

# Lubricating Ability of Aspirated Synovial Fluid from Emergency Department Patients with Knee Joint Synovitis

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**ABSTRACT. Objective.** To determine if joint effusions encountered in the emergency department (ED) requiring arthrocentesis possess normal lubricating ability or evidence cartilage degeneration. Chondroprotection of articulating joint surfaces is provided by lubricin, a mucinous glycoprotein that is a product of megakaryocyte-stimulating factor gene (GenBank U70136) expression. Loss of synovial fluid's (SF) lubricating ability has been implicated in the pathogenesis of degenerative joint disease.

**Methods.** A retrospective ED observational study from May 1, 1999, to October 1, 2000, of adult and pediatric patients presenting with radiographically negative knee joint complaints and clinical evidence of joint effusion. Knee joints were aspirated by the emergency physician and the synovial fluid tested for lubricating ability and collagen type II degeneration. Lubricating ability was assayed *in vitro* in an arthrotripsometer oscillating latex apposed to polished glass under a load of  $0.35 \times 10^6$  N/m<sup>2</sup>. Results were reported as the coefficient of friction ( $\mu$ ) relative to that of a 0.9% NaCl control; negative  $\Delta\mu$  value indicates lubrication. Comparisons of  $\Delta\mu$  were made to normal SF and aspirates from patients with osteoarthritis (OA) and rheumatoid arthritis. Collagen type II fragments were measured by a novel sandwich ELISA.

**Results.** Synovial fluid aliquots ( $n = 57$ ) lubricated poorly with  $\Delta\mu = -0.045$  (95% confidence interval =  $-0.006, -0.083$ ) compared to normal SF with  $\Delta\mu = -0.095$  (95% CI =  $-0.088, -0.101$ ). Only 20.6% of knee joint aspirates possessed normal lubricating ability. An association exists between nucleated cell count and  $\Delta\mu$  described by a logarithmic function. Collagen type II fragments were present in aspirates at a concentration of  $0.636 \mu\text{g/ml}$  (95% CI  $0.495\text{--}0.777 \mu\text{g/ml}$ ), significantly higher than  $0.173 \mu\text{g/ml}$  (95% CI  $0.154\text{--}0.193 \mu\text{g/ml}$ ) in the OA comparison group.

**Conclusion.** Knee joints with synovitis, commonly encountered in the ED, are frequently nonlubricated bearings and display catabolism of collagen type II. This may play a role in acute cartilage destruction ultimately resulting in posttraumatic OA. (J Rheumatol 2004;31:557–64)

## Key Indexing Terms:

JOINT LUBRICATION                      LUBRICIN                      SUPERFICIAL ZONE PROTEIN  
MEGAKARYOCYTE-STIMULATING FACTOR                      TRAUMATIC SYNOVITIS

Arthritis after joint injury involving a fracture extending into the synovial cavity is a well appreciated phenomenon<sup>1,2</sup>. Longitudinal studies support this observation, as well as a greater risk of knee osteoarthritis (OA) following fibula fracture and ligamentous and meniscal injuries<sup>3</sup>. Subclinical

and repetitive trauma also lead to early OA<sup>4</sup>. It is generally unknown if acute but mild forms of trauma could also lead to degenerative joint disease. We wished to determine if emergency department (ED) patients diagnosed with knee joint synovitis show evidence of synovial fluid (SF) and articular cartilage deterioration.

Emergency department management of weight bearing joint effusions is focused on determining the likelihood of fracture based on physical and radiographic findings, and the presence of infection or crystal induced synovitis<sup>5</sup>. Joint aspiration is also performed for patient comfort<sup>6</sup>. Suspicion of occult fracture in the traumatized knee joint that is radiographically negative prompts a diagnostic arthrocentesis<sup>7,8</sup>. In the absence of a history of trauma, aspirated SF is also examined for nucleated cells<sup>9</sup>, where counts exceeding 50,000/high powered field (hpf) may indicate a high probability of infection<sup>10</sup>.

SF is present to provide lubrication of apposed and pres-

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surized cartilaginous surfaces and also to nourish chondrocytes. SF contains hyaluronate, rendering it highly viscous. SF digested with hyaluronidase results in a nonviscous fluid that continues to lubricate<sup>11</sup>. SF digested with trypsin results in a viscous fluid that fails to lubricate<sup>12,13</sup>. SF is an ultrafiltrate of blood plasma, augmented by lubricin secreted by synovial fibroblasts<sup>14</sup>. This is a mucinous glycoprotein (Mr ~208 kDa)<sup>15</sup> with an apparent molecular weight of 240–280 kDa, which provides synovial fluid's lubricating ability. The origin of this protein has been linked to megakaryocyte-stimulating factor (MSF) gene expression (Genbank U70136)<sup>14,16,17</sup>. MSF is also expressed by superficial zone chondrocytes as superficial zone protein (SZP), directly lubricating the articular surface *in situ*<sup>16</sup>. MSF expression is increased by mechanical shear<sup>18</sup> and transforming growth factor- $\beta$  (TGF- $\beta$ ) and is decreased by interleukin 1 (IL-1)<sup>16</sup>.

