The Supraspinatus Tendon
Clinical and histopathological aspects

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Linköping 2001
To my parents Jan and Ulla

To my wife Gudrun and our children Henrike, Matthias, Kristina and Erik
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The supraspinatus tendon is an important structure of the rotator cuff. Subacromial impingement is a common reason for shoulder pain. Despite extensive scientific work in this field, the cause of impingement syndrome is still not fully understood. The general aim of the present thesis was to generate new knowledge with respect to pathogenesis and treatment of impingement syndrome. A combination of animal and clinical studies were performed. Different methods were used such as histology, immunohistochemistry, development and assessment of a novel measuring device and clinical and radiological assessment.

Thirty rats were injected with triamcinolone or saline into the subacromial bursa. After five corticosteroid injections, we found focal inflammation, degradation and fragmentation of collagen bundles in the supraspinatus tendon, whereas the control specimens were normal (p=0.035).

Subacromial bursitis was induced by injections of carrageenan into the subacromial space (n=28). Fibrocartilaginous metaplasia and bony metaplasia were found in the supraspinatus tendon. Even in specimens with no histologic changes of the collagen bundles the staining for fibronectin was significantly increased.

The distance between the anterolateral acromion and the supraspinatus tendon was measured in patients with impingement syndrome intraoperatively (n=30) and in controls (instability, n=15). The mean value of the subacromial distance in controls was 16 mm, the 95% mean confidence limits between 14 and 18 mm. The mean value in the group of patients with impingement syndrome was 8 mm before and 16 mm after the decompression.

Fifty patients were reviewed after arthroscopic subacromial decompression. Twenty-five showed calcific deposits in the rotator cuff on radiographs preoperatively. In 13 patients the calcific deposits totally disappeared postoperatively. In another six patients the calcifications had decreased in size. Four patients still showed calcifications, which were 5 mm or greater in size. The postoperative results measured by the Constant score were almost identical in the calcific and the non-calcific groups.
Human surgical supraspinatus tendon specimens were studied from patients with impingement (n=16), ruptured supraspinatus tendons (n=7) and controls (n=10). Degradation of tendinous tissue and fibrin were found only in some specimens from ruptures. The difference in fibronectin staining was significant between controls and patients with a rupture (p=0.002). Fibrosis and thinning of fascicles seemed to be a more non-specific finding, appearing in control, impingement and rupture specimens.

In conclusion, subacromial corticosteroid injections may cause rupture of the supraspinatus tendon. Metaplasia of the supraspinatus tendon may play a role in the pathogenesis of impingement and rupture of the supraspinatus tendon. The subacromial distance can be measured intraoperatively and was shown to be lower in patients with impingement than in patients with instability. Calcifications disappear or decrease in size after arthroscopic subacromial decompression and do not seem to influence the postoperative outcome in patients with impingement. Degradation of tendon tissue, fibrin and fibronectin appear to be signs of tendon degeneration, whereas fibrosis and thinning of fascicles were found also in controls.
LIST OF ARTICLES

Study I. Tillander B, Franzén L, Karlsson M, Norlin R.
Effect of steroid injections on the rotator cuff: an experimental study in rats.

Study II. Tillander B, Franzén L, Nilsson E and Norlin R.
Carrageenan-induced subacromial bursitis caused changes in the rat’s rotator cuff.

Study III. Tillander B and Norlin R.
Intraoperative measurements of the subacromial distance.

Study IV. Tillander B and Norlin R.
Change of calcifications after arthroscopic subacromial decompression.

Study V. Tillander B, Franzén L, Nilsson E and Norlin R.
Human biopsies in the rotator cuff disease.
STATEMENT

This work was carried out at the Division of Orthopaedics, Department of Neuroscience and Locomotion, Faculty of Health Sciences, Linköping University, Sweden.

Contributions were carried out at the Department of Pathology, University Hospital of Örebro, and at the Department of Pathology, University Hospital of Malmö, Sweden.

Description of contribution

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Table I. Description and contribution. BT=Bo Tillander, RN=Rolf Norlin, LF=Lennart Franzén, EN=Elise Nilsson, MK=Maria Karlsson. ( )=partial contribution.
**Ethical considerations**

Permission from the local ethical committee for animal research was obtained for paper I and II. Animal studies were performed since human subjects could not be used for the issues studied.

Permission from the local ethical committee for research on human subjects was obtained for paper V. No complications have been registered after similar biopsies in human subjects previously in other studies over the past 10 years.

In paper IV the patients were re-examined by one examiner. Surgery had been performed by other experienced shoulder surgeons as a routine operation for impingement syndrome prior to the study. The patients received written information and it was emphasized that their participation was voluntary. A regularly clinical examination without discomfort to the patient was performed. New radiographs of the shoulder were taken with negligible emission of X-rays. No invasive examinations were performed.

The measurements in paper III were performed during surgery. The standard one-centimeter skin incision for the motorized instruments was used. Hence, no further incision was necessary. The measurement itself caused no discomfort to the patient. The time of surgery was minimally prolonged.
### ABBREVIATIONS

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<td>AC-joint</td>
<td>Acromioclavicular joint</td>
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<td>ASD</td>
<td>Arthroscopic subacromial decompression</td>
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<td>DASH</td>
<td>Disabilities of the arm, shoulder and hand</td>
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<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
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<tr>
<td>MMP-1</td>
<td>Matrix metalloprotease-1</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>NSAID</td>
<td>Non-steroidal anti-inflammatory drugs</td>
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<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>PTAH</td>
<td>Phospho Tungstic Acid Hematoxyline</td>
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INTRODUCTION

Human shoulder anatomy
The rotator cuff consists of tendons from four separate muscles; supscapularis, supraspinatus, infraspinatus and teres minor. These muscles emerge from the scapula and blend in with the subjacent joint capsule as they attach to the lesser and greater tuberosity.

The supraspinatus tendon takes a fleshy origin from the supraspinatus fossa and inserts into the greater tuberosity. Its tendinous insertion fuses with the infraspinatus posteriorly and the coracohumeral ligament anteriorly. Because of its anatomical position, confined above by the acromion and the coracoacromial ligament, which connects two parts of the scapula; the coracoid process and the undersurface of the anterior acromion, and below by the humeral head, the tendon is at risk for compression and attrition.

The arterial supply originates from the sub- and suprascapular arteries as well as from the anterior humeral circumflex artery. The vascular bed of the supraspinatus tendon shows a zone of relative vascularity, as opposed to the subscapularis, the teres minor and the infraspinatus tendons, when the arm is adducted (Rathbun and Macnab 1970). The concentration of arterioles has been found to be lower in the articular portion of the supraspinatus tendon than in the bursal portion (Lohr and Uhthoff 1990, Yamanaka and Fukuda 1991). Each tendon fascicle is composed of tendon fibers and the tendon fascicles are surrounded by endotenons. Arterioles and venules are mainly found in the endotenon and elastic fibers around the arterioles and in the tendon fascicles. The tenocytes are located between the tendon fibers. In the area near the insertion chondrocyte-like cells are found (Yamanaka and Fukuda 1991).

The subacromial bursa lubricates motion between the rotator cuff and the acromion and AC-joint. The floor of the bursa is tightly adherent to the rotator cuff (Yamanaka and Fukuda 1991). It does not communicate with the glenohumeral joint as long as the rotator cuff is intact.
Impingement – clinical background

Pain disorders of the shoulder have been grouped under the diagnosis impingement syndrome. The patient suffers either acute or chronic pain in the shoulder region and upper arm and sometimes the pain radiates to the forearm. The range of motion is limited due to pain and/or subacromial pathology. The patient often suffers from pain at rest and at night. The symptoms may either resolve after some time or persist and become chronic.

The diagnosis is based on the history of the patient and clinical examination. The pain may have an acute onset, or gradually increase without any obvious cause, or begin subsequent to a trauma. Atrophy of the supra- and infraspinatus muscles may be present due to inactivity or tendon rupture. The active range of motion is in most cases reduced. Codman (1934) was the first to describe a relation between reduced ability to abduct the arm and rupture of the supraspinatus tendon. Especially abduction between 70° and 120°, often described as “painful arc”, is limited. Inward rotation may be limited due to posterior capsular tightness. The impingement sign is frequently positive. The Neer impingement sign induces pain when the arm is elevated by the examiner and supposedly the supraspinatus tendon is impinged against the anteroinferior acromion (Neer 1983). As an alternative method, the Hawkins impingement sign, the arm is rotated internally and adducted with the arm in 90° flexion (Hawkins and Abrams 1987). In order to establish the diagnosis of impingement syndrome, a local anaesthetic should be injected into the subacromial bursa. This is the so-called impingement test (Neer 1983). The impingement test is positive when the patient experiences pain relief following the injection. It indicates that the pain originates from the subacromial bursa.

Shoulder pain may be due to several other diagnoses, such as AC-joint arthrosis, arthrosis of the glenohumeral joint, cervical rhizopathy, myalgia, tumor or glenohumeral instability. In young athletes, who perform overhand activities in throwing, racquet and swimming sports functional impingement may arise; due to anterior instability the undersurface of the supra- and infraspinatus tendons may impinge along the posteroinferior border of the glenoid rim.

