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ANKLE

Marked pathological changes proximal and distal to the site of rupture in acute Achilles tendon ruptures

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Abstract A laboratory study was performed to evaluate the histopathological features of the macroscopically intact portion of the Achilles tendon in patients undergoing surgery for an acute rupture of the Achilles tendon. Tendon samples were harvested from 29 individuals (21 men, 8 women; mean age: 46 ± 12) who underwent repair of an Achilles tendon tear tear, and from 11 male patients who died of cardiovascular events (mean age: 61). Three pieces of tendon were harvested: at the rupture site, 4 cm proximal to the site of rupture, 1 cm proximal to the insertion of the Achilles tendon on the calcaneum. Slides were assessed using a semiquantitative grading scale assessing fiber structure and arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability, and hyalinization. Intra-observer reliability of the subscore readings was calculated. The pathological features were significantly more pronounced in the samples taken from the site of rupture than in the samples taken proximally and distal to it ($0.008 < P < 0.01$). There were no significant differences in the mean pathologic sum-scores in the samples taken proximally and distal to the site of rupture. Unruptured Achilles tendons, even at an advanced age, and ruptured Achilles tendons are clearly part of two distinct populations, with the latter demonstrating histopathological evidence of failed healing response even in areas macroscopically normal.

Keywords Achilles tendon · Rupture · Histology · Athletes · Sports

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Introduction

Acute ruptures of the Achilles tendon (AT) are the most dramatic injuries that affect the AT [1, 8, 39]. Although the strongest tendon in the body, the AT is also one of the most common tendons affected by spontaneous complete rupture [8, 16, 41]. Despite the relevance of the problem, causes and mechanisms of AT ruptures remain poorly understood [32].

Many possible etiological factors have been involved in AT rupture [9, 21, 37, 40], and these can broadly be divided into high-energy disruptions, changes compatible with a failed healing response, and mechanical imbalance [29, 30, 44]. Injuries acquired from participation in sports account for most ruptures [12, 28]. Acute injuries result from rapid force shifts to the lower limb in sports such as football, basketball, track and field, volleyball, squash, and badminton [51]. Direct injection of steroids [7, 54] and administration of systemic corticosteroids [17] and fluoroquinolones [5, 18] are associated with an increase in the risk of AT rupture. Failed healing response changes are found in most ruptured tendons, suggesting that there exists a pre-rupture phase and even a predisposition to rupture [19, 55]. Failed healing response intratendinous alterations

can be found in the tendons of people who are older than 35, and these changes have been found to be associated with spontaneous rupture [10–12]. Degenerative changes in 74 patients with a spontaneous AT rupture were hypothesized to be primary abnormalities present before the rupture [2]. Sixty-two percent of 891 tendon ruptures also revealed narrowing or obliteration of small arteries due to hypertrophy of the intima and media [11]. Most of these abnormalities had no etiologic explanation [27].

Biopsies from the site of rupture of the Achilles tendons in patients undergoing open repair for a subcutaneous rupture show profound histopathologic changes, while the tendons of aged persons with no known tendon abnormalities have, as a group, little histologic evidence of degenerative changes [33]. Macroscopically intact AT outside the rupture site showed histopathological changes in patients with spontaneous rupture [4]. Also, in ruptured human ATs, the tissue in the ruptured area undergoes marked rearrangement at molecular levels which involves matrix metalloproteases-2 activity [13].

In other tendons, histopathological studies confirmed that tendon changes are localized not only at the site of rupture, but also in the macroscopic intact tendon portion of the supraspinatus [23, 24], long head of the biceps tendon [22], and quadriceps tendon [20]. However, while in the supraspinatus tendon [23], in the long head of the biceps tendon [22], and quadriceps tendon [20], these findings were supported by a validated score for tendon histopathology [23, 38], this was not the case for the AT [4].

In the present study, the histopathological features of surgical specimens of AT tendon from patients with an acute AT rupture were analyzed.

The hypothesis of this study was that the macroscopically intact AT shows changes that may be shown by microscopic examination, as already demonstrated in the supraspinatus [23], long head of the biceps tendon [22], and quadriceps tendon [20].

