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**"Evaluating IL-11 and TGFβ1 Distribution Relative to Synovial Fibrosis Status"**

**Introduction:** Synovial fibrosis (SFb), a painful contracture limiting joint motion and quality of life, is a hallmark of arthrofibrosis (AF), a common complication after joint repair. SFb is categorized by low (<41%), moderate (42-54%), and high (>54%) collagen deposition levels and is a significant challenge in osteoarthritis (OA) patients. Transforming growth factor beta 1 (TGFβ1) drives SFb and regulates essential cell processes. Interleukin (IL) 11, synthesized downstream of the TGFβ1-mediated JAK/STAT3 cascade, promotes fibrosis if dysregulated. Novomedix's (NMX) novel inhibitors selectively target IL11 without disrupting TGFβ1-mediated functions. These inhibitors effectively reduce IL11-driven collagen deposition in OA-derived fibrotic synoviocytes. To assess the potential of NMX for in vivo SFb treatment, this study analyzes IL11 co-expression with TGFβ1 in banked knee OA samples, hypothesizing a correlation between IL11 expression and SFb severity.

**Methods**: Eighteen de-identified knee synovium samples from patients with end-stage OA were categorized into low (n=7) and high (n=11) SFb cohorts based on pre-defined histological scores. These samples underwent paraffin processing, embedding, and sectioning for co-detection of TGFβ1 and IL11 by indirect immunofluorescence (IIF). Following deparaffinization, heat-mediated antigen retrieval in citrate buffer (pH 6.0), and protein blocking, sections were incubated overnight with anti-TGFβ1 (mouse monoclonal) and anti-IL11 (rabbit polyclonal) antibodies. Sections were then stained with anti-mouse Alexa 594 and anti-rabbit Alexa 647 secondary antibodies for TGFβ1 and IL11, respectively, along with DAPI nuclear counterstain. Samples were mounted and imaged using a confocal microscope (Olympus) at 200x magnification. Co-expression of TGFβ1 and IL11 was quantified through background-corrected signal analysis using Slidebook™ software. Statistical comparisons between low and high SFb groups for adjusted TGFβ1 and IL11 levels were made using a two-tailed Student's t-test ($\alpha$=0.05).

**Results**: The mean expression of TGF1 observed in the synovium of patients classified with high SFb severity was 35% higher ($p = 0.0360$) compared to the signal measured from the low severity SFb group. Correspondingly, IL11 measures in the high SFb severity were registered at a 77% increase ($p = 0.0016$) from patients with less severe SFb. A moderate but significant correlation was calculated between TGFβ1 and IL11 ($R = 0.51$; $p = 0.0314$).

**Discussion**: Increased expression of IL11 relates to TGFβ1 in agreement with SFb severity. While this study does not prove causality, it suggests a relationship between IL11 and SFb, highlighting the diseased synovium as an effective target for NMX administration. The study is limited by sample size and doesn't account for confounding variables such as synovitis grade and presence of additional pro-fibrotic factors such as connective tissue growth factor. Further studies will investigate the effectiveness of NMX on aberrant collagen deposition, contraction, and myofibroblast differentiation rate of patient-derived synovial fibroblasts.

**Conclusion**: Evidence of TGFβ1-dependent expression of IL11 relative to SFb severity indicates the potential supplementation of NMX to assist manipulation under anesthesia and arthroscopic lysis of adhesions in the management of debilitating arthrofibrosis.