Purpose: Hand osteoarthritis (OA) is one of the most common localization of OA, affecting predominantly women. Patients with knee, hip or spine OA exhibit increased cartilage type II collagen degradation as detected by the urinary excretion of C-terminal crosslinking telopeptide of type II collagen (CTX-II), but data on hand OA are lacking. The aim of this study was to investigate the relationship between urinary CTX-II and hand OA in women.

Subjects and Methods: We investigated 590 women from the OFELY population-based study (mean age: 61.8 years ± 10.2) including 475 postmenopausal women. Clinical hand OA was defined according to the ACR criteria (Altman et al, 1990) slightly modified (without functional complains). At the same time of hand OA evaluation, knee and spine OA were assessed by radiographs and self-reported hip OA was recorded. Levels of urinary CTX-II measured by ELISA (Urine CartiLaps®, IDS) in the 186 women with hand OA (mean age: 67.2 years ± 8.4) were compared to those of the 404 other women without hand OA (mean age: 59.4 years ± 10.1). All analyses were adjusted for age and concomitant knee, hip or spine OA.

Results: Urinary CTX-II levels were significantly increased in women with hand OA (+ 45% vs controls, $p = 0.001$) after adjustment for age and for knee, spine and hip OA). When urinary CTX-II concentrations were considered in quartiles, subjects with levels in the highest quartile had an increased risk of presenting with hand OA, with an odds-ratio of 2.06 (95% CI: 1.3-3.2; $p = 0.002$) after adjustment for OA at the other anatomical sites.

Conclusion: Hand OA is characterized by increased type II collagen degradation. Urinary CTX-II could be a useful biomarker for the clinical investigation of hand OA.

118

TNF-α IS ELEVATED IN SYNOVIAL FLUID OF PATIENTS WITH AN ANTERIOR CRUCIATE LIGAMENT RUPTURE 5 YEARS AFTER THE INJURY. PRO-INFLAMMATORY CYTOKINES IN SYNOVIAL FLUID AND SERUM – A PROSPECTIVE LONGITUDINAL STUDY

M. Hansson [†], S. Larsson [†], R. Frobell [†], L.S. Lohmander [†,‡], A. Struglics [†]. [†] *Lund Univ., Clinical Sci. Lund, Lund, Sweden;* [‡] *Res. Unit for Musculoskeletal Function and Physiotherapy, and Dept.s of Orthopaedics and Traumatology, Univ. of Southern Denmark, Odense, Denmark*

Purpose: Trauma induced cytokine response in knee injuries may play a role in the development of osteoarthritis (OA) since knee injuries are often a gateway to OA. Cytokines regulate responses to infection, inflammation and trauma, attract leucocytes to the affected tissue, and are involved in tissue destruction mediated by matrix metalloproteases. Blocking of cytokines (e.g. TNF-α) has been successful in the treatment of patients with rheumatoid arthritis. As part of a longitudinal study (KANON study), where we aim to analyze different outcomes in patients suffering from ruptured anterior cruciate ligaments (ACL), we here report the pro-inflammatory cytokine levels in synovial fluid (SF) and serum at different times after injury.

Methods: SF and serum was collected from ACL injured patients (n=121) 0-3 weeks (baseline), at 16, 30, 52, 104 and 260 weeks after the injury. Mean age of the included patients was 26 years (range 18-35) and 74% were male. Samples from knee healthy subjects (reference group n=23) matched for age were included. Pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12 p70, IFN-γ, and TNF-α) were analyzed using the Human Pro-inflammatory 7-plex Ultra-Sensitive Kit (Meso Scale Discovery) according to manufacturers instruction. Recovery and dilution linearity tests were performed on randomly selected SF samples. Values below lower limit of quantification (LLOQ) were assigned half the LLOQ value for each cytokine. Concentrations were not normally distributed and thus between group differences were analyzed using the Mann-Whitney U-test and correlations using Spearman's rank test (r_S).

Results: The serum concentrations of IL-1β, IL-6, IL-12p70 and IFN-γ were below LLOQ for 73-100% of the analyzed samples and were therefore excluded. IL-10 was detectable in 50% of the samples, while TNF-α and IL-8 were detectable in all serum samples. Serum concentrations of IL-8 were significantly lower in the injured group at all time points, except at 16 weeks, when compared with the reference group (Table 1).

