

## REVIEW

## The role of skin and muscle biopsy in the diagnosis of main connective tissue diseases

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### Abstract

Systemic involvement in autoimmune diseases is often unclear and organ changes are confounding, thus making it difficult to have an early accurate diagnosis. In those situations, both clinical and paraclinical findings might orientate the diagnosis, but only histological or immunohistochemistry changes might be accurate enough. The skin histological changes are relevant and sometimes might have a tremendous role in the accurate diagnosis of autoimmune rheumatic diseases, due to the correlation with the clinical systemic manifestations of the diseases and through the accessibility of biopsy. In the same time, muscle biopsy can provide important support for physicians improving diagnosis and optimizing management of connective tissue diseases.

**Keywords:** skin biopsy, muscle biopsy, connective tissue disease, systemic lupus erythematosus, systemic scleroderma, myositis.

### Introduction

Systemic involvement in autoimmune diseases is often unclear and organ changes are confounding, thus making it difficult to have an early accurate diagnosis. Rheumatic diseases might benefit from the modern imaging methods, such as musculoskeletal ultrasonography [1, 2] or capillaroscopy [3], both for diagnosis and management of the diseases, but in some cases, the pathology remains unclear. In those situations, combined clinical and paraclinical findings might orientate the diagnosis, but only histological or immunohistochemistry (IHC) changes might be accurate enough. Skin is not only a protective layer, but it can be considered as a part of the immune system, as demonstrated by early studies [4] and as its involvement, along with systemic damage, in autoimmune diseases proves it. The skin histological changes are relevant and sometimes have a tremendous role in the diagnosis of autoimmune rheumatic diseases, both through the correlation with the clinical systemic manifestations of the diseases and through the accessibility of the biopsy. In a connective tissue disorder like systemic lupus erythematosus, dermatomyositis or scleroderma, the skin and muscles are affected because of autoimmune mechanism, compromising the functions, with the appearance of characteristic signs.

The role of histopathological (HP) findings in the connective tissue diseases arises from the possibility to accurately identify the diagnosis and to establish an

optimal treatment for the prevention of complications that are incompatible with life.

Systemic scleroderma (SSc) is a complex autoimmune disorder with various clinical manifestations and increased mortality, due to cardiovascular, pulmonary, renal and cutaneous complications, characterized by extensive fibrosis in the skin, vessels and organs, due to the immune system disorder [5].

The prevalence and incidence of SSc varies within ethnic and racial groups and with geographical areas, establishing a predominance of limited form in the Caucasian population and diffuse form in the African-American population [6]. Thus, an incidence of seven to 20 cases/1 000 000/year is estimated, with a prevalence rate ranging from 28 to 443 cases/1 000 000/year in the United States, where there has been an increase in the last 60 years and 120 cases/1 000 000/year in the UK [7–9].

The disease occurs more frequently in women, similarly to all connective tissue disorders, with the women: men ratio from 7:1, in the younger patients, to 2:1 in patients over 50 years old [10]. The survival expectancy of 10 and 20 years for diffuse cutaneous SSc is estimated to 86%, respectively 64% of patients [11].

Lupus erythematosus (LE) is a chronic inflammatory disorder with multisystemic impairment due to the imbalance between inflammatory and autoregulation factors, with increased mortality through organic failure. According to cutaneous, immunological and histological

changes, there are three subtypes of LE: chronic LE, subacute LE [12] and systemic one, with acute systemic involvement and skin changes. It is known that 12% of patients with chronic LE progress to SLE [13]. Cutaneous changes of systemic lupus erythematosus (SLE), by location in exposed areas, are often associated with a decrease in quality of life, social and psychological disabilities. Skin is involved in up to 80% of SLE patients, falling within the types of specific and non-specific lesions. Specific skin lesions represent an important criterion in differentiating SLE from other diseases.

Cutaneous and mucosal lesions are important for the rapid diagnosis of the disease and might be the first manifestation of the disease in 60–85% of SLE patients, especially in juvenile-onset of SLE (JSLE). Thus, early identification of skin lesions might allow early diagnosis and treatment of the disease before systemic damage. Patients without skin lesions are often diagnosed later in a more advanced stage of the disease [14].

Polymyositis (PM)/dermatomyositis (DM), represent a group of inflammatory autoimmune myopathies [idiopathic inflammatory myopathies (IIM)], whose symptomatology is represented by inflammation and weakness of proximal muscles associated to extramuscular manifestations, with severe prognosis in terms of mortality and morbidity [15]. Prevalence is estimated at 0.55–17.5/100 000 inhabitants [16].