Lubricating ability of lubricin is labile in the presence of small amounts of elastase<sup>13</sup>. This proteolytic enzyme is well known for its destructive effect toward connective tissue<sup>19</sup>. It follows that mild joint inflammation, commonly encountered among the emergency patient population, could prove deleterious to synovial fluid's ability to lubricate joint surfaces. Lack of chondroprotection could lead to premature microfibrillation of articular cartilage, placing patients at enhanced risk for acquiring degenerative joint disease later in life. Obesity<sup>20</sup>, strenuous occupations<sup>4</sup>, and injuries<sup>21</sup> have all been identified as risk factors in developing OA in longitudinal case-control studies. However, discrete and identifiable subclinical events that may initiate OA remain unknown.

This observational study was undertaken to determine if both diminished boundary lubricating ability and cartilage deterioration occur among ED patients diagnosed with knee joint synovitis (KJS). Aspirates from knee joints of patients with established OA and rheumatoid arthritis (RA) were also studied for comparison. A secondary objective was to identify if any relationship exists between observed SF lubricating ability measured *in vitro* and nucleated cell count. A coefficient of friction ( $\mu$ ) was assayed in a bearing system of latex apposed with polished glass as described<sup>22–25</sup>, which replicates the lubricating behaviors of cartilage-containing bearings but is more reproducible. Cartilage degeneration was indicated by a novel sandwich ELISA for collagen type II peptides, as this is the predominant collagen in articular cartilage.

## MATERIALS AND METHODS

This was a retrospective laboratory based observational study of the lubricating ability of aspirated knee joint SF from ED patients compared to patients with normal joint function and those with well established arthritides. Institutional review board (IRB) approvals were obtained prior to initiation of the study.

*Study setting and population.* Aliquots of SF from a convenience sample of pediatric and adult ED patients undergoing diagnostic knee joint arthrocentesis were collected from May 1, 1999, to October 1, 2000, following routine laboratory analysis for nucleated cell count, protein, glucose, gram

stain, and bacterial culture. Remaining SF was processed and stored for this investigation. Aliquots that met inclusion criteria below were studied in the friction apparatus. Aliquots of SF from patients with OA (n = 122) and RA (n = 13) were also collected for comparison, as were samples of normal SF from healthy donors (n = 6).

*Study protocol.* An ED chart review was conducted by the principal investigator to determine discharge diagnosis, age, and, in those cases where trauma played a role in knee joint effusion, the mechanism of injury. Patient history was evaluated for gout, pseudogout, hemophilia, sickle cell anemia, and history of degenerative joint diseases. Inclusion criteria were monoarticular knee joint effusions judged to be clinically significant, requiring arthrocentesis in the absence of radiographic evidence of fracture. Exclusion criteria included a diagnosis of occult fracture, documented joint instability, history of previous joint injury or degenerative joint disease, history of sickle cell anemia or hemophilia, patellar bursitis, samples contaminated with blood, or bacterial growth within 48 h. SF was aspirated by emergency medicine attending and resident physicians from the lateral aspect of the affected knee joint via an 18 gauge needle using standard aseptic technique following anesthesia of the site with 2% lidocaine injected subcutaneously. After analysis for nucleated cell count, protein and glucose concentration, and bacterial culture, samples were stored at 4°C. Within 12 h, the remaining SF was centrifuged at 12,000 g at 4°C for 60 min to remove cellular debris. The supernatant was decanted and stored at -80°C for subsequent lubrication assay in the friction apparatus.

Aliquots of SF from patients undergoing arthroscopy or total knee joint replacement for advanced OA were also collected and assayed in the friction apparatus as historical controls. Both OA and RA samples were collected prospectively and represented a convenience sample. Patients with OA typically presented with Noyes<sup>26</sup> criteria grade III or IV. SF was aspirated prior to procedure by orthopaedic surgeons as described above following general anesthesia. SF aspirates from patients with established RA were obtained following routine rheumatologic followup. Aliquots from both sources were processed as above on the day of collection and stored at -80°C. Collection of SF in this manner was approved by the IRB. Informed consent was not obtained as these were samples that would otherwise be discarded. Samples of normal human SF were obtained from donor human subjects undergoing allograft cartilage transplant surgery. These subjects were without evidence of gross degenerative joint disease and samples were obtained in gratis from Dr. M. Lotz at Scripps Research Institute. The Scripps Research Institute IRB approved the collection of normal human SF in this manner.

