Surg Radiol Anat (2014) 36:17–24
DOI 10.1007/s00276-013-1136-y

ORIGINAL ARTICLE

Multidisciplinary approach to the persistent double distal tendon of the biceps brachii

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Received: 23 July 2012 / Accepted: 4 May 2013 / Published online: 26 May 2013

Abstract
Purpose The aim of this study is to correlate the ultrasound (US) appearance of the persistent double or bifid distal tendon of the biceps brachii muscle with anatomical and histological data. This will provide a new model to study the pathological distal biceps brachii tendon (DBBT).
Methods The DBBT of 20 cadaveric elbows were examined with linear array broadband US transducers (frequency band 14–6 MHz) using an anterior approach. Trypan blue dye was injected underneath the paratenon under US guidance in 16 specimens. After they were dissected, five of them were processed to obtain histological slices stained with hematoxylin–eosin and antiserum to protein S100.
Results At US, the DBBT is a tendon in which the fascicles are organized in two different hyperechoic components separated by a hyperechoic septum related to the endotenon. The endotenon is lax, flexible, and makes folding and gliding of the two portions feasible. The DBBT is surrounded by a hyperechoic paratenon adjacent to the tendon surface, which is only differentiable by US when dye is interposed between such structures.
Conclusions The connective septum of endotenon located between the two main components of the DBBT is responsible for the US image of two separate tendons and functionally enables it to work as two separate entities, thus allowing respective folding and gliding. The paratenon surrounding the lacertus fibrosus and the DBBT plays an important stabilization role, enabling them to change shape and arrangement during joint motion. It is also an important conduit for nerves and blood vessels.

Keywords Distal brachii biceps tendon · Ultrasonography · Tendon anatomy · Tendon histology · Bifid tendons

Introduction

Several clinical literatures have dealt with the structural anatomy of the biceps brachii muscle (BBM) and the distal

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biceps brachii tendon (DBBT), focusing on different morphological aspects and clinically relevant features [1, 4, 6, 13, 19, 21]. From the morphological point of view, two main patterns of the DBBT have been described, a single DBBT and a DBBT with two separate components [7, 9, 20, 24]. In both cases, there is agreement in the literature that the fascicles corresponding to the long head (LH) of the muscle are characterized by a deeper insertion on the proximal aspect of the ulnar side of the radial tuberosity, whereas fascicles that correspond to the short head (SH) insert more superficial on the distal aspect of the ulnar side of the radial tuberosity. Due to this peculiar arrangement of the fascicles and the fact that the fingerprint is oriented perpendicularly to the myotendinous junction (MTJ) of the BBM, the DBBT rotates 90° assuming a helicoidal twisting [1, 5, 20].

Recent advances in imaging techniques have highlighted new features of the DBBT. Magnetic resonance imaging (MRI) and ultrasound (US) have demonstrated that a common division in two components of the DBBT, the bifid DBBT, exists. This seems to have implications in case of partial DBBT tears [7, 24]. In addition, US has shown independent gliding between LH and SH during real-time dynamic examination [24]. Also, there exists biomechanical data that confirms a different efficiency of the LH and SH during flexion and supination of the forearm [15].

The aim of the present study is to correlate the persistent US appearance of the double or bifid DBBT with anatomical and histological data. The tendon morphology is discussed pointing out functional and clinical perspectives.

Materials and methods

This study was based on 20 cryopreserved upper extremities from 13 cadavers (6 women and 7 men, aged 70–85, 9 right sides and 11 left sides). All specimens derived from liberal donation to the Faculty of Medicine. The upper extremities were analyzed with US, gross anatomy, and histology.

Ultrasound study

Ultrasound examination was performed in all specimens using an anterior approach with extended elbow and supinated forearm, in the area between the middle third of the arm down to the forearm. The BBM and surrounding structures were evaluated systematically. Special emphasis was given to the DBBT. Imaging was based on short-axis planes to identify the architecture of tendon fascicles. In order to avoid anisotropy (artifact related to an incorrect alignment of the probe relative to the tendon axis), the transducer was tilted along the tendon length to make the beam perpendicular to the tendon fibers to preserve the fibrillar echotexture.

In 16/20 specimens, 3 ml of trypan blue solution (1 ml of trypan blue and 2 ml of saline) was injected underneath the paratenon under US-guidance. In each of them, the injection site was established with US at the level of the elbow joint line. For this purpose, we used a conventional intramuscular needle and a Mindray M5 (Shenzhen, China) US scanner equipped with a broadband linear array electronic transducer (frequency band 14–6 MHz).