The role of HP changes in the IIM is even more important, compared to other connective tissue diseases, the muscle biopsy being included as diagnostic criteria along with elevation of serum skeletal muscle enzymes, electromyographic evaluation and specific clinical findings (Bohan & Peter 1975 criteria). The risk of association with malignancies enforces the need of an early and accurate diagnosis.

### ☐ Systemic lupus erythematosus

Skin involvement is present in up to 80% of SLE patients, skin lesions being an important hallmark of the disease, helping in differentiating SLE from other conditions; however, *Systemic Lupus International Collaborating*

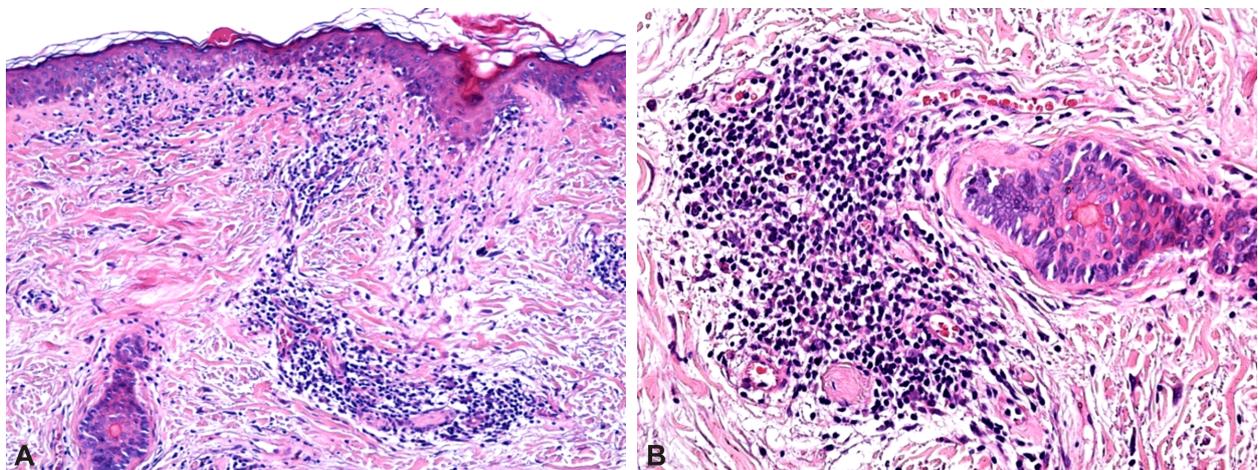
*Clinics (SLICC) Classification Criteria for Systemic Lupus Erythematosus* criteria are limited to acute cutaneous lupus, chronic cutaneous lupus, ulceration and non-scarring alopecia, making it difficult to associate non-specific skin lesions with SLE and LE subtypes [17]. The lupus skin changes, include specific and non-specific lesions; butterfly rash, mucosal lesions, bullous lupus, polycyclic and papulosquamous/psoriasiform variants are just few of the specific ones [12].

In SLE, the HP root of skin lesions is the apoptosis of keratinocytes through various apoptotic pathways, involving p53, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), Fas/FasL. Another hypothesis is the presence of major histocompatibility complex (MHC) anomalies in the keratinocytes, which leads either to the release of abnormal cytokines or to deficient synthesis of essential proteins involved in the regulation of the apoptosis, from keratinocytes, with incapacity to prevent cell death, induced by UV rays [18].

HP examination of the skin is sometimes the main diagnostic tool, especially in non-systemic forms. There are some patterns of HP changes in skin biopsies, based on the presence of specific features, which, associated with the clinical aspects, can sustain the diagnosis. Thus, the vacuolar alteration of basal keratinocytes and mucin present in reticular dermis can be present in all types of LE [19].

Baltaci & Fritsch [20] histologically classified skin changes of chronic lupus erythematosus (CLE) into early, fully developed and late lesions; in the same time, they pointed out the specific manifestations, like LE *profundus* and revealed that fully developed discoid lupus erythematosus (DLE) might be considered to be a model of lupus skin involvement, because it presents the majority of the histological characteristics of LE.

**Early histological lesions** include neutrophils and nuclear dust disposed below the dermoepidermic junction (DEJ) and rare perivascular infiltrates (Figure 1). Necrotic keratinocytic changes, focal alteration of the basal cells and extracellular mucin deposits between collagen bundles are situated in the reticular dermis.



