ORIGINAL PAPER

Morpho-functional changes in human tendon tissue

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SUMMARY

Insertion tissue biopsies of right arm common extensor tendons from 11 patients with chronic lateral epicondylitis were processed for light and electron microscopy. The subjects were aged between 38 and 54 years (only one was 25). The specimens showed a variety of structural changes such as biochemical and spatial alteration of collagen, hyaline degeneration, loss of tenocytes, fibrocartilage metaplasia, calcifying processes, neovascularization and vessel wall modifications. Tissue alterations were evident in limited zones of the tendon fibrocartilage in which the surgical resection was generally visible. The areas where the degenerative processes were localized, were restricted and in spatial contiguity with morphologically normal ones.

The observed cases presented histological and electron microscopic findings that characterize lateral epicondylitis as a degenerative phenomenon involving all tendon components.

INTRODUCTION

Normal tendons contain bundles of I-type collagen oriented along the long axis of the tendon; among them, there are thin tenocytes, arranged longitudinally, again parallel to the tendon long axis. Normally, tendon fibers are composed of collagen fibrils embedded in a matrix of proteoglycans, glycosaminoglycans and water (Reale *et al*., 1981; Woo *et al*., 1994; Teitz *et al.*, 1997; Kraushaar *et al.*, 1999).

With the ageing process, tendon tissue can undergo particular morphological changes, mostly represented by an increase in intercellular matrix, larger collagen fibre appearance, and a decrease in elastic fibre number. An increase of glycosaminoglycans (GAG) also occur (Ippolito *et al.*, 1980; Chard *et al.*, 1994; Tuite *et al*. 1997). Benazzo *et al.* (1996) in a study on athletes undergoing surgical exploration of Achilles and patellar tendons, observed a return to full activity in 79% of the younger patients, where a minor degree of tendon degeneration was present.

Mechanical load is regarded as the most important etiologic factor in disorders affecting human tendons (Khan *et al.*, 1999; Movin, 2000) and important changes affect tendon tissue in pathologies such as lateral epicondylitis (Organ *et al*., 1997; Kraushaar and Nirschl, 1999).

Previous studies correlated the term "tendon inflammation" to lateral epicondylitis; now "tendinosis" is more correctly used, implying tendon

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degeneration in the absence of clinical or histological signs of an inflammation (Goldie 1964; Nirschl 1988; Nirschl 1992; Jozsa and Kannus, 1997; Maffulli *et al*., 1998; Kraushaar *et al*., 1999).

Recent reports underlined the presence of vascular proliferation and focal hyaline degeneration. These aspects, together with patient insensivity to anti-inflammatory therapy, support the hypothesis of a degeneration process, rather than an inflammatory one (Coonrad *et al*., 1973; Regan *et al.*, 1992; Leadbetter, 1992; Mosier *et al*., 1999).

Pre-surgical magnetic resonance imaging confirmed histological patterns (Chard *et al*., 1994; Galliani *et al.*, 1997; Galliani *et al*., 1998) in terms of tendon tissue degeneration and progressive microdisruption of collagen fibres. To date, more powerful technical approaches, such as ultrasonography, add further information to tendon pathology understanding (Potter *et al.*, 1995; Astrom *et al.* 1996; Pfahler *et al.*, 1998; Paavola *et al.*, 1998; Kartus *et al.*, 2000).

Evidence of tendon degeneration has been referred to virtually all human tendons. According to Kainberger *et al.*(1997), tendons show different morpho-functional patterns, depending on focal hypovascularization, biomechanical overload or secondary degeneration.

Epicondylitis, also known as "tennis elbow" (lateral epicondylitis) can not be exclusively associated to mechanical, sport-dependent, insults (Guidotti, 1992), but a less serious form of tendinosis is evident in subjects frequently carrying heavy loads.

