, 1.01)	1.00 (0.99, 1.01)	0.73 (0.21, 2.57)
	IL-8	1.03 (0.98, 1.08)	1.00 (0.99, 1.01)	1.00 (0.99, 1.01)	0.67 (0.18, 2.53)
	TNF-α	1.16 (0.90, 1.48)	0.81 (0.58, 1.11)	0.83 (0.65, 1.06)	0.86 (0.23, 3.19)

atlas with loss of joint space and osteophyte progression evaluated separately as well as combined (to a radiographic score). Progression from first to second examination was defined by increase of one or more in grade or score. We evaluated patient-reported outcomes by separate analysis of the five subscales of the Knee injury and Osteoarthritis Outcome Score (KOOS; Table 1), with progression defined as a worsening in the subscale score by 10 units or more. To study associations between cytokine concentrations and progression of outcomes, we used multivariate logistic regression analysis. The odds ratios (OR) reflect higher concentration, or change in concentration between visits, in increments of 1 pg/ml, or having increased in concentration or not. We did not use multiple test adjustments due to the exploratory character of the study.

Results: At group level, synovial fluid concentrations of IL-6, IL-8 and TNF-α all increased between examinations (Fig. 1B). *At the first examination*, there was an overall trend for increased likelihood of progression of radiographic features of OA in the subsequent years with higher synovial fluid cytokine levels (mean OR 1.1; Table 1). This was most pronounced for osteophytes, for which higher concentrations of IL-6 (OR 1.05) and TNF-α (OR 1.35) were associated with progression (Table 1). For patient-relevant outcomes, the only association found was between IL-6 and worsening of the KOOS domain Sports and Recreation (OR 1.08; Table 1). *At the second examination*, higher concentrations of TNF-α (but not IL-6 and IL-8) was associated with having progressed in loss of joint space (OR 1.70) or worsened in function of daily living (OR 1.50) in the preceding years (Table 1). We further found that for the *change* in cytokine concentrations between examinations, increase in TNF-α (but not in IL-6 or IL-8) was associated with progression of loss of joint space (OR 1.34; Table 1). With change in cytokine concentrations dichotomized as 'increasing vs. not increasing', we found that those who had increased in concentrations of IL-6 or TNF-α from first to second examination had a 5-fold increased likelihood of having progressed in loss of joint space as compared to those with stable or decreasing concentrations (Table 1).

Conclusions: In middle-aged subjects with previous meniscectomy, having high or over time increasing synovial fluid levels of IL-6 and TNF-α was associated with increased risk for progression of radiographic features of OA over four to ten years. Although our results need confirmation in prospective studies, they indicate that IL-6 or TNF-α may represent valid treatment targets in knee OA.

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ELEVATED PERIPHERAL BLOOD LEUKOCYTE INFLAMMATORY GENE EXPRESSION IN RADIOGRAPHIC PROGRESSORS WITH SYMPTOMATIC KNEE OSTEOARTHRITIS: NYU AND OAI COHORTS

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Purpose: We and others have demonstrated low grade inflammation exists in OA joint tissues, where it may contribute to disease pathogenesis. In the current studies we assessed whether inflammatory events occurring within joint tissues were reported in the peripheral blood leukocytes (PBLs) of patients with symptomatic knee OA (SKOA).
Methods: PBL inflammatory gene expression (IL-1, TNFα, COX-2) was assessed in two independent cohorts of patients with SKOA, and a cohort of healthy control subjects: 1) 111 patients with tibiofemoral medial OA and 21 healthy volunteers from the NYUHJD Cohort, and 2) 200 patients from the OAI progression cohort who had "high quality radiographs", at both baseline and 24 months, and had KL2 or 3 in the signal knee at baseline. Radiographic progression was defined as narrowing of medial joint space width (JSW) in the signal knee between baseline and 24-months in each cohort. Radiographic progressors were defined as subjects who had JSN >0.0, 0.2 and 0.5mm over 24 months. For measuring predictive performance, we used the area under the curve (AUC) of a receiver operating characteristics (ROC). OAI SKOA subjects were dichotomized as radiographic non-progressors (JSN <0.0 mm) and progressors (JSN>0.0mm) for association studies.
Results: Elevated PBL expression of IL-1, TNFα or COX-2 identified SKOA patients who were "fast progressors" (mean JSN 0= 0.71, 0.75 and 0.71 mm / 24 months, respectively) compared to patients with levels below the median. In a multivariable model, anthropometric traits alone (BMI, gender, age) did not predict progression, whereas addition of PBL gene expressions improved prediction of fast progressors (JSN>0.5mm). We next examined inflammatory gene expression in PBLs of radiographic

