Summary. Background/Aim. In a condition of pain in the Achilles tendon insertion there are multiple structures involved, such as the Achilles tendon itself, the retrocalcaneal bursa and a bony protrusion at the calcaneal tuberosity called Haglund’s deformity. The innervation patterns of these structures are scarcely described, and the subcutaneous calcaneal bursa is traditionally not considered to be involved in the pathology. This study aimed at describing the innervation patterns of the four structures described above to provide a better understanding of possible origins of pain at the Achilles tendon insertion. Methods. Biopsies were taken from 10 patients with insertional Achilles tendinopathy, which had pathological changes in the subcutaneous and retrocalcaneal bursae, a Haglund deformity and Achilles tendon tendinopathy as verified by ultrasound. The biopsies were stained using immunohistochemistry in order to delineate the innervation patterns in the structures involved in insertional Achilles tendinopathy. Results. Immunohistochemical examinations found that the subcutaneous bursa scored the highest using a semi-quantitative evaluation of the degree of innervation when compared to the retrocalcaneal bursa, the Achilles tendon, and the calcaneal bone. Conclusions. These findings suggest that the subcutaneous bursa, which is traditionally not included in surgical treatment, may be a clinically important factor in insertional Achilles tendinopathy.

Key words: Insertional Achilles tendinopathy, Innervation, Subcutaneous bursa, Retrocalcaneal bursa

Introduction

Pain in the Achilles tendon (calcaneal tendon; tendo calcaneus) or its insertion into the calcaneus is known to be difficult to treat (Myerson and McGarvey, 1998; Kearney and Costa, 2010; Waldecker et al., 2012). The mid-portion of the Achilles tendon is the most common location for pain (55-65%), followed by the insertion (20-25%) (Jarvinen et al., 2001). Insertional Achilles tendon pain - commonly called insertional Achilles tendinopathy, comes with posterior heel pain and a prominent tendon insertion. The diagnosis is most often clinical, but ultrasound should be a standard tool in examining the Achilles tendon insertion, bursae and bone (Mahlfeld et al., 2001). This should be used to differentiate the condition from isolated retrocalcaneal bursitis, which is a state of fluid accumulation in the retrocalcaneal bursae (bursa tendinis calcanei) (van Dijk et al., 2011).

Apart from the pathological distal part of the Achilles tendon itself, other tissues are often associated with insertional pain. Bursitis in the retrocalcaneal bursa, as mentioned earlier in the text, where the pain originates from the amount of fluid in the bursa (Lohrer and Nauck, 2014), a skeletal posterosuperior prominence of the calcaneal tuberosity (Haglund’s deformity) causing a tendon-calcaneal impingement, and the
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presence of calcifications in the achilles tendon insertion, alone, or together, are often involved in this condition (Kang et al., 2002; Yodlowski et al., 2002). The retrocalcaneal bursa is located between the Achilles tendon and calcaneus, and another bursa - the subcutaneous bursa (superficial bursa) - is found between the tendon and the skin. Both these structures are thought to decrease local pressure and friction (Jozsa and Kannus, 1997) (Fig. 1). Although the retrocalcaneal and subcutaneous bursae can be visualised using ultrasound (US) in people with bursitis and possibly other conditions, it has been shown that it cannot be discerned by US in a healthy population (Mahlfield et al., 2001).

The structure and histopathology of the region for the Achilles tendon insertion, including the distal part of the Achilles tendon, the retrocalcaneal bursa and the skeletal posterosuperior prominence of the calcaneal tuberosity, has been clarified in a study on cadavers from older patients (Rufai et al., 1995), as well as in thorough morphological studies of the calcaneus, and the retrocalcaneal bursa on cadaveric material (Kachlik et al., 2008a,b). However, there is sparse information about the innervation patterns in the bursae and bone, and studies on the innervation patterns in patients suffering from insertional Achilles tendinopathy are lacking. For the Achilles mid-portion, a recent study on patients with chronic painful tendinopathy has shown multiple, especially sympathetic, but also sensory nerves, outside, but not inside, the ventral side of the tendon (Andersson et al., 2007).