In this study, tendon biopsies were analysed from 11 subjects involved in a variety of professional jobs (i.e. truck drivers, typists and others) all undergoing surgery. Fragments, from hand common extensor tendons were studied by light (LM) and electron (TEM) microscopy.

MATERIALS AND METHODS

Hand common extensor biopsies were withdrawn and fixed immediately after surgery. For LM, fragments were fixed with 10% formaldehyde, alcohol dehydrated and embedded in paraffin.

Histological examination was performed by means of staining with Azan-Mallory (specific for collagen fibres), Weigert- picric acid (differentially staining elastic and collagen fibrils, in brown and yellow,

respectively), Alcian blue at acid pH (to show GAG) and toluidine blue (for metachromasia).

For TEM examination, fragments were size-reduced, fixed with 2.5% phosphate buffer glutaraldehyde, post-fixed with 1% OsO_4, alcohol dehydrated and embedded in araldite. Thin sections, stained with uranyl acetate and lead citrate, were observed with a Philips CM10 electron microscope at 80 kV.

RESULTS

Large area LM observations allowed the detection of tissue lesions and changes, while TEM gave a more detailed insight of tendon component modifications. The characteristic pathological pattern present in all the examined cases was hyaline degeneration. It seemed to affect collagen fibres, which revealed the classic "glassy" aspect, and appeared size-increased and focally more homogeneous, frequently losing their periodicity. This feature is commonly correlated to the decreasing cell number and collagen fiber stainability (Maffulli *et al*., 2000) (Fig. 1a).

In patient A. (25 yr) wide domains of collagen-free cell matrix and neoformed capillaries, as well as areas containing calcium concretions, were evident (Fig. 1b). These calcium deposits did not always stain well with the von Kossa method, and this may indicate that the mineralization stage was still in progress (Chard *et al*., 1994). Needle-like crystals also appeared in tenocytes, probably associated with mitochondria (Fig. 2a) as previously described (Regan *et al*., 1992; Galliani *et al.*, 1997).

Progressive calcification, here described both with LM and TEM, had been previously identified in this case also by radiography (data not shown). It appears in ageing tendons, and is commonly considered a sign of degeneration (Ippolito *et al.*, 1980).

In another case (patient G., 42 yr), hyaline degeneration involved wide areas of tendon tissue, characterized by evident morphological and histochemical changes, which appeared as pale-blue regions with abnormal collagen and matrix production. Close to the surgical resection site, cavities appeared among collagen fibres and within fibrocartilage, filled with necrotic debris and small concretions undergoing calcification (Fig. 1c).

