MEASUREMENT AND CORRELATION OF INFLAMMATORY MEDIATORS IN SERUM AND SYNOVIAL FLUID FROM A CROSS-SECTIONAL OSTEOARTHRITIS COHORT

K. Ma1, S. Visvanathan1, P.A. MasterS1, M. Mascelli1, X-y. Song2, S. Lohmander2, S. Blake3, F. Baribaud1
1Centocor R&D Inc, Radnor, PA; 2Ethicon R&D Inc, Somerville, NJ; 3Dept. of Orthopedics, Clinical Sciences Lund, Sweden

Purpose: Evaluate the use of multiplex immunodetection assays to measure a broad range of inflammatory mediators in a cross-sectional osteoarthritis cohort. Examine relationships between markers in serum and synovial fluid.

Methods: A cross-sectional cohort composed of 6 sub-groups including healthy volunteers (REF n=23), patients with either acute pyrophosphate arthritis (APA n=17), anterior cruciate ligament rupture isolated or combined with another ligament (LI n=146), meniscal tear (MT n=70), osteochondral fracture (OC n=19), primary knee osteoarthritis (OA n=46) for a total of 321 patients. One sample of knee synovial fluid (SF) and serum (S) at 1 time point, varying between 0 days and 26 years after knee injury or onset of symptoms, were analyzed. Analytes measured using the Luminex platform were cytokines/chemokines, adipokines, and acute phase proteins (LINCOPlex assays, Millipore). Tukey's multiple comparison testing was used for all statistical analyses on logarithmic-transformed marker values.

Results: The intra- and inter-assay variability was below 10 and 20% respectively in both S and SF. The dynamic range of the standard curves spanned over a 4 logarithm range with correlation coefficients of at least 0.9 for all analytes tested with the exception of SAP, which in S had a correlation coefficient of 0.84. Data validation was determined by spiking recovery experiments at 6 concentrations over a 3 logarithm range using 70-130% of target concentration. Coefficients of variation (CV) for multiple measurements of the analytes were less than 9%.

Conclusions: Multiplex kits were validated for their use in measuring markers in S and SF from a cross-sectional OA cohort. Data from 24 and 14 analytes from S and SF respectively were collected for at least 50% of all samples assayed. A wide range of concentrations was found for all analytes across all sub-groups in the cohort. Finally, several markers correlated with each other whether in S or SF.

OSTEOCHONDRAL INJURY INCREASES TYPE II COLLAGEN DEGRADATION PRODUCTS (C2C) IN SYNOVIAL FLUID OF THOROUGHBRED RACEHORSES

A.B. Scarbrough, M.P. Brown, T.N. Trumble
University of Florida, Gainesville, FL

Purpose: To investigate the effects of exercise and osteochondral (OC) injury on type II collagen degradation (C2C) in synovial fluid (SF) from Thoroughbred (TB) racehorses and to compare these results with radiographic and arthroscopic scores of severity of joint injury.

Methods: SF samples were obtained from 3 groups of TB racehorses: (1) Rested: 20 TB horses (age range 14 to 20 months), confirmed normal by clinical and radiographic examination. SF samples were collected from metacarpophalangeal (MCP) joints (n=20) and middle (n=14) or radiocarpal joints (n=6) of these horses before the start of race training. (2) Exercised: horses from the rested group (group 1) had the same joints sampled at the end of 5 to 6 months of training. (3) OC injury: 28 Thoroughbred horses (age range 2 to 7 years) undergoing MCP (n=13), metatarsal/medial tarsal (MTP; n=1), middle carpal (n=9), or radiocarpal (n=5) arthroscopic surgery for removal of (OC) fragments, which were injuries resulting from racing. For group 3, radiographic and arthroscopic scores were determined. SF samples were assayed using a commercially available ELISA (C2C, IBEX Technologies, Inc). Differences between groups were determined by one-way ANOVA and a post hoc Tukey's multiple-comparison test. Positive and negative predictive value of SF C2C for identifying OC injury was determined by Fisher's exact test. Correlations were determined using the Spearman correlation. P < 0.05 was considered significant.

