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ORIGINAL PAPER

Variations of the micro-vascularization of the greater tuberosity in patients with rotator cuff tears

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Abstract
Purpose The aim of the study was to analyse greater tuberosity's (GT) micro-vascularization in the context of rotator cuff tear and to identify factors that could affect the rate and distribution of micro-vessels.
Methods Eighty-seven patients with supraspinatus and/or infraspinatus tendon tears were included in a prospective study. Mean age at surgery was 58 years (41–78) and clinical symptoms were lasting from an average of 20 months before surgery. A bone core of 1-cm depth was obtained from the GT during rotator cuff repair at two localizations, medial and lateral within tuberosity. Micro-vascularization was then analysed with an immunohistochemistry technique based on CD34 antigen tracking endothelial cells at two levels of depth for each sample (more and less than 5 mm). Epidemiologic and pathologic data were correlated with the rate of micro-vascularization measured.
Results Median rate of GT's micro-vascularization was 9.8 %, which ranged from 0.13 % to 33.4 %. This rate decreased with preoperative steroid injection (7.4 % vs 11.2) and with localization close to the cartilage of the humeral head (8.7 % vs 11.9 %). However, it remains almost homogenous along the depth's core. Moreover, no significant correlation was found regarding age at surgery, gender, context of previous trauma, smoking habits, duration of symptoms, and specific data regarding the tendon tear.
Conclusions This study highlighted the variability of GT's micro-vascularization in case of rotator cuff tear. A greater rate was observed at the lateral part of the footprint, whereas medical history of steroid injection has a negative influence on micro-vascularization.

Keywords Bone · Shoulder · Rotator cuff tear · Micro · Vascularization

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Introduction

Healing of rotator cuff tendons after repair remains problematic and improvement of healing rate is of ongoing investigation [1–5]. The strongest mechanical fixations of the tendons to bone have been shown to be correlated to better tendon healing. Indeed, quality of sutures, type of anchorage and surgical repair technique are more and more reliable [6]. However, two weak links remain of concern and are predictable of the anatomical result of the repair: the quality of the musculo-tendinous unit and the quality of the bone at the reinsertion site [7]. Fatty infiltration index, muscle atrophy and tendon retraction are reported to be major prognostic factors [7, 8]. The mechanical value of the bone seems also to be implied, as much as its biological value [9–15]. It has been demonstrated that initiation of the healing process of the tendon onto the bone are related to bone marrow-derived cells coming from the footprint, which differentiate into tendon tissues [9, 11, 12, 14, 16]. Therefore, micro-vascularity of the greater tuberosity (GT) could reflect the biological potential of the bone in cases of rotator cuff repair involving the supraspinatus and/or the infraspinatus tendons. To our knowledge, no study to date has investigated the micro-vascularization at the footprint in case of rotator cuff tear.

The aim of the study was to analyse the micro-vascularization of the GT in the context of rotator cuff tear and to identify factors that could affect its rate and distribution. Our hypothesis was that micro-vascularity at the GT was variable, depending on epidemiological data and the type of rotator cuff tendon tear.

Methods

Study design and patients

A prospective study was conducted in our department from January 2011 to January 2012 after being approved by the institutional review board. Inclusion criteria were: all patients who were operated for suprapsinatus and/or infraspinatus tendons tears after a failed conservative treatment, with an open or arthroscopic surgical technique, and who gave their agreement to enter the study. Exclusion criteria were: lack of preoperative MRI or arthro-CT scans focused on rotator cuff tendon (stage of retraction and muscle fatty infiltration), a subscapularis tear without involvement of supra or infraspinatus tendon, and a bone sample quality that did not allow a valuable immunohistochemical analysis.

One hundred three patients were consecutively enrolled in this study during the period of inclusion. Two of them were excluded because of an isolated subscapularis tendon tear and two others because of incomplete radiologic data; 12 bone samples were inadequate for biological analysis, leaving 87 patients (87 samples), who were available for statistical analysis.

Patients

Among the 87 patients, there were 43 men and 44 women with a mean age of 58 (range, 41–78). Twenty-four (27 %) patients were tobacco smokers and 16 had other arteriosclerosis risk factors such as diabetes, hypertension, obesity and hypercholesterolemia. Steroid injection in sub acromial space had been used preoperatively in 20 patients (23 %).

Clinical symptoms were of 20 months (range, one to 126) duration before surgery. Thirty-three patients (38 %) described a traumatic event as the beginning of their shoulder symptoms.

Tissue collection and preparation

Two senior surgeons performed surgery according to a standardized technique: subacromial decompression, biceps tenotomy or tenodesis and rotator cuff repair in anatomic position. The tendon was fixed with transosseous Mason-Allen non-absorbable sutures or lateral tension band anchorage during, respectively, open and arthroscopic surgery [17].