In another patient (S., 41 yr), damaged chondrocytes, surrounded by strongly dilated niches,
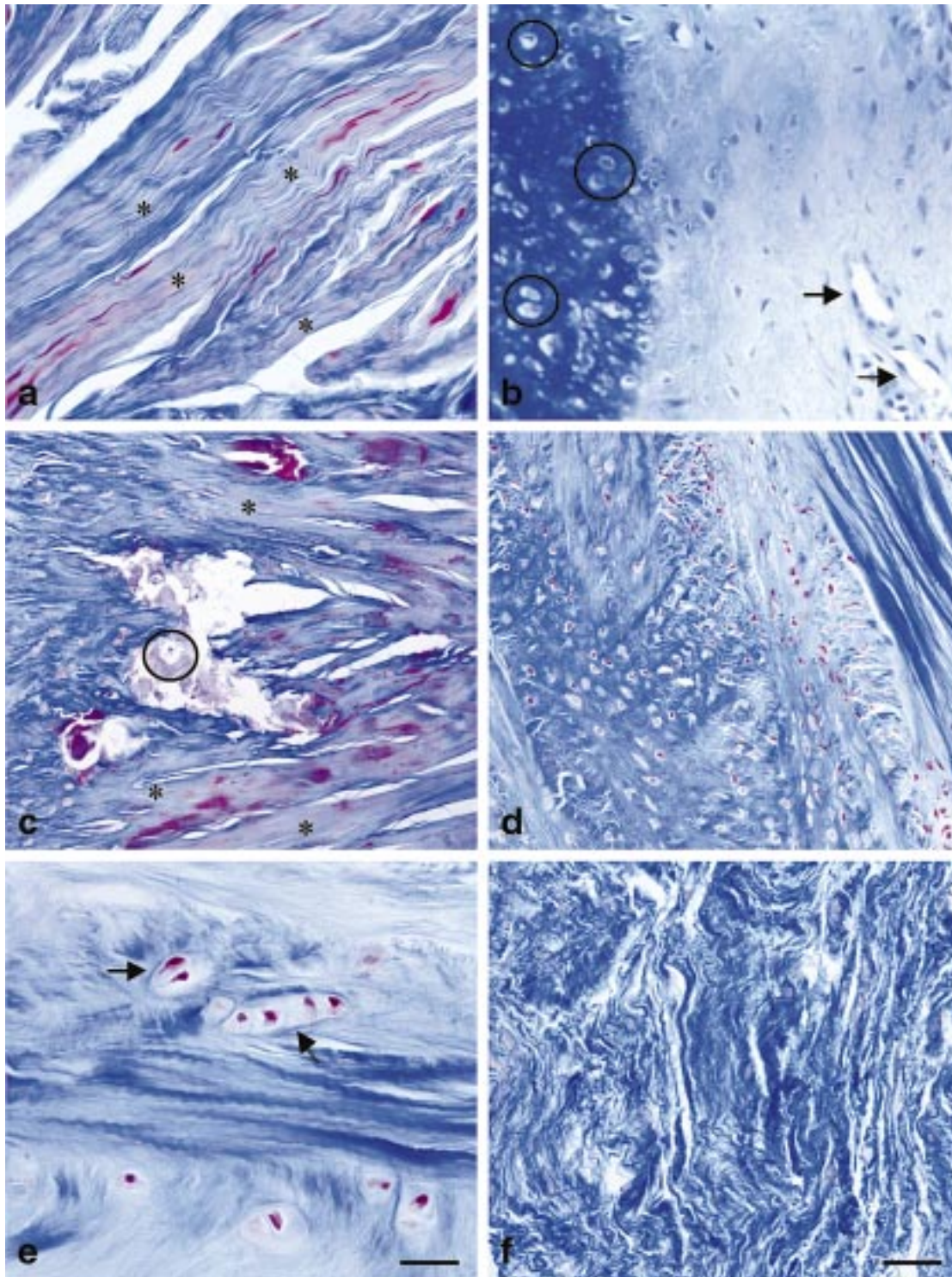
Fig. 1 - Hyaline degeneration (�֍) and decreasing cell number (a). Calcium concretions (○) and areas with increasing intercellular matrix, with neoformed capillaries (→) (b). Hyaline degeneration (✖) surrounding cavities with necrotic debris and microconcretions (○) (c). Metaplastic cartilage (d). Damaged chondrocytes in large