Radiography is helpful to rule out malignancy and arthrosis of the glenohumeral joint. It may also show signs of subacromial pathology, such as narrowing of the subacromial space, sclerosis and cystic changes of the tuberosities and osteophytes of the anterior acromion and AC-joint. MRI and ultrasound may also be helpful in diagnosing impingement, but their value in this context is still under debate. In the future open MR imaging systems combined with
other techniques may contribute to the understanding of muscle imbalance as an important cause of impingement in some patients (Brossman et al 1996).

**Pathogenesis of the impingement syndrome**

The pathogenesis of impingement syndrome is not fully understood. Multiple etiologic factors play a role such as age, trauma, overload, hypovascularity, glenohumeral instability, muscle imbalance, scapulothoracic dysfunction and congenital abnormalities. The most obvious pathological changes have been found in the subacromial space. Within this space extrinsic and intrinsic causes are involved in the pathogenesis of impingement and rotator cuff rupture (Neviaser and Neviaser 1990, Hyvönen et al 1998, Sano et al 1999). The term extrinsic refers to the tissues outside the supraspinatus tendon and the term intrinsic indicates primary changes within the tendon itself.

**Extrinsic**

The term impingement originates from a report by Neer (1972), in which he described mechanical wear on the supraspinatus tendon below the anteroinferior third of the acromion and the coracoacromial ligament. This mechanical insult eventually leads to proliferative spurs extending from the anterior undersurface of the acromion and rupture of the rotator cuff. According to Neer (1983 and 1990) the subacromial changes develop in continuum from acute bursitis to partial and complete tears of the supraspinatus tendon:

- **Stage I**: reversible inflammation and edema of the rotator cuff.
- **Stage II**: fibrosis and thickening of the subacromial bursa and rotator cuff.
- **Stage III**: bony spurs and partial or full thickness tear of the rotator cuff.

Furthermore, acromion shape has been associated with rotator cuff rupture. In a cadaveric study the acromion undersurface was categorized as flat, curved and hooked (type I, II and III). Hooked acromions were found in 70% of observed ruptures (Bigliani et al 1986 and 1991, Rockwood and Lyons 1993). However, both the significance of the hooked acromion and the reliability of radiographic acromial morphology are a matter of dispute (Jacobson et al 1995). The distance between the undersurface of the anteroinferior third of the acromion and
the upper border of the humerus has been measured by several authors. This distance is about 10 mm on radiographs in asymptomatic shoulders and reduced in patients with a rupture of the rotator cuff (Golding 1962, Cotton and Rideout 1964, Petersson and Redlund-Johnell 1984, Flatow et al 1994).

**Intrinsic**

Pathological changes affect the supraspinatus tendon first of all rotator cuff tendons. The acromial spurs may represent secondary changes as a result of, intrinsic, primary pathology of the supraspinatus tendon (Budoff et al 1998, Sano et al 1999). Age is regarded as the most important contributing factor (Milgrom et al 1995, Uhthoff and Sano 1997, Matsen III et al 1998). Referring to the supraspinatus tendon, Codman (1934) pointed out that "degeneration in the collagen of the tendinous fibers is so common that it is difficult to find in elderly subjects an example of normal tendinous substance". This "critical portion" is located 0.5 inch (1.3 cm) proximal to the insertion of the supraspinatus tendon as described by Codman. In older patients an age-dependent increase of disorganization and fragmentation of the human supraspinatus tendon was found (Brewer 1979). One cause of this degenerative process in the critical portion of the supraspinatus tendon may be hypovascularity, which primarily affects the articular side of the supraspinatus tendon (Rothman and Parke 1965, Rathbun and Macnab 1970, Ozaki et al 1988, Lohr and Uhthoff 1990). Rathbun and Macnab concluded from the results of their investigations that the avascular zone precedes the degenerative changes. Brooks et al (1992) has questioned these findings. They found hypovascularity in the distal 15 mm in both the supra- and infraspinatus tendons and concluded that factors other than vascularity are important in the pathogenesis of supraspinatus rupture. Most partial-thickness cuff tears are located on the articular side of the supraspinatus tendon according to Gartsman (1995) and the presence of articular side tears may speak in favour for the intrinsic theory (Sano et al 1999). However, Yamanaka and Fukuda (1991) found a majority of the partial tears in the intratendinous portion of the supraspinatus tendon of human cadavers. Intratendinous tears are defined as tears within the tendon and they reach neither the bursal nor the articular side.

Fibrocartilaginous metaplasia is a physiological response within the tendon at the site of compression and a normal feature at the insertion of tendon into bone (Giori et al 1993). Its role in the pathogenesis of impingement is, however, not fully understood. Chard et al (1994) found fibrocartilaginous metaplasia within the rotator cuff tendons in cadavers with no known
history of shoulder pain. The metaplasia seemed associated with increasing age. Fukuda et al (1990) studied bursal-side rotator cuff tears in patients with impingement syndrome and found abundant chondrocytes in the proximal stumps in all cases. They interpreted these findings as metaplasia from tenocytes to chondrocytes due to low oxygen environment. Metaplasia may take place within the tendon due to impingement of the supraspinatus tendon between the acromion and the humeral head and/or angular force where the supraspinatus tendon wraps around the convexity of the humeral head. The tendon may then, potentially, be less resistant to tensile stress, which may lead to rupture.

Codman stated as early as 1934 that the articular side tears account for most ”sore shoulders” and that they never heal. Yamanaka and Fukuda (1991) drew the conclusion, based on their own findings that spontaneous healing is very rare, and therefore, incomplete tears gradually progress into full thickness tears. On the other hand, increased staining of collagen III in specimens of torn rotator cuff tendons has been found, which may indicate that healing in the margins of a tear persists over time (Amiel et al 1987, Kumagai et al 1992).

**Calcifications**

Calcifications in the rotator cuff are most common in females between 40 and 50 years of age and the most frequent site of calcifications is the supraspinatus tendon. The prevalence of calcifications on radiographs of the shoulder has been reported to be about 7% up to 17% in symptomatic shoulders (Welfing et al 1965, Hedtmann and Fett 1989). The prevalence of calcifications in asymptomatic patients varies in the literature between 3% and 20% (Bosworth 1941, Ruttimann 1959, Welfing et al 1965).

De Palma and Kruper (1961) categorized the calcifications into two groups. Type I calcifications are fluffy in their appearance with poorly defined peripheries. Type II calcifications are homogeneous with well-defined peripheries.

The calcifications in the rotator cuff (Fig. 1) have been identified as calcium hydroxyapatite crystals. These are to be differentiated from crystals, which occur intraarticularly, characteristically in cartilage, namely monosodium urate and calcium pyrophosphate dihydrate (Resnick 1988). However, unlike gout and pseudogout there is no acute inflammatory component in the vicinity of calcifications of the rotator cuff (O’Connell 2000).
The cause of calcifications in the rotator cuff is, however, still under debate. In the past, it was stated that calcium was deposited in necrotic tissue (Codman 1934). The theory described by Uthhoff and Sarkar (1981) describes fibrocartilaginous transformation within the tendon i.e. metaplasia of tenocytes to chondrocytes, perhaps due to hypoxia. In the vicinity of the chondrocytes calcifications appear. During this formative phase the surrounding tissue is avascular and marked by the absence of vessels as opposed by the findings in endochondral bone formation, which is associated with the presence of blood vessels at the time of calcification (Uthhoff and Sarkar 1981, Hennigan and Romeo 1999). The crystals in calcifications of the rotator cuff are much larger than apatite crystals observed in bone (Faure and Daculsi 1983).

**Treatment**

**Non-operative treatment**

The initial treatment of patients with impingement is rest and avoidance of painful arm motions. Physical therapy is important to relieve stress on the rotator cuff and to correct imbalance of the shoulder girdle muscles. NSAIDs and subacromial corticosteroid injections may reduce pain and improve range of motion. This strategy of non-operative treatment is recommended for at least 6-12 months (Bokor et al 1993, Morrison et al 1997).
**Corticosteroids**

In the short-term, corticosteroid injections may be effective (Benjamin et al 1996). However, the value of subacromial corticosteroid injections for recovery is controversial (Withrington et al 1985, Vecchio et al 1993, Dalton 1994). In other regions of the human body the use of local treatment by corticosteroid injections have been related to adverse effects i.e. tendon rupture (Bedi and Ellis 1970, Gottlieb and Riskin 1980, Holmes and Mann 1992, Stannard and Bucknell 1993). Hence, physicians hesitate to inject corticosteroids in the vicinity of the Achilles and patellar tendons.

Earlier animal studies on the effects of corticosteroids have focused on the Achilles tendon, the patellar tendon and the medial collateral ligament of the knee (Balasubramaniam and Prathap 1972, Unverferth and Olix 1973, Matthews et al 1975, Kapetanos 1982, Wiggins et al 1994, Walsh et al 1995). However, no studies have been performed on the rotator cuff. Pettersson (1942) pointed out that the subacromial bursa and the tendons of the rotator cuff “are intimately connected with each other”. Thus, corticosteroids in the bursa could possibly have adverse affects on the supraspinatus tendon.