The aim of this study was to compare the histopathological features of different portions of the AT harvested from patients with acute AT ruptures, using a validated tendon score.

Materials and methods

All procedures described in this study were approved by the Ethics Committee of the University of Keele Medical School.

Surgical procedure

A 50:50 mixture of 10 ml of 2% lignocaine hydrochloride (Antigen Pharmaceuticals Ltd, Roscrea, Ireland) and 0.25% bupivacaine hydrochloride (Astra Pharmaceuticals Ltd, Kings Langley, England) is instilled into an area of between 8 and 10 cm around the ruptured Achilles tendon. The patient is placed prone, and a pillow is placed beneath the anterior aspect of the ankles to allow the feet to hang free. The operating table is angled down 20° cranially to reduce venous pooling in the feet and ankles. Three 2.5 to 3 cm transverse incisions are made over the Achilles tendon. The first is directly over the palpable defect. A small piece of tendon (see below) from the proximal stump at the rupture site is removed. Another incision is made 4 cm proximal to the first incision, medial to the midline to reduce the risk of damage to the sural nerve. The posterior surface of the Achilles tendon is exposed, and a small piece of tendon (see below) is removed. A third incision is made 1 cm proximal to the insertion of the Achilles tendon on the calcaneus. The posterior surface of the Achilles tendon is exposed, and a small piece of tendon (see below) is removed.

A small hemostat is used to free the tendon sheath from the overlying subcutaneous tissue. A 1 PDS II (Ethicon, Johnson and Johnson Intl, Brussels, Belgium) double strand suture on a long curved needle is passed transversely through the distal incision passing through the substance of the tendon and out through the same incision. The needle is then reintroduced medially into the distal incision through a different entry point in the tendon and passed longitudinally through the tendon, to lock the tendon, and is directed toward the middle incision and out through the ruptured tendon end. The suture that is still protruding from the distal incision is re-threaded onto the needle and reintroduced laterally into the distal incision and into the tendon. It is passed proximally through the tendon to exit from the middle incision. Traction is applied to the suture to ensure a satisfactory grip within the tendon. The same procedure is carried out for the proximal half of the ruptured tendon. A further 1 PDS II (Ethicon, Johnson and Johnson Intl, Brussels, Belgium) double stranded suture can be placed in the tendon ends as described above in order to produce an 8-strand repair. The sutures are then tied with the ankle in neutral plantar flexion. The tension is assessed by observing the contralateral limb as the sutures are tied.

Tendon samples

Ruptured ATs (N = 29 patients)

During surgery, three biopsies samples, each about $3 \times 3 \times 3$ mm, were taken: one from the proximal stump at the site of the rupture, one 4 cm proximal to the rupture site, and one 1 cm proximal to the calcaneal insertion of the AT were obtained from 29 consecutive patients (21 men, 8 women; mean age: 46 ± 12, range 28 to 64)

who had sustained an acute rupture of the AT and underwent a percutaneous repair. All patients underwent percutaneous repair of the AT tear under local anesthesia using the technique described by McClelland and Maffulli in the period 2002–2003 [46]. All patients were operated within 48 h of presentation to the senior author, and within 2 weeks of the original injury (4.6 ± 3.1 days, range 1 to 14 days).

Nonruptured Achilles tendons from deceased patients (N = 11 Tendons)

One AT was obtained from each of 11 male patients (4 right, 7 left tendons) who had died of cardiovascular events (mean age, 61 ± 10, range 44 to 81 years). The tendon was harvested in the post-mortem room under sterile conditions through a medial approach. The tendon was freed from surrounding tissue and as much muscle and fat as possible were removed. The tendon was cut horizontally at the superior and inferior ends. From questioning the patients' relatives and from consultation of the hospital notes, it was learned that no patient had sustained an acute or overuse injury to the Achilles tendon, no patient had taken corticosteroids [50] during the past 5 years, nor had had fluoroquinolones [52].