Table 1
Area under the curve (AUC) of a receiver operating characteristics (ROC) of PBL inflammatory gene expression for distinguishing radiographic progressors from non-progressors in the OAI cohort. For multivariable models, we used 10-fold stratified cross-validation repeated with 100 different splits of data into different splits of data into 10-folds.

Progressors (JSN>0.0mm) vs.non-Progressors (JSN<0.0mm)	AUC	CI	P value
IL-1β	0.750	0.66−0.84	0.0000
TNFα	0.674	0.58−0.77	0.0002
COX-2	0.621	0.52−0.72	0.0095
IL-1β + TNFα + COX-2	0.611	0.51−0.71	0.0170
Progressors (JSN>0.2mm) vs non-Progressors (JSN<0.0mm)	**AUC**	**CI**	**P value**
IL-1β	0.755	0.06 - 0.65	0.0000
TNFα	0.674	0.57 - 0.73	0.0002
COX-2	0.614	0.50 - 0.72	0.0204
IL-1β + TNFα + COX-2	0.719	0.62 − 0.82	0.0000
Progressors (JSN>0.5mm) vs non-Progressors (JSN<0.0mm)	**AUC**	**CI**	**P value**
IL-1β	0.758	0.65−0.87	0.0000
TNFα	0.675	0.56−0.79	0.0015
COX-2	0 611	0.49−0.73	0.0382
IL-1β + TNFα + COX-2	0.730	0.62−0.84	0.0000

progressors in the OAI cohort. Similar to the NYUHJD cohort, elevated expression of IL-1β, TNFα and COX-2 mRNA distinguished radiographic progressors from non-progressors (Table 1). PBL IL-1β expression found to be strongest predictor of all three radiographic progressors. In multivariate models that combine all three markers did not improve upon IL-1β predictivity. We thus conclude that either the signal in TNFα and Cox-2 is already subsumed by IL-1β and/or that it is not easy to capture the non-overlapping signals without increasing the sample size (i.e., fitting a stronger multivariate predictor will require more sample size).
Conclusions: We identified, and confirmed in two cohorts, increased inflammatory gene expression (IL-1, TNFα or COX-2) by PBLs that predict radiographic progression in patients with SKOA. The data indicate that inflammatory events within joint tissues of patients with SKOA are reported in the peripheral blood. These PBL transcriptome signals of local joint inflammation merit further study as potential biomarkers for OA disease progression.

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CHANGES IN ARGS-AGGRECAN, C-TERMINAL TYPE II AND N-TERMINAL TYPE I COLLAGEN TELOPEPTIDES, AND CYTOKINE CONCENTRATIONS OVER FIVE YEARS AFTER ANTERIOR CRUCIATE LIGAMENT INJURY