*Friction apparatus.* The friction apparatus<sup>13,25</sup> was an improved version of McCutchen's instrument<sup>11</sup> as modified by Davis, *et al*<sup>24</sup> (Figure 1). Natural latex was oscillated against a ring of polished glass with a constant contact area of 1.59 cm<sup>2</sup>. The bearing system was axially loaded within a gimbals system free to rotate around 2 perpendicular horizontal axes. Latex and glass as bearing materials were chosen since they offer a flat surface with small asperity heights on the order of 0.05 mm. Latex, like cartilage, is compliant. Within the gimbals system, these surfaces possess near perfect coplanarity. Accordingly, fluid wedges were not generated and only a thin layer of boundary fluid was present. The entraining velocity (i.e., sliding speed) was 0.37 mm/s with a constant contact pressure of  $0.35 \times 10^6$  N/m<sup>2</sup>.

The friction apparatus recorded displacements of the gimbals system around the vertical loading axis through a linear displacement voltage transducer, the output voltage of which was directly proportional to the magnitude of the frictional torque. The peak to peak amplitude of this signal was related to  $\mu$  by a previous calibration with known frictional torque.

Test surfaces were cleaned extensively before use. A 3.8 × 3.8 cm piece of latex strapped onto the stainless steel stud was washed under running distilled deionized water (DDW) for 2 min. It was then placed in a shallow bath of 0.9% NaCl physiological saline. The glass slide was scrubbed with a 1% (v/v) 7X detergent (Flow Laboratories, McLean, VA, USA) solution in DDW for 10 min and then allowed to soak in the same solution at 100°C. A 5 min scrubbing was also performed with the hot 7X solution followed by rinsing for 2–4 min under running DDW.

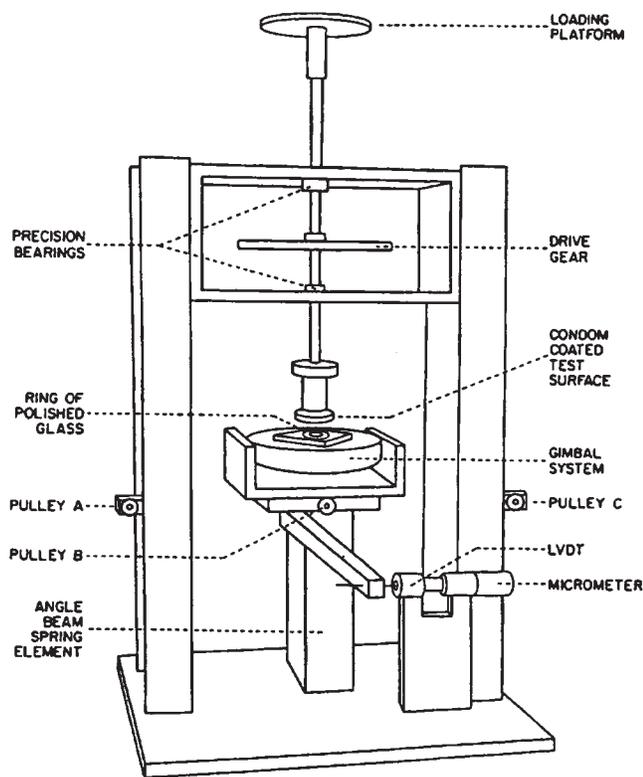


Figure 1. The friction apparatus.

The  $\mu$  was measured at 25°C and was preceded by a baseline measurement of the  $\mu$  with saline. Lubrication was manifested by a reduction in  $\mu$  relative to the  $\mu$  of saline. Negative  $\Delta\mu$  values indicate lubrication, whereas positive values indicate friction. In this way, each sample was calibrated against its own saline reference. Addition of 200  $\mu$ l of saline, and later 200  $\mu$ l of test lubricant, was followed by bringing the bearing surfaces close enough so that the solution wet both surfaces. After 5 min for equilibration, the latex-coated bearing was brought to rest on the glass as it was oscillating. Peak to peak voltages were automatically recorded after 1, 3, and 5 min. At this point the surfaces were separated for 2 min and then brought back together for 3 additional 5-min sessions. The 3 and 5 min  $\mu$  values of the last two 5 min sessions were recorded<sup>23,24</sup> and the mean calculated. This value was then compared to the corresponding mean  $\mu$  for baseline saline, resulting in the  $\Delta\mu$  calculation described above. Equilibration of the bearing surfaces and stabilization of  $\Delta\mu$  occurs during the last two 5-min sessions.

