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Blood flow in the anterior humeral circumflex artery reflects synovial inflammation of the shoulder joint in rotator cuff tears

Hiromi Asano, MD[a], Nobuo Terabayashi, MD, PhD[a,*], Kenji Kawashima, MD, PhD[b], Atsushi Goto, MD[a], Tsuneo Watanabe, PhD[c], Takuma Ishihara, MSc[d], Haruhiko Akiyama, MD, PhD[a]

[a]Department of Orthopaedic Surgery, Gifu University Graduate School of Medicine, Gifu, Japan
[b]Department of Orthopaedic Surgery, Yamauchi Hospital, Gifu, Japan
[c]Department of Medical Technology, Gifu University of Medical Science, Gifu, Japan
[d]Innovative and Clinical Research Promotion Center, Gifu University Hospital, Gifu, Japan

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Background: An association has been reported between rotator cuff tear and inflammation. We hypothesized that blood flow in the anterior humeral circumflex artery would reflect synovial inflammation in the shoulder. This study aimed to clarify the association of blood flow in the anterior humeral circumflex artery with synovial inflammation and shoulder pain in patients with rotator cuff tears.

Methods: In this prospective, cross-sectional study, tissue samples from the synovium in the rotator interval were obtained from 33 patients undergoing arthroscopic rotator cuff repair. Reverse transcription-polymerase chain reaction and real-time polymerase chain reaction were performed to determine the messenger RNA expression of inflammatory mediators, growth factors, and matrix metalloproteinases. Additional tissue samples were fixed for histologic evaluation. Before surgery, we measured the peak systolic velocity in the anterior humeral circumflex artery using pulse Doppler ultrasonography.

Results: The peak systolic velocity in the anterior humeral circumflex artery was positively correlated with the messenger RNA expression of interleukin 1β, interleukin 8, and matrix metalloproteinase 3 genes ($r = 0.49$, $P = .004$; $r = 0.55$, $P = .001$; and $r = 0.39$, $P = .026$, respectively), as well as histologic synovitis scores ($r = 0.48$, $P = .005$). Additionally, it was significantly higher in patients with resting pain than in those without resting pain ($P = .048$).

Conclusion: The peak systolic velocity in the anterior humeral circumflex artery is associated with the severity of synovial inflammation. Our results suggest that assessing the peak systolic velocity in the anterior humeral circumflex artery is useful for evaluating the severity of synovial inflammation.

Rotator cuff tears (RCTs) are common disorders that cause shoulder pain. Ultrasonography (US) and magnetic resonance imaging (MRI) have high accuracy for detecting RCTs[8]; however, the RCT size reportedly does not correlate with shoulder pain.[7] A noninvasive imaging modality for assessing the degree of inflammation has not yet been established.

Nighttime pain is a typical symptom in patients with rotator cuff pathology.[4] Although several studies have reported the factors associated with nighttime pain in shoulder disorders, including sleeping position,[14] skin temperature,[21] abnormal neovessels,[24] and melatonin,[11] its pathology remains unclear. Nighttime pain reportedly decreases after transcatheter arterial embolization of abnormal neovessels[24] and steroid injections.[9,13]

Based on these findings, we hypothesized that the inflammation associated with angiogenesis is one of the causes of nighttime pain. Power Doppler US can be used to evaluate synovial inflammation by detecting vascularity, especially in patients with rheumatoid arthritis.[27,38] However, the synovitis in RCTs is reportedly of low grade,[34] and power Doppler US was not suitable for detecting low-grade inflammation.[18,25]

Therefore, we focused on the blood flow in the anterior humeral circumflex artery (AHCA), whose branch supplies the anterior part of the lateral aspect of the capsule,[2] and the rotator interval (RI).[26] A previous study reported that the peak systolic velocity (PSV) in the AHCA

Ethical approval for this study was obtained from the Gifu University Institutional Review Board (no.: 2019-054).

This study was conducted at the Gifu University Graduate School of Medicine.

*Corresponding author: Nobuo Terabayashi, MD, PhD, Department of Orthopaedic Surgery, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu city, Gifu 501-1194, Japan.

E-mail address: nobutera@gifu-u.ac.jp (N. Terabayashi).

