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# Association of synovial inflammation and inflammatory mediators with glenohumeral rotator cuff pathology

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**Hypothesis:** We hypothesized that patients with full-thickness rotator cuff tears would have greater synovial inflammation compared with those without rotator cuff tear pathology, with gene expression relating to histologic findings.

**Methods:** Synovial sampling was performed in 19 patients with full-thickness rotator cuff tears (RTC group) and in 11 patients without rotator cuff pathology (control group). Cryosections were stained and examined under light microscopy and confocal fluorescent microscopy for anti-cluster CD45 (common leukocyte antigen), anti-CD31 (endothelial), and anti-CD68 (macrophage) cell surface markers. A grading system was used to quantitate synovitis under light microscopy, and digital image analysis was used to quantify the immunofluorescence staining area. Quantitative polymerase chain reaction was performed for validated inflammatory markers. Data were analyzed with analysis of covariance, Mann-Whitney *U*, and Spearman rank order testing, with significance set at $\alpha = .05$.

**Results:** The synovitis score was significantly increased in the RTC group compared with controls. Immunofluorescence demonstrated significantly increased staining for CD31, CD45, and CD68 in the RTC vs control group. CD45+/68– cells were found perivascularly, with CD45+/68+ cells toward the joint lining edge of the synovium. Levels of matrix metalloproteinase-3 (MMP-3) and interleukin-6 were significantly increased in the RTC group, with a positive correlation between the synovitis score and MMP-3 expression.

**Conclusions:** Patients with full-thickness rotator cuff tears have greater levels of synovial inflammation, angiogenesis, and MMP-3 upregulation compared with controls. Gene expression of MMP-3 correlates with the degree of synovitis.

**Level of evidence:** Basic Science Study; Molecular Biology

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**Keywords:** Rotator cuff; synovium; synovitis; inflammation; cytokine; MMP

The Standard University Institutional Review Board approved this study (Stanford IRB Protocol 30479).

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Rotator cuff tears are the most common cause of shoulder disability in the upper extremity[8] and account for most of the 4.5 million annual office visits for shoulder pain in the USA.[32] Extrinsic and intrinsic processes have both been proposed as the underlying cause of rotator cuff tears, but the

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exact etiology leading to the disease pathology remains unknown.[10,35] According to Neer,[30] impingement of the rotator cuff on the undersurface of the acromion can cause abrasion and tearing. Others have emphasized the role of intrinsic tendon degeneration caused by a variety of factors, including systemic patient factors and inflammatory mediators.[16,24,31]

Chronic inflammation has long been known to be a contributing factor in pathologies such as cardiovascular disease,[18,26] chronic gingivitis,[17,29] and rheumatoid arthritis.[4] Historically, chronic inflammation as a source of joint pain has been thought to encompass only the inflammatory arthropathies. Chronic inflammation has more recently been recognized as a source of joint pain and dysfunction in those with pathologies previously considered purely degenerative.[37,38]

Although many animal and basic science studies have investigated inflammatory mediators in rotator cuff pathology,[1,5,11,15,27] the study of this condition in an in vivo model has been limited because control samples have often been obtained from adjacent, nondiseased tendon within the same shoulder in those with known rotator cuff pathology[6,7,36,40] or from cadaveric specimens.[24] Other studies have examined only the synovial fluid[34,49] or the subacromial bursa,[3,45] often with conflicting results. Because the synovium is a major location for the production of inflammatory mediators, it is critical to understand the association between synovial inflammation and the generation of proinflammatory molecules to better understand the mechanisms behind the development rotator cuff pathology.

No prior studies have examined and correlated the amount of synovial inflammation with inflammatory mediator production in patients with full-thickness rotator cuff tears vs those with arthroscopically normal rotator cuff tissue. We hypothesized that patients with full-thickness rotator cuff tears would demonstrate increased synovial inflammation on microscopy and increased inflammatory mediators on gene expression analysis compared with those without rotator cuff pathology.

## Methods

The study enrolled 30 patients. Inclusion criteria consisted of a history of nontraumatic onset of shoulder pain of >6 months and age ≥18 years. The study excluded patients with a history of inflammatory arthritis, prior surgery to the involved shoulder, previous trauma coincident with the onset of shoulder pain, those receiving glucocorticoid or other intravenous/intramuscular anti-inflammatory medication within 6 weeks of surgery, and those receiving oral anti-inflammatory medication within 2 weeks of surgery.

Patients were divided into 2 groups. The control group consisted of those with shoulder pain with no radiographic glenohumeral arthritis (Weinstein grade I) as well as an intact rotator cuff confirmed by magnetic resonance imaging (MRI) and arthroscopy. The rotator cuff tear (RTC) group consisted of patients without radiographic glenohumeral arthritis (Weinstein grade I) but with MRI and arthroscopically confirmed full-thickness rotator cuff tear.

All patients underwent surgical treatment by a single surgeon, with a synovial biopsy specimen obtained from a common site within the rotator interval after anterior portal establishment. A diagnostic arthroscopy was performed in all patients to ensure there were no nonbiopsy location sites that demonstrated nonrepresentative areas of abnormal synovitis.

The preoperative MRI was examined for tear size and the degree of fatty infiltration using the MRI modification of the Goutallier classification.[12] Tear size was determined in the anterior-posterior (AP) dimension and by the amount of retraction. AP tear size was determined on sagittal oblique T2 sequences, and the largest dimension noted in medial-to-lateral scrolling was recorded. The amount of tendon retraction was determined on coronal oblique T2 sequences and was recorded as the maximum distance of any rotator cuff tendon edge during anterior-to-posterior scrolling as measured from the medial aspect of the rotator cuff footprint immediately adjacent to the humeral head articular cartilage. All measurements were made using the IntelliSpace 4.4 digital picture archiving and communication system (Phillips, Amsterdam, The Netherlands).

### Light microscopy analysis

Biopsy specimens were frozen at −80°C in optimum cutting temperature compound (Tissue-Tek, Torrance, CA, USA), and 10-μm cryosections were cut and affixed on glass slides. Sections from 3 different depths of the sample were used to obtain a representative sample of the entire specimen.

Tissue was stained with hematoxylin and counterstained with eosin, as previously detailed.[23] Light microscopy was used to calculate a synovitis score, which has been previously described and validated.[20,21] The synovitis score consists of 3 components—lining cell layer, synovial stroma, and inflammatory infiltrate—each graded on a scale of 0 to 3 points. Scores are added to achieve a final synovitis score. Samples were blinded and scored twice by 2 observers.

### Immunofluorescence analysis

Cryosections were cut and placed on slides, as described above. Slides were fixed with 4% paraformaldehyde in 1× phosphate-buffered saline. Blocking was performed with 1% bovine serum albumin and 1% normal goat serum. Slides were incubated with monoclonal anti-human CD31 (Thermo Fisher Scientific, Waltham, MA, USA) and anti-human CD45 antibodies (BioLegend, San Diego, CA, USA) overnight, followed by secondary conjugated antibodies of anti-mouse immunoglobulin (Ig)G1 and IgG2a, respectively (Alexa Fluor 555 and 488; Life Technologies, Carlsbad, CA, USA). Another set of slides was incubated with monoclonal anti-human CD45 and anti-human CD68 antibodies (Abcam, Cambridge, UK), followed by conjugated secondary antibodies of anti-mouse