Results: SF C2C concentrations in OC injured carpal and MCP/MTP joints were significantly different than rested and exercised joints (P < 0.01) (Figure 1). However, carpal and MCP/MTP SF C2C concentrations were not significantly different between rested and exercised groups. Arthroscopic scores were significantly higher for OC injured carpal than OC injured MCP/MTP joints.

Figure 1. Horizontal scatter plot of SF C2C concentrations for MCP/MTP (top) and carpal (bottom) joints in rested, exercised, and OC injured TB racehorses. The short vertical solid lines represent the mean value for each group. The vertical dashed lines represent SF concentrations (> 64 pmol/mL for MCP/MTP joints and > 75 pmol/mL for carpal joints) for which there is predictive value in discriminating OC injured horses from rested or exercised horses. Like letters represent no significant difference.
joints ($P=0.004$). OC injured SF C2C concentrations were positively correlated with radiographic scores ($R=0.43; P=0.03$) and arthroscopic scores ($R=0.52; P=0.03$). Arthroscopic scores were positively correlated with radiographic scores ($R=0.51; P=0.04$). SF C2C concentrations $> 64$ pmol/mL for MCP/MTP joints and $> 75$ pmol/mL for carpal joints were arbitrarily chosen to determine predictive value for discriminating OC injured horses from rested or exercised horses. This yielded a positive predictive value of 73% and a negative predictive value of 94% for the MCP/TP joints, and positive predictive value of 79% and negative predictive value of 95% for the carpus.

Conclusions: OC injury caused a significant increase in SF C2C concentrations in carpal and MCP/MTP joints compared to rested and exercised horses. SF C2C concentrations were correlated to severity of joint injury. Based on these findings, SF C2C analysis may be useful for evaluation of joint injury.

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### 105

**FOLLOW-UP OF COLL2-1, COLL2-1NO2 AND MYELOPEROXIDASE SERUM LEVELS IN MARATHON RUNNERS**

M. Deberg$^1$, A. Labasse$^1$, C. Sanchez$^1$, A. Bosseloir$^2$, Y. Henrotin$^1$

$^1$University of Liège, Liège, Belgium; $^2$Zentech, Liège, Belgium

**Purpose:** To determine the influence of marathon on the serum levels of two markers of cartilage degradation, Coll2-1, a peptide of type II collagen triple helix, and its nitrated form, Coll2-1NO2, and of a marker of neutrophils activation, the myeloperoxidase (MPO).

**Methods:** Coll2-1, Coll2-1NO2 and MPO were measured by specific immunoassays in 66 marathon runners without joint pain and aged in mean of 47 (min: 31 - max: 59) years. Sera were taken before and after the marathon. All the subjects were submitted to a questionnaire concerning their physical activity (i.e. training or best performance) and their life style (i.e. diet). The total running distance was 42 km. Their performance in the marathon was ranged from 149 to 270 min.

**Results:** Before the marathon, the Coll2-1 and Coll2-1NO2 values were not affected by age, body mass index, sex and performance (Coll2-1 median: 90.66 (26.77-234.68) nM and Coll2-1NO2 median: 0.16 (0.05-0.71) nM), while MPO levels were higher in female [median: 33.00 (15.60-84.60) ng/ml] (p<0.05). After the marathon, Coll2-1 levels were slightly decreased [median: 76.63 (28.89-185.06) nM], Coll2-1NO2 levels were unmodified [median: 0.15 (0.05-0.61) nM] and MPO levels were doubled [median: 71.80 (31.50-172.10) ng/ml] compared to the pre-marathon values [MPO median: 34.20 (15.60-96.50) ng/ml] (p<0.001). The variation of MPO during the marathon was negatively correlated with the training time per week ($r=-0.36; p=0.0045$). Before the marathon, there was no correlation between the different biological markers (Coll2-1, Coll2-1NO2 and MPO).

**Conclusions:** Coll2-1 and Coll2-1NO2 serum levels were not modified by marathon, suggesting that, at least at short-term, this intensive physical activity does not significantly modify cartilage metabolism. Interestingly, serum MPO levels were increased after the marathon and training reduced this elevation. These results suggest that neutrophils were activated during the marathon and that training reduces neutrophils activation during the marathon.