Staining procedures

All the tendon samples were placed in 20 ml of sterile 10% formalin in a universal container for transportation to the Pathology Department. Once fixed with buffered 10% formalin, the pieces were dehydrated, embedded in paraffin, and cut at 4-μm sections. Finally, sections were stained with hematoxylin and eosin and examined both under white light and under polarized light microscopy. The harvested samples from the areas outlined above were used for histologic examination, and were fixed in 10% neutral-buffered formalin for 24 to 48 h and processed to paraffin wax. Transverse 5-mm sections were then mounted onto 3-aminopropyltriethoxysilane-coated slides and dried at 37°C overnight. All procedures were performed in batches. Sections were dewaxed in two 10-min changes of xylene, followed by one change in absolute alcohol, 95% alcohol, and 70% alcohol, for 10 min each to rehydrate the sections. The sections were then rinsed under running tap water. Sections were stained using hematoxylin and eosin.

Assessment of tendon lesions

For each tendon and each staining technique, three slides were randomly selected and examined using a light microscope (3600, SM-LUX, Leitz, Wetzlar, Germany). The identification number on each slide was covered with a removable sticker, and each slide was numbered using randomly generated numbers. After one of the authors interpreted all the slides once, the stickers were removed, a new sticker was applied, and the slides were renumbered using a new series of randomly generated numbers. The degree of staining of all the slides was reassessed by the same author, and the two results were compared. If an inconsistency (more than one grade on the scoring system described in Table 1) existed between the two results, the slides were reassessed with the help of the senior author.

The area of each specimen showing the most advanced pathologic changes was selected, and the worst possible results for each slide were used in this study. The slides were interpreted using the modified semi-quantitative grading scale [3, 6, 33, 34, 43, 47, 55] which assesses various aspects of tendon tissue. The variables included in the scale are as follows: (1) fiber structure, (2) fiber arrangement, (3) rounding of the nuclei, (4) regional variations in cellularity, (5) increased vascularity, (6) decreased collagen stainability, and (7) hyalinization. In the four-point scoring system used, 0 indicates a normal appearance, 1 indicates a slightly abnormal appearance, 2 a moderately abnormal appearance, and 3 a markedly abnormal appearance. Overall, the total score for a given

Table 1 Kappa scores for each variable

Tendons	Kappa value						
	FS	FA	*N*	RVC	*V*	DCS	*H*
All	0.74	0.68	0.78	0.76	0.75	0.63	0.62
Site of rupture	0.68	0.65	0.81	0.80	0.71	0.78	0.62
4 cm proximal to the rupture site	0.75	0.70	0.91	0.67	0.72	0.60	0.65
1 cm proximal to the calcaneal insertion of the Achilles tendon	0.71	0.69	0.73	0.81	0.58	0.77	0.71
Control	0.80	0.72	0.70	0.72	0.64	0.64	0.58

1 indicates a perfect match, and 0 represents no match

FS fiber structure, *FA* fiber arrangement, *N* rounding of the nuclei, *RVC* regional variations in cellularity, *V* increased vascularity, *DCS* decreased collagen stainability, *H* hyalinization

slide could vary between 0 (normal tendon) and 21 (most abnormal appearance detectable).

Statistical analysis

Kappa statistics were used to assess the agreement between the scoring of the slides. The chi square test was used to ascertain the association between the type of tendon (control or ruptured) and the pathologic score. Because the pathologic scores were not normally distributed, the Mann–Whitney *U*-test was used to determine whether the sum-score difference between the two tendon groups was statistically significant. A chi square for trend test was performed to evaluate the pathological changes in the ruptured AT at different time intervals between the rupture and the repair. A probability level of <0.05 was considered significant.

Results

Using the kappa statistics, the agreement between the two readings ranged from 0.58 to 0.91. The mean pathologic sum-score of ruptured tendons was greater than the mean pathologic score of control tendons ($P = 0.001$) (Table 2). Within each specific category of tendon abnormalities, the chi square test showed significant difference between the control and ruptured tendons; all the variables were significantly different ($P < 0.05$).