One bone sample (a core) was obtained from each patient during the surgical procedure using 8-gauge diameter trocars (Jamshidi®, Carefusion, USA) inserted perpendicular to the exposed footprint of the GT at the rotator cuff site and before abrasion of the bone with a burr. Two localisations in the middle zone were investigated in a randomized design selection: close to the cartilage of the humeral head (medial footprint-group M) or at the top of the GT (lateral footprint-group L). A bone core of 1-cm depth obtained from each patient was immediately sent to the Pathology Department in a hydrated compress for specific fresh analysis.

Bone sections were obtained from paraffin-embedded blocks. Immunohistochemistry analysis was performed with an anti-CD34 antibody (clone QBEnd10, 1:100 dilution; Beckman Coulter, Marseille, France), which is a specific tracker of endothelial cells and micro-vessels, widely used in tumour vascularity characterization [18, 19]. Slides were processed automatically (Autostainer link 48; Dako, Courtaboeuf, France) according to the protocols supplied by the antibody manufacturers (Fig. 1).

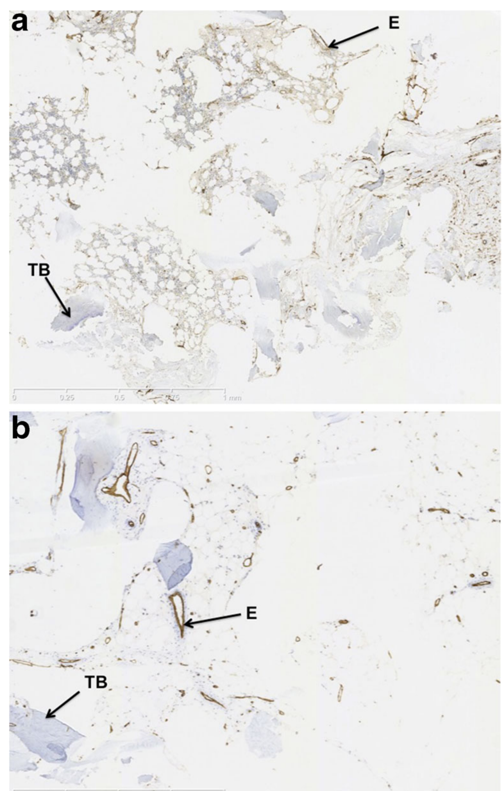

Fig. 1 Slides scanned with immunoexpression of CD-34 in bone samples coming from two different patients (**a** and **b**). *Brown clusters* represent microvessels. *E* endothelial cells immunostaining with anti-CD34 antibody, *TB* trabecular bone

After slides scanning (NanoZoomer; Hamamatsu, Massy, France), vascular structures labelled with anti-CD34 antibody were quantified using the imaging software NDP.view 2.3.1 (Hamamatsu) and NIS elements Basic Research 2.30 (Nikon, Champigny sur Marne, France) at the same scale (Fig. 2). The micro-vascularization resulted from the ratio automatically calculated between vascular structures' area and miscellaneous structures' area (trabecular bone, fat interstitial tissue, stromal cells, etc.). This relation was studied at two levels of depth for each sample: less than 5 mm (level 1) and more than 5 mm from the top of the core (level 2).

Statistical analysis

Univariate comparisons were performed to study the relationship between micro-vascularization and others variables such as demographic factors (age, sex), clinical history (smoker, arteriosclerosis risk factors, history of traumatic event or steroid injections) and pathological data (number of tendons involved, stage of tendon retraction and fatty degeneration index of the muscle, shape of acromion according to Bigliani classification). Median and interquartile-range (IQR) were given for GT's microvacularization. For categorical variables, we used Wilcoxon or Kruskal-Wallis nonparametric comparison tests and, for continuous variables, we calculated the Pearson's correlation coefficient. We considered statistical significance reached when p-value <0.05. Analyses were conducted using Stata software v11.1.