**Operative treatment**

Surgical treatment of impingement syndrome has changed considerably over the years. Some decades ago both complete acromionectomy (Armstrong 1949, Watson-Jones 1960, Hammond 1962) and lateral acromionectomy (Smith-Peterson et al 1943, McLaughlin 1944) were performed, both with disappointing results. Neer (1972) described open anterior acromioplasty in his classic report. He described resection of a wedge-shaped piece of bone from the anterior undersurface of the acromion along with the attachment of the coracoacromial ligament. Based on the same principles arthroscopic subacromial decompression (ASD) was introduced in the mid 1980s by Ellman.

The ASD is performed with the arthroscope in the posterior portal 1 cm distal to the posterior lateral acromion. The shaver is introduced through the anterolateral portal 3 cm distal to the anterolateral edge of the acromion. The inflamed bursa is resected with the shaver. Anterior acromioplasty is then performed with a burr, which replaces the shaver in the anterolateral portal (Fig. 2). As the acromioplasty progresses the coracoacromial ligament is detached from the acromion. To achieve an adequate bone resection the amount of bone to be resected can be estimated comparing the depth of the groove with the diameter of the burr, which is about 5
mm. It is also possible to switch the portals between the arthroscope and the burr; the burr should after bone resection lie flush with the undersurface of the anterior acromion (Ellman 1987, Hawkins et al 1988, Altchek et al 1990). However, no exact measurement is possible before and after bone resection.

In all patients anterior, posterior and inferior translation is assessed and a diagnostic arthroscopy of the glenohumeral joint is performed (Altchek et al 1990, Harryman II et al 1992, Hawkins and Bokor 1998, Tillander and Norlin 2001).

The long-term results after ASD have been reported to be as good as after open acromioplasty i.e. about 70-90% satisfactory outcome (Ellman 1987, Hawkins et al 1988, Altchek et al 1990, Ellman and Kay 1991, Levy et al 1991, Speer et al 1991, Lindh and Norlin 1993). The failure rate (10-30%) may be explained by several different factors. Rotator cuff rupture has been reported as a negative prognostic factor (Esch et al 1988, Altchek et al 1990, Levy et al 1991, Ellman et al 1993, Ogilvie-Harris and Demazière 1993). Central neural plasticity may result in pathological pain, which remains after ASD (Coderre et al 1993, Hoe Hansen et al 2001). Adolfsson (1992) found a tendency of inferior results in females compared to males. Some cases of failures are explained by inadequate bone resection since the subacromial distance is left too low. On the other hand, too much bone may be resected (Gartsman 1990, Ogilvie-
Harris et al 1990, Warner et al 1994). Most authors suggest, on empirical basis, a bone resection of about 6-15 mm (Altchek et al 1990, Paulos and Franklin 1990, Speer et al 1991, Ellman and Gartsman 1993, Olsewski and Depew 1994). However, the subacromial distance has previously never been measured intraoperatively. Hence, there are no control values and no exact measurements of the subacromial distance in the literature.

Several authors have reported good results after surgical excision of calcifications. However, in most of these reports, removal of calcifications was combined with other surgical procedures, such as bursectomy (McLaughlin 1963, Reichelt 1981, Ark et al 1992), bursectomy and division of the coracoacromial ligament (DePalma and Kruper 1961, Gschwend et al 1981), partial resection of the coracoacromial ligament (DePalma 1983, Uhthoff and Sarkar 1990) and/or acromioplasty (Ellman and Kay 1991, Ellman et al 1992, Huber 1992, Molè 1992, Esch 1993, Johannsen et al 1996). Removal of calcific deposits without any of the above mentioned measures was performed by Harmon (1958) and Litchman et al (1968). They reported good results, but had a great number of patients with acute symptoms. Acute symptoms, however, normally disappear within a few weeks without any treatment and the calcific deposit resolves.

According to McLaughlin (1946 and 1963) and Uhthoff and Sarkar (1981 and 1990) calcific deposits disappear spontaneously. This observation supports non-surgical treatment, and may even indicate that the calcification is an insignificant finding.

**Experimental background**

**Animal model for impingement**

The pathogenesis of impingement and rupture of the supraspinatus tendon has been investigated for many years but is still not fully understood. Until the mid 1990s there was no accepted animal model for studies of subacromial pathology, which partly explains the lack of knowledge in this field. The most appropriate animal model for study of the human subacromial space is the rat’s shoulder (Norlin et al 1994, Soslowsky et al 1996). It is easy to identify the anatomic landmarks such as the scapula with its distinct spine and well developed acromion, the coracoacromial ligament, the subacromial bursa, the AC-joint, the clavicle, the humerus, the supraspinatus and infraspinatus tendons, the biceps tendon and the other parts of the rotator cuff. The deltoid muscle covers the shoulder. Its insertion on the humerus is more
prominent than in humans. The rat uses the forepaws for ambulation, which is a disadvantage with this animal model. Still, the rat also uses the forepaws for object manipulation.

**Carrageenan-induced bursitis**

Carrageenan is a polysaccharide, which is derived from the seaweed Chondrus crispus by hot water extraction. Carrageenan has been used for many years in order to study the effects of inflammation on joints and tendons (Gardner 1960, Di Rosa et al 1971, Di Rosa 1972, Lowther and Gillard 1976). However, it has never been used in studies on subacromial pathology. The inflammation induced by carrageenan may be acute (Di Rosa 1972) or chronic (Benitz and Hall 1959, DiRosa 1972, Lowther and Gillard 1976, Santer et al 1983). Several authors have found that carrageenan induces a granulomatous inflammation (Benitz and Hall 1959, DiRosa 1972, Santer et al 1983) and that carrageenan is taken up within macrophages (Di Rosa 1972).

**Markers for tendon pathology**

Fibronectin, a high-molecular-weight glycoprotein, may be regarded as a link between different inflammatory cells, such as macrophages, and collagen through its ability to bind on cells and macromolecules (Stecher et al 1986, Mitchell and Cotran 1999). Furthermore, fibronectin is important in the tissue healing process, by promoting fibroblast migration and adhesion of fibroblasts to fibrin (Grinnell et al 1981, Grinnell 1984, Gelberman et al 1991). This matrix promotes collagen organization (Grinnell et al 1981). Fibrin has a stimulatory effect on forming granulation tissue and induces increased collagen formation (Hedelin et al 1983). In uninjured tendinous tissue, fibronectin is found at the outer synovial layer and to a lower degree inside the tendon (Banes et al 1988, Amiel et al 1989, Brigman et al 1994, Kannus et al 1998).

The fibronectin content has been found to be increased in chronic tendinitis and in freshly ruptured tendons (Williams et al 1984, Kvist et al 1988, Lehto et al 1990, Gelberman et al 1991, Jozsa et al 1989). In a normal healing process fibronectin eventually disappears (Kurkinen et al 1980, Hölund et al 1982, Williams et al 1984). However, when the granulation tissue reaction persists, the high levels of fibronectin persist (Grinnell 1984). Increased occurrence of both fibronectin and fibrinogen was found in the paratenon in patients with chronic Achilles tenonitis (Kvist et al 1988). Thus, an increased presence of fibronectin may indicate an immature nature of scar tissue (Kvist et al 1988).
Matrix metalloprotease-1 (MMP-1) belongs to a family of neutral metalloproteinases. MMP-1 breaks down collagen type I, II and III (Gotoh et al 1997). These authors investigated human supraspinatus insertions with immunohistochemistry and found MMP-1 “throughout the tendon”, mainly “in the granulation tissue of the torn supraspinatus tendon”. MMP-1 was also stained slightly in control specimens. They concluded that MMP-1 could play a role in the pathogenesis of rotator cuff rupture, but they investigated neither the presence of MMP-1 among patients with impingement, nor the medial part of the tendon in patients with ruptures.
PURPOSE

The general purpose of this work was to contribute to the understanding of supraspinatus tendon pathology from a clinical and histopathological point of view.

The following specific questions were addressed:

1. Do corticosteroid injections harm the tendon?

2. Does the subacromial bursitis cause tendon changes?

3. Can the subacromial distance be measured intraoperatively in a simple way and is the distance related to pathological conditions?

4. Do the calcifications in the rotator cuff disappear after arthroscopic subacromial decompression and do they influence the clinical result?

5. Are there any reliable histological markers for tendon pathology in patients with impingement and rupture of the supraspinatus tendon?
ANIMALS AND METHODS

Animals (I, II)
The subacromial space of the rat is a well-defined anatomic site. It is easy to identify both by palpation and through dissection. The animals are easy to handle. A total of 68 female Sprague-Dawley rats were used (Table II). The animals were kept in cages with free access to food and water. All injections were performed under a short anaesthesia induced by carbon dioxide.

<table>
<thead>
<tr>
<th></th>
<th>I</th>
<th>II</th>
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<tr>
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<td>Saline</td>
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Table II. Animals used in paper I and II.

Methods (I, II)

Dissection (I, II)
The animals were killed using carbon dioxide and exsanguinated prior to dissection. The AC-joint was identified and divided. The deltoid was then detached from the acromion, which was fractured manually. The supra- and infraspinatus tendons were separately removed from their bony insertions on the greater tuberosity and divided through its muscle belly.