Table 2 Summary of pathologic scores of control and ruptured tendons

	Control tendons	Ruptured tendons
Total tendon pathologic score at the rupture site		
Mean	4.1	19.8
Median	2	14
SD	2.0	2.2
Range	1–15	13–21
Total tendon pathologic score 1 cm proximal to the insertion of the Achilles tendon on the calcaneum		
Mean	3.5	15.2
Median	2	12
SD	1.8	1.5
Range	1–11	10–17
Total tendon pathologic score 4 cm proximal to the site of rupture		
Mean	3.8	16.2
Median	2	13
SD	2.1	1.8
Range	1–13	10–21

The worst scoring result was used for each situation

The overall semiquantitative histopathological scores were not significantly different from those of the Achilles tendons operated before the 10-day mark ($P > 0.05$). The pathological features were significantly more pronounced in the samples taken from the site of rupture than in the samples taken proximally and distal to it ($0.008 < P < 0.01$). There were no significant differences in the mean pathologic sum-scores in the samples taken proximally and distal to the site of rupture ($P > 0.05$).

The following is a description of the histopathological features at the site of rupture, and proximal and distal to it.

Fiber structure

In the control specimens, the fibers were arranged close and parallel to each other with slight waviness. Increased waviness and separation of the fibers accompany slight and moderate changes (Figs. 1, 2). Markedly abnormal specimens showed loss of the finer fiber structure.

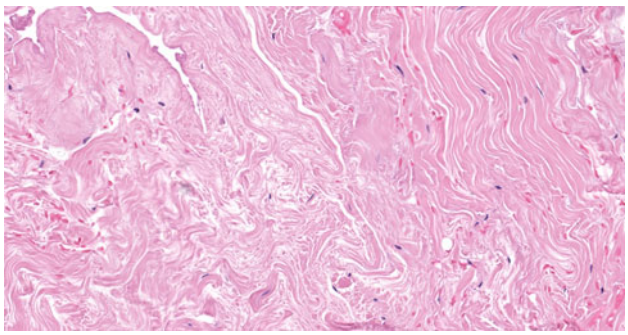

Fig. 1 Hematoxylin and eosin stain of a Achilles tendon harvested from the site of rupture. The collagen fibers have an undulating distribution, and the area is hypercellular

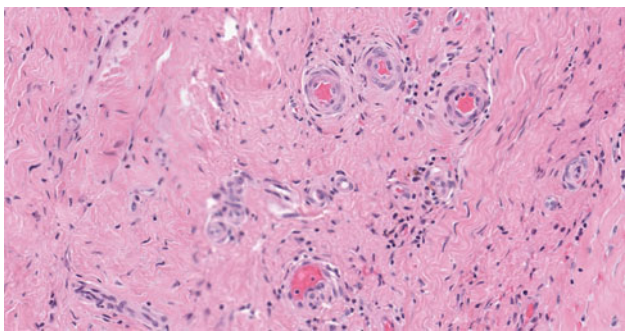

Fig. 2 Hematoxylin and eosin stain of a Achilles tendon harvested 4 cm proximal to the rupture site. The collagen fibers have an undulating distribution, and the whole area is hypercellular. Clusters of capillaries are present

Fiber arrangement

In the control tendons, the fibers were arranged parallel to each other. In ruptured and tendinopathic samples, this parallel arrangement was lost and haphazard (Figs. 1, 2).

Tenocyte nuclei

Normally, the tenocyte nuclei were flattened and spindle shaped, sometimes arranged in rows. In the ruptured and tendinopathic samples, the tenocytes first decreased in number; then, as the pathologic changes progressed, the nuclei became progressively rounded (Figs. 1, 2). In some instances, these tenocytes resembled chondrocytes.

Cellularity

The whole slide was assessed for areas of increased cellularity. The degree of cellularity was greater in the ruptured tendons than in the control tendons (Figs. 1, 2).

Vascularity

Vascular bundles usually run parallel alongside the collagen fibers. The number of these vascular bundles increases with the more advanced changes the tendon (Fig. 2).

Collagen stainability

Normal collagen colors a deep pink-red when hematoxylin and eosin stain is added. However, with degenerated collagen, the section stainability is reduced and appears paler. This pallor was graded.

