Histol Histopathol (2015) 30: 963-969

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Might the Masson trichrome stain be considered a useful method for categorizing experimental tendon lesions?

Tiziana Martinello[1], Francesco Pascoli[2], Giovanni Caporale[1], Anna Perazzi[3], Ilaria Iacopetti[3] and Marco Patruno[1]

[1]Department of Comparative Biomedicine and Food Science, University of Padua, [2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD) and [3]Department of Animal Medicine, Production and Health, University of Padua, Italy

Summary. Strain injuries of tendons are the most common orthopedic injuries in athletic subjects, be they equine or human. When the tendon is suddenly damaged, an acute inflammatory phase occurs whereas its repetitive overloading may cause chronic injuries. Currently the criteria used for grading injuries are general and subjective, and therefore a reliable grading method would be an improvement. The main purpose of this study was to assess qualitatively the histological pattern of Masson trichrome stain in healthy and injured tendons; indeed, the known "paradox" of Masson staining was used to create an evaluation for the matrix of tendons, following experimental lesions and natural repair processes. A statistically significant difference of aniline-staining between healthy and lesioned tendons was observed. Overall, we think that the Masson staining might be regarded as an informative tool in discerning the collagen spatial arrangement and therefore the histological characteristics of tendons.

Key words: Masson trichrome, Mesenchymal stem cells, Tendon injuries

Introduction

Tendon tissue is composed of a complex arrangement of extracellular proteins in which are embedded cells, blood vessels and nerves. Since the cellular component of tendon is scarce, the functional properties of tendon are determined mainly by the composition and the arrangement of proteins within the extracellular matrix or ECM. The matrix is synthesized by tendon fibroblasts (tenocytes) and is composed of collagen fibers associated with collagenous and non-collagenous proteins. The parallel-oriented dense collagen fibre bundles, together with very few elastic fibres, furnish the tendon with strength and resiliency, characteristics uncommon in other tissues but necessary to sustain great loads and speed. However, repetitive overloading of the tendon may cause chronic injuries in which there is no inflammation but tenocytes proliferate and synthetize proteoglycans, which leads to an increase of its thickness (Williams et al., 1980). By contrast, when the tendon is suddenly damaged an acute inflammatory phase occurs and the increasing expression of growth factors leads to the proliferative/remodelling phases (Sharma and Maffulli, 2005). Subsequently, tendon healing may occur by an extrinsic or intrinsic pathway (Bi et al., 2007; Spaas et al., 2012). In particular, acute tendinitis is characterized by an increase of tendon cross-sectional area (CSA) due to intratendinous hemorrhage and inflammatory fluid accumulation (Dehghan et al., 2007). The areas of intratendineous lesions are commonly called core lesions and have been defined as types 1-4 based on the ultrasound echogenicity of the lesion (Goodship et al., 1993; Reef, 2001; Dehghan et al., 2007). However, from a histological perspective the appearance of a core lesion depends on the staining used; moreover, it has been shown that in tendon lesions performed experimentally with enzymatic procedures, ultrasonographic observation and histological examinations led to different interpretation and results (Maia et al., 2009; Martinello et al., 2013).

In view of these facts, the capacity to objectively estimate the grade of tendon injuries is essential; current staining methods are easy to perform but need to be

Offprint requests to: Marco Patruno, Department of Comparative Biomedicine and Food Science, Università di Padova, Agripolis, Viale dell'Università, 16, 35020 Legnaro (PD), Italy. e-mail: marco.pat@unipd.it

DOI: 10.16470/HH-11-601

interpreted by a specialist in order to detect changes in the ECM due to increased GAG or altered collagen distribution (Movin et al., 1997). In this study, we set out to test the hypothesis that a simple histological stain (Masson trichrome), currently used to check collagen distribution (Kinoshita et al., 2013; Kwon et al., 2014), might be used to detect staining differences between healthy and lesioned tendons and to estimate the "phase" of the tendon lesion. The Masson stain was primarily used for distinguishing collagen fibers; after nuclei and cytoplasmic staining with Hematoxylin and Scarlet-Acid Fuchsin respectively, the background dye is displaced by phosphomolybdic acid so that collagen is coloured by the counterstain with Aniline Blue. Flint et al. (1975) demonstrated an apparent 'paradox' with the Masson method, and in contrast to other popular staining techniques, that displacement of the first colorant is associated with the level of tissue stretch: the greater the tension, the less displacement of Fuchsin was possible. In this study an induced lesion was performed in the digital deep flexor tendon (DDFT) of sheep, at two different time points, and all histological sections were examined with the Masson trichrome with the aim of detecting specific and different staining reactions for healthy and injured tendon tissue.