The aims of this investigation were to study the nerve distribution in the subcutaneous and retrocalcaneal bursae, the skeletal posterosuperior prominence of the calcaneal tuberosity, and the ventral side of the distal part of the Achilles tendon in patients suffering from chronic painful insertional Achilles tendinopathy, as diagnosed by ultrasound and Color Doppler (US and CD) examination. This study was performed by collecting tissue samples from the subcutaneous and retrocalcaneal bursae, the skeletal posterosuperior prominence of the calcaneal tuberosity and the ventral side of the distal part of the Achilles tendon, in patients undergoing surgical treatment for insertional Achilles tendinopathy. Insertional Achilles tendinopathy was defined as a combined pathology in the subcutaneous and retrocalcaneal bursae, a Haglund deformity and tendinopathy of the distal Achilles tendon, as visualised by US and CD.

Materials and methods

All patients included in this study had a combined pathology in the Achilles insertion, verified by US and CD, which affected the distal part of the Achilles tendon as well as the subcutaneous and retrocalcaneal bursae, and showed a skeletal posterosuperior prominence of the calcaneal tuberosity; i.e. Haglund’s deformity. Ethical approval was obtained from the regional ethical board for medical research at Umeå University, Sweden (dnr 2011-83-32M (addition to dnr 04-157M)). Informed consent was obtained from all patients included in this study.

Altogether 10 patients (7 men and 3 women, mean age 44 years) with a long duration (median 17 months) of pain in the Achilles tendon insertion were included. Patient characteristic is shown in Table 1. The activity levels included non actives (n=1), recreational athletes (n=8) and one elite level athlete (n=1). All patients had tried a period of rest and the majority had tried other treatment methods such as eccentric calf muscle training (n=5), non-steroid anti-inflammatory drugs (n=8), local sclerosing Polidocanol injections (n=4), or local cortisone injections (n=2) without satisfactory results, prior to surgery. Consecutive recruitment of patients who fulfilled our diagnostic criteria of insertional Achilles tendinopathy with pathology in all four mentioned structures, which had not responded to conservative treatment alternatives or injection therapy, was performed until 10 patients had been included. A single control was included to visualize the normal ultrasonic image of the area of interest (Fig. 2).

All patients had a combined pathology in the Achilles tendon insertion, which was seen clinically (swelling and tenderness) verified using ultrasound (US) and color Doppler (CD) examinations (thickening, structural changes, fluid accumulation and high blood flow in the distal part of the Achilles tendon and bursae, in combination with a skeletal posterosuperior prominence of the calcaneal tuberosity; i.e. Haglund’s deformity). In this study we referred to the patients as suffering from “Insertional Achilles Tendinopathy” although some other studies do not include pathology in the bursae in this diagnosis (van Dijk et al., 2011). Patients presenting with painful intratendinous calcifications or bone spurs in the insertion of the Achilles tendon were excluded from the study. The

![Fig. 1. Anatomical relationship of the Achilles tendon, bursae and calcaneus. The area where biopsies were taken from the Achilles tendon, calcaneus and retrocalcaneal bursa is marked in yellow.](image-url)
surgical procedure and postoperative rehabilitation was the same for all patients. A healthy control was included in the study to visualize the normal ultrasonic image of the Achilles tendon insertion and to visualize the lack of high blood flow at the healthy Achilles tendon insertion as previously shown by others (Knobloch et al., 2006). This control patient was only used in the ultrasound examination, and no biopsies were taken due to ethical considerations. For the same reason, biopsies were not taken from the healthy side of the operated patients.

The US and CD pre-operative examination was performed using a high-resolution grey scale US and CD examination (Acuson Sequoia 512, Siemens) was performed, using a linear multi-frequency (8-13MHz) probe.