Anatomic study

5/20 specimens were frozen after injection and cut transversally. 15/20 specimens were stratigraphically dissected. We systematically removed the skin, the superficial fascia including cutaneous nerve endings and the superficial venous plexus. The deep fascia was opened [trying to leave the lacertus fibrosus (LF) intact as much as possible] to access the different compartments and isolate the DBBT. After this preparation, the DBBT was detached. It was cut proximally at the level of the muscle belly and distally at its bone insertion. On 10/20 specimens, the paratenon was removed and the arrangement of tendon fascicles was observed. On five cases, the paratenon was preserved and the specimen processed histologically.

Histological study

Five DBBT specimens were processed histologically to obtain fine short-axis slices. Each specimen was initially divided in four transverse blocks of similar size and independently embedded in paraffin. 7 μm thick slices were obtained and stained with either hematoxylin–eosin (HE) for a panoramic view or processed for immunohistochemistry using antiserum against S100 protein to stain neural structures, as described elsewhere [8]. Images were recorded using transmitted light microscopy. We also took advantage of the fluorescent properties of eosin to obtain fluorescent images with a high degree of contrast among connective tissue structures. All preparations were examined under a Nikon Eclipse E800 Microscope equipped with epifluorescence accessories.

Cross-section area study

Cross-section area (CSA) was measured and compared in 10 specimens. All of the specimens were measured by ultrasound. Of these specimens, five were further studied by histological cross-section slices and five were further studied by anatomic transverse cuts. To establish a correlation across techniques, we considered the width and thickness of the DBBT at the level of the joint. Also, we considered the CSA by applying the ellipse area formula ($CSA = \pi \times width \times thickness$).

Results

Ultrasound study

All specimens showed an intramuscular tendon into which the LH and the SH of the BBM were seen converging. More distally, the LF arose medially and the DBBT laterally. At the point where muscle fibers were no longer detected, the LF image became undefined as it merged with the deep fascia of the forearm. In all specimens, the DBBT was recognized as two different hyperechoic cylindrical components of tendon fibers separated by a hyperechoic central septum. Distinguishing the central septum from the two tendon components was relatively unfeasible when the US beam was oriented 90° to the tendon axis. The septum became visible by tilting the probe and orienting the beam obliquely to the tendon fibers. With this maneuver, the two tendon bundles became hypoechoic as a result of anisotropy, whereas the central septum remained hyperechoic. From cranial to caudal, the more lateral component corresponded to the LH and, as it reached the fingerprint, it appeared deeper and located underneath the fibers of the SH. The SH was at first recognized in a more medial position and then became more superficial. A paratenon surrounding the DBBT was recognized although it was unfeasible to identify the limits with the epitenon. In all injected specimens (16/20), the needle tip was successfully inserted under the paratenon. During injection, the dye filled a virtual space between the paratenon and the DBBT and it was seen as an anechoic fusiform image (Fig. 1).

Anatomic study

At the level of the MTJ, the two components of the DBBT showed a spatial correlation with the LH and the SH of the BBM. The muscular portion of the LH of the BBM converged into the lateral aspect of the intramuscular DBBT and the muscular portion belonging to the SH of the BBM converged into the medial aspect of the intramuscular DBBT. The arrangement of the DBBT tendon along its course in two main components appeared on anatomic transverse cuts as previously described on US study (Fig. 2). A more clear view of tendon twisting was obtained. The tendon twisted externally in both arms. Nevertheless, the SH had a more straight course, while the LH folded from the lateral side of the tendon to the inferior side.

A closer look to the two tendon components showed a parallel fibrillar arrangement. The two components of the tendon were found to be joined together by a septum oriented longitudinally, which was an extension of the endotenon. In 3/15 dissected (2/3 bilateral) specimens, the two tendon components were well individualized and, after removal of the paratenon by dissection, they could be easily separated because of intervening adipose tissue, areolar connective tissue, and blood vessels in between them.

A paratenon investing the DBBT and the LF was always observed and it resulted to be more consistent proximally, at the level of the LF. In all the injected specimens, the dye solution remained confined underneath the paratenon and surrounded the DBBT and the proximal aspect of the LF (Fig. 3).