**Nucleated cell counts.** Prior to centrifugation an aliquot of SF was analyzed by standard technique using a hemocytometer under 40 $\times$  magnification. Wright stain was used to identify white blood cells (WBC), which were reported as the number of cells per mm<sup>3</sup>. This procedure was done by certified clinical laboratory staff.

**Hyaluronidase SF treatment.** SF samples were collected, aliquoted, and stored at -80°C. Sample aliquots used in the elastase activity assays were not treated with EDTA. Other aliquots were treated with 5 mM EDTA and centrifuged at 10,000 rpm for 15 min to remove debris. SF samples were treated with Streptomyces hyaluronidase (Sigma-Aldrich, St. Louis, MO, USA) to a final concentration of 200 U/ml for 40 min at 56°C and subsequently for 20 min at 80°C.

**Sandwich ELISA for CII peptides.** Two CII monoclonal antibodies, 18:6:D6 and 14:7:D8, were used in this assay<sup>27</sup>. Antibody 18:6:D6 was made against CII CNBr cleavage peptide 9.7 coupled to ovalbumin. Antibody 14:7:D8 was made using a 15 amino acid synthetic peptide GPOGPRGDKGEGEP

coupled to keyhole limpet hemocyanin and was conjugated to horseradish peroxidase using the EZ-Link maleimide activated horseradish peroxidase kit (Pierce Chemicals, Rockford, IL, USA). Microtiter plates (High binding Costar; Corning Inc., Corning, NY, USA) were coated with a 1:500 dilution of 18:6:D6 antibody in phosphate buffered saline (PBS) overnight. Each well received 100  $\mu$ l of the antibody solution. Hyaluronidase-pretreated SF samples (serial dilutions in PBS-Tween 20), CNBr-cleaved CII peptide-spiked SF samples, and standards (different dilutions of CNBr-cleaved CII peptides) were incubated on the precoated plates for 60 min at room temperature. Antibody 14:7:D8 conjugated with peroxidase in PBS-Tween, a 1:1000 dilution, was subsequently added for 60 min at room temperature. Fluorescence substrate developing solution (100  $\mu$ l) (QuantaBlu™; Pierce, Rockford, IL, USA) was added for 60 min, and the fluorescence was measured at an excitation wavelength 330 nm and emission wavelength 460 nm using a Packard Fluorocounter (Packard Instruments, Meriden, CT, USA).

**Total protein levels.** To control for variations in CII peptide concentrations resulting from differences in volume of aspirated SF samples among patient groups, CII peptide concentrations were normalized to total protein concentrations. Total protein concentrations of SF samples were determined colorimetrically using the Micro BCA™ Protein Assay Reagent Kit (Pierce). A series of bovine serum albumin standards, 100  $\mu$ l (2–100  $\mu$ g/ml), as well as SF samples diluted in distilled water, 100  $\mu$ l, were incubated with Micro BCA reagent, 100  $\mu$ l, in the wells of a 96-well plate for 60 min at 37°C. The absorbance was subsequently measured at 570 nm using a Dynéx MRX microtiter plate reader (Dynéx Technologies, Chantilly, VA, USA).

**Hyaluronate concentration determination.** SF aspirates were assayed by the manual carbazole reaction for uronic acid<sup>28</sup>. This involved layering 125  $\mu$ l of sample (diluted 10-fold with saline) onto 750  $\mu$ l of concentrated H<sub>2</sub>SO<sub>4</sub> containing 25 mM sodium borate that had been cooled to -70°C. After heating at 100°C for 10 min and cooling to room temperature, 25  $\mu$ l of 0.125% (w/v) carbazole in absolute ethanol was introduced. Upon heating at 100°C for 15 min the presence of uronate was indicated by a pink color, which was quantified by spectrophotometry at 530 nm against a standard curve. Hyaluronate concentration was reported in units of mg/ml after factoring in the 10-fold dilution.

**Data analysis.** Comparisons of  $\Delta\mu$  between lubricants were by graphical assessment of median and 95% confidence intervals and one-way analysis of variance (ANOVA) across normal, KJS, OA, and RA groups. Pairwise comparisons were conducted by t tests using the ANOVA error term. Nucleated cell count and  $\Delta\mu$  for KJS samples were tested for association by least-squares regression in predicting  $\Delta\mu$  from WBC. Both linear and logarithmic models were applied to this analysis. Hyaluronate and CII peptide concentrations between the KJS and OA groups were compared by the independent-samples t test. The significance level for all statistical tests, determined a priori, was 0.05.

