ANALYSIS OF SYNOVIAL FLUID BIOMARKERS IN PATIENTS WITH RHEUMATOID ARTHRITIS, OSTEOARTHRITIS, AND NORMAL DONORS

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**Purpose:** To compare synovial fluid (SF) biomarker levels in samples from patients with rheumatoid (RA), osteoarthritis (OA), and normal organ donors for prognostic/diagnostic purposes.

**Methods:** Synovial fluid (SF) was collected from the knees of 45 (6 males and 39 females) OA, 22 (14 females and 8 males) RA patients and 20 (15 females and 5 males) normal organ donors. Eight biomarkers analyzed by ELISA methods were evaluated: interleukin (IL)-1, IL-8, IL-11, leukemia inflammatory factor (LIF), cartilage oligomeric protein (COMP), osteocalcin, and osteogenic protein-1 (OP-1). Multivariate analysis assessed the effects of gender and disease activity; WOMAC scores for OA samples, and SF WBC, ESR, CRP for RA samples. Multivariate Kruskal-Wallis and Mann-Whitney Tests were used; p < 0.05 was considered significant.

**Results:** The mean (±SD) age was: 53 ±9 years for OA, 54 ±11 for RA, and 52 ±7 for normals. No gender differences were identified between markers. In RA SF, the levels of 4 out of 5 tested cytokines/chemokines were higher than in OA and normal SF. The most significant differences were found for IL-6 and IL-8, where IL-6 concentration was 2.5-fold higher than in OA, 2587.59 ±6393.69 vs. 1045.68 ±5451.13 pg/ml, respectively (p < 0.005), and 8-fold higher than in normal, 2587.59 ±6393.69 vs. 327.06 ±698.28, respectively (P < 0.002). The levels of IL-8 were 8-fold higher than in OA, 6490.04 ±16550.74 vs 849.48 ±906.28, respectively (P < 0.002). The levels of IL-1 were statistically different from normal. SF from both OA and RA had higher biomarker levels than normals, regardless of disease state, though there was a trend of higher chemokine levels in less active RA, and higher II-1, II-6, and II-8 levels in active OA.

**Conclusions:** Our findings suggest that the levels of pathophysiologically important biomarkers in SF of patients with OA and RA differ according to the mechanisms that drive each disease. Thus, II-1, II-6, LIIF and OP-1 appear to be significant for OA, while IL-6, II-8, and OP-1 may have significance for RA. As previously observed in cartilage, a strong negative correlation between the levels of OP-1 and II-6 family of chemokines was seen. Larger studies are necessary to develop a biomarker algorithm that would be of diagnostic/prognostic use.

VASCULAR ABNORMALITIES PLAY KEY ROLES IN OSTEOARTHRITIS: IMPLICATIONS FOR DETECTION AND TREATMENT

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**Purpose:** Osteoarthritis (OA) is a multifactorial disease, the causes of which remain contentious. The studies presented were designed to seek evidence to link vascular abnormalities with inflammation, matrix degradation and bone turnover as indicators of joint destruction. Based on this evidence, the role of vascular abnormalities was evaluated and the potential for development of novel biomarkers was explored.

**Methods:** Histological studies: Femoral heads removed from 11 OA patients at hip arthroplasty were examined histologically for evidence of microvascular thrombosis and occlusive intravascular lipid. They were compared with seven control femoral heads obtained at post mortem from donors in whom OA was excluded.

Haematological studies: Global and specific markers of coagulability, fibrinolysis, inflammation, matrix degradation and bone turnover were measured in peripheral blood from 44 patients with OA of the hip and 52 control subjects.

Use of combinations of “markers” to detect OA: A subset of 12 potential haematological markers and clinical measures was refined and applied to 45 subjects with OA and 47 control subjects to determine if the number of markers per subject could be used as a guide to discriminate between OA and non-OA subjects.

**Results:** Histological studies confirmed the presence of occlusive intravascular lipid and thrombosis in the vessels of the femoral head of patients with OA. These changes were not observed in the control femoral heads. The haematological studies and clinical data showed increased coagulation tendency in OA with significantly increased blood pressure, BMI, blood lipids, platelet aggregation, clotting factors, blood viscosity and fibrinopeptide A levels. There was hypofibrinolysis as shown by prolonged euglobulin clot lysis time and raised plasminogen activator inhibitor, alpha 2 antiplasmin and LP(a) levels. Mild inflammation was demonstrated with increased levels of II-1, TNF alpha and CRP. Leukocyte elastase degrades collagen and elastic fibres and it was elevated in OA. There were also increased levels of markers of bone turnover including ALP, BGP and DPD. Crosslinks. All parameters were statistically significant. Seventy percent of OA patients had four or more markers versus only 15% of controls and there was generally a combination of both increased coagulation risk and hypofibrinolysis in the osteoarthritis subjects.

**Conclusion:** Our findings led to the formation of a “Vascular Concept of OA Causation” that links vascular insufficiency to the clinical and laboratory findings in OA. Based on this we propose that anti-arthritic agents that act on the actual disease process ought to enhance the joint microcirculation and in addition to being anti-inflammatory they should also have core activities that include anti-coagulant, fibrinolytic and lipolytic activities that will promote haemostasis.

**Purpose:** We have observed that African Americans (AA) are more likely to have osteoarthritis at the hip and knee compared to Whites. Elevated TGF-β1 has been associated with osteophyte formation in the OA joint. Therefore, the purpose of the current study was to assess the utility of serum TGF-β1 as a biomarker of roA in AAs and Whites, men and women, with and without hip and knee roA.

**Methods:** Baseline data from 330 participants (42% AA; 39% men) in the Johnston County Osteoarthritis Project Biomarker Substudy were used in the analysis. Natural logarithm transformation was used to produce near-normal distributions for TGF-β1 (lnTGF-β1) in analyses. Descriptive statistics were calculated for demographic variables, knee and hip roA (defined as K/L grade ⩾ 2 at each joint), and lnTGF-β1; differences were assessed by two-sample t-tests. Generalized linear models were used to obtain adjusted means estimates for lnTGF-β1 and roA presence, laterality (none, unilateral, or bilateral), and severity (K/L grade 0, 1 = none, K/L grade 2 = mild, K/L grade 3, 4 = moderate/severe). Models were adjusted for age, gender, race, and body mass index (BMI). Interactions by race and gender were considered significant at a p-value < 0.1. Analyses were performed using SAS version 9.1 (Cary, NC).

**Results:** The mean ± SD of the sample was 60.5 ± 9.4 years, with a mean BMI of 30.3 ± 6.9 kg/m²; 54% had knee roA and 22% had hip roA. The mean for lnTGF-β1 was 2.82 ± 0.34 ng/ml. Mean lnTGF-β1 was higher among AA participants compared to Whites (p = 0.0085), and women compared to men (p = 0.0006). There were no significant associations.