Also, during dissection, we could observe anatomical variations in three specimens, all concerning to a supernumerary head of the proximal origin of the BBM. These variations did not modify the morphology of the DBBT.

Histological study

In all specimens and along the whole DBBT, tertiary fiber bundles (in which tendon fascicles are organized), assumed

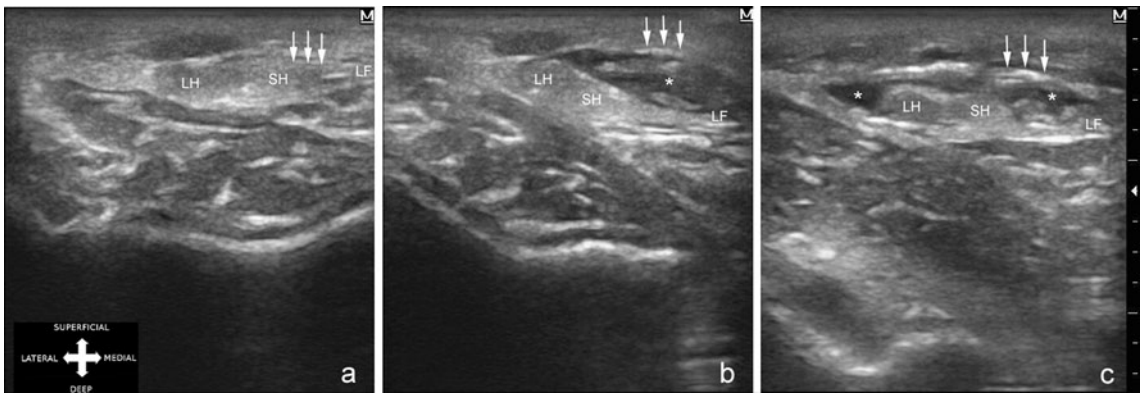

Fig. 1 Ultrasound images before, during, and after trypan blue solution injection between paratenon and DBBT. **a** Visualization of the elbow articular line and the DBBT. **b** Trypan blue solution diffusion pattern when injected under the paratenon. **c** Trypan blue solution distribution after injection. *LH* long head of the DBBT, *SH* short head of the DBBT, *LF* lacertus fibrosus, *arrows* paratenon, *asterisk* trypan blue solution