## RESULTS

A total of 80 patients (59 male, 21 female) were identified with monoarticular knee effusion following some form of trauma (n = 16), swelling of unknown etiology (n = 14), possible infectious synovitis (n = 13), or history of recurrent crystal induced synovitis (n = 23). Another 14 patients did not have the reason for arthrocentesis documented in the ED record. All patients underwent either diagnostic or therapeutic joint aspiration. Overall, 23 patients (28.7%) (18 female, 5 male) were diagnosed with crystal induced synovitis: 17 (73.9%) had gout and 6 (26.1%) had pseudogout and were thus excluded. Two patients had a history of sickle cell anemia and were also excluded. A total of 57 patients met inclusion criteria.

A total of 57 patients appeared to have KJS that was neither infectious nor crystal induced. A total of 16 of these (28%) were evidently a result of trauma, based on chart review. These patients presented with blunt trauma (n = 5; 31.2%), a sprain/strain mechanism (n = 4; 25%), or lacerations (n = 2; 12.5%), and another 5 patients did not have a mechanism documented (n = 5; 31.2%). Patients averaged 46.9 years of age and ranged in age from 2 to 87 years. The time from onset of joint symptoms to ED presentation from chart review was 6.7 days (95% CI 4.7–8.7 days) and ranged from 1 to 32 days. This was not recorded in the medical record of 8 (14%) patients.

**Lubricating ability.** Samples of KJS lubricated poorly with a  $\Delta\mu$  of  $-0.045$  (95% CI  $-0.006$  to  $-0.083$ ) compared to normal synovial fluid, which lubricated with a  $\Delta\mu = -0.095$  (95% CI  $-0.088$  to  $-0.101$ ) (Figure 2). SF from patients with OA and RA had a  $\Delta\mu = -0.068$  (95% CI  $-0.054$  to  $-0.088$ ) and  $\Delta\mu = +0.031$  (95% CI  $+0.011$  to  $+0.053$ ), respectively. One-way ANOVA revealed a main effect of group ( $F(3,134) = 28.08$ ,  $p < 0.0001$ ). Followup pairwise comparisons revealed significant differences between all group pairs, except between normal SF samples and those from patients with OA (Table 1). Overall, 20.6% of aspirates from patients

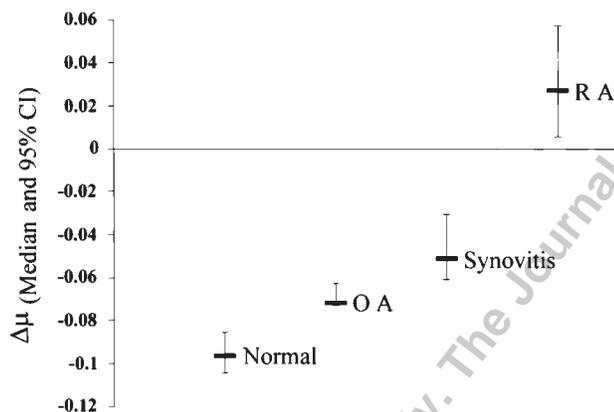


Figure 2. Lubricating ability ( $\Delta\mu$ ) of synovial fluid from patients without degenerative joint disease (Normal) and with osteoarthritis (OA), noninfectious knee joint synovitis (KJS), and rheumatoid arthritis (RA) assayed in the latex:glass friction bearing under a pressure of  $0.35 \times 10^6$  N/m<sup>2</sup>.

Table 1. Pairwise comparisons revealed significant differences between all group pairs, except between normal SF samples and those from patients with OA. Pairwise t tests used ANOVA error term to estimate the standard error of the difference.

	OA	KJS	RA
Normal	t(194) = 1.63 p = 0.1039	t(194) = 2.91 p = 0.0041	t(194) = 6.47 p < 0.0001
OA		t(194) = 3.52 p = 0.0005	t(194) = 8.60 p < 0.0001
KJS			t(194) = 6.33 p < 0.0001

diagnosed with KJS possessed normal lubricating ability as  $\Delta\mu$  values of these aspirates fell within the 95% CI of  $\Delta\mu$  for normal SF.

The concentration of hyaluronate in SF from patients with KJS and OA was 3.04 mg/ml (95% CI 2.78–3.30 mg/ml) and 2.84 mg/ml (95% CI 2.67–3.02 mg/ml), respectively (Student t test 1.39;  $p = 0.16$ ). SF from patients with KJS had a slightly higher concentration of hyaluronate, indicating that synovial effusion in KJS aliquots was not diluting MSF/lubricin and thus accounting for the loss of lubricating ability. The published normative value<sup>29</sup> of synovial hyaluronate is 3.3 mg/ml, from which the KJS aliquots did not differ significantly (Student t test 1.89;  $p = 0.06$ ).