**Figure 1 – (A and B) Skin biopsy showing large areas of epidermal atrophy, rare colloid bodies in the basal layer of the epidermis, vacuolar interface dermatitis; moderate perivascular and periadnexal inflammatory infiltrate principally containing lymphocytes and histiocytes, located in upper and lower dermis. Hematoxylin–Eosin (HE) staining:  $\times 100$  (A);  $\times 200$  (B).**

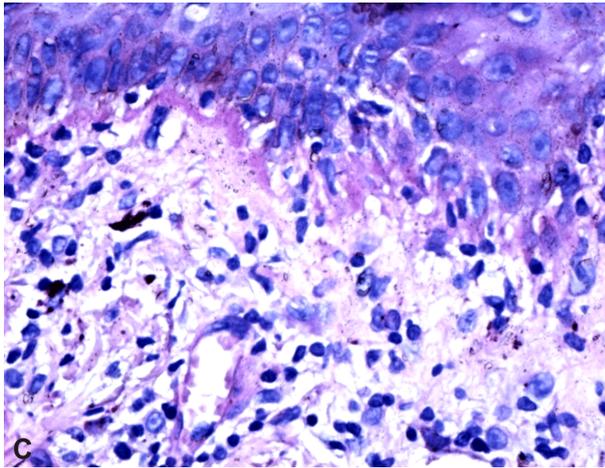


Figure 1 (continued) – (C) *Inflammatory infiltrate passing through disrupted basal layer. Periodic Acid-Schiff (PAS) staining, ×400.*

The fully developed lesions are characterized by perivascular lymphocytic infiltrates located in the papillary

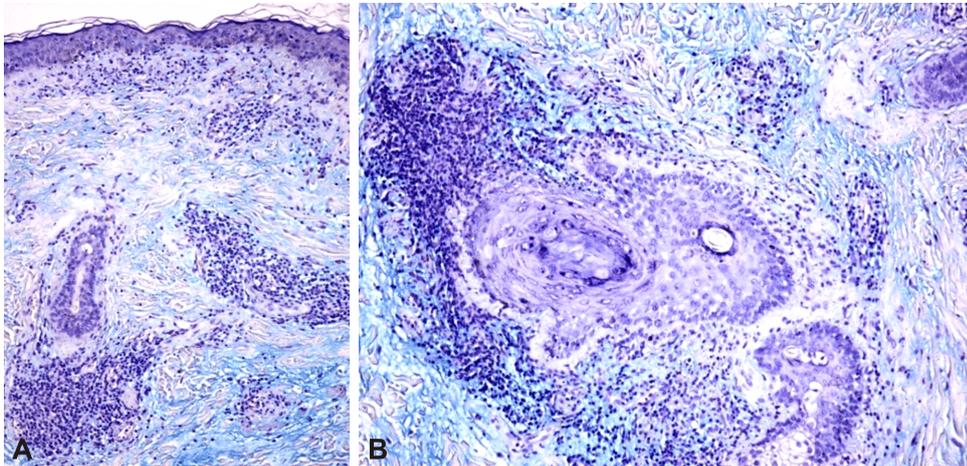


Figure 2 – (A and B) *Skin biopsy showing significant mucinosis in the reticular dermis and focal enlargement of the basal membrane. Periadnexal inflammatory infiltrate. Alcian Blue staining at pH 2.5: ×100 (A); ×200 (B).*

Bullous LE presents with leukocytes at the DEJ level, superficial perivascular lymphocyte infiltrates, mucin deposits in the reticular dermis, nuclear dust and neutrophils in the upper dermis [20].

The histological aspects of subacute cutaneous lupus erythematosus (*SCLE*) are similar to those of *DLE* (fully developed and late lesions), with the exception of hair follicles, which are not affected.

*Lupus erythematosus tumidus* has findings from fully developed lesions and just few elements to differentiate: perivascular dermal lymphocytic infiltrates, polymorphous light eruption (PLE), which means a papillary edema and absence of mucin deposits.

There are some histological changes specific for *LE profundus*, which occur in subcutaneous cellular tissue with fat necrosis (foamy fat cells) and degenerative fibrinoid changes that give the appearance of fibrosclerosis in evolution, with hyalinization of the adipose lobules and calcification.

*Chilblain lupus erythematosus* shows similar aspect with fully developed and late lesions, the changes including an interface dermatitis, perivascular lymphocytic infiltrates and dermal edema, necrotic keratinocytes and epidermal atrophy.