Triamcinolone (I)
Five µl (0.3 mg/kg body weight) triamcinolone (Lederspan®, Wyeth Lederle, St Davis, Philadelphia, USA) (n=16) or 5µl physiological saline (n=14) was injected in the left shoulder of the rat. All injections were performed under sterile conditions. To ensure that the injections were placed correctly, subacromial injections with diluted methylene blue were performed in five rats. After dissection of these shoulders, the dye was found in the subacromial bursa each time. No intratendinous deposition of dye could be found.
**Carrageenan (II)**

The animals randomized to carrageenan injections (n=28) were given one subacromial injection with 5-10 µl 3% carrageenan (Type II Sigma, USA) in saline in order to induce subacromial bursitis of varying degree. Control animals were injected subacromially with 5 µl physiological saline (n=4).

**Histology (I, II)**

The tendon specimens were harvested and immediately fixed in 4% paraformaldehyde for two days, dehydrated in ethanol, embedded in paraffin and sectioned parallel to the collagen bundles in 3 µm sections. Staining with hematoxyline eosin and Van Giesons hematoxyline was done for histological assessment. All sections were analyzed blindly by the senior pathologist together with the first author. The sections were analyzed using a standard light microscope.

**Immunohistochemistry (II)**

Immunofluorescent staining of fibronectin and fibrinogen was performed using rabbit polyclonal fibronectin (A0245) and fibrinogen (A0080) antibodies together with a swine anti-rabbit, FITC-labelled antibody (F0205). Antibodies were purchased from DAKOPATTS AB (Stockholm, Sweden). Sections of skin wounds were used as positive controls for fibronectin and fibrinogen. For each section studied a negative control was stained according to the protocol but with the primary antibody omitted. Before application of antibodies, the specimens were stained with 0.05% pontamine sky blue 5BX (BDH Chemicals Ltd, Poole, United Kingdom) in PBS with 1% dimethylsulfoxide to change the autofluorescence of collagen to wavelengths above 590 nm (Cowen et al 1985). All sections were analyzed blindly by the senior pathologist together with the first author. The intensity and extent of the immunofluorescent staining of fibronectin and fibrinogen was assessed semiquantitatively in a fluorescence microscope. The following scores were used: 0 = no detectable staining, 1= slight, 2= moderate and 3= pronounced and widespread increase in intensity and extent of the immunofluorescent staining.

**Statistics (I,II)**

Statistical evaluation was performed according to Table IV (see page 38).
PATIENTS AND METHODS

Detailed description of the material and methods is given in the individual papers. A brief summary is presented below.

<table>
<thead>
<tr>
<th>III</th>
<th>IV</th>
<th>V</th>
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| Controls | 10/5 | 28 | 6/4 | 28 |
| Impingement | 20/10 | 53 | 9/16 | 49 | 7/9 | 51 |
| Impingement with calcifications | 9/16 | 49 |
| Rupture | 5/2 | 57 |

Three patients with frozen shoulder are not shown in the table.

Table III. Patients included in study III-V.

Paper III

Patients (Table III)

Fifteen patients (five females) served as controls. Average age was 28 years (range 21-41). The preoperative radiographs were normal. In all these patients the rotator cuff was intact. Furthermore, there was no subacromial bursitis and no osteophyte on the anteroinferior part of the acromion. The coracoacromial ligament was normal in all cases. Nine patients had instability problems, three had a labral lesion, one a post-traumatic dislocation of the AC-joint and two patients were examined because of shoulder pain but no intra-articular or subacromial pathology was found.
In 30 patients (ten females) with impingement syndrome stage II arthroscopic subacromial decompression (Ellman 1987, Ellman and Kay 1991) was performed. Average age was 53 years (range 31-72). A positive impingement test prior to surgery was found in all cases. The radiographs showed neither signs of arthrosis in the glenohumeral joint nor superior migration of the humeral head.

Methods

The subacromial distance was measured between the undersurface of the anterolateral part of the acromion and the upper surface of the supraspinatus tendon intraoperatively (Fig. 3 A and B). The measurements were performed through a portal 3 cm below the anterolateral acromion. The subacromial distance was measured with the device twice intraoperatively: after bursectomy and then after bone resection. The arthroscopic subacromial decompression aimed at restoring normal subacromial distance regardless of gender.

Figure 3A. Device used in paper III for measurements of the subacromial distance.

Figure 3 B. Arthroscopic view, showing measurement of the distance between the anterolateral undersurface of the acromion (above) and the supraspinatus tendon (below).
All operations were performed with the patient in the lateral decubitus position. The arm was positioned in 20° abduction and in 10° flexion in all cases. In the standard setting a four-kg traction was used. Traction varying from 1 to 6 kg was tested.

The pump system “Arthro FMS 4” (Future Medical Systems S.A., Geneva, Switzerland) was used. Varying fluid pressure between 130 mm Hg and 230 mm Hg, as measured inside the pump, was tested.

In order to test the reliability of the device, we performed repeated measurements by the same surgeon three times sequentially right after one another (intraindividual assessment) and repeated measurements by different surgeons (interindividual assessment). The interindividual measurements were blinded. The tip of the device remained in the subacromial space between the measurements.

Paper IV

Patients (Table III)

Between February 1992 and July 1994 a total of 194 patients underwent ASD at our department. A protocol was completed after each operation (Fig. 4). Open surgery was never performed. The preoperative diagnosis was in all cases impingement syndrome: chronic pain for more than one year, which was exacerbated by activity, night pain and a positive impingement test. Indications for surgery were shoulder pain and impairment of function for more than one year despite conservative treatment, including physical therapy, NSAID and corticosteroid injections. There were no cases with signs of instability. Thirty-two (16 %) of these patients had calcific deposits on preoperative radiographs. Radiographs of the affected shoulder were taken in 4 positions: anteroposterior views in internal and external rotation, lateral view in the scapular plane and axillary view. The size and structure of each calcific deposit was recorded in order to compare pre- and postoperative findings on radiographs (DePalma and Kruper 1961). The preoperative radiograph showed calcific deposits type 1 in 4 cases and type 2 in 28 cases. The time between onset of symptoms and surgery was on average 5 ½ years (range 1-18 years). The calcific deposits were left untouched at surgery. Five patients were excluded from the study: one patient with arthrosis in the glenohumeral joint, one patient who formerly had surgery with open biceps tenodesis, one patient, who could only be interviewed by telephone and two patients with a total rupture of the
supraspinatus respectively the supra- and infraspinatus tendons. Two patients were not available for re-examination.

Figure 4. Protocol used for documentation of surgical procedures of the shoulder.
Methods

The remaining 25 patients with calcific deposits were re-examined by the first author. Each one of these patients was matched with another patient without calcification according to the state of the rotator cuff, date of surgery, age and gender. In 18 of the 25 cases the rotator cuff was described as normal at surgery and in 7 cases a partial articular side rupture of the supraspinatus tendon was found. The date of surgery within each matched pair of patients was the same ± 3 months. The follow-up time after surgery was 2 years (9-39 months, median 24 months).

The Constant score was used for evaluation of shoulder function (Constant and Murley 1987). Point allocation includes subjective assessment (35 points) and medical metrics (65 points). Pain is allocated 15, activities of daily living 20, range of motion 40 and power 25 points (total 100) (Fig. 5). The follow-up examination was blind regarding the presence or absence of calcific deposits.

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</table>

Figure 5. The Constant score (in Swedish).
Twenty-four patients with calcifications before surgery were re-examined with radiographs 12-42 months after surgery. One patient did not approve a postoperative radiograph to be taken. The same views were used at follow-up as before surgery. The calcifications were classified according to size in the greatest dimension (Bosworth 1941, Harmon 1958). The measurements of the calcifications were performed with a ruler directly on the radiograph. The greatest size in any dimension was chosen and compared before and after surgery.

Paper V

Patients (Table III)

Thirty-six consecutive patients were included in the study. Sixteen (nine females) patients with impingement syndrome and intact rotator cuff were assessed. Average age was 51 years (range 30-61). The patients had a history of more than one year of shoulder pain and a positive impingement test.

In seven patients (two females) a rupture was found in the supraspinatus tendon arthroscopically. Five patients had a total rupture and two a partial. Average age was 57 years (range 41-73).

Three patients had an arthroscopic release due to primary frozen shoulder. One of these patients also had diabetes mellitus.

Ten (four females) patients without any history, clinical or arthroscopic signs of subacromial pathology served as controls. In all controls the supraspinatus tendon appeared normal both from the bursal and the articular side view and there were no signs of bursitis. Average age was 28 years (range 20-37). Nine underwent surgery due to glenohumeral instability: six cases had posttraumatic anterior instability and three cases multidirectional instability. One patient was treated by an arthroscopic lateral resection of the clavicula because of arthrosis of the acromioclavicular joint.

Methods

Histology

Under arthroscopic control a full thickness tendon biopsy about 4 x 4 mm in size, was excised en bloc in the middle portion of the tendon, between the insertion to the greater tuberosity and the muscle-tendon junction. When a rupture was present the biopsy was taken within the
tendon medial to the rupture. The specimens were immediately fixed in 10% buffered formaldehyde for 24-48 hours, dehydrated in ethanol, embedded in paraffin and sectioned in 4 µm sections. Staining with hematoxyline eosin and Van Giesons hematoxyline was done for histological assessment. PTAH-staining was used for fibrin visualization. The senior pathologist and the first author together analyzed histology sections in a blinded fashion using a standard light microscope.