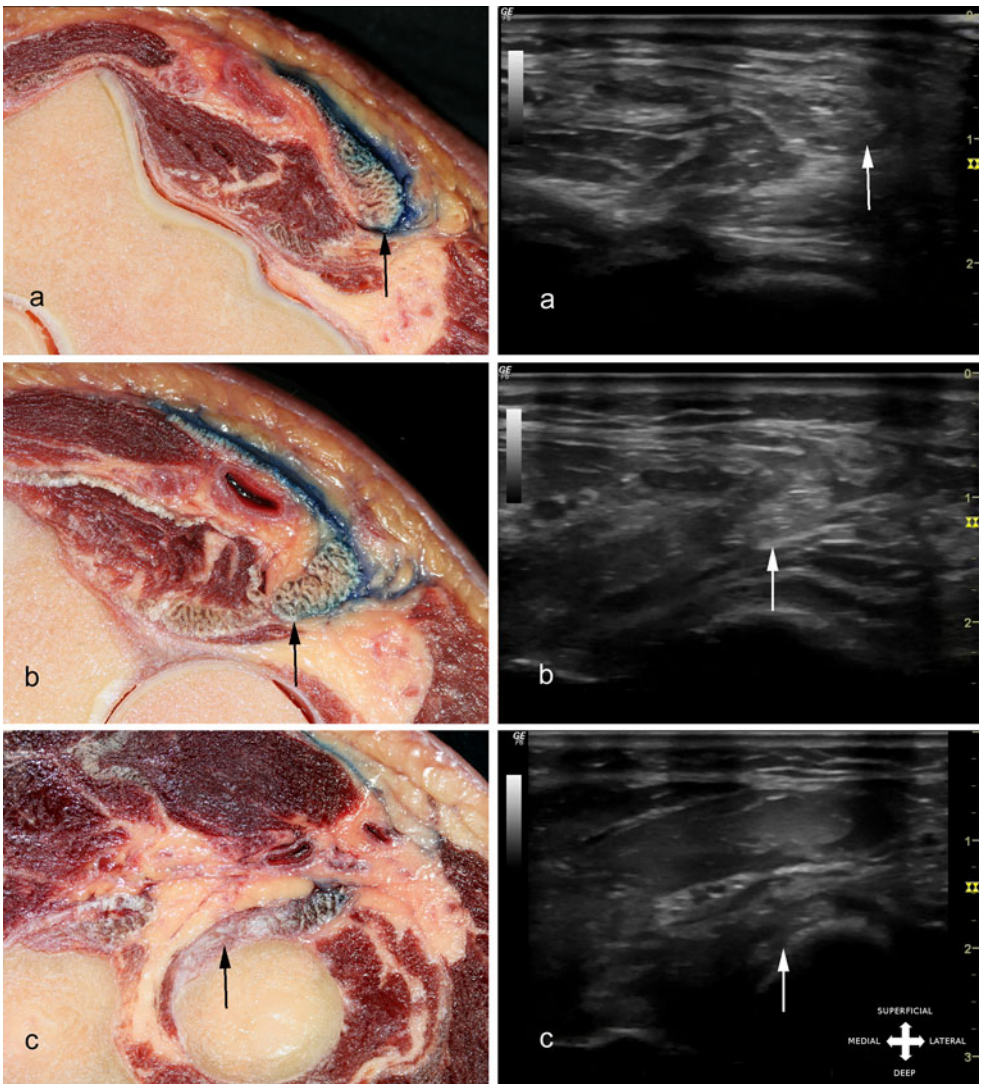

Fig. 2 Ultrasound and anatomic transverse cuts comparison of DBBT cross-section sequence from proximal to distal (**a**–**c**). The LH migrates from a lateral position (**a**) to a deeper position (**b**) and it inserts more proximally (**c**), in relation to the SH component. The SH component migrates from a medial position (**a**) to a more superficial position (**b**) and it inserts more distally and superficially (**c**), in relation to the LH component. *Arrows* LH component

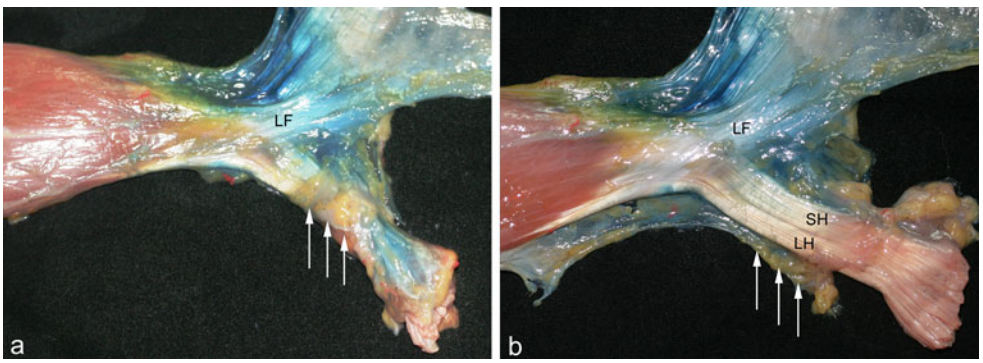

Fig. 3 Paratenon dissection: anterior view. **a** DBBT and LF with paratenon, injected with trypan blue. **b** Paratenon dissected through midline, two tendon components are visible, united by endotenon. *LH* long head of the DBBT, *SH* short head of the DBBT, *LF* lacertus fibrosus, *arrows* paratenon