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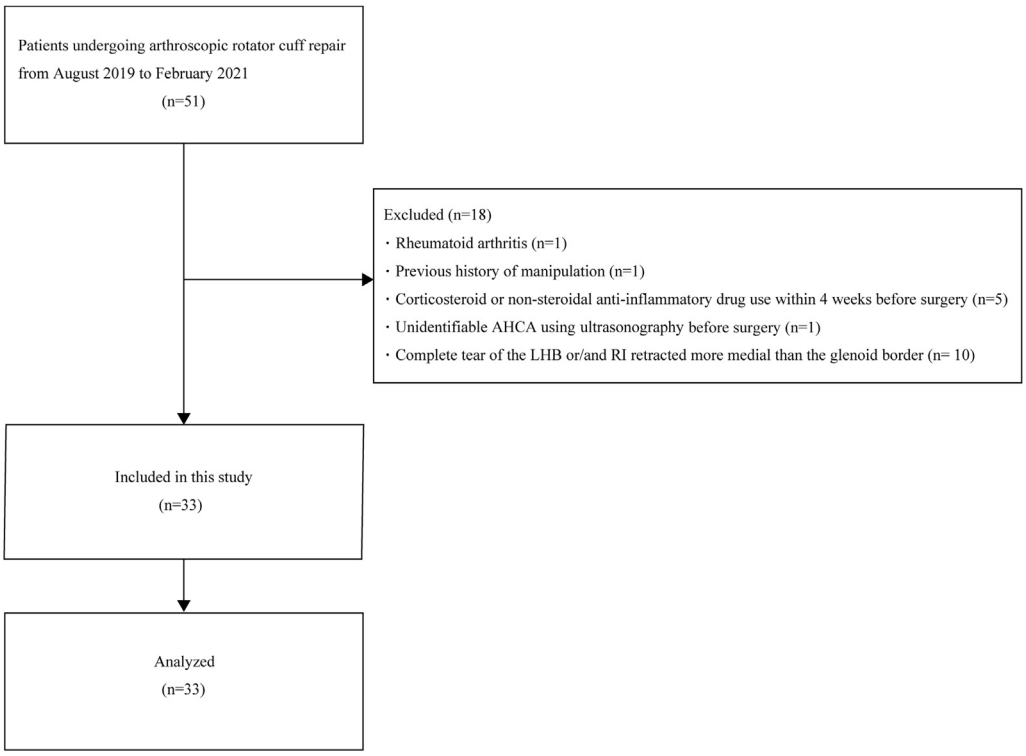

Figure 1 Flow diagram of patient selection. *AHCA*, anterior humeral circumflex artery; *LHB*, long head of biceps tendon; *RI*, rotator interval.

measured using pulse Doppler US was increased in RCTs with nighttime pain[37]; however, the molecular, biological, and histopathological evaluations were not demonstrated. Thus, the association between blood flow in the AHCA and synovial inflammation remains unclear.

This study had a 2-fold purpose: (1) to clarify the association between PSV in the AHCA and synovial inflammation and (2) to clarify the association between PSV in the AHCA and shoulder pain.

Materials and methods

Ethical approval and study design

This study was approved by the relevant institutional review board, and informed consent was obtained from all patients. This was a prospective, cross-sectional study.

Patients

This study included patients with RCTs who underwent arthroscopic rotator cuff repair between August 2019 and February 2021 (Fig. 1). Of the 51 patients who underwent arthroscopic rotator cuff repair, 18 were excluded for one of the following reasons: rheumatoid arthritis or other collagen diseases; previous surgery or manipulation of the affected shoulders; history of corticosteroid, hyaluronic acid, or nonsteroidal anti-inflammatory drug use, including oral forms or injections, within 4 weeks before operation; patients in whom the AHCA could not be confirmed using US before surgery; and complete tear of the long head of biceps tendon because the ascending branch of the AHCA runs alongside the long head of biceps tendon.[12] Furthermore, to harvest the synovial sample at a consistent point, cases in which the RI retracted more

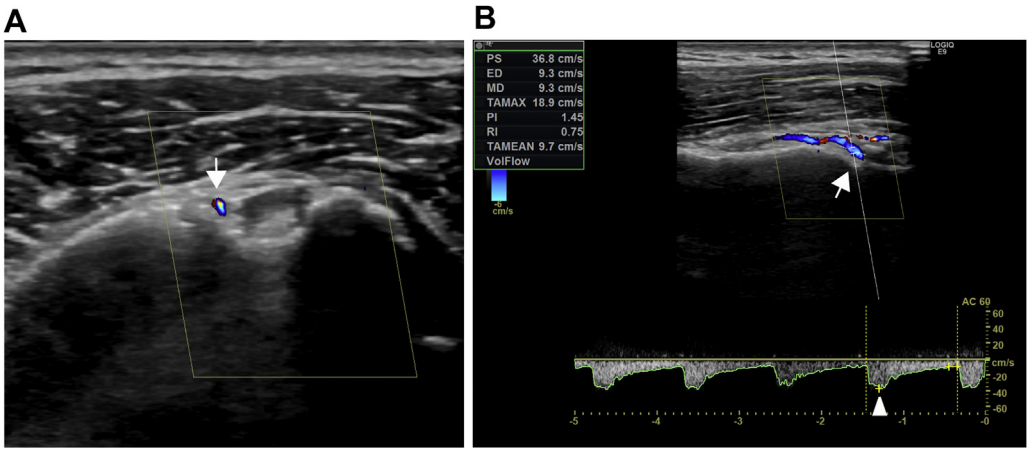

Figure 2 Method of measuring the peak systolic velocity (PSV) in the anterior humeral circumflex artery (AHCA). (**A**) Short-axis color Doppler ultrasound (US) image of the bicipital groove. The ascending branch of the AHCA (*arrow*) is confirmed. (**B**) Pulse Doppler US image measuring the PSV (*arrowhead*). The *arrow* points to the long-axis color Doppler US image of the ascending branch of the AHCA.