niches (→) (e). Collagen fibril size variability and fragmentation (f). Bar: 50 µm (a, b, c, d, f). Bar: 25µm (e) Azan-Mallory (a,c,d,e,f) and toluidine blue (b) stain.
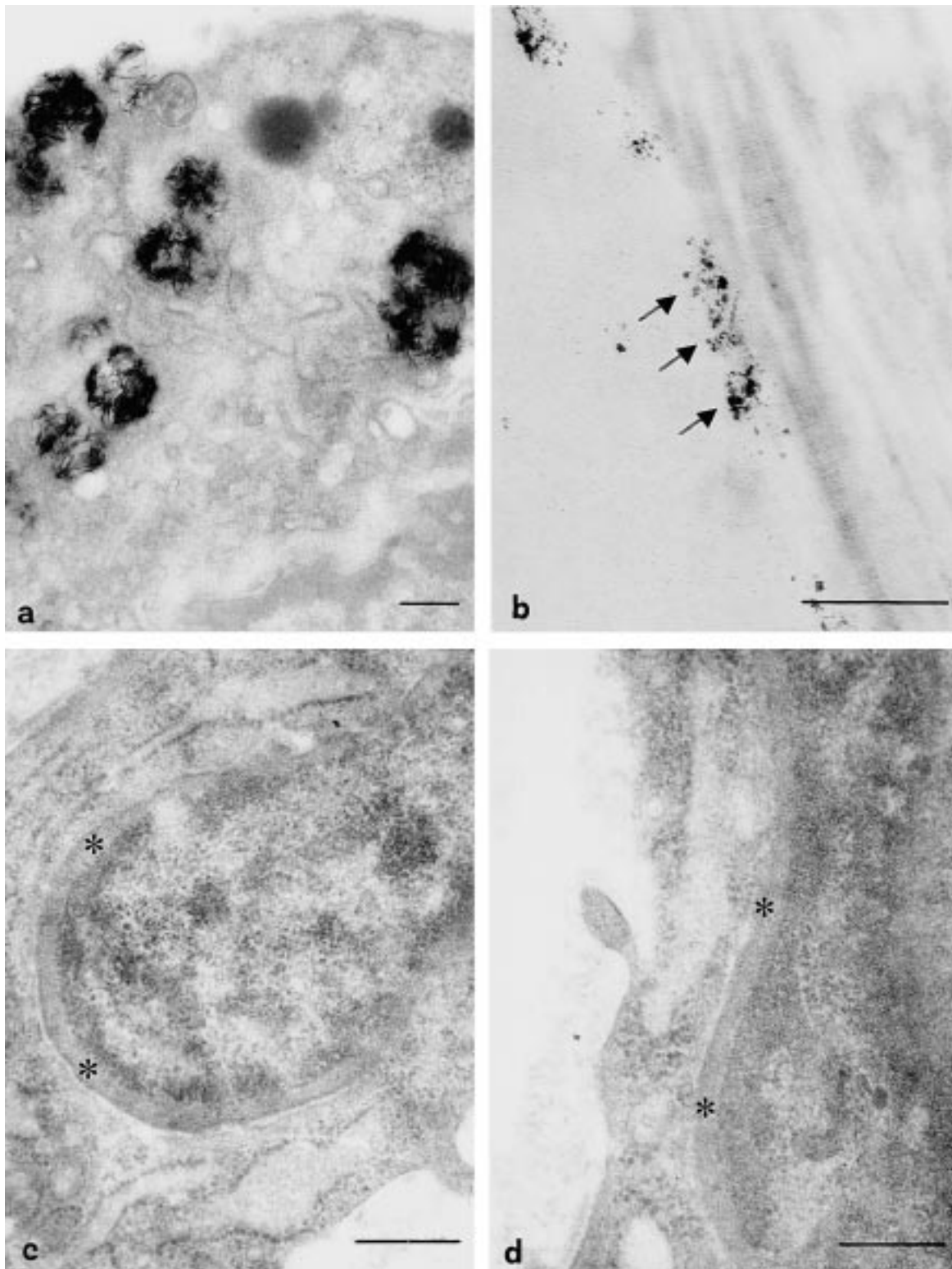
Fig. 2 - TEM observation of tendon. Needle-like, electron-dense crystals in tenocyte cytoplasm (a). Mineral crystals (→) in the extracellular space, along collagen fibrils (b). Thickened nuclear lamina (�֎) in epicondylitis tenocytes (c and d). Bar: 0.25 µm.