Materials and methods

Animals employed and induction of lesions

Deep digital flexor tendons (DDFT) of 9 Bergamasca sheep were examined. Animals homogeneous for size and age were used in this study. Sheep were acclimated to a box 4 weeks prior to beginning the experimental study. Parasitological and biochemistry examinations were carried out to ensure the good health of the subjects. The integrity of tendons was assessed by clinical and ultrasound examination. The Ethic Committee of the University approved protocols that involved live animals for Animal Experimentation (CEASA), as well as the Italian Ministry of Health (DM no. 97/2010-B). Tendinitis was induced by collagenase injection in the DDFT of 9 selected hindlimbs of each sheep. Animals were sedated by intravenous administration of 0.2 mg/kg of Metadone® (Eptadone®) togliere and 10 mg/kg Medetomidine (Sedator®), and positioned in lateral recumbency. A 23-gauge was inserted into each tendon under ultrasonographic guidance. The needle was inserted into the thickness of the DDFT with a lateral approach 15 cm in the proximal–distal direction from the calcaneal bone. 500 IU (0.1 ml) of filter sterilized bacterial Collagenase type 1A (C-9891; Sigma, Milan, Italy) was injected to create the lesions. A suture was applied to each lesioned limb where needle was inserted, in order to mark the precise point for treatment and tendon harvesting. Antibiotic therapy (penicillin-diidrostreptomycin Combiotic®, 10 ml/sheep) was given for 5 days. All animals were clinically assessed daily and analgesia was provided by the administration of 0.01 mg/kg of Buprenorphine (Temgesic®) BID for 5 days.

Masson Trichrome staining

30 and 120 days after the collagenase injection, the sheep (4 animals for each group) were euthanised and the DDFT of lesioned hindlimbs were harvested for the histological analysis. The DDFT of contralateral hindlimbs were harvested as healthy controls. One animal did not receive any collagenase injection and was euthanised as a supplementary control. Harvested DDFT were cut to 1 cm and the proximal-distal orientations were marked. Tissue samples for histology were fixed in 4% paraformaldehyde (PFA) and embedded in paraffin. Sections were cut at 5 μm, mounted on microscope slides and Masson Trichrome Staining (HT15; Sigma, Milan, Italy) was performed. After deparaffinization of slides, Bouin's Solution was applied for 15 minutes at 56°C; the slides were washed to remove yellow color and after Hematoxylin staining and washing, Biebrich Scarlet-Acid Fucshin was applied for 5 minutes; then 5 minutes of treatment with Phosphotungstic/Phosphomolybdic Acid Solution and finally collagen was demonstrated by staining with Aniline Blue Solution (5 minutes). Finally, to render the shades of color more delicate and transparent the sections were rinsed in acetic acid, before dehydrating and mounting in Eukitt.

Image analysis of tendon lesions

After staining, samples were analyzed with an imaging analysis software (Olympus Cell B, Japan), associated with a digital camera and a light microscope (Olympus photomicroscope Vanox, Japan), to evaluate the area occupied by single stains. This quantitative assessment proceeded as follows: (1) Each haul was represented by 5 non-serial sections from each tendon. (2) Three fields from each section were captured and the images analyzed using ImageJ software (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012), with specific plugins (Color Deconvolution, Gabriele Landini e 3D Color Inspector, Kai Uwe Barthel). Color Deconvolution plugin implements stain separation using Ruifrok and Johnston's method (2001). The plugin assumes images generated by colour subtraction. Prior to processing, background subtraction with colour correction was performed as stated by Landini (How to correct background illumination in brightfield microscopy, ImageJ Documentation, Wiki) and the stain vectors were set on Masson's Trichrome. After that, with the 3D Color Inspector plugin, the area stained by single stains (Aniline Blue, Fucsin and Light Green) were evaluated and expressed as area of single stain on total area of the field. As Light Green percentage did not

differ significantly among different images ($p<0.05$), it was excluded from analyses.