Table I
List of primer sequences.

Gene	Sequence (5′-3′)	Reference
VEGF	F: ATCAGTTCGAGGAAAGGGAAA R: CTGCGGATCTTGTACAAACAAA	Shindle et al, 2011[32]
NGF	F: CCCATCCCATCTTCCACAGG R: GGTGGTCTTATCCCCAACCC	Takano et al, 2017[35]
IL1B	F: GTACCTGTCCTGCGTGTTGA R: GGGAACTGGGCAGACTCAAA	Takano et al, 2017[35]
IL6	F: TGCAGAAAAAGGCAAAGAATC R: TAAAGCTGCGCAGAATGAGAT	Shindle et al, 2011[32]
IL8	F: ACTGAGAGTGATTGAGAGTGGAC R: AACCCTCTGCACCCAGTTTTC	Spandidos et al, 2010[33] PrimerBank ID 10834978a2
TNFA	F: CTTCTGCCTGCTGCACTTTG R: GTCACTCGGGGTTCGAGAAG	Takano et al, 2017[35]
MMP3	F: TGGGCCAGGGATTAATGGAG R: GGCCAATTTCATGAGCAGCA	Klatte-Schulz et al, 2018[15]
MMP13	F: CTTTTGGAATTAAGGAGCATGG R: TTGTCCAGGTTTCATCATCATC	Shindle et al, 2011[32]
GAPDH	F: GAGTCAACGGATTTGGTCGTATT R: GACAAGCTTCCCGTTCTCAGCCT	Shindle et al, 2011[32]

VEGF, vascular endothelial growth factor; *F*, forward; *R*, reverse; *NGF*, nerve growth factor; *IL*, interleukin; *TNFA*, tumor necrosis factor-α; *MMP*, matrix metalloproteinase; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

medial than the glenoid border due to the complete tear of subscapularis were also excluded. Finally, 33 patients were included.

Clinical evaluation

We defined nighttime pain as an aching pain that disturbs sleep. We questioned the patients about the presence of nighttime pain or resting pain before surgery. The RCT size was evaluated intraoperatively using the classification introduced by Cofield.[5]

Ultrasonographic examination

Ultrasonographic examinations were performed independently by 3 experienced sonographers with extensive experience in musculoskeletal US, using a LOGIQ E9 scanner (GE Healthcare, Bensalem, PA, USA) with a 6- to 15-MHz linear transducer. Blood flow in the AHCA was assessed using pulse Doppler US, as previously described.[37] First, the color Doppler mode was used to identify the AHCA (Fig. 2, *A*). Pulse Doppler mode was used to measure the PSV in the AHCA on a longitudinal scan (Fig. 2, *B*). After waveforms appeared similar to each other, PSV was measured at the tallest waveform. On the nonoperative side, the presence or absence of RCT and PSV in the AHCA was also evaluated.

In a pilot study, intrarater and inter-rater reliabilities were assessed by examining 5 healthy volunteers.

Table II
Clinical characteristics of patients.

Characteristic	n = 33
Age* (yr)	62 (58-71)
Sex (male), n (%)	22 (66.7)
Duration of symptoms, n (mo)*	6 (3-12)
Preoperative ROM (deg)*	
Active FE	100 (80-160)
Passive FE	150 (110-160)
Active ER	40 (30-60)
Passive ER	40 (40-60)
Tear size, n (%)	
Small	7 (21.2)
Medium	11 (33.3)
Large	8 (24.2)
Massive	7 (21.2)
Involved tendons, n (%)	
SSP	4 (12.1)
SSP + ISP	3 (9.1)
SSP + SSC	3 (9.1)
SSP + ISP + SSC	23 (69.7)
Traumatic onset, n (%)	27 (81.8)
Nighttime pain, n (%)	18 (54.5)
Resting pain, n (%)	11 (33.3)
PSV in the AHCA* (cm/s)	22.3 (18.6-28.3)

ROM, range of motion; *FE*, forward elevation; *ER*, external rotation; *SSP*, supraspinatus; *ISP*, infraspinatus; *SSC*, subscapularis; *PSV*, peak systolic velocity; *AHCA*, anterior humeral circumflex artery.
*Data are presented as the median (interquartile range).

Arthroscopic synovitis score

The degree of glenohumeral synovitis was graded as previously described.[6] Retrospectively, videos of the arthroscopic surgery were independently evaluated by 2 shoulder surgeons in a blinded fashion to assess interobserver reliability. Glenohumeral synovitis was graded based on the following 4 characteristics: color of capsule, villous projections, capillaries, and axillary recess. The score of each objective criterion was summed, and the total synovitis score ranged from 0 to 6.

Tissue samples

During surgery, the synovial tissue samples harvested from the RI were immediately frozen in liquid nitrogen at −80°C for polymerase chain reaction (PCR) analysis. The remaining samples were fixed with 4% paraformaldehyde for histologic analysis.