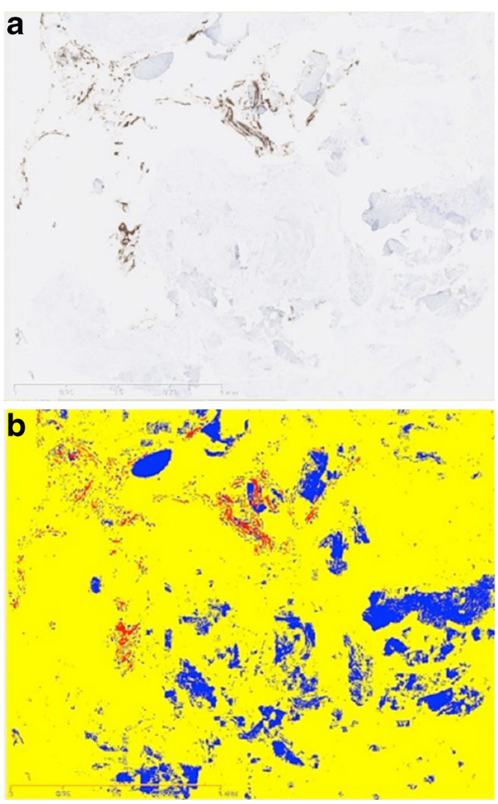

Fig. 2 Slide scanned (**a**) that fit the slide built by the software NDP.view 2.3.1 and NIS elements Basic Research 2.30. (**b**). In this example, microvessels were rated at 8.78 %

Results

Data distribution was not normal and ranged from 0.13 % to 33.4 %. Median rate of GT's micro-vascularization was 9.8 % (mean, 11.5; SD, 7.8; IQR, 5.5-16.2) (Fig. 3).

There was no significant difference in the micro-vascularization rate at different depths of the bone cores: level 1 was 9.49 % (IQR 0.18–36.5) and level 2 was 9.91 % (IQR 0.09–50.63) (p=0.56). The micro-vascularization rate tended to be higher at the lateral footprint than at the medial footprint, respectively, 11.9 % (IQR 6.2–18.0) and 8.7 % (IQR 4.3–14.5) (p=0.16).

No statistically significant correlation was identified according to demographic characteristics, i.e. age at surgery or duration of symptoms did not seem to affect micro-vascularization. However, patient population with previous steroid injection trended to have a lower rate of micro-vascularization (7.4 % vs 11.2 %; p=0.13) (Table 1).

No significant correlation was found between bone micro-vascularization and type of rotator cuff tendon tears, fatty degeneration index and acromion shape (Table 2).

Discussion

To our knowledge, this is the first study which has investigated the bone biological patterns at the GT with a reproducible technique of measurement of micro-vascularization based on anti-CD34 antibodies, which is commonly used to assess angiogenesis in human solid tumours [17, 18]. The rate of the micro-vascularization at the footprint has been shown to

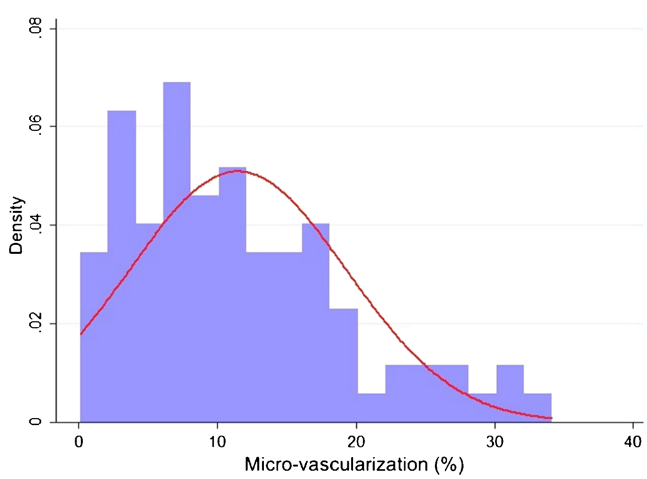

Fig. 3 Distribution of the results without a Gaussian distribution

Table 1 Demographic data

Variable	Microvascularization (median %)	Interquartile range	*p-value*
Sex			0.51
Female (n=43)	8.7	[4.8–16.4]	
Male (n=44)	11.2	[6.0–16.2]	
Smoker			0.76
No (n=63)	9.0	[5.3–17.3]	
Yes (n=24)	11.6	[6–15.0]	
Age at surgery	(Linear regression model)	Pearson's correlation coefficient: −0.075	0.49
Arteriosclerosis risk factors (others than tobacco)			0.58
No (n=66)	8.9	[5.8–14.7]	
Yes (n=21)	12.0	[4.3–17.3]	
Traumatic event			0.94
Yes (n=54)	9.6	[5.8–15.9]	
No (n=33)	11.0	[3.9–17.3]	
Steroid injection			0.13^{INT}
No (n=67)	11.2	[5.8–17.3]	
Yes (n=20)	7.4	[4.4–12.7]	
Number of steroid injections			0.32
0 (n=67)	11.2	[5.8–17.3]	
1 (n=17)	7.6	[4.0–11.7]	
2 (n=3)	6.2	[6.7–13.7]	
Duration of symptoms	(Linear regression model)	Pearson's correlation coefficient: −0.029	0.79

INT intermediate risk of significance (statistical tendency)

be highly variable from less than 1 % to more than 33 % of tissue, with a median rate of 9.8 %, in the context of rotator cuff tendon tears. This rate was almost homogenous at any level of depth into the GT (in the limits of 10 mm in depth). However, micro-vascularization tended to decrease with steroid injection performed before surgery. Bone close to the humeral head cartilage (medial footprint) had a lower rate of vascularization than at the top of the GT (lateral footprint). No significant statistical correlation has shown relationship between micro-vascularization and age at surgery, gender, context of previous trauma, smoking habits or others arteriosclerosis risk factors, number of torn tendons, stage of retraction or fatty degeneration index and duration of symptoms.