Late lesions like pseudoepithelioma hyperplasia,

and reticular dermis, dense mucin deposits in the papillary dermis, thinning of the epidermis, smudged appearance of DEJ, vacuolar degeneration of basal cells, necrotic keratinocytes, thickening of the basal membrane, pigmentary incontinence in the upper dermis, follicular plugs, compact orthokeratosis.

The late lesions include epidermal and follicular full atrophy, the disappearance of lymphocyte infiltrates, vacuolization of basal cells, basal membrane thickening, interface dermatitis (an inflammatory process at the basal membrane of interfollicular epidermis), dermal fibrosclerosis, increased mucin deposits in the reticular dermis (Figure 2) [19, 20].

The involvement of the skin in *acute LE* is histologically overlaid with early lesions, expressing alteration of keratinocytes, which are the diagnostic sign, upper dermis edema, perivascular lymphohistiocytic infiltrate, nuclear dust, dilated vessels with extravasation of the erythrocytes and mucin deposits in the reticular dermis.

hyperkeratosis, vacuolar degeneration of the basal cells, a thickened basement membrane in the epidermis and the dermis, reveal perivascular lymphocytic infiltrates, interface dermatitis and mucin deposits, changes that might be frequently found in *verrucous (hypertrophic) lupus erythematosus* [20].

Immunoglobulin granule deposits (IgG, IgM, IgA) and complement (C3) can be observed by direct immunofluorescence (DIF), along the basal membrane in patients with LE and this method was named, therefore, the lupus band test (LBT). Anyway, it is not a specific test, as it might be falsely positive in other connective tissue diseases (SSc, mixed connective tissue disease) and its sensitivity and sensibility are related to the body area tested, as the exposure to sun might change the results. Thus, the presence of LBT in skin biopsies varies between 36–90%, depending on the exposure to sun, being lower in sun-protected areas. Moreover, LBT might be helpful in distinguishing CLE from SLE in some confusing situations, as in SLE patients, LBT has been described both in lesion areas and non-lesion ones, in contrast to CLE, where only the lesion areas are LBT positive. To date, LBT might be identified in healthy subjects' sun exposed skin, but the predictive value for SLE increases with complement value [19, 21].

The pathophysiological mechanism of skin depositing of immunoglobulins (Ig) and complement is supported by two hypotheses: first, that they are the result of circulating immune complexes, similar to those in the kidney, which are diffusing through the terminal arterioles in DEJ; the second hypothesis is that Ig and complement deposits appear under the influence of local factors (epidermal blood flow, proliferation and exposure to UV rays), releasing nuclear epidermal DNA that diffuses through the basal membrane and interacts with anti-nuclear antibodies at the DEJ level. The latest one explains the increased incidence of LBT in biopsies of the sun exposed skin [22].

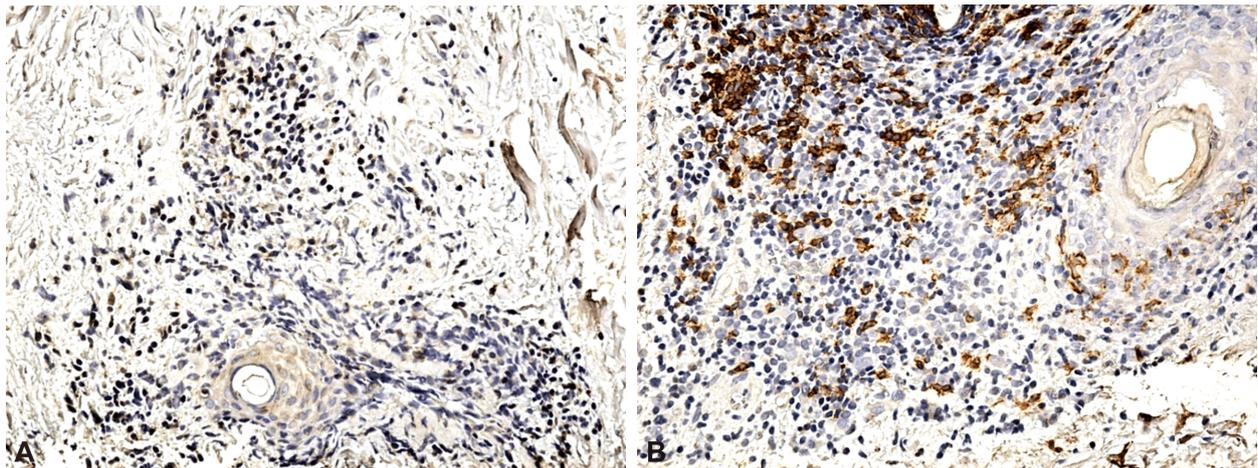
Over time, it has been attempted to correlate the presence of LBT in skin, with LE clinical activity, but without conclusive results, as only the decrease of C3 could be correlated with the presence of LBT. Also, immunosuppressive therapy (Cyclophosphamide, Azathioprine) and glucocorticoids (GCs) do not seem to influence the disappearance of LBT, which may persist for several months after treatment.