Reverse transcription PCR and real-time PCR

Total RNA was extracted using the RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). Reverse transcription PCR was performed using a high-capacity complementary DNA reverse transcription kit that included an RNase inhibitor (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR for the vascular endothelial growth factor, nerve growth factor, interleukins (*IL1B*, *IL6*, and *IL8*), tumor necrosis factor-α, and matrix metalloproteinases 3 and 13 (*MMP3* and *MMP13*) was performed using TB Green Premix Ex Taq II (Takara Bio, Shiga, Japan). The expression level of each gene was normalized to that of glyceraldehyde-3-phosphate using the comparative C_T method. Primers other than *IL8* were obtained from past reports,[15,32,35] and *IL8* primers were obtained from the PrimerBank database[33] (PrimerBank ID: 10834978a2). The PCR primer sequences are listed in Table I.

Histopathological assessment

Tissue samples were fixed with 4% paraformaldehyde for 24 hours and embedded in paraffin for histologic analysis. The samples were cut into 5-μm-thick sections. Hematoxylin and eosin staining was performed following the standard protocol. The degree of synovitis was graded using the synovitis score, as described by Krenn et al.[19,20] The synovitis score comprises 3 morphological parameters (enlargement of the synovial lining cell layer, the density of the resident cells, and inflammatory infiltrate). Three parameters were scored from 0 to 3 and summed to establish a total synovitis score ranging from 0 to 9. Each slide was independently evaluated in a blinded fashion by 2 trained and experienced orthopedic surgeons to assess interobserver reliability.

Immunohistochemistry

Immunohistochemistry was performed to determine *IL-8* and *MMP-3* expression. Sections were pretreated and subsequently

Table III
Comparison of PSV in the AHCA between the operated and nonoperated sides.

Presence of RCT on the nonoperated side	n	PSV in the AHCA on the operated side (cm/s)	PSV in the AHCA on the nonoperated side (cm/s)	*P* value
With RCT	12	20.1 (18.9-26.6)	17.7 (13.2-18.4)	.005†
Without RCT	17	22.5 (18.6-32.0)	14.5 (12.4-21.2)	.017*

PSV, peak systolic velocity; *AHCA*, anterior humeral circumflex artery; *RCT*, rotator cuff tear.
*$P < .05$.
†$P < .01$.

incubated with primary antibodies against *CXCL8/IL8* (27095-1-AP; Proteintech North America, Chicago, IL, USA; dilution 1:500) and *MMP3* (ab52918; Abcam, Cambridge, UK; dilution 1:200) at 4°C overnight. After rinsing, the sections were incubated with an antirabbit secondary antibody containing horseradish peroxidase for 30 minutes (Dako North America, Inc., Carpinteria, CA, USA). Antibody binding was visualized using a diaminobenzidine substrate (Dako North America Inc., Carpinteria, CA, USA), and counterstaining was performed with hematoxylin.

Statistical analysis

This study sought to estimate the correlation coefficient with reliable accuracy. Therefore, the sample size was designed to include 33 patients, assuming a Spearman's correlation coefficient of 0.6 and a 95% confidence interval (CI) width of <0.5.[3] Patient characteristics were summarized as the median and interquartile range for continuous variables and as numbers and percentages for categorical variables. The inter-rater reliability for measuring PSV among the 3 examiners was confirmed using the intraclass correlation coefficient (ICC) of the 2-way random-effects model. The intrarater reliability for measuring PSV was confirmed by using the ICC of the 1-way random-effects model. To evaluate the validity of the arthroscopic and histologic synovitis scores, the ICC was calculated for values measured by 2 surgeons.

The PSV in the AHCA was compared between the operated and nonoperated sides using the Wilcoxon signed-rank sum test according to the presence or absence of RCT. To confirm the association between the PSV in the AHCA and synovial inflammation, Spearman's correlation coefficient was calculated. The Mann-Whitney U test was used to compare the difference in the PSV and the histologic synovitis scores between groups with and without resting pain, with and without nighttime pain, or traumatic and atraumatic RCTs.

The receiver operating characteristic curve and the best cutoff point were calculated to estimate the diagnostic performance of the PSV in the AHCA for resting pain. The cutoff point was calculated according to the Youden index, and its sensitivity and specificity were also determined. The Kruskal-Wallis test was performed to compare the PSV in the AHCA between RCT sizes. If the Kruskal-Wallis test result was statistically significant, a Steel-Dwass test was planned for paired comparisons. The association between nighttime pain and resting pain was confirmed using Fisher's exact test. A 2-sided *P* value of <0.05 was considered statistically significant, and all statistical analyses were performed using the R software program, version 4.1.1 (www.r-project.org).

Table IV
Correlation between the PSV in the AHCA and gene expression.