The SF concentrations of IL-1β and IL-12 p70 were below LLOQ for 59-100% of the samples and were excluded. Compared to the reference group, SF concentrations of IL-6, IL-8, IL-10, IFN-γ and TNF-α were significantly elevated in the injured group at baseline. IL-6 levels at baseline were about 1800 times higher compared with the reference group, thereafter levels decreased, and after 30 weeks and later no significant differences were observed. The SF concentration of TNF-α was elevated directly after injury and remained significantly elevated over 5 years compared to the reference group (Table 1). Further, a positive correlation was observed between SF and serum for IL-8 (r_S=0.156, p=0.004) and TNF- α (r_S=0.109, p=0.043) concentrations.

Table 1
Cytokine concentrations (pg/ml) in SF and *serum* expressed as medians (25th–75th percentile range), *P*-values for group comparisons between references against ACL patients at different time. Half LLOQ values: IL-10, 1.0; IL-6, 0.3; IFN-γ, 0.6.

Groups	n	IL-8		IL-10		TNF-α	
		Conc.	*P*-values	Conc.	*P*-values	Conc.	*P*-values
Ref	20	13.8 (9.6-83.2)	-	1.0 (1.0-2.4)	-	2.2 (1.0-3.6)	-
	23	*9.0 (5.8-14.9)*	-	*2.2 (1.0-6.5)*	-	*5.9 (4.9-7.3)*	-
Baseline	51	89.2 (47.8-189.7)	<0.001	20.1 (8.8-41.1)	<0.001	12.4 (9.8-15.2)	<0.001
	119	*5.8 (4.5-7.6)*	*<0.001*	*1.0(1.0-3.0)*	*0.205*	*6.3 (5.2-7.7)*	*0.303*
16 weeks	50	29.4 (16.2-77.1)	0.091	2.8 (1.0-4.7)	0.089	5.8 (4.8-7.2)	<0.001
	64	*6.1 (4.4-10.9)*	*0.067*	*2.0 (1.0-2.8)*	*0.483*	*6.3 (5.3-7.7)*	*0.240*
30 weeks	47	23.4 (16.2-37.9)	0.279	1.0 (1.0-2.4)	0,889	4.7 (3.9-6.2)	0.001
	63	*6.5 (4.8-9.2)*	*0.041*	*1.0 (1.0-3.6)*	*0.271*	*6.5 (5.4-7.3)*	*0.185*
52 weeks	47	20.1 (10.9-37.1)	0.722	1.0 (1.0-2.1)	0.368	4.4 (3.4-5.6)	0.001
	63	*6.3 (4.6-8.9)*	*0.018*	*1.0 (1.0-2.6)*	*0.191*	*6.3 (5.3-6.9)*	*0.461*
104 weeks	81	15.8 (11.6-26.2)	0.878	1.0 (1.0-2.3)	0.396	4.0 (3.2-4.8)	0.002
	118	*5.5 (4.4-7.4)*	*<0.001*	*1.0 (1.0-2.8)*	*0.163*	*6.3 (5.4-7.5)*	*0.237*
260 weeks	64	10.6 (7.4-14.4)	0.046	1.0 (1.0-1.0)	0.070	3.7 (2.5-4.5)	0.044
	114	*5.8 (4.6-7.5)*	*0.002*	*2.0 (1.0-3.6)*	*0.530*	*6.5 (5.1-8.0)*	*0.199*

Groups	n	**IL-6**		**IFN-γ**	
		Conc.	*P*-values	Conc.	*P*-values
Ref	20	0.7 (0.3-8.2)	-	0.6 (0.6-0.6)	-
Baseline	51	1271.1 (321.0-3614.7)	<0.001	8.0 (2.9-28.1)	<0.001
16 weeks	50	11.2 (3.3-33.1)	0.005	0.6 (0.6-1.4)	0.372
30 weeks	47	3.2 (0.6-16.5)	0.209	0.6 (0.6-0.6)	0.387
52 weeks	47	1.3 (0.3-5.7)	0.528	0.6 (0.6-0.6)	0.670
104 weeks	81	1.0 (0.3-4.0)	0.895	0.6 (0.6-0.6)	0.564
260 weeks	64	0.7 (0.3-2.5)	0.588	0.6 (0.6-0.6)	0.446

Conclusions: Pro-inflammatory cytokines are elevated in SF, but not in serum, immediately after knee ACL injury. SF TNF-α level stay elevated over the first 5 years after ACL injury and this high TNF- α concentration may contribute to a later development of OA.