However, patients with positive LBT have a three-fold increased incidence of lupus nephritis, but the absence of LBT does not exclude renal disease [18]. In the same time, the severity of kidney damage seems to correlate with

the presence of LBT. Thus, patients with LBT have a more aggressive form of renal involvement, as a 10-year long study, conducted by Davis & Gilliam, on 51 SLE patients biopsied before, within and six months after treatment, concluded. Patients with absent LBT before treatment remained negative over 10 years after the end of treatment; in the same time, LBT present leads to a lower 10-year survival rate (54%), compared to patients with absent LBT (95%) [23].

DIF of skin biopsies also showed the presence of C5b-9 at the DEJ level, but the presence of the membrane attack complex along the blood vessels and the absence of LBT is predictive for the diagnosis of DM, with a specificity of 93.5% and a sensitivity of 78.3%, making it difficult to differentiate it from CLE under the conditions of an amyopathic form [24].

IHC studies on lupus specific lesions of SLE patients, showed that perivascular lymphocyte infiltrate contains mostly T-lymphocytes (CD3), while B-lymphocytes (CD20) represent 5% of the infiltrate and macrophages (CD163) are only occasionally detected (Figure 3). Similarly, in DLE, CD3 is strongly expressed in dermal cell infiltrates and there is a predominance of CD4+, compared to CD8+. The B-lymphocytes (CD20) are arranged in perivascular aggregates surrounded by T-lymphocytes [25, 26].



**Figure 3 – Dermal inflammatory infiltrate composed of both CD3+ T-lymphocytes (A) and CD20+ B-lymphocytes (B), ×400.**

### ☐ Polymyositis/dermatomyositis

Polymyositis (PM)/dermatomyositis (DM) represent the most common inflammatory myopathies (IM), defined by inflammation and weakness of proximal skeletal muscles, extramuscular manifestations and severe prognosis in terms of mortality and morbidity. Diagnostic criteria for polymyositis and dermatomyositis, established by Bohan & Peter, include, besides proximal muscle weakness, raised serum levels of muscle enzymes, electromyography with a myopathic pattern and characteristic lesions of the skin; however, the muscle biopsy still represents the golden standard as a diagnostic tool [15, 27, 28]. In some situations, in dermatomyositis, muscle biopsy is not available and the clinical findings are not enough for the diagnostic, so skin biopsy, more superficial and easy to perform, might prove itself very helpful.

However, HP aspect of early skin changes in DM is sometimes hard to differentiate from SLE one, as it shows

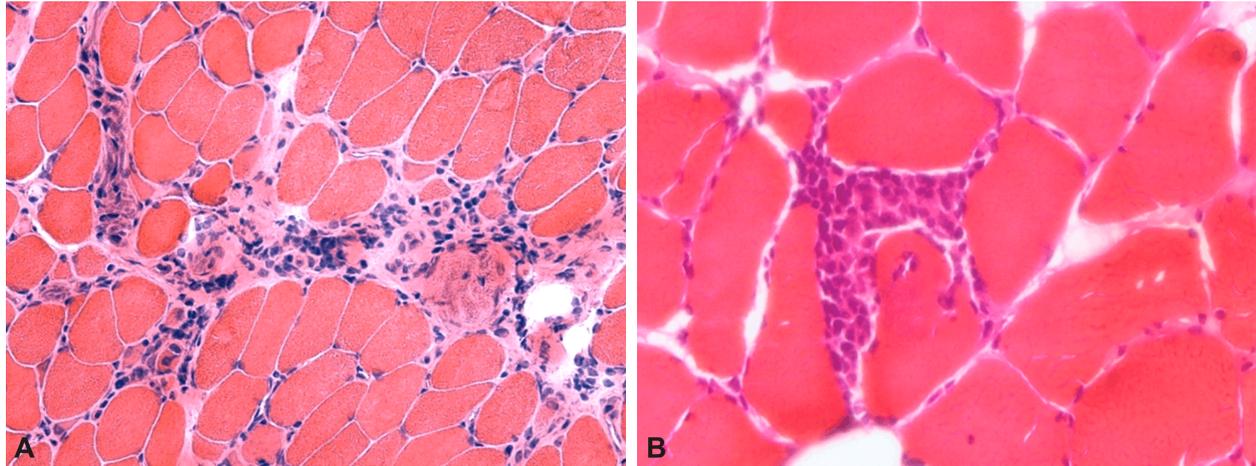
non-specific inflammation, epidermal atrophy, basal membrane degeneration, basal keratinocytes vacuolization, inflammatory lymphocytic infiltration around blood vessels and mucin interstitial deposition [29].