Gene	Correlation coefficient	95% CI	*P* value
VEGF	−0.21	−0.51, 0.15	.252
NGF	0.03	−0.32, 0.37	.882
IL1B	0.49	0.18, 0.71	.004†
IL6	0.31	−0.03, 0.59	.077
IL8	0.55	0.26, 0.75	.001†
TNFA	−0.06	−0.4, 0.29	.73
MMP3	0.39	0.05, 0.65	.026*
MMP13	0.32	−0.03, 0.6	.072

PSV, peak systolic velocity; *AHCA*, anterior humeral circumflex artery; *CI*, confidence interval; *VEGF*, vascular endothelial growth factor; *NGF*, nerve growth factor; *IL*, interleukin; *TNFA*, tumor necrosis factor-α; *MMP*, matrix metalloproteinase.
*$P < .05$.
†$P < .01$.

Results

Clinical characteristics

This study included 33 patients comprising 22 men and 11 women, with a median age of 62 (58-71) years. The clinical characteristics of the patients are shown in Table II.

Intrarater and inter-rater reliability for measuring the PSV in the AHCA

The intrarater reliabilities for measuring the PSV in the AHCA were 0.996 (95% CI, 0.981-0.999), 0.989 (95% CI, 0.945-0.998), and 0.993 (95% CI, 0.965-0.999) for the 3 examiners. The inter-rater reliability for measuring the PSV in the AHCA was very good (0.985; 95% CI, 0.935-0.998).

Inter-rater reliability for arthroscopic and histologic synovitis score

The ICC for the arthroscopic synovitis score was 0.756 (95% CI, 0.564-0.871), whereas it was 0.590 (95% CI, 0.318-0.773) for the histologic synovitis score.

Comparison of the PSV in the AHCA between the operated and nonoperated sides

Of the 33 patients, 4 were excluded for 1 of the following reasons: previous surgery of the nonoperated shoulder and unconfirmed AHCA of the nonoperated shoulder using US before surgery. PSV was significantly higher on the surgical side than on the nonsurgical side, regardless of the presence or absence of RCT on the nonoperated side (Table III).

Association between the PSV in the AHCA and synovial inflammation

The PSV in the AHCA significantly and positively correlated with the mRNA expression levels of *IL1B*, *IL8*, and *MMP3* (*IL1B*: $r = 0.49$, $P = .004$; *IL8*: $r = 0.55$, $P = .001$; *MMP3*: $r = 0.39$, $P = .026$; Table IV). The PSV in the AHCA significantly and positively correlated with the arthroscopic synovitis score ($r = 0.40$, $P = .022$; Fig. 3, *A*). In patients with low PSV, the color of the capsule was pale, and a few villous

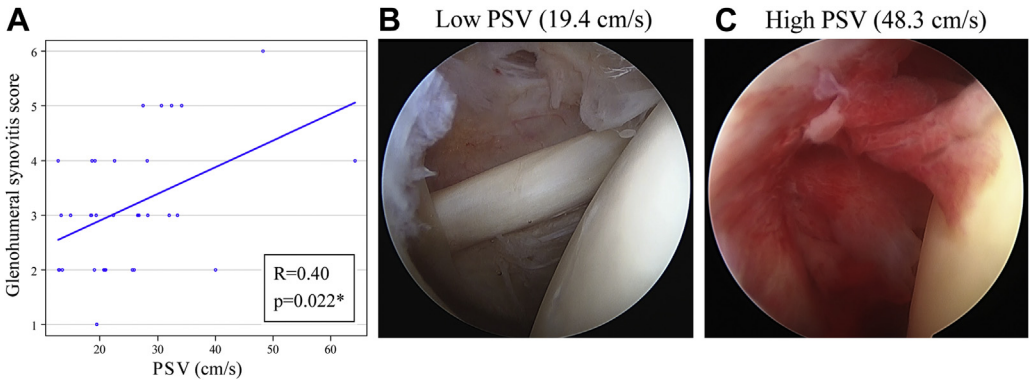

Figure 3 Arthroscopic score of glenohumeral synovitis. (**A**) Peak systolic velocity (PSV) in the anterior humeral circumflex artery (AHCA) significantly and positively correlated with the glenohumeral synovitis score. R is Spearman's rho. *$P < .05$. (**B**) Arthroscopic image of a patient with a low PSV in the AHCA. The color of the capsule is pale, and a few villous projections are observed. (**C**) Arthroscopic image of a patient with a high PSV in the AHCA. The color of the capsule is red, and extensive villous projections are observed.

projections were observed (Fig. 3, *B*). In patients with high PSV, the color of the capsule was red, and extensive villous projections were observed (Fig. 3, *C*).

The PSV in the AHCA also significantly and positively correlated with the histologic synovitis score (r = 0.48, $P = .005$) (Fig. 4, *A*). More enlargement of the synovial lining cell layer and inflammatory infiltration were observed in patients with high PSV than in those with low PSV (Fig. 4, *B*, *C*).

Immunohistochemical staining of the synovium demonstrated increased staining for *IL8* and *MMP3* in samples from patients with high PSV (Fig. 5). *IL8* was expressed around the vessels, whereas *MMP3* was expressed in the synovial lining cells.

Association of shoulder pain with the PSV in the AHCA and with synovial inflammation

No significant difference in the PSV in the AHCA was observed between patients with and without nighttime pain ($P = .708$; Table V). The PSV in the AHCA was significantly higher among patients with resting pain than that among those without resting pain ($P = .048$; Table VI).