**Surgical procedure**

Local anaesthesia (5-10 ml of Xylocaine 10 mg/ml with adrenaline 5 µg/ml) was distributed into the subcutaneous tissues, inside and around the subcutaneous and retrocalcaneal bursae, towards the periosteam of the skeletal posterosuperior prominence of the calcaneal tuberosity, and on the ventral side of the distal part of the Achilles tendon. The surgical procedure was started 10-15 minutes after the local anaesthesia had been given. The subcutaneous tissues were visualized through a lateral longitudinal skin incision about 4-6 cm in length. The subcutaneous bursa, localized between the insertion of the Achilles tendon and the skin, was first carefully dissected from the skin, and then separated from the tendon before being removed completely. The retrocalcaneal bursa, located between the posterior smooth surface of the superior calcaneal tuberosity and the ventral side of the distal part of the achilles tendon, was then removed by carefully starting laterally and moving all the way over to the medial side. The procedure was focused on extraction of the medial and lateral wall, the roof, and the synovial fold, of the retrocalcaneal bursa, as the thin dorsal and ventral walls of the bursa are continous with the calcaneal bone and the Achilles tendon respectively. A tendon tissue sample (0.5x0.5 cm) was extirpated from the ventral portion of the distal Achilles tendon at the same time as the loose connective tissue and infiltrating fat and fibrous tissue (including the blood vessels and accompanying nerves) ventral to the distal part of the Achilles tendon was scraped using a scalpel. Part of the proximal portion of the calcaneus (the posterosuperior prominence of the calcaneal tuberosity; i.e. Haglund’s deformity) (0.5-1.0 x1.0-2.0 cm) was finally resected by use of an osteotome. Finally the skin incision was sutured with non-resorbable sutures, careful hemostasis was achieved and the cavities were flushed with 4-5 ml of Xylocaine with Adrenaline (Alfredson and Isaksson, 2014).

**Tissue specimens and immunohistochemistry**

These biopsies were directly transported to the laboratory and chemically fixed in a 4% solution of formaldehyde in 0.1 M phosphate buffer, pH7.4 at 4°C overnight to prevent the degradation of the tissues, followed by a wash in 10% sucrose-containing Tyrode’s solution. The biopsies from the calcaneus were decalcified, from 4 hours to overnight depending on the size of the biopsy, in Decalcifying Solution-Lite (D0818, Sigma, Saint Louis, MO, USA) following the fixation. The biopsies were then mounted on thin cardboard in OCT embedding medium (Miles Laboratories, Naperville, IL, USA) and snap-frozen at -80°C in liquid nitrogen-chilled propane. Using a cryostat, sections of 7 μm were cut and mounted on pre-coated chrome-alun gelatin slides before being processed for immunofluorescence or H&E stain.

In short, the immunohistochemical staining was performed by an initial step of potassium permanganate-

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**Nerve distributions in insertional Achilles tendinopathy**

| Table 1. Characteristics of 10 patients with painful insertional Achilles tendinopathy. |
|------------------------------------------|------------------|
| Age (years) | 45 (24-55) |
| Weight (kg) | 71 (55-93) |
| Height (cm) | 175 (158-198) |
| BMI (kg/m²) | 25 (19-36) |
| Duration (months)* | 17 (5-144) |

Results are expressed as mean. (Range). *: median is shown due to outlier with 144 months duration.

**Fig. 2.** Ultrasound and Color Doppler examination of healthy Achilles tendon insertion. The calcaneus is clearly visible (C), and a single subcutaneous vessel is visualized by Color Doppler (arrow). No clear demarcation of the bursae is evident (asterisks). The dashed line marks the contour of the Achilles tendon.
submersion, followed by three washes in 0.1 M phosphate buffered saline (PBS). 1% Triton X-100 was used to achieve permeabilization of the sections for 20 minutes before another set of washes in PBS. Blocking was done by use of normal swine serum before incubation with the primary antibody directed towards PGP9.5 (7863-0504; AbD Serotec, Oxford, UK) was done at 37°C for 60 minutes. This was followed by another set of washes in PBS and another blocking step using normal swine serum. The secondary was a TRITC-conjugated swine anti-rabbit antibody (code: R0156; Dako, Copenhagen, Denmark) which the sections were incubated with for 30 minutes at 37°C. After a final set of PBS-washes, the sections were mounted with coverslips using a DAPI-containing mounting agent (H-1500; Vector Laboratories, Burlingame, CA, USA).