119
MULTIPLEX ANALYSIS OF OSTEOARTHRITIC SYNOVIAL FLUID: A COMPARISON OF LUMINEX & MESOSCALE DISCOVERY

C. Jayadev, S. Snelling, A. Price, P. Hulley. *Univ. of Oxford, Oxford, United Kingdom*

Purpose: Multiplex immunoassay platforms are indispensible for biomarker discovery. They allow high-throughput, multiple parallel analysis of precious small volume samples. The 2 dominant technologies are Luminex (LX) and MesoScale Discovery (MSD). Neither platform is validated for synovial fluid (SF) analysis. The cross-comparison of multiplex formats and comparison against the existing gold standard of ELISA are required to facilitate quality control and guide optimum assay selection for biomarker discovery. Previous comparison studies in culture medium and serum/plasma cannot be extrapolated to the complex biological matrix of SF. **The aim was to make a detailed comparison between LX and MSD platforms during the analysis of real clinical SF samples from end-stage knee OA.**
Methods: SF aliquots from patients with end-stage knee OA (N=31) were analysed for the inflammatory cytokines IL1β, TNFα, IL6 & IL8 using magnetic-bead LX and MSD multiplex assays. All SF samples underwent identical collection, processing, storage and hyaluronidase treatment. Aliquots of an additional SF sample were analysed by each platform after spiking with 2 known concentrations (high & low) of the respective assay calibrator. ELISA for IL6 & TNFα was performed on aliquots from patients previously analysed by the LX (n=8) and MSD (n=11) platforms. The LX and MSD assays were compared for limits of detection (LOD) & quantification (LOQ), spike recovery, and intra-assay precision. Agreement of cytokine measurements between platforms and against ELISA was tested by: weighted Deming Regressions (WDR), concordance correlation coefficient (CCC) for absolute agreement and intraclass correlation coefficient (ICC) for consistency. $P<0.05$ was considered significant.
Results: LOD: The MSD platform had a significantly lower LOD for all 4 analytes ($p<0.01$). IL6 & IL8 were >LOD in all samples on both platforms. IL1β & TNFα were >LOD in significantly more samples on the MSD platform: IL1β 81% vs 3% ($p<0.001$); TNF-α 100% vs 3% ($p<0.001$). **LOQ:** The MSD platform had the lowest LOQ for all 4 analytes, which was significant for all except IL6. Both platforms were able to quantify IL6 & IL8 in >96% of samples. IL1β & TNFα were not quantifiable by the LX assay compared to 29% ($p=0.003$) and 87% ($p<0.001$) by the MSD assay. **Spike-recovery:** Spike recoveries on the MSD platform were acceptable (100±20%) for both concentrations except for the IL6 low-spike (76.6%). IL6 & IL8 had acceptable recoveries for both spikes concentrations on the LX platform. Recoveries at either spike concentrations were below acceptable for IL1β & TNFα. **Intra-assay precision:** The coefficient of variation (CV) for replicate measurements for all analytes was acceptable (<20%) irrespective of platform. There was no significant difference in median CV between platforms. **ELISA validation:** All samples were <LOD on the TNFα ELISA. There was no significant systematic or proportional error for IL6 measurements by the either platform vs ELISA (WDR). There was moderate concordance for MSD vs ELISA (CCC=0.901) and poor concordance for LX vs. ELISA (CCC=0.768) The consistency of measurements for both platforms vs ELISA was excellent (>0.90). **Cross-platform agreement:** There was proportional bias but no systematic bias in IL6 & IL8 measurements between platforms. The concordance of IL6 (CCC=0.62) and IL8 (CCC=0.53) measurements was poor, but the relative agreement was excellent (ICC>0.95).
Conclusions: The MSD platform is better able to detect and quantify low-level analytes (IL1β & TNFα) in OA SF samples than LX. There is poor absolute agreement but excellent relative agreement between platforms. This is most likely due to differences in antibody pairs and kinetics. Cytokine measurements in OA samples are at best semi-quantitative and depend on the platform, assay and manufacturer, thus making comparisons between studies difficult. Biomarker studies should be consistent and explicit regarding assays.