Statistical analyses

All statistical analyses regarding the ImageJ documentation were performed using STATISTICA 9 (StatSoft, USA). All data are expressed as Mean±SE. Data were checked for normality by the Shapiro-Wilk test. To highlight differences among different groups, an ANOVA and HSD-Tukey post-hoc test were performed. Where assumptions were not respected, non-parametric tests were applied. In all analyses, a p-value less than 0.05 was considered as significant. The data relative to the histologic variables classified by scores (0 up to 3) were analysed and compared between groups using the Wilcoxon nonparametric test. The results were shown as median scores obtained in each DDFT belonging to both healthy and injured groups. Examined variables and scores were the following: fibroblastic density (0=few, 3=many), fibroblast characteristics (shape), vessel numbers (0=few, 3=many), tissue organization (0=none, 3=well organized) and characteristics (arrangement of collagen fibres).

Results

Clinical evaluation and follow-up

After the collagenase 1A injection, all sheep showed a mild localized thickening of DDFT and peritendonous tissue. Pain detected by palpation remained evident for

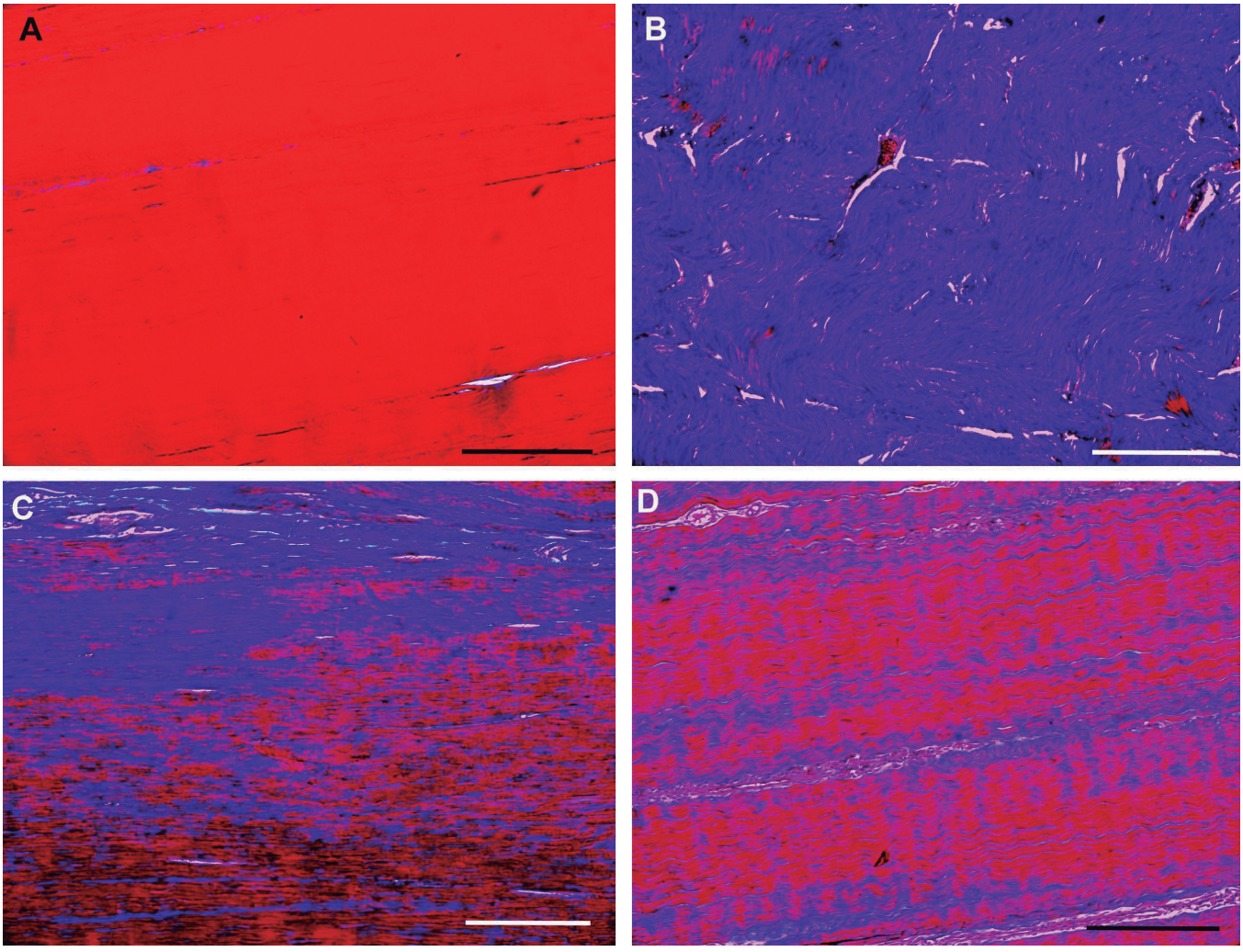

Fig. 1. Representative histological sections of sheep DDFT stained with Masson trichrome. **A.** Healthy tendons in which the section appears completely stained with fucsin. **B.** At 30 days from the lesion, tendon sections stained completely with aniline at the level of the core-lesion. **C.** At 120 days from the lesion, the area of the core-lesion was a mixture of red (fucsine) and blue (aniline). **D.** At 120 days from the lesion, the adjacent areas to the core-lesion stained mainly with fucsin. Scale bars: 500 µm.