In the receiver operating characteristic analysis, the diagnostic accuracy of the PSV in the AHCA for resting pain yielded an area under the curve of 0.715 (95% CI, 0.519-0.910), with 0.636 sensitivity and 0.773 specificity. The best cutoff value of the PSV in the

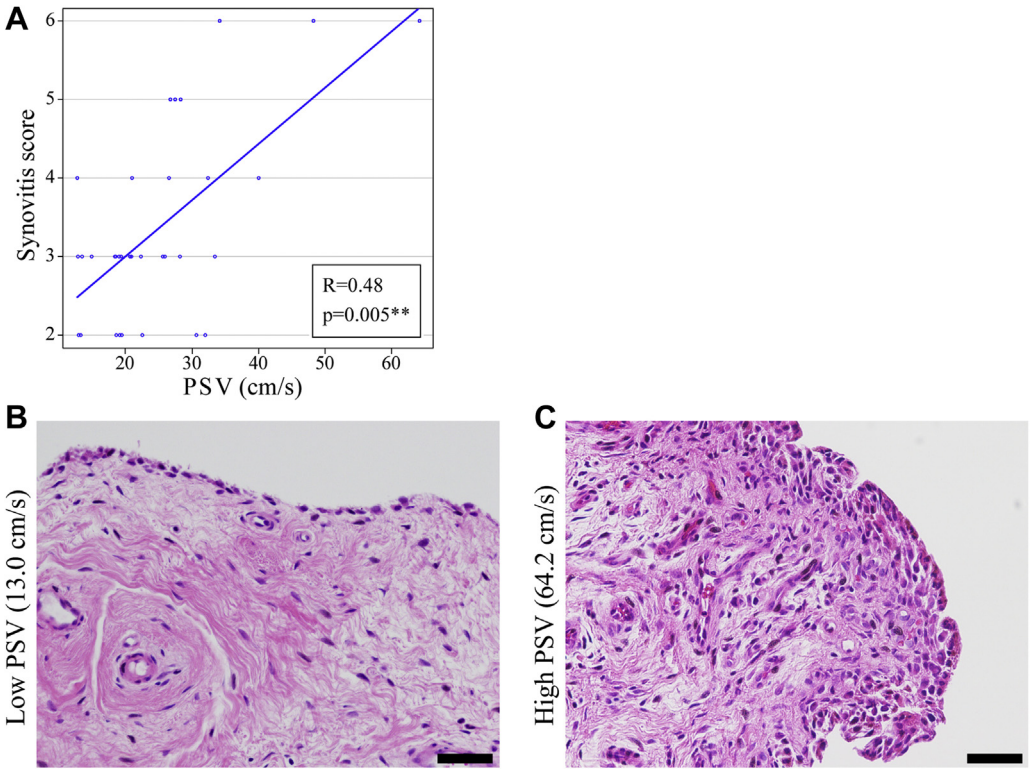

Figure 4 Histologic evaluation of synovitis. Scale bars = 50 μm; hematoxylin and eosin stain. (**A**) The peak systolic velocity (PSV) in the anterior humeral circumflex artery (AHCA) significantly and positively correlated with the histologic synovitis score. R is Spearman's rho. **$P < .01$. (**B**) Light microscopy images of the synovium in a patient with a low PSV demonstrate no enlargement of the synovial lining cell layer and only a slight inflammatory infiltration. (**C**) Light microscopy images of the synovium from a patient with a high PSV demonstrate enlargement of the synovial lining cell layer and inflammatory infiltration.