**Biopsy evaluation**

The evaluation of the biopsy sections was performed by three microscopists (JC, GA, LB). Both patient identity as well as the tissue studied were coded and the evaluations were performed in a blinded manner. The general morphology was studied using H&E staining of one section. For the immunohistochemical evaluation, one section from each biopsy was given a semi-quantitative grade depending on the amount of PGP 9.5-positive structures, ranging from 0 to 3 (0 = very few or no PGP 9.5-positive structures, 1 = a few PGP 9.5-positive structures were seen in the section, 2 = PGP 9.5-positive structures were frequently occurring throughout the section, 3 = PGP 9.5-positive structures were seen in all fields of view throughout the section). The relationship of the nerve fibres to other structures was noted and is also presented in the Results. The localization (i.e. perivascular, intratendinous, peritendinous etc.) of the reactive fibres did not affect the scoring of the sections. PGP 9.5 is a general nerve marker and can visualize nerve fibres and nerve fascicles, but does not differentiate between different nerve types. All sections were evaluated using the same magnification (20x) when the grading described above was performed. Following the grading the mean and median was calculated for each of the groups (tendon, calcaneus, retrocalcaneal bursa, and subcutaneous bursa) based on the grade of each section. After the grading was finished, the sections were decoded. The results are presented as median and inter-quartile range as the data collected is based on an ordinal scale. Statistics were performed in SPSS 20.0, using non-parametric tests (Kruskal-Wallis One Way Analysis of Variance, and the Mann-Whitney U Test), a p-value of <0.05 was considered significant.

**Results**

**Ultrasound and color doppler findings**

Ultrasonography of the Achilles tendon insertion showed high blood flow inside and outside the bursae of the demarcated subcutaneous and retrocalcaneal bursae. In the single healthy control in this study, a high blood flow was not seen at the region of the Achilles tendon insertion (Fig. 2). Structural tendon changes were seen in the thickened, distal part of the Achilles tendon. These changes were seen primarily in the ventral and central parts of the tendon. A high blood flow was noted inside and outside the ventral part of the tendon. In all patients a prominent bone formation in the proximal calcaneus (Haglund’s deformity) was noted.

**Tissue innervation patterns**

In all of the four groups evaluated (subcutaneous bursa, retrocalcaneal bursa, bony tissue of calcaneus and distal part of the Achilles tendon) the PGP 9.5-positive reactions were most often seen as perivascular fibres (Fig. 3a). However, not all vessels showed clear perivascular innervation (Fig. 3b). In some of the sections, larger nerve fascicles were noted, however these were only seen in the sections from the subcutaneous or retrocalcaneal bursae. The sections containing tendon tissue showed very few nerve fibres in the tendon tissue proper (median score below), whereas in the paratendinous tissue some nerve fibres were seen. The few intratendinous nerve fibres that were seen were related to vessels coursing between the tendon fascicles. The calcaneus showed similar results with the innervation consisting primarily of perivascular nerve fibres in the Haversian canals (Fig. 3c). No biopsies were available from healthy control, nor the unaffected contralateral side of the patients, due to ethical considerations.

Structures reactive for PGP 9.5 scored the highest using a semi-quantitative evaluation of the degree of innervation and the lowest evaluation was seen in the tendon group (median score below).

The grading of the biopsies resulted in a median value of 1 (IQR=0-1.5) for the retrocalcaneal bursa; 2 (IQR=1-2.3) for the subcutaneous bursa; 0 (IQR=0-1) for the tendon, and 1 (IQR=0-1) for the calcaneus (Table 2). Although the median for the subcutaneous bursa was higher, no statistical significant differences were seen between the groups.