KJS SF cell count was 22,033 cells/mm<sup>3</sup> (95% CI 355–29,025 cells/mm<sup>3</sup>) and ranged from 10 to 195,840 cells/mm<sup>3</sup>. The median was 3352.5 cells/mm<sup>3</sup>, with 8 KJS samples exceeding 50,000 cells/mm<sup>3</sup>. Normal and OA SF aspirate cell counts were negligible. All KJS SF aliquots in this study, including aspirates intended to rule out infection, had negative bacterial growth after 48 h.

The lubricating ability of KJS aspirates decreased as a function of nucleated cell count; however, this relationship was not linear (Figure 3). Rather, it was better described using a logarithmic function, whereby as nucleated cell count increased, lubrication decreased, but with diminishing returns as lubricating ability approached that of saline. This logarithmic function accounted for 37.3% of variance in contrast to a linear function, which accounted for only 12.4% (data not shown).

**Collagen type II peptides.** Normal SF samples had undetectably low CII peptide concentrations. The CII peptides in SF from patients with KJS showed a wide range of concentrations, between 0.068 and 3.369  $\mu\text{g/ml}$ , a mean of 0.636  $\mu\text{g/ml}$  (95% CI 0.495–0.777  $\mu\text{g/ml}$ ). The OA SF aspirates had a mean of 0.173  $\mu\text{g/ml}$  (95% CI 0.154–0.193  $\mu\text{g/ml}$ ). CII peptide concentrations were significantly higher in SF samples from patients with KJS compared to SF samples from patients with OA ( $t = 6.60$ ,  $p < 0.001$ ). Hyaluronic acid concentration showed no significant difference between the 3 patient groups. When CII peptide concentrations were normalized to total protein concentrations, the significant elevation of CII peptide concentrations in patients with KJS compared to patients with OA ( $p < 0.005$ ) was still apparent (Figure 4).

## DISCUSSION

Studies have identified injury<sup>20</sup> and occupation<sup>4,30</sup> as risk factors in the pathogenesis of OA, but laboratory evidence of the earliest sentinel events leading to OA remain under investigation within the orthopedic and rheumatologic research communities. These investigators have understandably restricted their studies to patients with well documented disease processes and not to those at the earliest

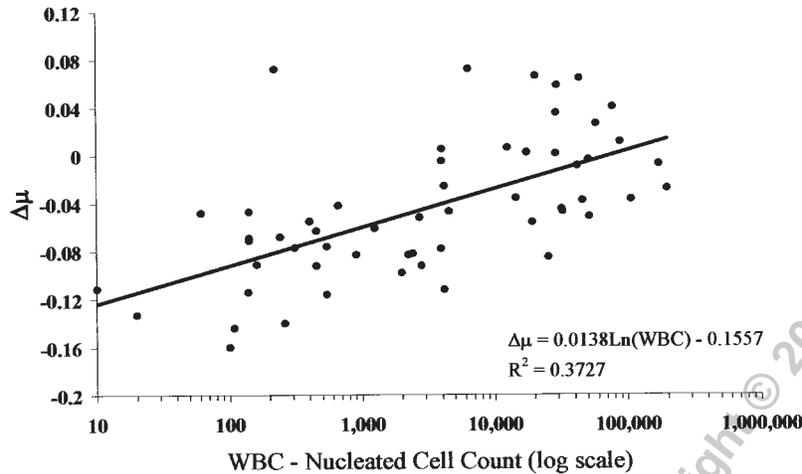


Figure 3. Correlation between lubricating ability ( $\Delta\mu$ ) and nucleated cell count (WBC) of synovial fluid aspirates from ED patients with KJS.

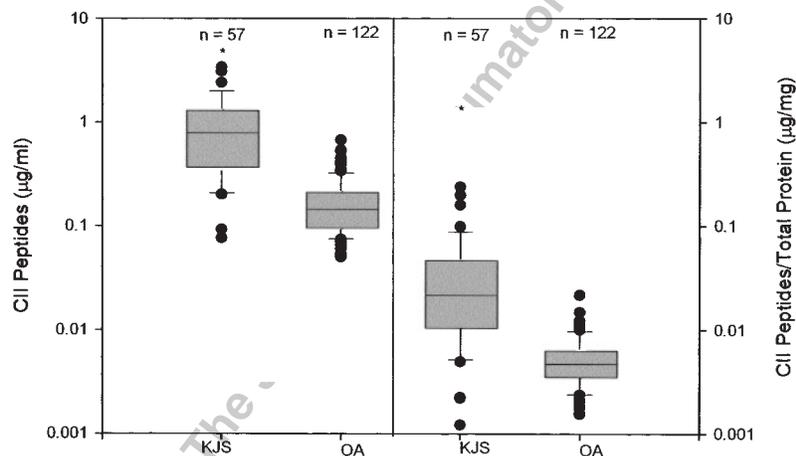


Figure 4. Concentration of CII peptides normalized to total protein among patients with KJS and OA. \*  $p < 0.005$ .