**Immunohistochemistry**

A mouse anti-human MMP-1 monoclonal antibody (MAB 3307; CHEMICON International Inc., Temecula, California, USA) together with a FITC-labelled rabbit anti-mouse antibody (DAKOPATTS, F0205) was used for MMP-1 identification. Immunofluorescent staining for fibronectin was performed using rabbit polyclonal fibronectin (A0245) antibodies together with a swine anti-rabbit, FITC-labelled antibody (F0205). Antibodies were purchased from DAKOPATTS AB (Stockholm, Sweden). Sections of gastric wounds were used as positive controls for fibronectin and MMP-1. For each section studied a negative control was stained according to the protocol but with the primary antibody omitted. Before application of antibodies, the specimens were stained with 0.05% pontamine sky blue 5BX (BDH Chemicals Ltd, Poole, United Kingdom) in PBS with 1% dimethylsulfoxide to change the autofluorescence of collagen to wavelengths above 590 nm (Cowen et al 1985). The first author analyzed blindly the intensity and extent of the immunofluorescent staining of fibronectin in a fluorescence microscope. This was done in 10 randomly selected images from each specimen. Images were captured with a Leica DC 200 digital camera mounted on a Leica DMRXE microscope (Leica microsystems, Wetzlar Gmbh, Germany) and stored in a computer. The following scores were used: 0 = no detectable staining, 1= slight, 2= moderate and 3= pronounced and widespread increase in intensity and extent of the immunofluorescent staining. The senior pathologist and the first author assessed MMP-1 fluorescence in the microscope. Such fluorescence was only occasionally seen and therefore not suited for semiquantitative evaluation.

**Statistics (III, IV, V)**

Statistical evaluation was performed according to Table IV.
<table>
<thead>
<tr>
<th>Paper I</th>
<th>Paired and unpaired $t$-tests (2-tailed) and Fisher’s exact test (1-tailed) were used for statistical evaluation. $p&lt;0.05$ was considered significant.</th>
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<tbody>
<tr>
<td>Paper II</td>
<td>The non-parametric Kruskall-Wallis test was used initially for evaluation of the overall significance. As a post hoc test, we carried out a Mann-Whitney U test combined with Bonferroni’s correction. $p&lt;0.01$ was considered significant.</td>
</tr>
<tr>
<td>Paper III</td>
<td>The Pearson product-moment correlation test and the confidence interval for the mean (95%) were used for statistical evaluation.</td>
</tr>
<tr>
<td>Paper IV</td>
<td>The chi square- and paired $t$-tests were used for statistical evaluation of the data. $p&lt;0.05$ was considered significant.</td>
</tr>
<tr>
<td>Paper V</td>
<td>The non-parametric Kruskall-Wallis test was used for evaluation of difference between all groups. As a post hoc test we used the Mann-Whitney U test with Bonferroni’s correction. $p&lt;0.01$ was considered significant.</td>
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**Table IV. Summary of statistical methods used in paper I-V.**
RESULTS

Paper 1
The purpose was to evaluate whether triamcinolone given repeatedly into the subacromial space of the shoulder would be deleterious to the supra- and infraspinatus tendons.

Macroscopic morphology (Table V)
There were no macroscopic changes in the structure of the tendon, neither among the control rats, nor in specimens from rats, which received triamcinolone three times. In specimens from rats, which received triamcinolone five times, 2/7 showed macroscopic changes. The tendon had become more whitish and less smooth.

<table>
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</table>

*p=0.035 (Fisher’s exact test).

Table V. Number of pathological findings with different subacromial treatment in rats.
Histology (Table V)

Microscopic changes were observed in 4/7 rats which were injected with triamcinolone five times. In these specimens degradation and fragmentation of collagen bundles were observed (Fig. 6).

Figure 6. Degradation of collagen bundles with fragmentation and discoloration (arrow).

Inflammatory cells, predominantly macrophages and giant cells, were found in great numbers in the tendons between the collagen bundles (Fig. 7). The histopathological changes differed significantly between the triamcinolone and saline treated rats (p=0.035).

Figure 7. Focal inflammation with macrophages and giant cells (arrow) at the junction between tendon and muscle.

Body weight

The rats, which were injected with triamcinolone lost weight, whereas those injected with saline, gained weight (p<0.001 in both groups).
Paper II

The purpose was to evaluate the rotator cuff after carrageenan-induced bursitis and to evaluate the possibility of using fibronectin and fibrinogen as markers for early tendon involvement following subacromial bursitis.

Evaluation of the subacromial bursa

In control rats injected with saline no inflammatory cells were seen in the subacromial space. In the three groups of rats injected with carrageenan, the inflammatory response in the subacromial space was associated with the total amount of carrageenan injected. In group A (carrageenan x 5), inflammatory cells were found in 2/8 cases. In group B (carrageenan x 10), there were signs of inflammation in 6/8 rats. In group C (double dosis carrageenan), the inflammatory response was present in all 12 rats. The inflammatory response was characterized by a granulomatous reaction with macrophages, which often had phagocytized carrageenan. There were also lymphocytes, plasma cells and scattered giant cells (Fig. 8).

Figure 8. Granulomatous inflammatory response after carrageenan injections (hematoxyline eosin). Numerous macrophages are seen, some of which have ingested carrageenan. Arrows indicate two multinucleated giant cells.
**Macroscopic evaluation of the tendons**

There were no macroscopic changes of the tendons, in the controls or in groups A or B. However, in group C, the bursal side of the tendons was more yellow in color compared to the controls, and its surface had lost its shiny appearance, whereas the joint side appeared normal in all cases. In some cases, the tendons were difficult to identify from a bursal view, which was due to the marked bursitis. The joint side of the tendon was always easy to identify.

**Microscopic evaluation of the tendons**

In controls, there were neither signs of degeneration of collagen fibers nor metaplasia. In one tendon there was a slightly increased number of vessels and damage to the collagen fibers in a minor part of the tendon.

In group A no signs of inflammation, metaplasia or degeneration in the tendons were seen. In group B macrophages were seen between the collagen fibers of the supraspinatus tendon in 3/8 rats (Fig. 9).

![Image of microscopic view of tendons](image)

**Figure 9.** Inflammatory cells, mostly macrophages, were seen in the supraspinatus tendon in some animals (3/8) in group B (hematoxyline van Gieson). Arrows indicate streaks of inflammatory cells between bundles of tendinous collagen.
In these rats there were neither signs of collagen degeneration nor metaplasia within the tendon itself. In group C the microscopic changes were characterized by marked infiltration of macrophages, and fibrocartilaginous metaplasia (Fig. 10) in all 12 rats and bony metaplasia (Fig. 11) in two thirds of the specimens within the supraspinatus tendon. There were no signs of deposits of calcium or other crystals.

No fibrocartilaginous metaplasia was found in the infraspinatus tendon but rather fibrosis within the tendon with derangement of the orderly collagen structure.

![Figure 10. Cartilaginous metaplasia in the supraspinatus tendon after injections of carrageenan (hematoxyline van Gieson). The asterix in the upper left corner indicates an area with metaplastic hyaline cartilage. Tendinous collagen is seen in the lower right corner and in between is a transitional zone of fibrocartilage.](image)
Fibronectin, as well as fibrinogen (see below), was analyzed in order to detect signs of an inflammatory reaction. The intensity and extent of the immunofluorescent staining for fibronectin in the tendon seemed associated with the degree of bursitis. The difference in the intensity and extent of the immunofluorescent staining for fibronectin was significant between group A and B (p=0.009), non-significant between controls and group A (p=0.86) and controls and group B (p=0.03).

Figure 11. Bony metaplasia (arrows) in carrageenan-injected supraspinatus tendon (hematoxyline van Gieson). The unstained areas with some scattered cells represent bone marrow. The asterix indicates an area with cartilage. Tendinous collagen is seen in the lower part of the figure.

Fibronectin (Table VI)
Fibronectin, as well as fibrinogen (see below), was analyzed in order to detect signs of an inflammatory reaction. The intensity and extent of the immunofluorescent staining for fibronectin in the tendon seemed associated with the degree of bursitis. The difference in the intensity and extent of the immunofluorescent staining for fibronectin was significant between group A and B (p=0.009), non-significant between controls and group A (p=0.86) and controls and group B (p=0.03).
<table>
<thead>
<tr>
<th></th>
<th>Degree of fibronectin staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Controls (n=4)</td>
<td>2</td>
</tr>
<tr>
<td>Group A (n=8)</td>
<td>5</td>
</tr>
<tr>
<td>Group B (n=8)</td>
<td>1</td>
</tr>
</tbody>
</table>

Table VI. Number of rats distributed for intensity and extent of the immunofluorescent staining for fibronectin (graded 0-3) The rats were injected 5 or 10 times with carrageenan or saline (controls). There was significantly more fibronectin staining after 10 than after 5 injections (p=0.009).