the first 5 days as well as a slight lameness and a local increase in temperature that was observed for 3 days. The lesions were detectable in DDFT, by ultrasonography, as a *core-lesion* with hypoechoic areas a few days from the collagenase injection and less evident at 30 days from the lesion (data not shown). A strong inflammatory reaction was not detected in any sheep after the treatment. During the progression of the study, all treated sheep showed a uniform infilling of the lesions. No significant differences were found among the treated sheep with regard to echogenicity, CSA and collagen fibre alignment.

Histological evaluation at 30 and 120 days from the lesion

The Masson trichrome staining revealed that the control "physiologically normal" tendon appeared uniformly red (Fig. 1A) while the collagenase-treated tendon (30 days post-lesion) stained completely blue both at the level of the core-lesion (Fig. 1B) and in adjacent areas (data not shown). By means of H/E staining (Fig. 2), the DDFT was characterized 30 days after the lesion by a disorganized extracellular matrix (ECM), a high cellularization and fibroblast-like cells with plump nuclei, as well as increased vascularization (Fig. 2B). All treated sheep showed increased cellularization compared to the healthy tendon. After 120 days post-lesion Masson trichrome staining revealed that the area of the core-lesion was a mixture of red and blue (Fig. 1C), while adjacent areas were mainly red, similarly to healthy tendon (Fig. 1D); this was also confirmed with H/E staining since the tissue appeared well organised and with less cellularity (Fig. 2C). Table

Table 1. Scores and criteria used for each variable during the histologic evaluation.

Variables	Scores and Criteria
Fibroblastic density	0: sparse (normal) 1: slight increase 2: moderate increase 3: sheets of fibroblasts
Fibroblast characteristic	evaluation of fibroblast predominant shape (rounded or elongated) observed in the injured area
Neovascularization (vessel numbers)	0: normal 1: slight increase 2: moderate increase 3: severe increase
Tissue organization	0: None 1: Discretely organized 2: Moderately organized 3: Organized (normal)
Tissue characteristics	Evaluation of collagen fibres and fibroblasts disposition in the tendon matrix

The data relative to the histologic variables (fibroblastic density, neovascularization, and tissue organization), classified by scores (0 to 3), were analysed and compared between groups using the Wilcoxon nonparametric test. The obtained results were shown under the median form from the scores obtained in each DDFT belonging to both healthy and injured groups. The score and variables were modified and adapted by Maia et al., 2009.

