HYALURONAN-LINKED INTER-α-TRYPSIN INHIBITOR HEAVY CHAIN (SHAP), INTERLEUKIN 8, MMP-3 AND HYALURONAN IN HUMAN SYNOVIAL FLUID AND SERUM IN OSTEOARTHRITIS, JOINT INJURY AND INFLAMMATION

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PURPOSE
Inter-α-trypsin Inhibitor (IAI) occurs in plasma and has protease inhibitory activity. Heavy chains of IAI can bind covalently to hyaluronan (HA) to form a serum derived hyaluronan associated protein, SHAP, releasing bikunin (1). The transfer of IAI heavy chain to HA is catalyzed by enzyme factors such as TNFα-stimulated gene 6 protein (TSG-6) (2). SHAP potentiates CD44-mediated leukocyte adhesion to hyaluronan substratum, and has putative roles in HA cross-linking and inflammation (3). The CXC chemokine interleukin 8 (IL-8), a potent neutrophil recruiting and activating factor, is a pro-inflammatory cytokine present in many disease conditions. Matrix metalloproteinase-3 (MMP-3) synthesis and secretion in chondrocytes and synovial cells is upregulated by enzyme factors such as TNFα, recruiting and activating factor, is a pro-inflammatory cytokine present in many disease conditions. Matrix metalloproteinase-3 (MMP-3) synthesis and secretion in chondrocytes and synovial cells is upregulated by MMP-3 (5). IL-8 synthesis was quantified by LINCO® human cytokine multiplex kit (HCYTO-60K-PMX22, and HCYTO-60K-PMX21).

METHODS
Paired knee SF and serum samples were from a convenience sample of patients with primary knee OA (POA n=43, mean age 49), knee injury (INJ n=212, mean age 34), pseudogout (PPA n=10, mean age 38), and knee-healthy persons (REF n=8, mean age 30). SHAP and HA were quantified by ELISAs, using plates coated with HA-binding region of bovine aggrecan (4). MMP-3 protein was assayed by ELISA (5). IL-8 was quantified by LINCO® human cytokine multiplex kit (HCYTO-60K-PMX22, and HCYTO-60K-PMX21).

RESULTS
Group median levels of SHAP in SF were 500-2500 times higher than those in serum, with overall levels highest in pseudogout (p<0.001, Table 1). The amount of SHAP relative to HA was higher in serum than in SF. SHAP levels in SF were highly correlated with MMP-3 protein (R=0.7, p<0.001, Fig 1). IL-8 levels correlated with SHAP (R=0.44, p<0.001, Fig 2) and MMP-3 (R=0.40, p<0.001). HA levels in SF were low in pseudogout, normal or high in OA. SHAP concentrations in SF were elevated for 3 months after knee injuries that commonly lead to OA, while HA levels were low (p<0.005) (Fig 3).

DISCUSSION
We find very high concentrations of SHAP in synovial fluid, suggesting local production in the joint. Concentrations of SHAP in joint fluid were highly correlated with MMP-3 and IL-8, suggesting synovial activation in joint injury and OA, as well as in arthritis. In contrast to some previous reports, HA levels in OA joints were normal or high, compared to those in healthy knee joints. The observed changes of these biomarkers are consistent with an upregulation of joint inflammation after joint injury and in OA, with increased levels of agents that support leukocyte recruitment, activation, and adhesion to HA.

REFERENCES

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