**Fibrinogen**

In three animals among the controls the staining was graded 1 and in one animal 0. In group A two animals were graded 2, two 1 and four 0. In group B three animals were graded 2, three animals 1 and two 0. None of the sections were graded 3. There was no difference between the groups (p>0.01).

**Paper III**

The purpose of this study was twofold: to document the accuracy of a novel measuring device and to compare the subacromial distance for a control group with patients with impingement syndrome, before and after ASD.

**Subacromial distance in relation to applied traction on the arm**

The subacromial distance was measured repeatedly with increasing weight applied to the arm in the lateral decubitus position (n=5). Two patients with impingement before ASD and three controls (glenohumeral instability) were used for this purpose. The subacromial distance increased between 1-3 mm when the traction was successively increased from 1 to 6 kg. The increase in subacromial distance was never more than 1 mm when traction was increased from 4 to 5 kg.
Subacromial distance in relation to pressure applied within the subacromial space

The subacromial distance was measured after bursectomy but before bone resection in two patients with impingement. The pump pressure was increased from 130 mm Hg up to 250 mm Hg over ten minutes. During this time the subacromial distance increased one mm.

Intraindividual measurements

The subacromial distance was measured by the surgeon three times sequentially for each patient. Three patients with impingement and two controls were used. The difference between two measurements never exceeded one mm.

Interindividual measurements

The subacromial distance was measured by the two authors on the same patient. Four patients with impingement and two controls (glenohumeral instability) were used. The measurements by the two examiners never differed more than one mm on the same patient. The correlation coefficient between the measurements by both authors was 0.99.

Controls

The mean value of the subacromial distance in the group of controls (n=15) was 16 mm (SD=4) (Fig. 12). The 95% confidence limits for the mean were 14 and 18 mm (Table VII).
Figure 12. The subacromial distance (mm) was measured in patients with impingement syndrome before (A) and after (B) resection of the coracoacromial ligament and bone resection. These values are compared with the values of the controls (C).

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Confidence limits* (95%)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=15)</td>
<td>No ASD</td>
<td>16</td>
<td>14-18</td>
<td>8-22</td>
</tr>
<tr>
<td>Impingement (n=30)</td>
<td>Before ASD</td>
<td>8</td>
<td>7-9</td>
<td>3-15</td>
</tr>
<tr>
<td>Impingement (n=30)</td>
<td>After ASD</td>
<td>16</td>
<td>15-17</td>
<td>11-22</td>
</tr>
</tbody>
</table>

*Confidence limits for the mean

Table VII. The subacromial distance (mm) between the supraspinatus tendon and the anterolateral undersurface of the acromion in all patients intraoperatively.

Patients with impingement (Fig. 12 and Table VII)

Among the patients with impingement syndrome (n=30) the subacromial distance after bursectomy was on average 8 mm (SD=4). All but four of these values were below the 95% mean confidence limits of the controls. The subacromial distance after bone resection measured 16 mm (SD=4). After bone resection 24 patients were within the confidence limits or above its higher limit. The subacromial distance was increased by 8 mm with a range between 5 and 13 mm.
Paper IV

The purpose of this study was to investigate whether the untouched calcifications remain postoperatively and to evaluate whether they play a role for the outcome after ASD.

Clinical outcome (Table VIII)

The postoperative results evaluated with the Constant score were almost identical in both groups. The median value in the calcific group (n=25) was 78 points (range 55-100) and in the non-calcific group (n=25) 79 (range 37-100).

The patients’ postoperative active range of motion did not differ between the calcific (n=25) and the non-calcific groups (n=25).

At 2-year follow-up examination, the patients who had calcifications preoperatively improved abduction compared with patients without calcifications (mean 9°; 95% confidence interval: 12° worse to 28° better). Outward rotation in patients with preoperative calcium deposits were 1° lower (95% confidence interval: 9° worse to 7° better). Thus, large differences between the groups can be excluded.

Seventy-two percent of the patients without calcific deposits on radiographs were satisfied with the result, compared to 80 per cent of the patients with calcifications. This difference was non-significant.
Table VIII: Clinical outcome measured by the active range of motion and the Constant score at 2-year follow-up examination.

**Calcifications on radiographs**

In 13 out of 24 patients the calcific deposits disappeared. In another six patients the calcifications decreased. The calcifications were unchanged after surgery in four cases.

There was no difference in shoulder function as evaluated by the Constant score when comparing the patients with increased or unchanged calcifications to those with decreased or resorbed ones (Table IX). In one patient the calcific deposit increased. In spite of that, the Constant score was 98 points.
<table>
<thead>
<tr>
<th>Calcification at follow-up</th>
<th>Number of patients (type I-calcifications)</th>
<th>Constant score (points) (median, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased</td>
<td>1 (0)</td>
<td></td>
</tr>
<tr>
<td>Unchanged</td>
<td>4 (0)</td>
<td>75 (55-100)</td>
</tr>
<tr>
<td>Decreased</td>
<td>6 (1)</td>
<td>78 (62-100)</td>
</tr>
<tr>
<td>Resorbed</td>
<td>13 (2)</td>
<td></td>
</tr>
</tbody>
</table>

### Table IX. Change in size of the calcifications after ASD and Constant score at 2-year follow-up examination (n=24).

Preoperatively 17/24 patients had calcifications of 5 mm or more. Seven patients had calcific deposits less than 5 mm. At follow-up 20 patients had none or just small (< 5 mm) calcifications (p< 0.001). Four patients had greater calcifications (≥ 5 mm) (Table X).

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>2-year follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcifications &lt;5 mm</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>Calcifications ≥5 mm</td>
<td>17</td>
<td>4</td>
</tr>
</tbody>
</table>

### Table X. Number of patients with different size of calcification, preoperatively and at 2-year follow-up examination (n=24).

#### Paper V

The purpose of this study was to investigate human surgical supraspinatus tendon specimens from patients with impingement, ruptured supraspinatus tendons, frozen shoulder and controls with respect to histological changes and the presence of fibronectin and MMP-1.

**Histology**

In 2/7 rupture specimens the normal appearance of the collagen fibers was changed and they obtained an amorphous, homogenous appearance with a yellowish tint. Such findings were not observed in controls or in patients with impingement. Fibrin was found in 4/7 ruptures (Fig. 13). No fibrin was observed in controls and patients with impingement.
Fibrosis within the supraspinatus tendon was found among all patient categories: in 6/10 controls, in 8/16 patients with impingement, in 3/7 patients with a rupture and in one patient with frozen shoulder.

Thinning of fascicles was found in 2/16 patients with impingement, in one patient with a rupture and in 2/10 controls.

Calcifications were found in one control specimen only. Lipomatosis was found in one specimen from each group. Chondrocyte-like cells were not found. Inflammatory cells were found only one specimen from a non-diabetic frozen shoulder.

**Fibronectin**

Fibronectin was found in 6/7 ruptures and 4/16 impingement cases (Fig. 14). The staining for fibronectin differed between all groups (p=0.008) and was significantly increased among patients with a rupture compared with controls (p=0.002). The difference between controls and impingement (p=0.5) and between controls and frozen shoulder (p=0.5) was non-significant (Table XI).
Figure 14. Ruptured supraspinatus tendon. A: increased, patchy staining for fibronectin (arrows). B: negative control for fibronectin immunohistochemistry. C: degeneration of collagen bundles with altered, yellowish staining in the center of the micrograph (arrows; Van Giesons hematoxyline staining).

<table>
<thead>
<tr>
<th></th>
<th>Degree of fibronectin staining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Controls (n=8)</td>
<td>1</td>
</tr>
<tr>
<td>Rupture (n=7)</td>
<td>0</td>
</tr>
<tr>
<td>Impingement (n=16)</td>
<td>3</td>
</tr>
<tr>
<td>Frozen shoulder (n=3)</td>
<td>1</td>
</tr>
</tbody>
</table>

Table XI. Number of patients distributed for immunofluorescent staining of fibronectin (graded 0-3). The difference between controls and rupture was significant (p=0.002).
MMP-1
MMP-1 was found in 1/16 patients with impingement and in 1/7 ruptures, both within the tendon and at the edge of ruptured collagen fibers. MMP-1 was not found in controls and among patients with frozen shoulder.
GENERAL DISCUSSION

Corticosteroid effects on the supraspinatus tendon
In patients with impingement, with or without calcifications, the treatment often includes repeated subacromial corticosteroid injections. Short-term clinical effects as pain reduction and increased range of motion after one subacromial injection of triamcinolone was shown by Benjamin et al (1996) in patients with impingement without rupture of the rotator cuff. The value of subacromial corticosteroid injections for recovery is, however, controversial (Withrington et al 1985, Vecchio et al 1993, Dalton 1994). Animal studies have shown that corticosteroids may have adverse effects on tendons (Wrenn et al 1954, Krahl and Langhoff 1971, Balasubramaniam and Prathap 1972, Matthews et al 1975, Kapetanos 1982, Wiggins et al 1994). Furthermore, ruptures of tendons and ligaments in humans have been reported after local deposition of corticosteroids (Lee 1957, Bedi and Ellis 1970, Gottlieb and Riskin 1980, Halpern et al 1977, Holmes and Mann 1992). Physicians therefore do not inject corticosteroids in the region of the Achilles or the patellar tendon. However, repeated subacromial corticosteroid injections is common. The lack of scientific reports referring to the subacromial space and corticosteroids may contribute to this practice.