point after a joint injury or complaint. Our results demarcate a group of ED patients with incipient injury attributable to lack of chondroprotection provided by synovial fluid's lubricating ability or inflammation in general. It is unknown how long SF displays deficient lubricating ability in synovitis with a traumatic or unclear etiology. The implication, however, is that early degenerative joint disease may have a causal relationship with mild joint trauma<sup>31</sup> or subclinical joint trauma.

Deficient lubricating ability among patients with synovitis stands paradoxically in contrast to SF aspirated from joints of patients with OA. It has been reported<sup>32</sup> in a smaller study that these aspirates have normal lubricating

ability. These intriguing observations are partly explained by the fact that the lubricating moiety is produced by superficial zone articular chondrocytes<sup>16</sup> and synovial fibroblasts<sup>14</sup>, secreting superficial zone protein and lubricin, respectively. Both are identical protein products of megakaryocyte-stimulating factor expression<sup>33</sup>. Patients with advanced OA lack superficial zone chondrocytes and yet continue to have normal SF lubricating ability *in vitro*, indicating that the synovial fibroblast contribution to joint lubrication in the form of lubricin endures. Disease states such as trauma and RA exemplified by SF deficient in lubricating ability have both cell types affected. The histopathologic appearance of traumatic synovitis is similar to RA but

less intense and extensive<sup>34</sup>. Inflammatory processes can lead to IL-1 $\alpha$  expression, which in the case of superficial zone articular chondrocytes<sup>16</sup> downregulates expression of MSF *in vitro* and can ultimately lead to proteolysis. In the absence of lubrication, chondrocyte apoptosis, as a result of excessive mechanical loading<sup>35</sup>, is a possible outcome.

The association between nucleated cell count in SF and loss of its lubricating ability suggests that proteolytic destruction of lubricin is occurring possibly by neutrophil elastase. This enzyme has been implicated in the catabolism of extracellular matrix in a number of pathological processes<sup>19</sup>. Neutrophil elastase is found at higher concentrations in SF from patients with RA as compared to OA<sup>36</sup>. The plot of  $\Delta\mu$  against the logarithm of nucleated cell count in Figure 3 produced a convincing association between these 2 independent variables in contrast to the more conventional linear scaling. Since many of the data points corresponded to nucleated cell counts less than 1000 cells/hpf and the range extended to 50,000 cells/hpf or more, a nonlinear pooling of data points near the origin was resolved by a logarithmic function. This "model" is one of diminishing returns in which incremental increases in the concentration of proteases, released by neutrophils, result in geometrically cumulative protein digestion (and loss of lubrication) as a given enzyme will digest many copies of substrate.

A large number of enzymes including serine proteases have been identified in SF from patients with RA and OA including thrombin, trypsin-like protease, Factor Xa, collagenase, plasmin, elastase, and urokinase<sup>37</sup>. Indeed, thrombin appears to be most abundant. Serine proteases are also involved in the activation and deactivation of metalloproteases (MMP), which have been shown to have both collagenase and elastase-like activity and thus are also destructive to extracellular matrix<sup>38</sup>. The interplay between serine proteases and metalloproteinases and the contribution of each to connective tissue destruction remains unclear<sup>36</sup>. Antagonism by the universal protease inhibitors alpha-1-protease inhibitor ( $\alpha_1$ PI) and alpha-2-macroglobulin ( $\alpha_2$ m) adds a further layer of complexity<sup>39</sup>. Neutrophil elastase is completely inhibited by  $\alpha_1$ PI and incompletely by  $\alpha_2$ m<sup>40</sup>.