Hyalinization

Very few specimens showed any evidence of hyalinization, and analytical statistics showed that this histopathological criterion was poorly reproducible.

Discussion

The most important findings of this study are that the whole of the Achilles tendons of patients undergoing percutaneous repair for an acute rupture show profound histopathologic changes, while the tendons of aged persons with no known tendon abnormalities have, as a group, little histologic evidence of pathological changes. Moreover, tendon changes are localized not only at the site of rupture, but also in the macroscopic intact proximal and distal tendon portion.

This study has a few limitations. For example, the tendons that we considered normal came from patients with various degrees of vascular disease. However, the AT is normally a relatively avascular structure, and it is thus likely that the tendon samples were representative of normality, given the age of the patients. The ideal control should not have AT pathologies. However, for ethical and practical reasons, no alternatives were possible, as it is impossible to take surgical biopsies from healthy individuals. Furthermore, we believe that the differences between the control and ruptured ATs are strong enough to justify the conclusions of this study.

Also, as aging causes at least some morphologic changes in the tendons, and, as control tendons were harvested from donors older than patients with a torn AT, the use of an age-matched control population would have further highlighted the histologic differences that we have described. When interpreting the results of this study, it should be considered that only one staining method (hematoxylin and eosin) was used. Obviously, the fact that more advanced histochemical and immunohistochemical techniques and electron microscopy—to detect, for example, extra lipids, calcium deposits, collagen denaturation, pathologic tenocyte metabolism, collagen types, and foreign materials—were not used may have resulted in an underestimation of tendon abnormalities in the control group. However, the staining employed in the present study is widely available, is cost effective, and requires little technical ability. Also, most pathologists are familiar with hematoxylin and eosin staining and are used to interpreting a variety of specimens stained in this fashion.

All surgeries performed under local anesthesia. In vitro tenocyte proliferation and extracellular matrix component production have been shown to be significantly lower in bupivacaine-treated tenocytes when compared with controls [53]. We took great care to inject the local anesthetic solution in the subcutaneous tissue, although we cannot be sure that the solution did not infiltrate the tendon. However, it is unlikely that the local anesthesia may have induced any histopathological changes in the patients of this study, because of the short-term period of time that it was in contact with the tendon tissue. The synthesis of extracellular matrix by and the proliferation rate of tenocyte can be affected by local anesthetic agents, but this is unlikely to have been the case in the patients of this study, given the short exposure time to the drugs. Further histopathological studies are required to clarify the influence of anesthetic substances on tendons.

Pathological changes may develop in the ruptured Achilles tendon in a time-dependent fashion. It would therefore be possible that, if there was a marked time interval between the rupture and the repair, some of the changes documented might not have been present at the time of rupture and be instead secondary to this time lag. To test this hypothesis, a chi square for trend test was

performed. The tendons operated more than 10 days from the rupture exhibit a statistically significant trend toward greater cellularity and collagen disorganization, but the overall semiquantitative histopathological scores were not significantly different from those of the Achilles tendons operated before the 10-day mark.

The general characteristics of the samples harvested from the ruptured tendons were in accordance with other previous reports [3, 6, 33, 34, 43, 47, 55]. The pathological features were significantly more pronounced in the samples taken from the site of rupture than in the samples taken proximally and distal to it ($0.008 < P < 0.01$). There were no significant differences in the mean pathologic sum-scores in the samples taken proximally and distal to the site of rupture.

The biopsy site was standardized with by harvesting the samples 4 cm proximal to the rupture and 1 cm proximal to the insertion of the AT. The distance from the rupture to the distal biopsy site is not always symmetrical to the tendon rupture. In some patients, 1 cm proximal to the insertion of the Achilles tendon may be closer to the tendon rupture than in other patients. However, one of the major limitations of previous histopathological studies on the AT is the lack of standardization in the site of biopsy and in the use of a validated histopathological score. Therefore, we tried to standardize the site of the harvesting. In most patients, the distance between rupture and the site of harvesting was symmetric. It should also be kept in mind that the biopsy sites reflect the region where the transverse incisions were made to expose the Achilles tendon to perform a percutaneous repair. The Ethics Committee would not have allowed other additional incisions for the purposes of harvesting a tendon sample.