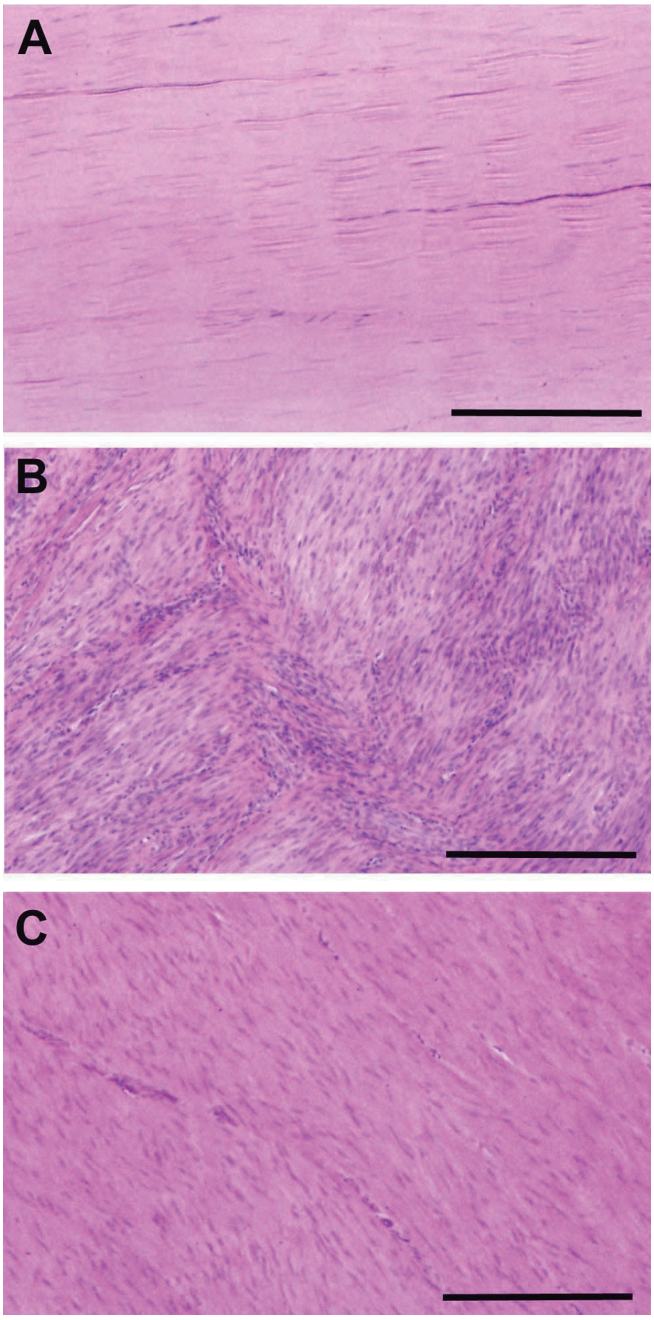

Fig. 2. Representative histological sections of sheep DDFT stained with H&E. **A.** Healthy tendon. **B.** At 30days from the lesion, tendons present a disorganized extracellular matrix and an high cellularization. **C.** At 120 days from the lesion, tendons tissue appeared with less cellularity and a better collagen orientation. Scale Bars: 500 μm.

1 shows scores and criteria used for each variable during the histologic evaluation for confirming the pathological status of injured tendons. The histologic findings in the injured tendon showed cell density composed of fibroblast sheets at 30 days (median, 3.0), a moderate to severe increase in neovascularization (median, 2.5), and a slight to moderate tissue organization (median, 1.5). At 120 days the cell number was moderate (median, 1.0) as well as the neovascularization (median, 1.5) while the tissue organization was better than at 30 days (median, 2.5). The healthy tendon shows normal tendon structure (0 for cell numbers and neovascularization and 3 for tissue organization). Furthermore, differing from the healthy tendons, the injured group at 30 days showed predominately rounded fibroblasts, as well as less regularly arranged collagen fibres and fibroblasts. Number of cells and vessel area between healthy and injured tendons confirmed the cellularization and neovascularization (Table 2).

Table 2. Cell number and vessel area.

Number of cells in healthy tendons:	20±5,1
Number of cells at 30 days:	540,8±24,3
Number of cells at 120 days:	330±13,1
Vessel area in healthy tendons:	3,2±1,1
Vessel area at 30 days:	90±6,2
Vessel area at 120 days:	54±7,3

Cell number and vessel areas are expressed as the mean ±SEM. Statistical analysis for cell number was carried out with STATISTICA 9 (StatSoft) software. The Mann–Whitney's U-test was performed to compare experimental groups at the two sampling periods (30 and 120 days). Differences among the experimental groups within each sampling were confirmed using a Kruskal–Wallis ANOVA. In all analyses, a $p<0.05$ value was accepted as significant.

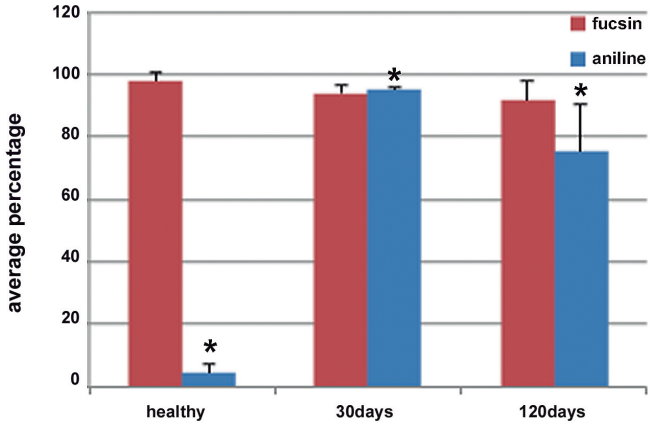

Fig. 3. The graph shows a statistical significant difference of Masson staining in healthy and lesioned tendons. The fucsin remains constant in the three types of tendons analysed. In contrast, aniline staining increases significantly (*) at 30 and 120 days after the lesion ($p<0.05$), in comparison to healthy tendons. Y-axis numbers indicate the average percentage of pigments analysed with the imaging software. Statistical analyses were performed using STATISTICA 9 (StatSoft, USA). All data are expressed as Mean±SE. Data were checked for normality by Shapiro-Wilk test. To highlight differences among different groups an ANOVA and HSD-Tukey post-hoc tests were performed. Where assumptions were not respected, not-parametric tests were applied.