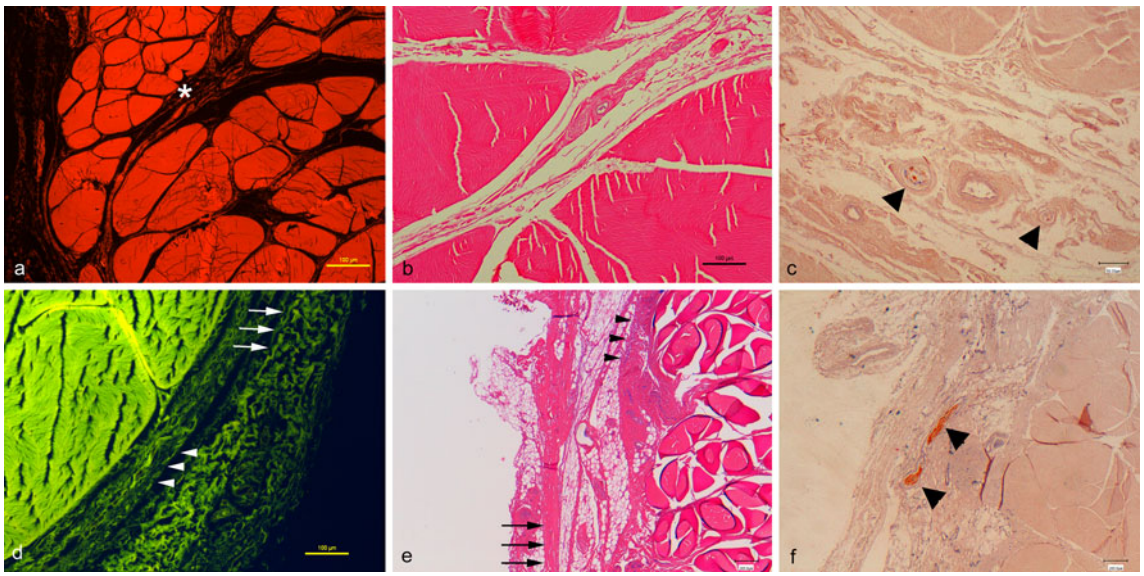

Fig. 4 Histological details of the DBBT. **a** Organization of tertiary fiber bundles (in which tendon fascicles are organized) in two components separated by an endotenon septum observed under light fluorescence microscopy (HE stain). **b** Endotenon septum with small blood vessels (HE stain). **c** Endotenon septum with a small nerve and small blood vessels (S-100 stain). **d** Detail of the epitenon and paratenon under fluorescence light microscopy (HE stain). **e** Hypertrophy of a paratenon with fat infiltration between the latter and the epitenon (HE stain). **f** Detail of a longitudinal nerve and several small blood vessels paratenon (S-100 stain). *Small arrowheads* epitenon, *arrows* paratenon, *asterisk* endotenon septum, *big arrowheads* nerves

a peculiar arrangement consisting of two main components separated by an engrossment of the endotenon, mimicking a lax septum (Fig. 4a, b), which was in continuity with the outer epitenon (transverse view). In all cases, this bridging structure consisted of areolar connective tissue, blood vessels, and neural structures (Fig. 4c). Adipocytes were found within it in 3/5 cases. Under light and fluorescence microscopy, the paratenon was recognized in all specimens (5/5) as a dense well-organized connective tissue layer of variable thickness containing several blood vessels and nerves (Fig. 4d–f). In one specimen, some adipose tissue was detected under it (Fig. 4e).

Cross-section area study

The results show a better correlation between histological CSA: 25.2 mm^2 (SD 7.3 mm^2) and US CSA: 24.6 mm^2 (SD 8.3 mm^2), than anatomic transverse cuts CSA: 41.9 mm^2 (SD 10.1 mm^2) and US CSA: 29.3 mm^2 (SD 11.1 mm^2). Full measurements are shown in Table 1.

Discussion

With a multidisciplinary approach, the present study shows that the DBBT is formed by two components of strictly parallel fascicles, joined by a common paratenon. These components are in continuity with the LH and the SH of the BBM (although both muscle bellies are interwoven distally).

To correctly analyze the DBBT using various processing techniques, we have used an anterior approach because it enables correct visualization of the paratenon and the bifid US appearance of the DBBT (Fig. 5). This approach has been also previously used to identify some landmarks of interest for US-guided needle placement [18]. Also, CSA results have confirmed a good correlation across techniques. Moreover, it is possible to use other approaches [14, 16, 18, 23] described in the literature to obtain better visualization of additional DBBT features, especially the radial insertion and the tendon gliding between radius and ulna during pronation.

Our findings are in agreement with the current literature which indicates that the DBBT is divided in two components, corresponding to the LH and SH of the BBM [24]. Nevertheless, we did not find any DBBT that was either totally separated or bifurcated in our specimens during posterior dissection. Considering the arrangement of the LH and SH of the DBBT, many authors have described this tendon twisting externally [1, 4, 6]. In addition, the tendon has been described to rotate clockwise on the left elbow and counterclockwise on the right elbow [18]. However, we would point out that the SH component inserts more distally and superficially and the LH component inserts more proximally and deeply. It is also important to consider that the LH component folds under the SH