### 106

**QUANTITATIVE WESTERN BLOTTING SHOWS DIFFERENCES IN SYNOVIAL FLUID AGGRECAN FRAGMENT PATTERNS BETWEEN HUMAN JOINT DISEASES**

A. Struggilis, S. Larsson, M. Hansson, S. Lohmander

Lund University, Lund, Sweden

**Purpose:** Aggrecan proteolysis is an early key event in arthritis. Aggrecanases cleave the interglobular domain of aggrecan releasing N-terminal ARGS fragments, and the chondroitin sulfate rich domain releasing C-terminal G3 domain fragments. Several reports suggest a role for more than one ADAMTS activity and possibly other proteases in the release of aggrecan fragments from joint cartilage. Differences between animal models and human disease, and differences between human joint diseases and disease stages suggest a role for a method to map in detail aggrecan proteolytic fragments in multiple samples. We have developed a quantitative Western blot method for analysis of aggrecan fragment patterns in synovial fluids (SF) from patients with different joint diseases.

**Methods:** Dissociative CsCl gradient centrifugation was used for sample preparation from SF of 5 knee-healthy subjects (reference) and 27 patients with 4 different knee joint diseases. Aggrecan fragments (D1-fractions) separated by SDS-PAGE and transferred to PVDF membranes were screened, using a digital luminescence imager, with an ARGS neo-epitope antibody or an antibody recognizing the G3-domain. ARGS-standards were generated by complete aggrecanase digestion of intact human aggrecan. Quantification was by co-running either ARGS-standards or a control sample (in G3 quantification) on each SDS-gel. Results were compared and expressed as mol (ARGS) or relative AU (G3) per mg glycosaminoglycan (GAG) determined by Alcian Blue precipitation.

**Results:** GAG recovery in D1-fractions was $>75\%$, and Western blot CVS between experiments for anti-ARGS and anti-G3 were 19% and 28%. Total ARGS fragment quantity in SF samples by Western blot correlated well with ARGS-ELISA ($R^2=0.87$, n=19), while the correlation between ARGS and GAG content was only moderate ($R^2=0.31$, n=31). SFs contained two major ARGS-bands, ARGS-SELE and ARGS-GVED, and three major G3-bands (GRGT-G3, OGLS-G3 and AGEG-G3) as a result of aggrecanase proteolysis. Median values of total ARGS fragments in the diagnostic groups were significantly higher in the acute arthritis and acute injury groups compared to the reference, and these SF samples contained the highest total ARGS values per mg GAG (Table 1; $^*P<0.05$, $^{**}P<0.01$ compared to reference). The differential between reference and disease groups was greatest for the ARGS-SELE fragment. Median values of total G3 per mg GAG were significantly lower in the acute arthritis and osteoarthritis groups than in the reference. The ratios of total-ARGS to total-G3 fragments varied markedly between the diagnostic groups.

**Table 1.** Western blot analysis in diagnostic groups. Median values normalized to reference

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ARGS-SELE</th>
<th>ARGS-GVED</th>
<th>Total ARGS</th>
<th>Total G3</th>
<th>Total ARGS/G3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute arthritis</td>
<td>8</td>
<td>25***</td>
<td>10.1**</td>
<td>10.7*</td>
<td>0.5*</td>
<td>22.3**</td>
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<tr>
<td>Acute injury</td>
<td>9</td>
<td>20.2**</td>
<td>10.2**</td>
<td>10*</td>
<td>0.6</td>
<td>9.5**</td>
</tr>
<tr>
<td>Chronic injury</td>
<td>5</td>
<td>4.2</td>
<td>2.2</td>
<td>2.1</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>5</td>
<td>8.4</td>
<td>2.9</td>
<td>3.4</td>
<td>0.3*</td>
<td>2.6</td>
</tr>
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</table>

**Conclusions:** We have developed a quantitative Western blot method for aggrecan fragment screening of synovial fluids. It provides reliable results as judged by relatively low CV and good correlation between Western and ELISA methods. Anti-ARGS...