could be noted. Therefore, metaplastic cartilage was probably formed - as also revealed in other cases - characterized by cells enclosed in large cavities, containing a homogeneous matrix (Fig. 1d,e). In this case, TEM evidentiated the presence of calcium crystals along collagen fibrils (Fig. 2b*)*, possibly representing an early stage of the calcium deposition phenomenon.

Tenocytes also occasionally showed thickened nuclear lamina (Fig. 2c), comparable to that observable in other patients (Fig. 2d) and previously reported in phlogistic chronic diseases and tendon sheath tumour (Ghadially *et al*., 1980; Borghetti *et al*., 1995; Galliani *et al*., 1998; Goldberg *et al.*, 1999; Hutchinson *et al*., 2001).

Sometimes, tenocyte nucleus displayed the typical chromatin changes suggestive of apoptosis (Fig. 3a).

In case V. (48 yr) hyaline degeneration occurred again and collagen fibres appeared size-variable, waved, separated and occasionally fragmented (Fig. 1f). Characteristic collagen fibril scarcity and extracellular matrix abundance have been also demonstrated by TEM (Fig. 3b).

In some cases, Alcian blue specifically stained a pericellular area in fibrocartilage (Fig. 4a*,*) indicating a marked proteoglycan reaction.

TEM observations (Fig. 3c) showed altered collagen bundles which appeared focally resistant also to electron microscopy stains, thus suggesting their biochemical rearrangement (Putz *et al.*, 1995; Birch *et al.*, 1999).

Case D. (47 yr) presented the strongest and most diffuse signs of degeneration. Normal tendon tissue was indeed replaced by compact altered collagen fibres (Fig. 4b) or, differently, by an Alcian blue positive collagen network, with a wide interposed cell matrix (Fig. 4c). Numerous small necrotic cavities were also observable and the fibrils appeared fragmented into short segments. Also TEM analysis confirmed deep cell alterations (Fig. 3d).

The last cases (F.,49 yr; G.,49 yr; B., 47 yr) showed patterns of hyaline degeneration, irregularly spaced collagen fibrils, fibrocartilage niches containing damaged chondrocytes and little cavities filled with necrotic debris (Fig. 4d).

Elastic fibres, as identified by specific Weigert technique in paraffin embedded sections and TEM analysis (data not shown), are the only tendon component which does not seem to undergo relevant morphological changes, even if the evaluation of their possible quantitative variations is still in progress. However, when the spatial organization of the collagen fibres is altered, the elastic fibres also undergo a deep spatial rearrangement (Fig. 4e).

When peritenonion septa, which, in physiological conditions, draw vessels into the inner tendon, appear at LM, a number of vessel wall alterations can be seen, also generating their obliteration (Fig. 4 f,g).

DISCUSSION

In the present study, close to all observed specimen areas, normal regions could be carefully analysed, which represented a basis for comparison with normal tissue morphology.

The degenerative process underlined by LM and TEM affects all tendon components, i.e. tenocytes, collagen fibres and extracelllar matrix. Most important structural changes lead to a general alteration of tendon organization, particularly concerning collagen and matrix.

Hyaline degeneration is present in numerous and wide biopsy areas. Collagen bundles reveal a deep histochemical alteration which determines fibril thickening and homogeneity as well as their scarce stainability to LM and TEM methods.

The fibrils do not form fascicles and often are visibly fragmented into short segments. The loss of collagen fibre organization affects the function of tendon , and, particularly, its responsiveness to tension stimuli, which is closely dependent on tendon collagen component.

Further damage is caused by cartilage metaplasia; in some cases, with the progressive tendon degeneration and concomitant loss of function, we have found that the cartilage metaplasia results in fibrocartilagineous changes it was characterized by tenocyte rounding and pericellular expression of GAG. Cells appeared included in large niches, containing even more than four cells. When compared to degenerative mechanism related to collagen fibre rearrangement, cartilage metaplasia can be considered as an intermediate stage, which reduces tendon mechanical resistance and increases its fragility (Wren *et al*., 2000).