Table 1 Comparison of cross-section area values of the DBBT across techniques

Specimen		Histological measurements			Ultrasound measurements		
		Width (mm)	Thickness (mm)	Cross-section area (mm^2)	Width (mm)	Thickness (mm)	Cross-section area (mm^2)
1		8.7	2.8	19.1	8.8	3	20.7
2		10	3	23.6	8.8	3.6	24.9
3		6.8	3.5	18.7	9.1	2	14.3
4		9.8	4.7	36.2	10.7	4.4	37
5		13.9	2.6	28.4	8.8	3.8	26.3
	Mean	9.84	3.32	25.2	9.24	3.36	24.6
	Standard Deviation	2.6	0.8	7.3	0.8	0.9	8.3
		Anatomic transverse cuts measurements			Ultrasound measurements		
		Width (mm)	Thickness (mm)	Cross-section area $(mm)^2$	Width (mm)	Thickness (mm)	Cross-section area $(mm)^2$
6		13	5	51	12	5.1	48
7		14	4	44	9	3	21.2
8		8	5	31.4	10.6	3.1	25.8
9		10	4	31.4	13.7	2.8	30.1
10		11	6	51.8	10.8	2.5	21.2
	Mean	11.2	4.8	41.9	11.22	3.3	29.3
	Standard Deviation	2.4	0.8	10.1	1.7	1	11.1

Ten specimens were first measured by ultrasound

Specimens 1–5 were also measured by transmitted light microscopy. Specimens 6–10 were also measured on anatomic transverse cuts

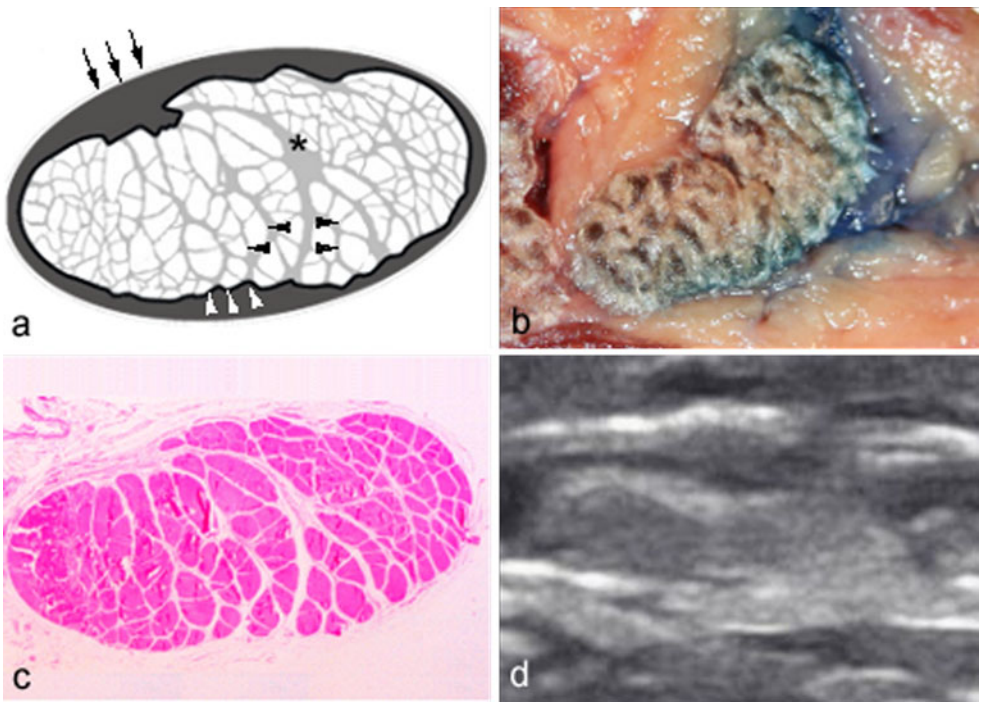

Fig. 5 Diagram of the multidisciplinary approach to the DBBT. Tertiary fiber bundles are distributed in the endotenon in two main components. Between the components, there exists an endotenon septum. **a** Diagram. **b** Dissection transverse section of a specimen injected with trypan blue. **c** Histological sample stained with HE. **d** US detail of a specimen injected with trypan blue. *Black arrowheads* tertiary fiber bundles, *white arrowheads* epitenon, *arrows* paratenon, *asterisk* endotenon septum

component, since a continuity of connective tissue exists between these components. This criterion may explain why the LH component invariably twists (Fig. 2).