It is difficult to compare the results of the present study with other study in literature, as we do not know of other study to evaluate the histopathological findings of the AT outside of the site of rupture with a well-established and validated score [33]. The histopathological appearance of AT rupture specimens demonstrated a condition of tendinous pathology similar to described by previous authors [4]. However, relatively few studies have tried to quantify the histopathological findings of tendinopathy [22–24], and the histopathological changes are currently described in a subjective or, at best, semiquantitative fashion. This may result in uncertainty about the histopathological findings of tendinopathy and has produced a lack of diagnostic uniformity among surgical pathologists. Probably, the pathological diagnoses in the different studies should follow an accepted classification scheme, thus allowing data comparison and combination. Several centers are undertaking studies on the tendinopathy [42, 48, 49], and it is possible that the individual studies may not be large enough to result in significant power for reliable evaluation. Therefore, combining the data from those studies with a similar study design is essential. Consistent high-quality pathology data are thus remarkably important for the success of the studies.

Aging may result in functional and structural changes in human tendons, with an increase in total collagen content and collagen fiber diameter and a decrease in collagen turnover. The increase in collagen fiber diameter is probably a consequence of several smaller fibrils becoming mechanically coupled so they can transmit mechanical stresses in concert [14, 15]. However, there is little proof that tendons from healthy, older persons exhibit histologic evidence of degeneration, and this is confirmed by the results of the present investigation. The changes in both cellular and fibrous components, with decrease in the average maximum diameter and density of collagen fibrils and an increase of fibril concentration, are most likely related to the decreased functional requirements [25, 26]. In healthy animals, the mechanical properties of tendons remain constant after the end of growth well into senescence [33].

In the present study, each slide was scored twice by one of the authors who has great experience in this field. Despite specific training, the agreement of blinded assessment for the various components of the scoring system is, at best, acceptable (Table 1). This underlines how difficult it can be to recognize specific patterns in tendon abnormalities, and the importance of having well-trained individuals to interpret the slides, especially if only a limited number of histologic techniques are used [33]. To improve the reproducibility of these readings, the assessment would have to be performed several times, with the slides being randomly reordered each time. Also, large populations of samples or other methods of assessing performance, possibly with weighted outcomes, would be required. Finally, two or more researchers scoring the tendons would decrease observer bias. Whether these methods could be implemented in clinical practice or in research studies is open to discussion.

In concert with previous investigations [3, 6, 33, 34, 43, 47, 55], we used a semi-quantitative assessment of the tendinopathic lesions observed. We are conscious of the limitations of this assessment system, as a qualitative evaluation of several aspects of the histopathological appearance of the tendon section examined is categorized into four classes (from 0, i.e. fully normal, to 3, i.e. markedly abnormal). It is desirable that the fully automated image analyses systems used in other fields of musculoskeletal medicine will be used in this field as well and thus allow a more objective quantification of the abnormal appearance of tendinopathic tendons.

In the present study, tendon changes were not only localized at the site of rupture, but also occur in the

macroscopically intact AT both proximal and distal to the site of rupture. A clinically relevant finding of this study is that surgical procedures that include a turn down flap may not be the optimal option [31, 32], as the macroscopically intact AT exhibits pathological features as well, and the failed healing response is not limited to the site of rupture. Probably, the tendon itself does not contribute to healing [35, 36].

Conclusions

In conclusion, unruptured ATs, even at an advanced age, and ruptured ATs are clearly part of two distinct populations. In ruptured ATs, the collagen appearance is abnormal [45]. Tenocytes from ruptured tendons produce greater quantities of type III collagen than tenocytes from normal tendons [34]. This altered production of collagen may be one reason for the histologic alterations described in this study and may result in the tendon being less resistant to tensile forces, and thus at increased risk of rupture. The pathological features are more marked at the site of rupture but are widespread throughout the tendon, with areas proximal to the site of rupture being more affected, from a histopathological view point, that the areas distal to it. This may have implications in the choice of reconstructive procedures.

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