Table 2 Characterization of the rotator cuff tears

Variable	Microvascularization (median %)	Interquartile range	*p*
Number of tendons involved			0.25
1(n=54)	11.6	[5.3–17.3]	
>1 (n=33)	8.0	[5.8–14.1]	
Supraspinatus retraction			0.56
Stage 1 (n=46)	11.7	[4.8–17.6]	
> Stage 1 (n=41)	9.3	[6.2–14.4]	
Supraspinatus fatty degeneration			0.66
Stage 0 (n=41)	11.7	[6.7–17.3]	
Stage 1 (n=31)	8.5	[4.0–16.2]	
Stage 2 (n=15)	12.0	[6.2–14.7]	
Acromion (Bigliani classification)			0.9
Type 1 (n=26)	8.7	[7.2–16.4]	
Type 2 (n=34)	9.0	[4.0–15.9]	
Type 3 (n=27)	12.8	[4.3–16.2]	

Many authors have investigated prognosis factors that could influence the healing process in case of rotator cuff tendon repair, including epidemiologic and histo-morphometric features [7, 19–24]. Therefore, the success of tendon healing seems to depend on patient's age, comorbidities, the tendon itself (quality, tear size, degree of retraction) and the muscle (fatty degeneration index and atrophy). Bone quality at the site of tendon insertion remains likely a major component as well, but few studies have investigated this link of the healing process. Biologically, cells coming from the footprint initiate the healing process [12, 14]. However, no significant difference could be shown in our study regarding the micro-vascularization at any depth of the GT. It seems from these results that bone spongialization could not be required for the footprint preparation before tendon anchorage. On the other hand, radiofrequency burning of the footprint commonly performed before tendon anchorage could result in damaging micro-vessels and should be avoided if possible [25]. Mechanically, it has been previously reported that an optimal fixation of the tendon is related to a higher healing rate, and that double row techniques using heavy sutures and anchors seem to improve tendon healing [4–6, 26]. However, fixations of the tendon seem to be better at the border of the articular surface, where the medial row is commonly placed [27–29]. Several authors have pointed out that bone density decreases in case of rotator cuff disease, which could affect bone stock distribution at the GT [10, 15, 30, 31]. According to this study, the tendency of greater micro-vascularization at the top of the GT is an argument for application of the tendon as lateral as possible onto the footprint. Doing so, the double row technique should offer some guarantees with respectively strong medial anchorage and lateral application for a better healing combining theoretical mechanical and biological advantages [5]. However, implications of double row techniques on clinical outcomes at follow-up remain controversial to date [26].

Our study supported that subacromial corticosteroid injections may play a role in lowering micro-vascularization's rate at the GT. These injections were known to induce adverse effects on the tendon structure [32–34]. In case of rotator cuff tear, corticosteroids directly impregnate the GT and could induce apoptosis of endothelial cells, as it has been shown in cases of femoral head osteonecrosis [35].

Limitations

This study has several weaknesses. There was no control group, with intact rotator cuff tendons, to validate normal bone micro-vascularization at the GT. Indeed, a control-group was ethically difficult to obtain in order to get bone samples in a healthy patient population. On the other hand, the number of patients included was low and did not allow us to perform multivariate analysis. Because of the lack of data in the literature, we could not perform evaluation of the number of subjects required based on a specific difference we wished to stress; this could explain in part the lack of statistical significance detection according to the confidence interval in some variables analyses. Furthermore, only two sites were explored at the supraspinatus footprint: the lateral footprint at the top of the GT and the medial footprint, close to the humeral head cartilage. An intermediary site could have been sampled but it would have decreased the contact bone/tendon area during the repair with an adverse affect on tendon healing onto the GT.

Conclusions

This study has shown that the rate of micro-vessels is variable and ranged from 1 % to about 33 % at GT, with a median rate of 9.8 %. Previous corticosteroid injections seem to lower this rate. Greater vascularisation was found close to the top of the GT, at the lateral footprint. Further investigations are necessary to understand how quality and quantity of micro-vessels could influence tendon healing after rotator cuff repair.

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