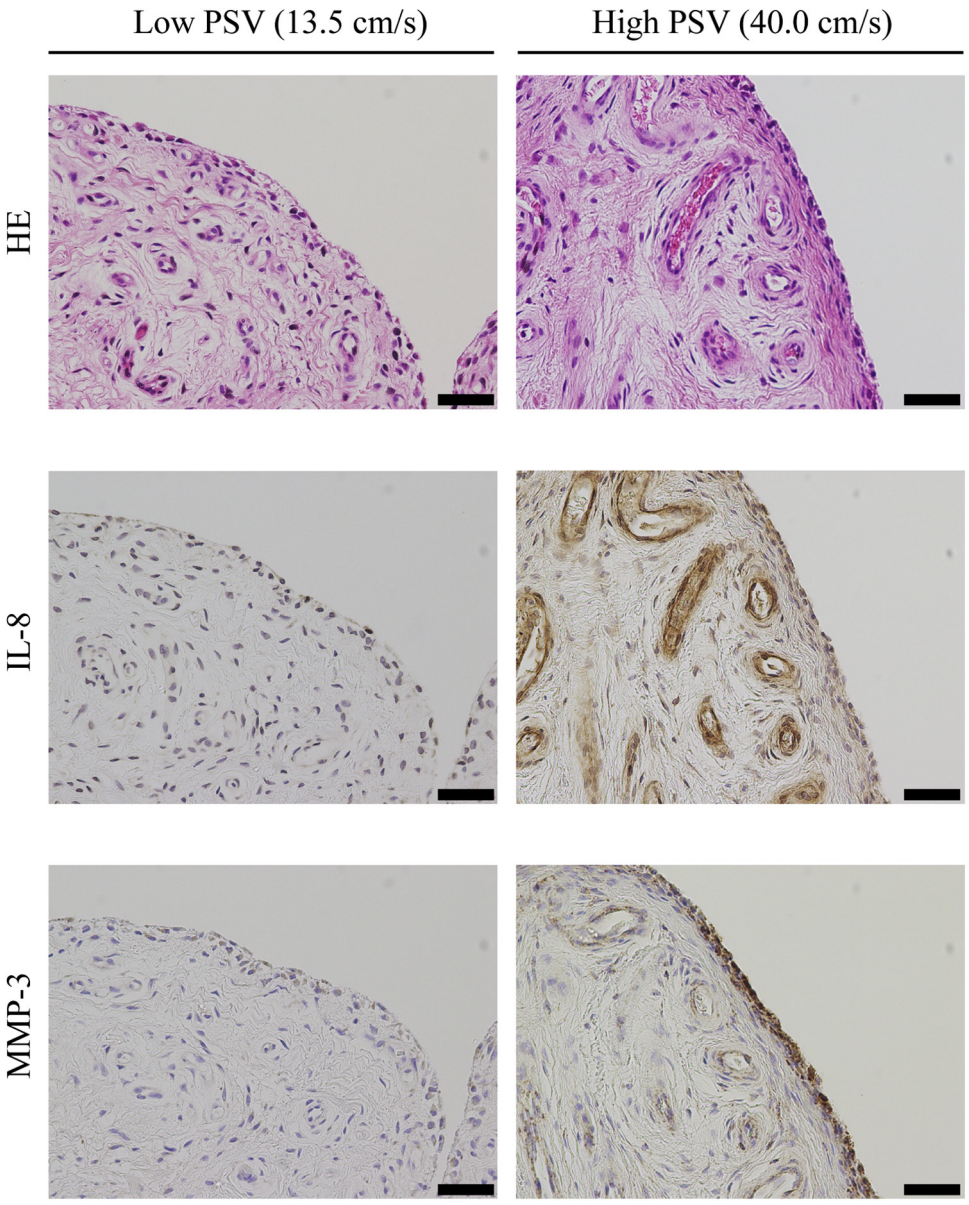

Figure 5 Histologic and immunohistochemical images of the synovium from patients with a low peak systolic velocity (PSV) and a high PSV. Immunohistochemical staining of the synovium demonstrates increased staining for interleukin 8 (*IL8*) and matrix metalloproteinase 3 (*MMP3*) in a sample obtained from patients with high PSV. *IL8* is expressed around vessels, and *MMP3* is expressed in the synovial lining cells. Scale bars = 50 μm. *HE*, hematoxylin and eosin stain.

AHCA was 26.7 cm/s. No significant association was observed between the PSV in the AHCA and RCT size ($P = .58$). Similarly, no significant difference in the PSV in the AHCA was observed between traumatic and atraumatic RCTs ($P = .48$).

The histologic synovitis score was significantly higher among patients with nighttime pain than that among those without nighttime pain ($P = .040$; Table V). The histologic synovitis score was significantly higher among patients with resting pain than that among those without resting pain ($P = .023$; Table VI). No significant difference in gene expression was observed between patients with and without nighttime pain or between those with and without resting pain.

In total, 61.1% (11/18) of patients with nighttime pain experienced resting pain, whereas no patient (0/15) experienced resting pain among those without nighttime pain. In those with nighttime pain, the PSV in the AHCA was significantly higher in patients with resting pain than that in those without resting pain ($P = .02$).

Discussion

The results of this study suggest that the PSV in the AHCA was associated with synovial inflammation in the glenohumeral joint. The PSV in the AHCA positively correlated with *IL1B*, *IL8*, and *MMP3* mRNA expression of the synovial samples as well as arthroscopic and histologic synovitis scores in RCTs. The PSV in the AHCA was higher in patients with resting pain than that in those without resting pain.

Several studies have suggested that synovial inflammation is observed in cases of RCT. A previous histologic study reported synovitis of the RI in patients with RCTs.[1,34] Gotoh et al reported that *IL1B* mRNA expression in RI samples was increased in patients with perforating RCTs.[10] Noh et al reported that the levels of *IL1B* and *IL8* were elevated in synovial fluids among patients with RCT.[22] *IL8* is a member of the CXC chemokine family that is induced under inflammatory conditions and is a potent angiogenic factor.[17,28]

Table V
Comparison of the PSV in the AHCA and histologic synovitis score between patients with and without nighttime pain.

	Patients without nighttime pain (n = 15)	Patients with nighttime pain (n = 18)	*P* value
PSV (cm/s)	20.9 (18.9-27.0)	26.2 (15.9-31.1)	.708
Histologic synovitis score	3.0 (2.0-3.0)	3.5 (3.0-4.8)	.040*

PSV, peak systolic velocity; *AHCA*, anterior humeral circumflex artery.
*$P < .05$.