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Purpose: Rupture of the anterior cruciate ligament (ACL) leads to an increased risk of developing knee osteoarthritis (OA). Mechanisms behind this development are not fully understood, and analysis of biomarkers could be used for elucidating these biological processes but also to detect and predict joint pathology and post traumatic OA. We prospectively monitored levels of synovial fluid and serum pro-inflammatory cytokines and aggrecan ARGS neoepitope, and urine C-terminal type II (CTX-II) and N-terminal type I (NTX-I) collagen telopeptides over five years after ACL rupture.
Methods: Synovial fluid, serum and urine were collected from 121 adults at baseline (0-6 weeks after injury), 16, 30 and 52 weeks, and two and five years after an acute ACL injury (the KANON trial, ISRCTN 84752559, http://www.controlled-trials.com). Reference samples: synovial fluid (n = 21), serum (n = 23) and urine (n = 62) were from knee healthy volunteers. Synovial fluid and serum concentrations of interleukin (IL)-6, IL-8, IL-10, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α were measured by a multiplex immunoassay from Meso Scale Discovery (MSD), and ARGS-aggrecan was analyzed by an in-house immunoassay assay. Urine levels of CTX-II and NTX-I were measured by ELISA assays from IDS and Osteomark, and were corrected for urine creatinine levels (CREP2, Roche). Concentrations of cytokines, ARGS-aggrecan and collagen telopeptides were not normally distributed. We used Mann-Whitney tests for group comparisons and Spearman's rank test (r_S) for correlation analysis. To estimate time until the synovial fluid biomarker concentrations had decreased to half that of the value at baseline ('concentration half-life') a mixed variance components model was used.
Results: At baseline (0-6 weeks after injury), synovial fluid cytokine concentrations were elevated 6- (TNF-α, p < 0.001), 7- (IL-8, p < 0.001), 13- (IFN-γ, p < 0.001), 21- (IL-10, p < 0.001) and 1050-fold (IL-6, p < 0.001) compared to knee healthy reference levels. This increase was not observed in serum cytokine concentrations. Synovial fluid and serum ARGS-aggrecan, and urine CTX-II concentrations were elevated 8- (p < 0.001), 1.4- (p = 0.002) and 1.5-fold (p = 0.009) respectively, while baseline urine NTX-I levels were no different from the reference. At time periods after baseline, concentrations of synovial fluid cytokines and ARGS-aggrecan, and urine CTX-II and NTX-I decreased rapidly, where ARGS-aggrecan and cytokines showed the following statistically significant mean concentration half-lives, in years: IL-6 0.9, IL-8 2.2, IL-10 2.3, IFN-γ 3.1, TNF-α 3.6, ARGS-aggrecan 4.0. Five years after injury, the synovial fluid TNF-α concentration was still higher (1.7-fold, p = 0.044) than the reference level, the urine NTX-I level was lower (0.5-fold, p < 0.001), while the concentration of the other markers were at the same level as seen in references. Over the five year period, there was a correlation between synovial fluid and serum ARGS-aggrecan concentrations (r_S = 0.36, p < 0.001), and between serum and synovial fluid ARGS-aggrecan and urine CTX-II levels (r_S = 0.36, p < 0.001 and r_S = 0.26, p = 0.006, respectively) (Table 1). Synovial fluid cytokine concentrations correlated with synovial fluid ARGS-aggrecan level (r_S = 0.41 to 0.49, p < 0.001) and with levels of urine collagen telopeptides (r_S = 0.21 to 0.31, p = 0.001 to 0.028) (Table 1).
Conclusions: Acute ACL injury induced high levels of proinflammatory cytokines in the joint which were associated with proteolysis of aggrecan and type II collagen. This trauma induced joint inflammation persisted many years after ACL injury, and together with the reduction of bone turnover, seen several years after injury as decreased urine NTX-I levels, may eventually result in permanent cartilage damage and the development of post traumatic OA.

Table 1
Correlation analysis between biomarkers. Spearman's rank order correlation (p-values) between biomarkers in different body fluids from ACL injured subjects (n = 121). To avoid repeated observations, each subject contributed samples at one time point only. Statistically significant correlations are in bold. ARGS, ARGS neoepitope of aggrecan.

	Synovial fluid ARGS	Serum ARGS	Urine CTX-II	Urine NTX-I
Synovial fluid IL-6	**0.479 (<0.001)**	0.130 (0.173)	**0.263 (0.006)**	**0.211 (0.028)**
Synovial fluid IL-8	**0.409 (<0.001)**	0.089 (0.353)	**0.276 (0.004)**	0.181 (0.059)
Synovial fluid IL-10	**0.492 (<0.001)**	0.073 (0.443)	**0.264 (0.006)**	0.146 (0.129)
Synovial fluid IFN-γ	**0.460 (<0.001)**	**0.257 (0.006)**	**0.309 (0.001)**	**0.278 (0.003)**
Synovial fluid NF-α	**0.438 (<0.001)**	0.075 (0.434)	**0.297 (0.002)**	**0.232 (0.015)**
Synovial fluid ARGS	—	**0.359 (<0.001)**	**0.258 (0.006)**	0.112 (0.243)
Serum ARGS		—	**0.356 (<0.001)**	0.086 (0.353)
Urine CTX-II			-	**0.691 (<0.001)**
Urine NTX-I				—