Imaging analysis

Data obtained from the histological observation of all areas of healthy and lesioned tendons, stained with the Masson trichrome, were analyzed with an imaging software and plotted in a graph; figure 3 shows there is a statistically significant difference for the aniline (average percentage of pigments) between healthy and lesioned tendons ($p<0.05$). The fucsin remains constant in the three types of tendons analysed (98.3% in healthy tendons; 94.2% at 30 days; 91.6% at 120 days, left bar of Fig. 3). In contrast, the blue colour (aniline) increases significantly at 30 days (95.3%) and at 120 days (75.3%), compared to healthy tendons (4.5%). Light Green percentage did not differ (about 4% in each sample) between healthy and lesioned tendons (data not shown). When the core lesion areas of the two groups (30 and 120 days) of tendons were analyzed separately, we did not observe a statistical significance but only an increasing trend for aniline at 30 days (data not shown).

Discussion

The main purpose of this study was to examine the histological pattern of Masson trichrome stain in healthy and in injured tendons. Overall, we think that the Masson staining might be regarded as a simple informative tool able to reveal the histological characteristics of an injured tendon. In particular, this study indicates a correlation between the healthy status of tendons and the percentage of expression of the blue-aniline colour; our results showed an increase of aniline staining at 30 days after the lesion, a reduction of the same stain at 120 days after the lesion and almost its absence in healthy tendons. As Light Green percentage did not differ significantly among different samples it was excluded from analyses in order to avoid increasing complexity of statistical calculations.

Generally, to score a tendon lesion with a histological analysis several parameters are considered such as fibroblast density, infiltrate characteristic or tissue organization (Maia et al., 2009; Maffulli et al., 2012; Smith et al., 2013). However, scores and criteria used in these evaluations are quite general and subjective. Therefore, a method able to assess the level of tendon damage with a standardized colorimetric quantification would be a valuable tool. Strain-induced injury of the tendons is the most common orthopedic injury in athletic subjects, be they equine or human (Aström and Rausing, 1995); in fact, during athletic activity, repetitive forces on tendon predispose them to injuries, which culminate in partial or total rupture of

tendons (Smith et al., 2013). In human patients detailed studies regarding the use of different histological stainings were performed mainly in Achilles tendon (Maffulli et al., 2000a,b) but in Veterinary Medicine data are quite scarce and limited mainly to horses (Smith, 2008). In this study it has been decided to detect changes at 30 and 120 days after the lesion since the acute phase of traumatic tendinitis in horses and sheep occurs few days/weeks after the trauma while the remodelling phases occur later and for a long period of time (Patruno and Martinello, 2013). Our proposed evaluation is based on an apparent paradox of Masson staining observed in tendons and demonstrated for the first time in 1975 by Flint et al. The Masson trichrome stain consists of a fiber stain solution and a "cytoplasm" stain solution, but the resulting reaction is associated with the tensional state of the collagen. Indeed, differences in the staining response of human dermal collagen and rabbit tendon with the Masson trichrome stain appeared to be associated with the state of tension of the collagen fibres (Flint, 1975). In other words, it is known that in the stretched state, a biological piezo-electric effect exposes positively charged dye-binding sites (Basset, 1971) and this fact might be reflected in the different binding of histological stains. To our knowledge this is one of the first attempts to use the "Flint" observations for distinguishing between healthy and injured tendons of sheep. Other staining techniques (Tallon et al., 2001; Maffulli et al., 2004; Longo et al., 2008) or observations with polarized microscopy (Vieira et al., 2012) are useful in detecting crimp pattern or pathological features of tendons but we found that the Masson was the simplest and ideal staining technique to obtain data with the imaging software used.