Severe injuries, contrariwise, are associated with sub-epidermal fibrin deposits, and in old lesions appear as band infiltrate under the atrophic epidermis with hydropic degeneration of the basal layer; Gottron papules exhibit HP changes suggestive for vacuolar degeneration of the basal layer. Subcutaneous cellular tissue presents focal areas associated with panniculitis with mucoid degeneration of the adipocytes; in late stages, frequent subcutaneous calcifications might be found.

Muscle biopsy is mandatory in inflammatory myositis, if possible and the specific lesions in active disease should be recognized, as follows: interstitial inflammatory infiltration (consisting of lymphocytes and macrophages), segmental muscle tissue necrosis, hyalinization of the

sarcoplasm, fragmentation and phagocytosis of the degenerate muscle fragments; older lesions appear as muscle fiber atrophy, with diffuse interstitial fibrosis and

vasculopathy due to deposits of immune complexes in the vessels walls, which occurs more frequently in juvenile DM (Figure 4).



**Figure 4 – Muscle biopsy cryosection. (A) Skeletal muscle biopsy revealing perifascicular atrophy of the muscle fibers as the hallmark of dermatomyositis, increased fiber size variability with basophilic regenerated myofibers and increased internal nuclei. Perivascular inflammatory infiltrates and reduced number of capillaries. (B) Endomysial inflammatory infiltrate surrounding and partially invading non-necrotic fibers in polymyositis. HE staining: ×200 (A); ×400 (B).**

Electron microscopy reveals focal disintegration of myofilaments and myofibrils, vacuolization and accumulation of lipids and lysosomes between muscle fibers and intracytoplasmic tubuloreticular structures in the skin and muscle [29, 30].

IM have some aspects in common regarding the presence of muscle fibers necrosis and mononuclear cell infiltration, but there are HP aspects characteristic for each entity [28]. Thus, in PM, the perimysium infiltrating mononuclear cells are mostly CD8<sup>+</sup> cytotoxic lymphocytes, along with macrophages and only a reduced number of CD4<sup>+</sup> lymphocytes; CD8<sup>+</sup> lymphocytes invade muscle fibers expressing MHC I antigen and release cytotoxic molecules, such as perforin and granzyme, leading to local necrosis [31].

In DM, the infiltrate consists in CD4<sup>+</sup> T-helper (Th) lymphocytes, B-lymphocytes, plasmacytoid dendritic cells, macrophages and a low number of CD8<sup>+</sup> lymphocytes and natural killer (NK) lymphocytes; the infiltrate is disposed interfascicular and perivascular in DM, compared to PM, where the infiltrate is disposed intrafascicular. Perifascicular atrophy, which is common in DM and involves types I and II muscle fibers, disposed in two to 10 layers, can support by itself the diagnosis, even in the absence of inflammatory infiltrate. Angiopathy affects intramuscular blood vessels and presents with deposits of Ig and complement (including C5b–C9-membrane attack complex) in the endothelial capillaries and small blood vessels, leading to reduced number of capillaries, intimal hyperplasia and widening of the lumen of the remaining capillaries; tubuloreticular inclusions of endothelial cell cytoplasm might be observed in electron microscopy of DM patients [31].

Correlation of the HP aspect with the presence of antibodies related to PM/DM revealed specific morphological phenotypes; consequently, it was observed that patients with PM have a specific pattern of perifascicular necrosis, while those with DM and anti-Jo-1 positive antibodies, a pattern of perifascicular atrophy, with

complement deposits in sarcolemma of the perifascicular fibers [32].