Age-related changes affecting the collagen component, have been described by Riley *et al.* (1994) in rotator cuff adult tendon, where GAG composi-
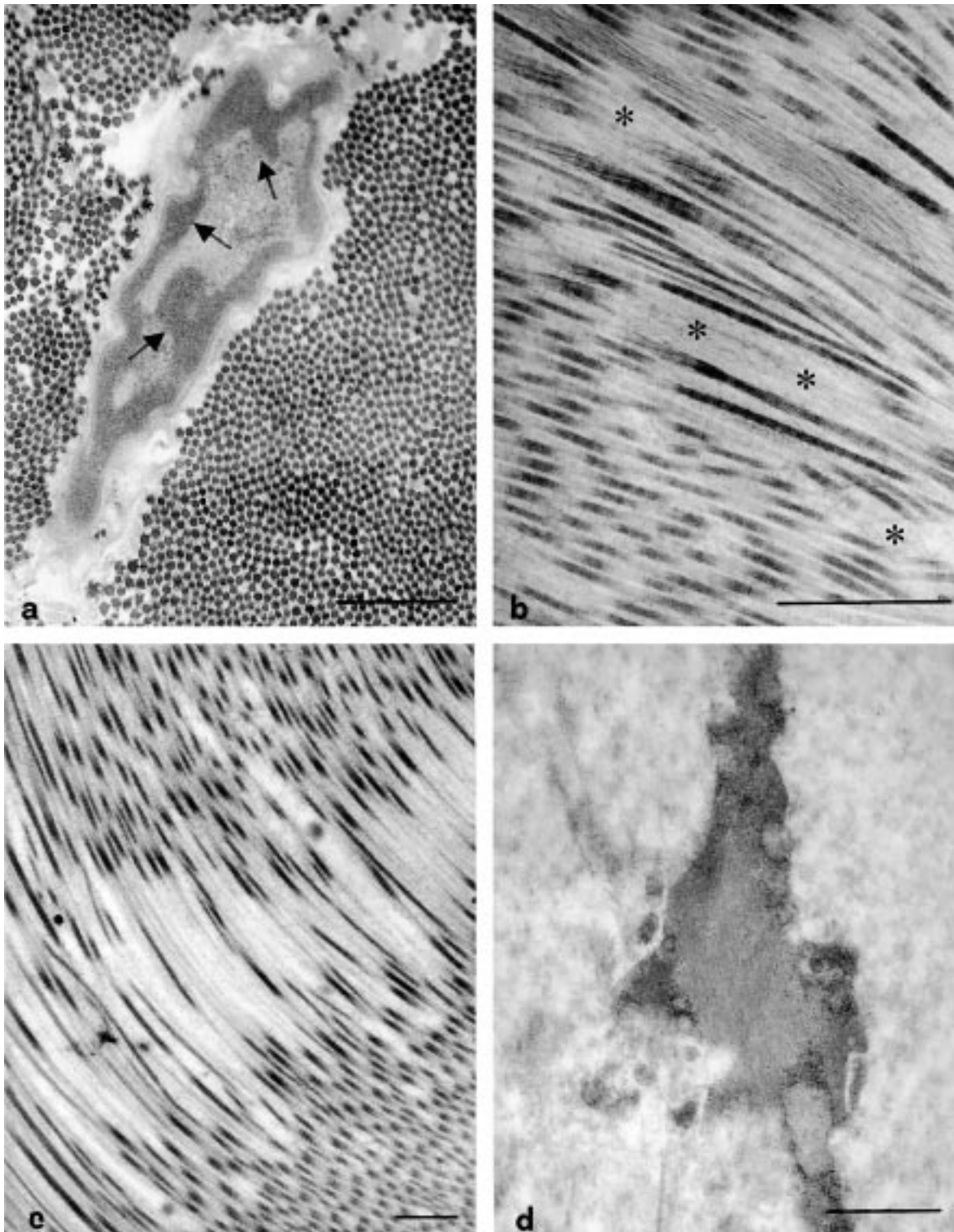
Fig. 3 - TEM features in epicondylitis tendon tissue. Tenocyte with apoptotic-like chromatin nuclear changes (→) (a). Abundant extracellular matrix within collagen fibrils (✳) (b). Focally unstained, modified collagen fibrils (✳) (c). Deeply altered tenocyte (d). Bar: 0.50 µm.
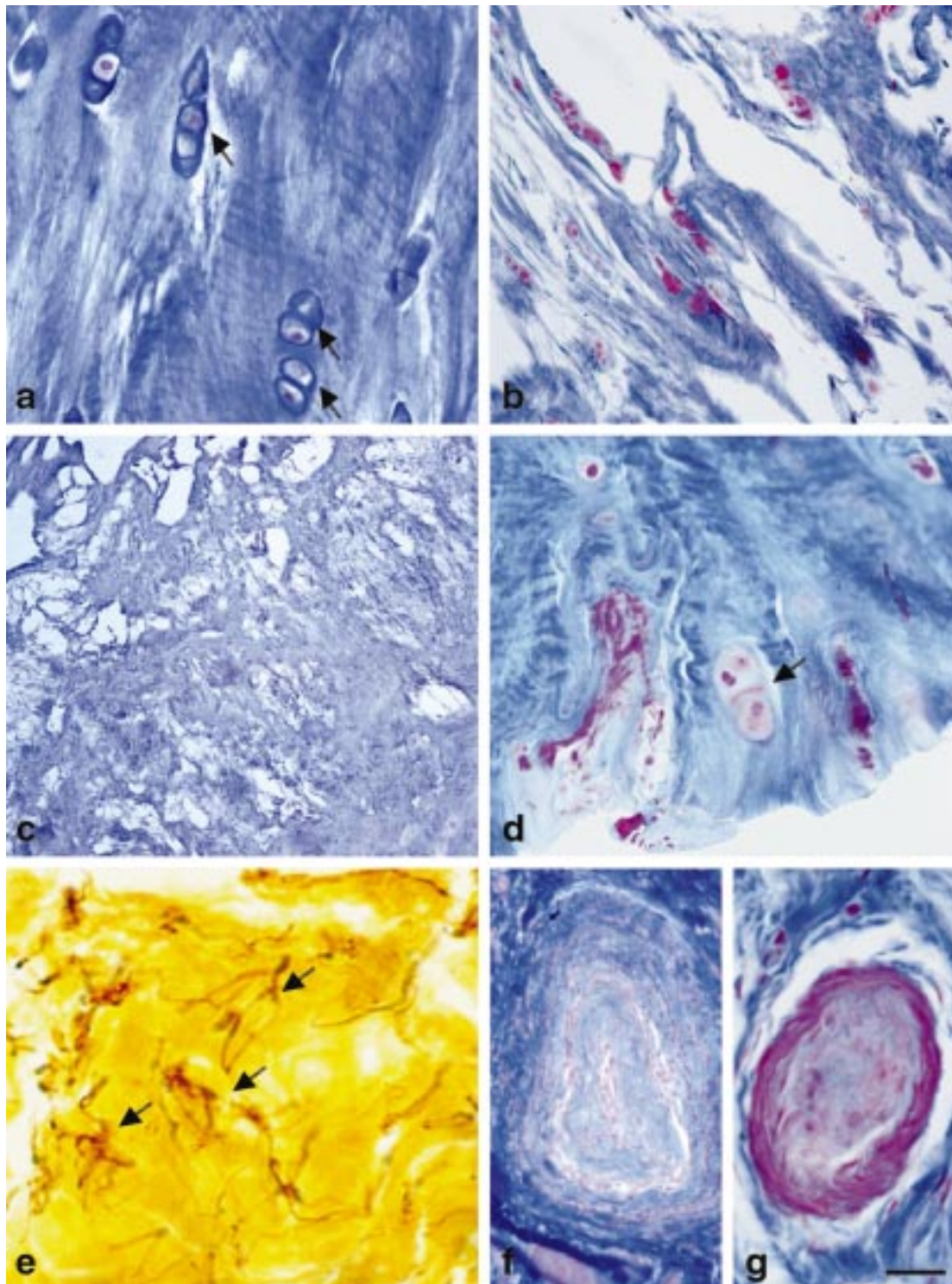
Fig. 4 - Fibrochondrocyte pericellular positivity to proteoglycans (→) (a). Very strong alterations of tendon morphology (b). Diffuse proteoglycans reaction (c). Small necrotic cavities and damaged chondrocytes (→) (d). Visible spatial rearrangement of elastic fibres (→), stained with Weigert-Picric Acid (e). Vessel obliteration and wall alterations (f,g). Bar: 25 µm (a, d, e, f, g). Bar: 50 µm (b, c). Alcian blue (a,c); Azan-Mallory (b,d,f,g) and Weigert-picric acid (e) stain .