Investigation is now focused on identifying the enzyme(s) responsible for the loss of lubricating ability in SF from patients with noninfectious synovitis. The sensitivity of lubricin to trypsin, elastase<sup>13</sup>, chymotrypsin<sup>33</sup>, and serine proteases is well established. These experiments have also offered clues to the structure-function relationship of lubricin/MSF. MSF is composed of 12 exons, the largest of which, exon 6, is heavily glycosylated and provides for lubrication. Chymotrypsin digestion would theoretically leave the central exon 6 region intact as no aromatic residues exist, indicating that both N and C-terminal ends of lubricin/MSF are as important to boundary lubricating activity as exon 6<sup>33,41</sup>. Digestion by elastase may be directed

against exon 6, acting on the alanine and proline within the degenerate repeating sequence of KEPAPTT. Collagen type II antibodies directed against articular cartilage *in vitro* failed to interact unless the surface of cartilage was first pretreated with polymorphonuclear elastase<sup>42</sup>. The same phenomenon observed *in vivo* was inhibited by the non-specific serine-esterase inhibitor phenylmethyl sulfonic acid, but not by the divalent metal chelator EDTA<sup>43</sup>. A parallel study of this same KJS population detected epitope 9A4, indicative of MMP-13 activity that was positively correlated with undifferentiated total elastase activity<sup>27</sup>. There was a mean 43% inhibition of elastase activity by EDTA, indicating that both elastase and elastase-like activity due to MMP-13 were present. After digestion of the lubricating mucinous glycoproteins (SZP and lubricin), these same enzymes would hypothetically go on to disrupt the most superficial layers of collagen in articular cartilage. These CII peptides were detectable by the novel sandwich ELISA described above.

Presumably the association between nucleated cell count and lack of *in vitro* boundary lubricating ability by aspirated SF could be used in the future to guide medical decision making in the use of antiinflammatory medications or devices to help restore SF. The opportunity to instill a new device presents itself as emergency physicians remove synovial effusions routinely and our rheumatologic colleagues inject SF analogs in the form of hyaluronic acid hydrogels.

Unlike lubricin, which is a boundary lubricant, hyaluronic acid provides for hydrodynamic lubrication in an unloaded joint and is naturally present in SF at a concentration of 3.3 mg/ml. Lubricin acts between apposed cartilaginous surfaces, which flatten and reciprocate under a load of  $1-2 \times 10^6$  N/m<sup>2</sup> at a sliding speed in the range of 1-5 mm/s<sup>44</sup>. The lubrication regime operating under these conditions is independent of viscosity and hyaluronate, and therefore does not appear to play a major mechanical role. However, hyaluronate is a major component of healthy SF, and its replacement or augmentation with a highly cross-linked hyaluronate is hypothesized to increase the hydration of SF. Low molecular weight constituents of hyaluronate may also have an antiinflammatory effect<sup>45</sup>. The manual carbazole assay used in this study is a measure of total uronate, and did not provide identification of long- versus short-chain hyaluronate among the synovial aspirates.

**Limitations.** It is unknown whether any patient with KJS actually had torn ligaments or menisci due to the lack of prospective followup in this preliminary investigation. A prospective study that quantifies resultant disability and extent of underlying injury, if any, is now proceeding. If the diminished lubricating ability is due to proteolytic activity, it is possible that some loss of activity may have occurred after the sample was aspirated. However, care was exercised in the handling of samples, as they were always stored at

4°C prior to centrifugation. This would significantly lessen the likelihood of this occurring.

An underlying assumption in this investigation is that an equilibrium exists between lubricin/SZP that is bound to articular cartilage and that which is aspiratable from the joint cavity. Assaying lubricating ability of aspirated SF *in vitro* or the concentration of lubricin by ELISA is a surrogate for *in vivo* activity. However, lubricin appears to interact with both cleaved and degenerative articular cartilage, suggesting that accumulation of lubricin on damaged articular cartilage from a dilute solution is possible<sup>46</sup>. The latex:glass friction apparatus in this investigation utilized bearing materials that are foreign to the biomolecular components of SF. The friction apparatus is a model of the tribological conditions thought to exist in cartilaginous bearings. In the hands of different investigators<sup>22,23</sup>, data obtained this way paralleled those obtained with cartilage–cartilage bearings<sup>47,48</sup> and were more reproducible. Finally, the volume of SF aspirated and the estimated size of synovial effusions were not recorded. The possibility of lubricin dilution was partly controlled for by determining the concentration of hyaluronate. Hyaluronate is a locally produced factor within the synovium, in contrast to total protein, which originates from both the synovium and serum. Samples of SF collected from patients with OA and normal joints were obtained under general anesthesia, which differed from patients with KJS and RA.

Many patients presenting to the ED with knee joint synovitis appear to be ambulating on nonlubricated cartilage surfaces and may be at risk for premature OA. These patients have laboratory evidence of cartilage deterioration as evidenced by the liberation of collagen type II peptides. Whether this is indicative of the microfibrillation of cartilage and cell death of the superficial chondrocytes is unknown. Inflammatory acute phase reactants may be responsible for this through the catabolic effects of neutrophil elastase and other proteases. Presently, the longterm effects of enhanced friction and resultant wear effects in patients with knee joint synovitis are unknown.

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