The presence of anti-signal recognition particle (SRP) antibodies, in PM patients, is associated with predominance of type I muscle fibers and necrotic myofibers, without inflammatory infiltrate, but these HP changes may also occur in non-inflammatory myopathies, like congenital muscular dystrophy, myotubular myopathy, nemaline myopathy, infantile acid maltase deficiency, infantile myotonic dystrophy, cerebellar hypoplasia and Krabbe disease [33].

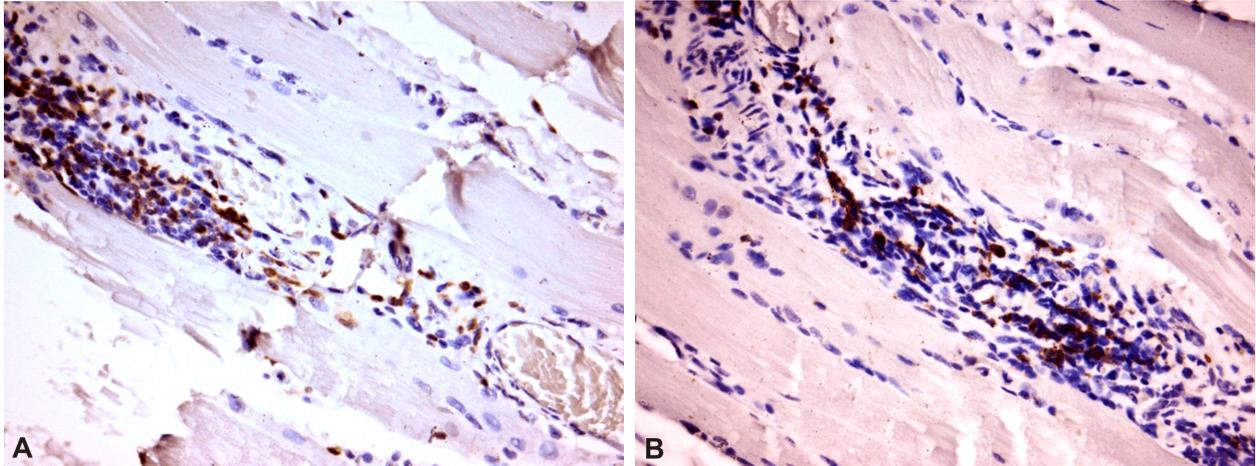
In order to standardize HP evaluation as an instrument to be used in clinical trials, a muscular biopsy international consensus score – HP visual analogue scale (hVAS) was agreed, which includes four domains – inflammatory, vascular, muscle and connective tissue, along with the Likert scale for involvement in each area. The highest score indicates the greatest severity. Consequently, possible correlation of muscle pathology scores with the clinical and laboratory variables, like creatine phosphokinase (CPK) and erythrocyte sedimentation rate (ESR), were searched with no significant results [34].

On the other hand, the association of lower hVAS scores with the myositis-specific antibodies might be predictive for the risk of remaining on treatment in juvenile DM. Thus, it seems that patients with anti-melanoma differentiation-associated gene 5 (MDA5) antibodies tend to have a lower hVAS score and a higher than 50% probability of remaining on treatment five years after diagnosis, while patients with anti-Mi-2 antibodies, with severe hVAS scores (8 points) have the probability of remaining on treatment five years after diagnosis of only 6%; patients with anti-transcription intermediary factor 1-gamma (TIF-1 $\gamma$ ) antibodies and anti-nuclear matrix protein-2 (NXP-2) and those with no antibodies detected, had lower hVAS scores, association that was predictive for the need of treatment for a longer time [35].

IHC shows, by monoclonal antibodies (mABs) staining,

that T-lymphocytes (CD3) and activated T-lymphocytes (CD45Ro+), are dominant in the inflammatory perivascular infiltrate of the papillary and reticular dermis and around