Subacromial corticosteroid injections may possibly have adverse effects on the supraspinatus tendon due to the close relationship between the subacromial bursa and the tendons of the rotator cuff (Pettersson 1942, Yamanaka and Fukuda 1991). We demonstrated this in a rat model. From a clinical point of view it was relevant to perform injections repeatedly because this is common and the complication rate probably increases with increasing number of injections. The chosen time interval between injections was short. However, according to allometric scaling laws the metabolic rate in the rat is about twice as high as in humans (West et al 1997). Thus, the chosen time intervals in this study would correspond to twice as long time periods in humans.

In order to assess the location of the injection, methylene blue was injected into the subacromial space. The dye was found in the subacromial bursa each time. Palpation of the subacromial space on the rat and insertion of the needle under the anterior part of the acromion was rather easy and reproducible. Therefore we felt confident that the injections were placed in the subacromial bursa and not in the tendon.
We chose to use triamcinolone (Lederspan®), which was commonly used in Sweden. The dose was calculated in relation to the weight of the rats in order to give doses equivalent to those given to humans.

We avoided using the untreated shoulder of the corticosteroid rats as control, because of the potential risk of systemic effects leading to corticosteroid-induced changes in the tendons of the non-injected shoulder.

Obvious signs of tendon damage were demonstrated in this study. Degradation and fragmentation of collagen bundles as well as inflammatory cells, predominantly macrophages and giant cells, were seen in specimens treated with triamcinolone. These results are in accordance with earlier studies on the side effects of corticosteroids on connective tissue (Balasubramaniam and Prathap 1972, Kennedy and Baxter Willis 1976, Unverferth and Olix 1973, Krahl and Langhoff 1971, Björkenheim et al 1988). In paper I these side effects were demonstrated for the first time on the rotator cuff. It should lead to a more careful policy for clinicians giving repeated subacromial corticosteroid injections.

**Carrageenan-induced subacromial bursitis in rats**

In paper II the rat model was used again. Subacromial bursitis was induced in order to study the effects on the supraspinatus tendon. The purpose was to investigate to what extent bursitis could evoke changes in the tendon. Through the arthroscope one can often observe changes in the bursa in patients with impingement i.e. the normal spin-web like bursa is replaced by strands of thick tissue, the wall is thickened and the number of vessels appears increased. However, observations of the inflamed subacromial bursa in humans from a histopathological point of view are very sparse in the literature. In calcifying tendinitis, rotator cuff rupture and AC-joint arthritis no inflammatory cells were detected by Sarkar and Uhthoff (1983) but they assessed only eight specimens in all. Rahme et al (1993) found fibrosis in most impingement cases, however, mononuclear cells in only about ¼ of all specimens. The bursitis may be localized only to the site of the lesion in the supraspinatus tendon and mainly characterized by a proliferation of fibroblasts, new collagen formation and by angioneogenesis. Ishii et al (1997) found these changes in the bursa of patients with rotator cuff rupture, impingement and calcifying tendinitis. However, to a significant lesser degree in the latter two groups. Lymphocytes, but no plasma cells, were observed. The presence of lymphocytes,
macrophages and activated fibroblasts in the bursa from patients with impingement and rupture was also found in an earlier study (Santavirta et al 1992).

The carrageenan-induced inflammation has been described as both acute and chronic. Bradykinin, kinin and prostaglandins are involved in the acute form, whereas the chronic form is characterized by a granulomatous reaction with histiocytes and macrophages. The number of fibroblasts and collagen turnover and formation increase (Benitz and Hall 1959, Pérez-Tamayo 1970, DiRosa 1972). Thus, on a histological level there appears to be some similarities between subacromial bursitis in humans and carrageenan-induced inflammation but they differ in request to the granulomatous response.

We found an increased staining of fibronectin in tendon specimens associated with the presence of subacromial bursitis. Our findings are similar with the results of other animal and human studies (Williams et al 1984, Kvist et al 1988, Lehto et al 1990, Gelberman et al 1991). Kvist et al (1988) found increased staining of fibronectin in the paratenon in chronic Achilles tenonitis. Subsequently, fibronectin may be an important factor in the impingement syndrome of the shoulder as well.

Furthermore, we found marked infiltration of macrophages, fibrocartilaginous metaplasia and bony metaplasia within the supraspinatus tendon. There were, however, no signs of deposits of calcium or other crystals. Recent studies indicate that fibrocartilaginous metaplasia may play a role in the pathogenesis of a rupture (Fukuda et al 1990, Yamanaka and Fukuda 1991, Chard et al 1994). Yamanaka and Fukuda (1991) reported chondrocyte-like cells in the vicinity of the rupture. Chard et al (1994) described similar findings in cadavers assessing the whole tendon. Metaplasia as found in paper II was associated with subacromial bursitis and may represent a direct reaction between the inflamed bursa and the supraspinatus tendon. An alternative explanation could be increased compression within the subacromial space (Vogel and Koob 1989, Giori et al 1993). The inflamed bursa may be impinged under the acromion and secondarily the supraspinatus tendon. These mechanisms could be relevant also in humans with impingement and rotator cuff rupture.
Subacromial distance

In patients with impingement syndrome the supraspinatus tendon impinges between the undersurface of the anterior acromion and the humeral head. This occurs especially in abduction and flexion. The purpose of the arthroscopic subacromial decompression is to restore the distance between the acromion and the supraspinatus tendon in order to leave enough space for the supraspinatus tendon and the subacromial bursa in abduction and flexion. The amount of bone, which is removed by the surgeon, is important for the postoperative results (Gartsman 1990, Ogilvie-Harris et al 1990, Warner et al 1994). The purpose of this paper was to evaluate an instrument, which enables intraoperative measurements of the subacromial distance before and after bone resection and, furthermore, to measure the subacromial distance in patients with impingement syndrome and in controls.

The measurement landmarks were the same as the ones used when performing an ASD; i.e. the narrowest distance between the undersurface of the anterolateral acromion and the upper surface of the supraspinatus tendon. Patients with a total rupture of the supraspinatus tendon were excluded because the measurement of the subacromial distance is difficult and less accuracy was expected. Intraindividual measurements showed a high reproducibility and blinded interindividual measurements a high correlation.

The confidence interval for the mean was calculated for the controls. This was used as a reference. However, the age differed between the control and the impingement groups. The population of interest was middle-aged men and women with signs of impingement syndrome. The age of the controls in this study averaged 28 years. However, no patients older than 40 years were examined arthroscopically unless symptoms such as pain were present. This means that the only achievable control values were those from younger patients with instability problems of the glenohumeral or the acromioclavicular joints. Age may, however, play a role due to tissue degeneration and altered laxity.

It was not possible to compare the measurements in our study with the results of a reference criterion standard, since such a standard is lacking. There is no report in the literature describing quantitative measurements of the subacromial distance intraoperatively. The values of the subacromial distance in both controls and patients with impingement syndrome were consistent with those observations reported earlier in radiograph and MRI studies, although our values were somewhat higher (Table XII).
<table>
<thead>
<tr>
<th>Method</th>
<th>n</th>
<th>Diagnosis</th>
<th>Distance measured</th>
<th>Subacromial distance in mm (* abduction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Golding 1962</td>
<td>150</td>
<td>normal distance</td>
<td>acromial undersurface – humeral head</td>
<td>7-13 (0°)</td>
</tr>
<tr>
<td>Cotton and Rideout 1964</td>
<td>6</td>
<td>normal distance</td>
<td>acromial undersurface – humeral head</td>
<td>6-14 (0°)</td>
</tr>
<tr>
<td>Petersson and Redlund-Johnell 1984</td>
<td>175</td>
<td>normal shoulders</td>
<td>acromial undersurface – humeral head</td>
<td>10 (0°)</td>
</tr>
<tr>
<td>Matthews and Fadale 1989</td>
<td>12</td>
<td>cadaver</td>
<td>coracoacromial ligament – humeral head</td>
<td>8-10 (0°), 2-5 (45°)</td>
</tr>
<tr>
<td>Flatow et al 1994</td>
<td>8</td>
<td>cadaver, no pathology</td>
<td>acromial undersurface – humeral head</td>
<td>11 (0°), 8 (60°)</td>
</tr>
<tr>
<td>Chen et al 1996</td>
<td>29</td>
<td>impingement syndrome</td>
<td>acromial undersurface – humeral head cartilage</td>
<td>6 (0°)</td>
</tr>
<tr>
<td>Tillander and Norlin</td>
<td>15</td>
<td>controls</td>
<td>acromial undersurface – supraspinatus tendon</td>
<td>16 (20°)</td>
</tr>
<tr>
<td>Tillander and Norlin</td>
<td>30</td>
<td>impingement syndrome</td>
<td>acromial undersurface – supraspinatus tendon</td>
<td>8 (20°)</td>
</tr>
</tbody>
</table>

Table XII. Subacromial distance measurements in the literature.