tion was studied, to score its change with ageing and inflammatory processes. These authors also analysed specimens from "supraspinatus" and "biceps" and the corresponding values of chondroitin sulphate, keratan sulphate, dermatan sulphate and hyaluronic acid, but the results are not comparable to one another.

Complex interactions relate cell and matrix, and it could be hypothesised that repeated damage leads to matrix synthesis alterations and also produces an irregular spatial organisation, rich in collagen fibres (Holmes *et al.*, 2001).

Alterations in fibrocartilage, which represents the terminal tendon portion close to bone insertion, have been detected by LM findings. Numerous small cavities, filled with necrotic material, appear indeed among tenocyte-chondrocyte rows. Damaged chondrocytes are also frequently observable.

In the present study, the vascularity in tendinosis was frequently abnormal. Blood vessels appeared to be indeed deeply altered and were frequently obliterated.These patterns could justify, by themselves, the tendon degeneration or disruption (Uhthoff *et al.*, 1976; Wirth *et al.*, 2000).

The thickening of the nuclear lamina, already described by others (Ghadially *et al.*, 1980; Borghetti *et al.*, 1995) and ourselves (Galliani *et al.*, 1998), represents an unusual and interesting pattern. Peripheral lamina is a component of the nuclear matrix, a proteic structure supporting and regulating nuclear shape and functions (Berezney and Coffey, 1977; Falcieri *et al.,* 1992). In particular, it supports the nuclear envelope and, on the other hand, allows chromatin attachment. Moreover, it mediates nuclear-cytoplasmic molecular traffic, being spatially and functionally correlated to nuclear pore complexes (Goldberg *et al.*, 1999). The significance of its peculiar alteration, here described in a number of patients undergoing epicondylitis, is still unclear. On the other hand, the nuclear lamina represents a highly dynamic structure, possibly deeply modified by chronic diseases (Hutchinson *et al.*, 2001), such as epicondylitis. Further studies are in progress to investigate, together with lamina ultrastructural features, its possible qualitative changes, in terms of distribution of lamins (A, B, C), its main components.

Apoptotic features in tenocytes also show an intriguing pattern. Although this phenomenon has been described in chondrocytes and other connective tissue cells (Hashimoto *et al.*, 1998; Kouri *et al.*, 2000), few reports are available about its role in tendon. Apoptosis seems indeed to occur in this tissue too, after injury (Kakar *et al.*, 1998) or in development (Sulik *et al.*, 2001). We suggest that this could be correlated to a tissue response to a chronic pathological condition. In any event, morphological features have to be confirmed by biochemical or flow-cytometric analysis, giving further information, particularly concerning cell DNA (Falcieri *et al.*, 2000).

To conclude, the observed cases present histological and electron microscopic findings that characterize lateral epicondylitis as a degenerative phenomenon, involving all tendon components. In addition, in people who do not practice sports, lateral epicondylitis is caused by repetitive injuries that arouse chronic microtraumatic events in the tendon tissue (Clements *et al.*, 2001).

ACKNOWLEDGEMENTS

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