To better address the double or bifid US appearance of the DBBT [7, 24], we have also compared the two components with corresponding histological preparation. The main histological structure of tendons has been reviewed by many authors [3, 17, 22]. Morphologically, the endotenon packs collagen fibrils in primary, secondary, and tertiary bundles, the latter visible on gross anatomy and at US scanning. The endotenon is a thin reticular network of connective tissue inside the tendon that has a well-developed crisscross pattern [17]. The arrangement of the collagen fibers within the endotenon is essential to prevent the tendon from being a rigid structure and enable movement between primary, secondary, and tertiary bundles, so the tendon is capable of adapting itself to different functional needs and giving the best tensile strength and transmission of the force [3, 10, 12]. Surprisingly, in the DBBT, we have observed a persistent distribution of the tertiary bundles in the two components which could account for two quaternary fiber bundles (Fig. 5). We propose that the endotenon thickening that separates these two components could be referred to as endotenon septum. This septum is, therefore, part of the endotenon and continuous with it.

Depending on the consistency of the endotenon septum, the two tendinous components can be separated when removing the paratenon by dissection, thus demonstrating its importance in tendon stabilization. Based on our findings, we believe that the endotenon septum between the tendinous component of the LH and the SH of the DBBT enables them to fold in each other and move independently during elbow movements, making them two proper functional units, which is concordant with recent literature [15]. Owing to the different echotexture of the endotenon septum relative to the tendinous bundles, we can demonstrate a tendon composed of two separate tertiary fiber bundle components on US. In our study, we did not encounter any DBBT tendon characterized by two completely independent cords, each of which invested by an individual paratenon. Similarly, we did not find bundles of fibers crossing from the LH portion to the SH portion or viceversa, as previously mentioned in the literature [11]. Another relevant finding related to this endotenon septum relies on the presence of small blood vessels and occasionally adipocytes. Some authors describe similar features and the presence of fibrocartilage at the level of DBBT insertion [2, 7]. In these papers, two separable portions or laminae of the DBBT have been described at the radial tuberosity. In our study, we did not find any fibrocartilage, probably due to the fact that we did not examine the enthesis. Further studies are needed to establish as to what extent this endotenon septum plays a role in cases of partial DBBT tears involving only one of the two components of the tendon.

The paratenon is a sheath of connective tissue surrounding the tendon, but anatomically separated from it [3, 17]. A definite paratenon surrounding the DBBT and LF was demonstrated in the present study using different techniques. With US, dye injection delineated a virtual space between the paratenon and the tendon itself. Histology showed two independent dense connective tissue structures, corresponding to the paratenon and the epitenon. In some specimens, adipocytes were encountered in between the aforementioned structures. It is important to note that the paratenon is intensely vascularized and contains neural structures. We can hypothesize that the paratenon plays a role to reduce friction between the DBBT and the surrounding anatomical structures, but at the same time, it seems ensuring vascular and neural supply to the tendon, playing a possible role in proprioception and protecting the tendon during joint motion, pronation and supination movements, and muscle contraction.

In the context of DBBT partial ruptures, our model of a single tendon consisting of two components that are histologically separated by an endotenon septum could explain the cases of partial tendon rupture involving the SH or the LH of the DBBT in isolation [7, 24]. Retraction of a single component would depend on the fact that all its tertiary fiber bundles are ruptured. Degeneration or rupture of a single portion of the DBBT could be related to different modalities in the application of shear forces during elbow and forearm movements.

Conclusion

Anatomic and histological data demonstrates that the US image of the two components of the DBBT is seen as a separate double tendon because the lax endotenon septum joining the two tendinous components has a different echotexture. The connective tissue forming the endotenon septum is made of areolar connective tissue, and it is continuous with the endotenon that surrounds all tendon fascicles. This could enable folding and independent movement of such components, but nevertheless maintain a close structural relation between them. The paratenon surrounding both the LF and DBBT plays an important role in stabilizing and maintaining both DBBT components together, enabling movement of DBBT in relation to surrounding structures, and represents an important conduit for nervous structures and blood vessels.

Acknowledgments The authors are thankful to body donors that enable the study of anatomy. We also thank Nieves Cayuela and Eva

Sanchez for their help and assistance in the laboratory. Supported by grants ACESB08/09 (Faculty of Medicine, C. Bellvitge, University of Barcelona) and 2009SGR152 (AGAUR, Generalitat de Catalunya, Spain).

Conflict of interest The authors declare that they have no conflict of interest.

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