In conclusion, the measurements with the device seem valid, although a criterion-related validity (Greenfield et al 1998) has not been established due to the lack of a standard. The results show a significant difference in subacromial distance between controls and patients with impingement syndrome. Furthermore, the subacromial distance after ASD was in most cases within the confidence interval of the controls. Measuring the result of the surgical performance intraoperatively with this novel instrument may be helpful to surgeons in order to resect an adequate amount of bone. The surgeon can also document the subacromial distance before and after bone resection.

Calcifications in the supraspinatus tendon
Calcifications on radiographs are a quite frequent finding among patients with shoulder pain (Bosworth 1941, Ruttimann 1959, Welfing et al 1965, Hedtmann and Fett 1989). Some authors regard calcifying tendinitis as a self-limiting disease with very limited indications for surgical excision (McLaughlin 1963, Uhthoff and Sarkar 1990), whereas other authors report
good results after surgery (Ellman and Kay 1991, Ellman et al 1992, Huber 1992, Molè 1992, Esch 1993, Johannsen 1996). Surgical excision was, however, combined by other procedures, such as ASD. What happens with calcifications after ASD without surgical excision of the calcifications? Is the clinical outcome different from patients without calcifications?

In paper IV we evaluated a consecutive series of patients with calcifications in the rotator cuff and a matching group of patients without calcifications, who all underwent an ASD between 1992 and 1994. This study is retrospective, lacking randomization to different treatment options. However, because of the limited number of patients with calcifications it would be difficult to find these patients within a reasonable period of time. The strength of this study is the fact that all calcifications were untouched, i.e. not surgically removed. Such a cohort of patients is unique in the literature. The follow-up time both for the clinical postoperative evaluation and the radiological re-examination was, however, quite short.

The postoperative result at 2-year follow-up evaluated with the Constant score revealed no significant difference between the patients who had calcific deposits on preoperative radiographs compared to patients without. If the untouched calcification itself had a negative influence on the postoperative outcome, one would expect a lower value of the Constant score in the group of patients with calcifications on preoperative radiographs. The patients’ active range of motion postoperatively did not differ between the patients with and without calcifications. Furthermore, the Constant score did not differ significantly between those patients with unchanged or even increased calcifications and those with decreased or resorbed calcifications.

The Constant score has been widely used for the assessment of impingement and is regarded as the golden standard by the European Society for Surgery of the Shoulder and Elbow (ESSSE) and was therefore used in this study. However, a thorough evaluation of the score seems to be lacking. Assessment of validity has not been performed, the reliability is low with moderate high inter- and intraobserver variability and the accurate measurements of power may be difficult (Conboy et al 1996). Furthermore, quality of life, ability to work, social activities and general health are not assessed. In order to assess these important aspects selfadministered evaluation instruments may be more appropriate, as for instance the DASH (disabilities of the arm, shoulder and hand)-questionnaire (Atroshi et al 2000).
More than half of the patients in the calcific group had no calcification on radiographs at follow-up, although the calcifications had not been removed during surgery. Furthermore, in 6 of the remaining patients the calcific deposits decreased in size. On preoperative radiographs, 7 patients had small calcifications (<5 mm). At follow-up 20 patients had none or only small calcifications (p<0.001). Bosworth (1941) found a disappearance of the calcific deposits in only 6.4%. This may indicate that the calcifications disappear more rapidly after ASD, possibly following a reduction in pressure in the subacromial space.

This study shows that untouched calcifications in the rotator cuff disappear or decrease in size after ASD. The results indicate further, that the postoperative outcome when ignoring calcifications is as good as in patients without calcifications. The natural course of calcific deposits supports this strategy of treatment.

**Surgical biopsies of the supraspinatus tendon**

In this study surgical specimens of the supraspinatus tendon were analyzed for comparison between the histologic features and the clinical diagnosis and surgical findings, which is not possible in cadaver studies (Fukuda et al 1994). Assessment of surgical specimens also excludes possible irrelevant post-mortem changes.

Degradation and fibrin were only found in specimens from patients with a rupture of the supraspinatus tendon. The presence of fibrin at the edge of ruptured fibers of the supraspinatus tendon as shown by Fukuda et al (1994) was confirmed in our study. However, since our biopsies were taken from intact tendon, medial to the rupture, our findings imply that tendon changes are widespread, not only localized in the rupture area.

The collagen fibers showed thinning and fibrosis among patients with impingement and rupture of the supraspinatus tendon. However, thinning of fibers as described by other authors (Uhthoff and Sano 1997, Sano et al 1999) was also present in several control specimens in paper V. This could possibly be due to actual pathology in the supraspinatus tendon among patients with instability. The presence of fibrosis may indicate this and was actually found in some of the controls. However, thinning of fibers should be interpreted with caution, as it may be due to fixation artefacts. In addition, the size of tendon fibers may vary (Williams et al 1984). The finding of disrupted fascicles (Uhthoff and Sano 1997) may be overestimated, since this finding may resemble oblique sections in intact tendons.
In most papers dealing with histology on the rotator cuff no controls were assessed (Fukuda et al 1990 and 1994, Schmelzeisen 1990, Ishii et al 1997, Uhthoff and Sano 1997, Sano et al 1999). Brewer (1979) described disorganization of collagen fibers with increasing age, but investigated only one control specimen. Gotoh et al (1997) used cadaveric supraspinatus tendons as controls versus surgical specimens of ruptured tendons. Our control group consisted of patients with instability, but no history or clinical signs of subacromial pathology, and they had normal arthroscopic findings of the rotator cuff and bursa. However, we cannot exclude the possibility of microscopic changes of the supraspinatus tendon in the controls, because of recurrent dislocations. For ethical reasons, it is not possible to take surgical biopsies from patients without any shoulder complaints.

In accordance with earlier studies (Uhthoff and Sano 1997, Budoff et al 1998), we did not find any inflammatory cells in the specimens from patients with impingement syndrome or supraspinatus tendon rupture.

Calcifications were neither found in impingement nor in ruptured tendons. In only one control specimen a calcific deposit was found. These findings are in accordance with other studies considering calcifications as a self-limiting disease with subsequent healing of the tendon and occuring also in the asymptomatic shoulders (McLaughlin 1946 and 1963, Uhthoff and Sarkar 1981, Uhthoff and Sano 1997).

The site and size of the biopsies (middle part of the tendon proximal to the rupture) may explain why chondrocyte-like cells were not found in the present study. One may speculate that fibrocartilaginous metaplasia is confined to the critical zone as defined by Codman and can therefore only occasionally be detected in the remainder of the tendon.

Fibronectin staining was significantly increased in specimens from ruptured tendons. This indicates that there has been a process within the tendon with an increased inflammatory activity. However, using antibodies we cannot determine whether the antibodies bind to the intact fibronectin molecule or its breakdown products (Schmidtchen 2000). In normal healing, fibronectin eventually disappears (Hölund et al 1982, Kurkinen et al 1980, Williams et al 1984, Gelberman et al 1991), but in case of defect wound healing or chronic inflammation fibronectin may remain (Grinnell 1984, Kvist et al 1988, Babu et al 1989). Babu et al (1989)
found increased intra- and extra-cellular fibronectin in keloid scars. They suggested that keloid fibroblasts might lack the ability to transform and thereby resemble embryonic cells, which exhibit an accelerated fibronectin production. This may be interpreted as an ongoing healing process within the tissue and may be paralleled in the case of a supraspinatus tendon rupture. The decrease in fibronectin, as shown in normal healing (Gelberman et al 1991), is counteracted by the continuous futile strive to heal the tendon. One may therefore perhaps regard fibronectin as a marker of a chronic repair reaction and incomplete healing of the supraspinatus tendon as it was proposed for chronic Achilles tenonitis (Kvist et al 1988).

Gotoh et al (1997) found MMP-1 staining in patients with a supraspinatus tendon rupture and concluded that MMP-1 could play a role in the pathogenesis of rotator cuff tears. MMP-1 staining was found in specimens from patients with impingement and supraspinatus tendon rupture also in our study.

In conclusion, degradation of collagen fibers, fibrin and fibronectin seem to be reliable signs of tendon degeneration, whereas fibrosis and thinning of fascicles were more non-specific findings.
CONCLUSIONS

1. Repeated subacromial corticosteroid injections result in degenerative and inflammatory reactions in the supraspinatus tendon.

2. The supraspinatus tendon responds to subacromial bursitis in terms of increased fibronectin content and fibrocartilaginous and bony metaplasia.

3. The new instrument for intraoperative measurements of the subacromial distance enables reproducible data collection.

4. The mean intraoperative subacromial distance in the lateral decubitus position is between 14-18 mm (confidence interval for the mean) in patients with glenohumeral instability and in patients with impingement syndrome 8 mm before bone resection and 16 mm after arthroscopic subacromial decompression.

5. Calcifications left untouched in the rotator cuff usually disappear or decrease in size after arthroscopic subacromial decompression.

6. The postoperative outcome after arthroscopic subacromial decompression in patients with impingement syndrome is not influenced by the presence of calcifications in the rotator cuff.

7. In human biopsies, degradation of collagen fibers, fibrin and fibronectin seem to be reliable signs of tendon degeneration. Patients with rupture have increased content of fibronectin.
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