Abrams et al reported that *MMP3* gene expression correlated with the degree of synovitis.[1] These previous reports indicated that synovial inflammation along with angiogenesis is associated with the pathophysiology of RCTs. In our study, the PSV in the AHCA positively correlated with *IL1B*, *IL8*, and *MMP3* mRNA expression and synovitis. Therefore, we suggest that the PSV in the AHCA is a good indicator of synovial inflammation severity.

Clinical studies have reported an association between the blood flow around the shoulder and shoulder pain using a dynamic MRI. Tamai et al assessed the coefficient of enhancement in the glenohumeral synovium using dynamic MRI, and clinical improvement was associated with a decrease in the coefficient of enhancement.[36] Sasanuma et al reported that increased abnormal blood flow (burning sign) in the RI was observed in 53% of patients with symptomatic RCTs, and burning sign was associated with shoulder pain.[29] These reports suggested that angiogenesis is involved in the pathology of shoulder disorders. Therefore, we suggest that evaluating blood flow around the shoulder is important for understanding the pathophysiological conditions of RCTs.

Pulse Doppler US has many advantages over dynamic MRI. Pulse Doppler US is noninvasive and can be evaluated in real time; therefore, it is suitable for follow-ups. Furthermore, results are expressed numerically, and the intrarater and inter-rater reliabilities were high in this study; thus, it allows objective evaluation of synovial inflammation. Accordingly, we believe that measuring blood flow in the AHCA using pulse Doppler US is useful for evaluating synovial inflammation in the shoulder. As a conservative treatment for RCTs, intra-articular injection of steroids and hyaluronic acid is used to suppress inflammation.[30] Objective methods for assessing the degree of inflammation have not yet been established; therefore, we estimated the effect of the injection by observing the changes in pain. By measuring blood flow in the AHCA, we can probably objectively evaluate whether inflammation was controlled by the injection. Therefore, further longitudinal studies are needed to investigate the association between the changes in clinical symptoms and the PSV in the AHCA.

In this study, the PSV in the AHCA and the histologic synovitis score were significantly higher in RCT patients with resting pain than those in patients without resting pain. Our results indicate that synovial inflammation is associated with resting pain; however, the expression of inflammatory cytokines was not significantly different between those with and without resting pain. Among previous studies using enzyme-linked immunosorbent assay, Shih et al reported that *IL1B* levels in the shoulder synovial fluid were significantly and positively correlated with shoulder pain,[31] and Okamura et al reported that *IL8* levels in the shoulder joint fluid were associated with resting pain.[23] Contrarily, Gotoh et al reported that the *IL1B* mRNA expression levels in the RI were inversely correlated with the degree of pain.[10] Therefore, the association between shoulder pain and inflammatory cytokines is controversial. In this study, various phases of RCTs were included, particularly shoulder stiffness. A previous study reported that *IL1B* levels in the joint fluids were significantly higher in RCTs with stiffness than those in cases without stiffness.[16] This study included various disease states of RCTs such as shoulder stiffness, arthritis, and impingement; therefore, further investigation wherein study participants are classified based on disease states is needed to clarify the association between inflammatory cytokines and shoulder pain.

Regarding nighttime pain, no significant difference in the PSV in the AHCA was observed between patients with and without nighttime pain. However, the histologic synovitis score was significantly higher in patients with nighttime pain, and more patients with nighttime pain also experienced resting pain than those without nighttime pain. Consequently, we suggest that nighttime pain is multifactorial and that synovial inflammation is one of its causes. In this study, the effect of sleeping position and turning over on pain could not be completely eliminated; thus, a more detailed analysis is needed to investigate the pathophysiological mechanisms of nighttime pain.

This study had some limitations. First, a selection bias in the study population was present as we only studied patients who underwent surgery. Most patients received conservative treatment before surgery; therefore, patients with RCTs immediately after injury were not included. Second, this was a cross-sectional study, and changes in the clinical symptoms and PSV in the AHCA were not investigated. Therefore, further longitudinal studies are needed to investigate the changes in clinical symptoms and the PSV in the AHCA after conservative treatment.

Conclusion

This study indicates that the PSV in the AHCA is associated with the severity of synovial inflammation in RCTs. The PSV in the AHCA was significantly higher in patients with resting pain than that in those without resting pain. Our results suggest that the PSV in the AHCA is useful for evaluating synovial inflammation in patients with RCTs.

Table VI
Comparison of the PSV in the AHCA and histologic synovitis score between patients with and without resting pain.

	Patients without resting pain (n = 22)	Patients with resting pain (n = 11)	*P* value
PSV (cm/s)	20.8 (18.4-26.4)	27.5 (20.2-33.3)	.048*
Histologic synovitis score	3.0 (2.3-3.0)	4.0 (3.0-5.5)	.023*

PSV, peak systolic velocity; *AHCA*, anterior humeral circumflex artery.
*$P < .05$.

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