During the tendon healing process the ECM is constantly under rearrangement and therefore the collagen turnover might influence the penetration of the stains used in the Masson trichrome, as well as the abnormal tenocyte morphology, observed in our study and in previous reports (Cook et al., 2004). Three phases are described in the healing process (Spaas et al., 2012): the first (inflammatory phase), is characterized by increased vascular permeability and an influx of local inflammatory cells including platelets, macrophages, monocytes, and neutrophils that release chemotactic agents to recruit blood vessels, fibroblasts, and intrinsic tenocytes (Sharma and Maffulli, 2005). During the second (proliferative phase), as observed around 30 days from the injury, fibroblasts start to produce new matrix and angiogenesis provides an increased local vascular supply (Oakes, 2004); here the blue colour was the dominant. In the third (remodeling phase), it is possible to observe a decrease in cellularity and an increase in collagen spatial arrangement (Gott et al., 2011) that probably caused a better penetration of the red colour, as seen at 120 days after injury and in the healthy tendon. Here it is important to note that our model was intended to distinguish mainly between healthy and injured tendons without exploring all differences in the pathological status of the tendon; indeed, we did not consider naturally strained lesions because of the lack of cases, but instead chose a collagenase treatment since it mimics the natural lesion and, more importantly, provides uniformity among subjects (Dehghan et al., 2007). However, in a future study we will consider strain lesions that have occurred naturally (data obtained from other species showed that Masson staining behave in the same way in both natural and induced lesions, Patruno et al., unpublished results), as well as lesions obtained with inflammatory agents as carrageenan (Vieira et al., 2013).

In this study the apparent paradox of the Masson staining was used to create a staining record for the matrix of tendons after an experimental lesion and a natural repair process. The lesions were experimentally induced and a correlation between the pathological status of a tendon and the Masson trichrome has been observed in our animal model, probably because this staining is able to detect tiny changes in the charge density of tissue proteins. Indeed, we might suggest that injured tendons would stain mainly blue with this staining, contrary to healthy ones. We are aware of the limitations of our investigation such as the small populations of animals used and the lack of mechanical tests but we do hope more researchers will be encouraged to use commercially available software to detect histological staining changes, after our observations.

In conclusion, we think that Masson staining should be regarded as a precise and quick tool for discerning between healthy and lesioned tendons, although detecting the different physiological tensions of collagen fibres, which correspond to several regenerating/remodeling phases, represents a task that is very difficult to achieve using a single staining method.

Aknowledgements. We would like to thank Prof. A. Rowlerson (King's College London, UK) for the English revision of the paper that improved the quality of the manuscript.

References

Aström M. and Rausing A. (1995). Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. Clin. Orthop. Relat. Res. 316, 151-164.

Basset C.A.L. (1971). Effect of force on skeletal tissues. In: Physiological basis of rehabilitation medicine. Downey J. and Darling R.E. (eds). pp, 283-316.

Bi Y., Ehirchiou D., Kilts T.M., Inkson C.A., Embree M.C., Sonoyama W., Li L., Leet A.I., SeoB.M., Zhang L., Shi S. and Young M.F. (2007). Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat. Med. 13, 1219-1227.

Cook J.L., Feller J.A., Bonar S.F. and Khan K.M. (2004). Abnormal tenocyte morphology is more prevalent than collagen disruption in asymptomatic athletes' patellar tendons. J. Orthop. Res. 22, 334-338.

Dehghan M.M., Mehrjerdi H.K., Masoudifard M., Sharifi D. and Vajhi A.R. (2007). Clinical and ultrasonographic findings of collagenase

induced tendinitis in the horse. Iran. J. Vet. Surg. 2, 47-58.

Flint M.H., Lyons M.F., Meaney M.F. and Williams D.E. (1975). The masson staining of collagen - an explanation of an apparent paradox. Histochem. J. 7, 529-546.

Goodship A.E., Watkins P.E., Rigby H.S. and Kenwright J. (1993). The role of fixator frame stiffness in the control of fracture healing. An experimental study. J. Biomech. 26, 1027-1035.

Gott M., Ast M., Lane L.B., Schwartz J.A., Catanzano A., Razzano P. and Grande D.A. (2011). Tendon phenotype should dictate tissue engineering modality in tendon repair: a review. Discov. Med. 12, 75-84.

Kinoshita H., Umezawa T., Omine Y., Kasahara M., Rodriguez-Vazquez J.F., Murakami G. and Abe S. (2013). Distribution of elastic fibres in the head and neck: a histological study using late-stage human fetuses. Anat. Cell. Biol. 46, 39-48.

Kwon S.Y., Chung J.W., Park H.J., Jiang Y.Y. Parl J.K. and Seo Y.K. (2014). Silk and collagen scaffolds for tendon reconstruction. Proc. Inst. Mech. Eng. H. 228,388-396.

Longo U.G., Franceschi F., Ruzzini L., Rabitti C., Morini S., Maffulli N. and Denaro V. (2008). Histopathology of the supraspinatus tendon in rotator cuff tears. Am. J. Sports Med. 36, 533-538.