120
LONGITUDINAL CHANGE IN SYNOVIAL FLUID AND SERUM LEVELS OF ARGS-AGGRECAN OVER 5 YEARS AFTER ANTERIOR CRUCIATE LIGAMENT INJURY

S. Larsson[†], M. Hansson[†], R. Frobell[†], L.S. Lohmander[†,‡], A. Struglics[†]. [†]*Dept. of Orthopaedics, Clinical Sci. Lund, Lund Univ., Lund, SWEDEN;* [‡]*Res. Unit for Musculoskeletal Function and Physiotherapy, and Dept. of Orthopaedics and Traumatology, Univ. of Southern Denmark, Odense, Denmark*

Purpose: Aggrecanase cleavage at the 373Glu-374Ala bond in the interglobular domain of aggrecan, releasing N-terminal 374ARGS fragments into synovial fluid (SF-ARGS), is an early key event in arthritis and joint injuries. We have previously shown that SF-ARGS is associated with radiographic progression of knee osteoarthritis (OA) as well as with progression of self-reported pain after meniscectomy. As a part of a randomized controlled study (KANON), initiated to investigate structured rehabilitation of anterior cruciate ligament (ACL) injuries with or without surgical reconstruction, we here report ARGS-aggrecan levels in SF and in serum over 5 years after ACL injury.
Methods: One hundred and twenty-one subjects (26% women, mean age 26 years, standard deviation [SD] 4.9 years) with an ACL rupture to a previously un-injured knee were followed within 3 weeks from trauma (baseline), at 16 weeks, 30 weeks, 1 year, 2 years and 5 years. Blood samples were obtained from all subjects and SF was collected from a subset of subjects (6 subjects had intact series of both SF and serum samples). Twenty-one knee-healthy individuals (38% women, mean age 28 years, SD 9.4 years) were used as reference. Levels of ARGS-aggrecan were measured in all SF and serum samples with an electrochemiluminescence immunoassay using an anti-aggrecan antibody (AHP0022; Invitrogen) for capture, and a monoclonal anti-ARGS (OA-1) for detection. We used Mann-Whitney rank sum test for comparison of ARGS-concentrations at different time points compared to knee-healthy references, and Spearman's rank order correlation for assessment of correlation, based on Shapiro-Wilk test indicating skewed distributions at all time points.
Results: Levels of ARGS-aggrecan in SF and serum correlated (Spearman's rho = 0.245, $p < 0.001$, n = 356) and were overall 10 times higher in SF than in serum: median nM ARGS in SF (25th and 75th percentiles) in ACL injured at all time points combined 1.16 (0.83, 1.94) and 0.13 (0.10, 0.17) in serum.
SF-ARGS levels in the ACL injury group were elevated compared to knee-healthy references at baseline and at 16 weeks after injury, but after 30 weeks or longer, levels were not significantly different from those observed in the reference group (Table 1). ACL injury serum-ARGS levels compared to knee-healthy reference level were significantly elevated at baseline but not at any other time point (Table 1).

Table 1
Cross-sectional ARGS-aggrecan concentrations (nM) in synovial fluid (SF) and serum after ACL injury and in knee-healthy subjects (REF). Values are medians (25th, 75th percentiles) with fold difference of the medians compared to REF. P-values, Mann-Whitney rank sum tests against uninjured references.

	n	SF-ARGS	fold dif.	p-values	n	serum-ARGS	fold dif.	p-values
REF	21	0.85 (0.57, 1.30)	-	-	19	0.112 (0.075, 0.138)	-	-
Baseline	51	6.85 (4.41,17.25)	8.1	<0.001	120	0.158 (0.114, 0.198)	1.4	0.002
16 weeks	50	1.22 (1.00, 1.89)	1.4	0.005	64	0.134 (0.099, 0.168)	1.2	0.073
30 weeks	48	1.10 (0.77, 1.77)	1.3	0.068	63	0.137 (0.107, 0.164)	1.2	0.075
1 year	49	0.95 (0.74, 1.32)	1.1	0.349	63	0.129 (0.105, 0.157)	1.2	0.148
2 years	85	1.07 (0.79, 1.28)	1.3	0.138	118	0.125 (0.094, 0.153)	1.1	0.290
5 years	63	1.01 (0.69, 1.39)	1.2	0.266	115	0.113 (0.091, 0.143)	1.0	0.784