**Discussion**

In this study on patients suffering from chronic painful insertional tendinopathy, analyses of the subcutaneous and retrocalcaneal bursae, the skeletal posteroverior prominence of the calcaneal tuberosity and the ventral side of the Achilles tendon showed that the highest score of innervation, based on our semi-quantitative scale, was found in the subcutaneous bursa although this was not statistically significant. This innervation pattern is nonetheless interesting and one may speculate that it is of clinical importance as the traditional types of surgery for this condition do not
involve extirpation of the subcutaneous bursa (Wiegerinch et al., 2012). There have been thorough examinations of the retrocalcaneal bursa and its relationship with surrounding tissues (Kachlik et al., 2008a), but the subcutaneous bursa is hardly ever mentioned in studies of the Achilles tendon insertion, and could potentially be a “neglected structure” of significant importance for pain in patients with chronic

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**Fig. 3.** Immunohistochemical staining with PGP 9.5. **A.** Positive perivascular reactions in a subcutaneous bursa. Arrows indicate nerve fibres, asterisk indicates lumen of vessels. **B.** Reactions in nerve fascicle (arrow) and vessels without any reactive perivascular nerve fibres (hollow arrows) in a retrocalcaneal bursa. **C.** Haversian canal (dashed line) in bone (asterisk) with vessels and nerve fibres (arrow). a, x 60; b, c, x 40
in this present study, however, because these four patients still suffered from pain it was assumed that the expression of the PGP 9.5 positive nerve fibres analysed and signs of sonographic remodelling of the tendon, insertional Achilles tendinopathy, result in pain relief and accompanying nerves in Achilles tendinopathy and which suggest that the treatment damages the pain signalling nerves (Alfredson and Lorentson, 2007). This injections of polidocanol. It has been shown that injections of polidocanol, targeting the region of vessels and the subcutaneous bursa, a structure that is seldom mentioned to the nerve rich subcutaneous bursa. Interestingly, with the strongest expression of PGP 9.5 can be assumed to play a role in the pathology of insertional Achilles tendinopathy. Furthermore, it is known that nerves follow the vessels (Andersson et al., 2007) and that the intensity of vasodilatory nerves may belong to the autonomic nervous system and regulate the vasodilation and/or constriction, there are studies describing a role for sympathetic innervation in pain regulation (Baron et al., 1999; Teasell and Arnold, 2004), which could also be the case for insertional Achilles tendinopathy. Furthermore, it is known that nerves follow the vessels (Andersson et al., 2007) and that the intensity of vasodilation correlates to pain in tendinopathy patients (Cook et al., 2005). A similar study showed that individuals with abnormal tendon structure, but without increased amounts of neovessels, had less pain (Cook et al., 2004). Bearing this in mind it is likely that the structures examined in this study, with a high blood flow combined with the strongest expression of PGP 9.5 can be assumed to play a role in the pathology of insertional tendinopathy.

In this study four of the included patients that suffered from insertional Achilles tendinopathy had previously been treated with US and DP guided injections of polidocanol. It has been shown that injections of polidocanol, targeting the region of vessels and accompanying nerves in Achilles tendinopathy and insertional Achilles tendinopathy, result in pain relief and signs of sonographic remodelling of the tendon, which suggest that the treatment damages the pain signalling nerves (Alfredson and Lorentson, 2007). This treatment could therefore potentially affect the expression of the PGP 9.5 positive nerve fibres analysed in this present study, however, because these four patients still suffered from pain it was assumed that the nerve fibres were not significantly affected by the polidocanol treatment and the patients were not excluded from the study.

Due to ethical reasons it was not possible to include control biopsies, i.e. biopsies from patients that do not suffer from tendinopathy, including pain. This is a limitation in the study because comparison between the expression of PGP 9.5 positive nerve fibres in tendinopathy and control biopsies could not be performed, thus the relevance of the findings could be questioned.