Maffulli N., Barrass V. and Ewen S.W. (2000a). Light microscopic histology of achilles tendon ruptures. A comparison with unruptured tendons. Am. J. Sports Med. 28, 857-863.

Maffulli N., Ewen S.W., Waterston S.W., Reaper J. and Barrass V. (2000b). Tenocytes from ruptured and tendinopathic achilles tendons produce greater quantities of type III collagen than tenocytes from normal achilles tendons. An in vitro model of human tendon healing. Am. J. Sports Med. 28, 499-505.

Maffulli N., Testa V., Capasso G., Ewen S.W., Sullo A., Benazzo F. and King J.B. (2004). Similar histopathological picture in males with Achilles and patellar tendinopathy. Med. Sci. Sports Exerc. 36, 1470-1475.

Maffulli N, Del Buono A, Spiezia F, Longo UG and Denaro V. (2012). Light microscopic histology of quadriceps tendon ruptures. Int. Orthop. 36, 2367-2371.

Maia L., de Souza M.V., Ribeiro Junior I., de Oliveira A.C., Alves G.E.S., Benjamin L. A., Silva Y.F.R.S., Zandim B.M., Moreira J.C.L. (2009). Platelet-rich plasma in the treatment of induced tendinopathy in horses: histologic evaluation. J. Equine Vet. Sci. 29, 618-626.

Martinello T., Bronzini I., Perazzi A., Testoni S., De Benedictis G.M., Negro A., Caporale G., Mascarello F., Iacopetti I. and Patruno M. (2013). Effects of in vivo applications of peripheral blood-derived mesenchymal stromal cells (PB-MSCs) and platlet-rich plasma (PRP) on experimentally injured deep digital flexor tendons of sheep. J. Orthop. Res. 2, 306-314.

Movin T., Gad A., Reinholt F.P. and Rolf C. (1997). Tendon pathology in long-standing achillodynia. Biopsy findings in 40 patients. Acta Orthop. Scand. 68, 170-175.

Oakes B.W. (2004). Orthopaedic tissue engineering: from laboratory to the clinic. Med. J. Aust. 180 (5 Suppl), S35-38.

Patruno M. and Martinello T. (2014). Treatments of the injured tendon in Veterinary Medicine: from scaffolds to adult stem cells. Histol. Histopathol. 29: 417-422.

Reef V.B. (2001). Superficial digital flexor tendon healing: ultrasonographic evaluation of therapies. Vet. Clin. North Am. Equine Pract. 17, 159-178.

Ruifrok A.C. and Johnston D.A. (2001). Quantification of histochemical staining by color deconvolution. Anal. Quant. Cytol. Histol. 23, 291-299.

Sharma P. and Maffulli N. (2005). Tendon injury and tendinopathy: healing and repair. J. Bone Joint Surg. Am. 87, 187-202.

Smith R.K. (2008) Mesenchymal stem cell therapy for equine tendinopathy. Disabil. Rehabil. 30, 1752-1758.

Smith R.K., Werling N.J., Dakin S.G., Alam R., Goodship A.E. and Dudhia J. (2013). Beneficial effects of autologous bone marrow-derived mesenchymal stem cells in naturally occurring tendinopathy. PLoS One. 8, e75697.

Spaas J.H., Guest D.J. and Van de Walle G.R. (2012). Tendon regeneration in human and equine athletes: Ubi Sumus-Quo Vadimus (where are we and where are we going to)? Sports Med. 42, 871-890.

Tallon C., Maffulli N. and Ewen S.W. (2001). Ruptured Achilles tendons are significantly more degenerated than tendinopathic tendons. Med. Sci. Sports Exerc. 33, 1983-1990.

Vieira C.P., de Aro A.A., Almeida Mdos S., De Mello G.C., Antunes E. and Pimental E.R. (2012). Effects of acute inflammation induced in the rat paw on the deep digital flexor tendon. Connect. Tissue Res. 53, 160-168.

Vieira C.P., de Aro A.A., Rè Guerra F., De Oliveira L.P., De Almeida M.D.S. and Pimentel E.R. (2013). Inflammatory process induced by carrageenan in adjacent tissue triggers the acute inflammation in deep digital flexor tendon of rats. Anat. Rec. 296, 1187-1195.

Williams I.F., Heaton A. and McCullagh K.G. (1980). Cell morphology and collagen types in equine tendon scar. Res. Vet. Sci. 28, 302-310.

Accepted March 3, 2015