In general, when discussing pathology in the Achilles tendon insertion the skeletal posterosuperior prominence of the calcaneal tuberosity (Haglund deformity), retrocalcaneal bursa, and the distal part of the Achilles tendon are mentioned as the possible sources for pain (Yodlowski et al., 2002; Kearney and Costa, 2010; Wiegerinck et al., 2012). It is also speculated that the paratenon is the origin of pain in tendinopathy, since it is shown to be more innervated than the tendon (Steece et al., 2014). We noticed that in the paratenon some nerve fibres were seen. Interestingly, the results of our study showed that the subcutaneous bursa, a structure that is seldom mentioned as relevant in chronic painful conditions (Josza and Kannus, 1997), was graded as the tissue with a high degree of innervation, in this group of patients with chronic painful insertional Achilles tendinopathy. To the best of our knowledge, our study is the first comparing the nerve distribution in these different tissues in patients with this chronic painful condition. It should be mentioned that the retrocalcaneal bursa has been morphologically described by Kachlik and collaborators as having a close relation to the calcaneus and ventral side of the Achilles tendon, and the biopsies collected in this study may be primarily delineating the innervation patterns in the roof, lateral and medial wall, as well as the synovial fold of the retrocalcaneal bursa (Kachlik et al., 2008a). Most often the retrocalcaneal bursa could be extracted in one piece, but a few times it had to be taken out in pieces.

Chronic painful insertional Achilles tendinopathy is well known to be difficult to treat (Myerson and McFarvey, 1998), and it might be that the varying results after surgery are due to not addressing treatment to the nerve rich subcutaneous bursa. Interestingly, with the exception of our own recently published study (Alfredson and Isaksson, 2014), we have not been able to find any reports on surgical treatment methods in which the subcutaneous bursa was removed. Not all patients with insertion tendinopathy have changes in these four different anatomical locations i.e. the proximal portion of the calcaneal bone, distal part of the Achilles tendon, subcutaneous and retrocalcaneal bursae. Therefore, we believe it is of significant importance to use US and DP in the pre-operative evaluation, to determine in which tissues there are pathological findings like thickening, structural abnormalities and

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<th>Nerve distributions in insertional Achilles tendinopathy</th>
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<td><strong>Table 2. Median score of innervation patterns in the tissues of interest.</strong></td>
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painful insertional Achilles tendinopathy. However, there are some studies describing the sonographic morphology in relation to the Achilles tendon and which mention the subcutaneous bursa in abnormal conditions (Mathieson et al., 1988), and discuss its clinical presentation with isolated bursitis (Singh et al., 2008; van Dijk et al., 2011). In this study we used the general nerve marker PGP 9.5, which is well known to be a reliable marker for nerve fibres (Thompson et al., 1983). This method provides information about the occurrence of nerves in the tissue samples, but gives no information about what type of nerves they are. It has however been shown that the number of visible blood vessels using ultrasound and Doppler is correlated to pain (Malliaras et al., 2010), and as the majority of nerve fibres in this study were found perivascularly one would expect that these fibres can signal pain to some extent. Although the majority of perivascular nerve fibres may belong to the autonomic nervous system and regulate the vasodilation and/or constriction, there are studies describing a role for sympathetic innervation in pain regulation (Baron et al., 1999; Teasell and Arnold, 2004), which could also be the case for insertional Achilles tendinopathy.

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localized high blood flow. US has been demonstrated to be a reliable method to study bursae and tendon tissue, and a prominent and impinging upper calcaneus can be detected during dynamic US examination (Mahlfeld et al., 2001). The findings from the current study are in line with previous results of biopsies taken from regions with US and DP-verified high blood flow in chronic painful mid-portion Achilles tendons, where the majority of nerves were shown to be located in close relation to blood vessels outside the painful tendons (Andersson et al., 2007).

Conclusion

In this study on patients with chronic painful insertional Achilles tendinopathy the innervation of the tendon, bone and bursae was studied. The subcutaneous bursa is of clinical interest because surgical treatment methods described for this condition in general never include removal of the subcutaneous bursa, but we found a high degree of innervation also in this structure. There is need for further clinical studies evaluating the importance of the subcutaneous bursa in patients with chronic painful insertional Achilles tendinopathy, and to study the long-term effects of removing it surgically.

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