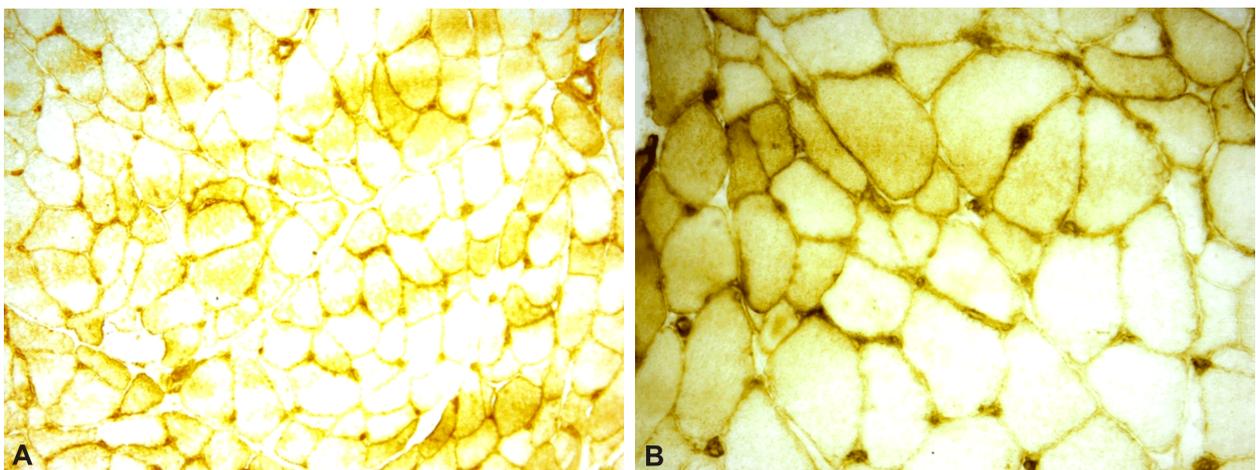
the hair follicles; the proportion of CD3 is higher in PM compared to DM, which is characterized by a higher number of CD20 and CD4 cells (Figure 5) [36, 37].



**Figure 5** – Immunohistochemistry on paraffin-embedded muscle tissue sections showing inflammatory infiltrates with CD3+ T-lymphocytes (A,  $\times 400$ ) and CD20+ B-lymphocytes (B,  $\times 400$ ).

Type I interferon (IFN-I) is involved in the pathogenesis of DM, by inducing the expression of *Myxovirus* resistance (MxA) protein, a biomarker for DM, with perifascicular disposal pattern. Another protein triggered by IFN-I is retinoic acid-inducible gene-I (RIG-I, the product of the *DDX58* gene), with the same perifascicular disposition, which induces response of IFN-I and adjusts MHC I. These two proteins, RIG-I and MxA, can facilitate and improve the diagnosis of DM, as specific biomarkers [38]. Also, interleukin (IL)-1 is expressed on endothelial cells of capillaries, venules and arterioles, but also in muscle tissue without inflammatory infiltrate; IL-1, along with other proinflammatory cytokines, influences MHC expression in muscle fibers [39, 40].

The expression of MHC I and MHC II on muscle fibers is specific to inflammatory myopathies, the presence of MHC I being necessary in order to establish the diagnosis of juvenile DM, while in adult DM, the muscle fibers express more MHC II. MHC I seems to be poorly expressed on muscle fiber sarcolemma (Figure 6) [28]. Both MHC I and MHC II require expression of intercellular adhesion molecule-1 (ICAM-1), which facilitates interaction between the cell receptor and MHC [39, 41]. The MHC I expression seems to be diffusely positive, while MHC II being more frequently observed in the patients with anti-synthetase syndrome (82.8%) *versus* DM (27.6%) [42].



**Figure 6** – Immunohistochemistry for MHC I on muscle cryosections showing up regulation of MHC I on the sarcolemma (A,  $\times 200$ ; B,  $\times 400$ ). MHC I: Major histocompatibility complex class I.

### ☒ Systemic and localized scleroderma

Systemic scleroderma (SSc) is a chronic autoimmune disease, whose dominant manifestation is cutaneous and visceral fibrosis, with pulmonary, cardiac, gastrointestinal, tendon and ligament involvement, through the chronic activation of fibroblasts and myofibroblasts.

The more common form of scleroderma, the localized one, only affects the skin, without any organ involvement. The type of skin lesions might be in the form of patches (morphea), linear, or *en coup de sabre*.

In the SSc, at the skin level, there is excessive production of extracellular matrix (ECM) proteins and replacement of the normal skin with connective tissue

rich in collagen, resulting in thickening of the skin and obliteration of exocrine glands, hair follicles and blood vessels.

ECM includes the cell compartment (resident cells, circulating cells) and acellular connective tissue, rich in collagen, proteoglycans (decorin, lumican), elastin fibers and adhesion molecules (fibronectin, vitronectin). ECM functions as a latent reservoir and produces through the cellular compartment, transforming growth factor-beta (TGF- $\beta$ ), and connective tissue growth factor (CTGF/CCN2), regulating, in this way, the differentiation of mesenchymal cells into fibroblasts [42–44].

It is known that commonly, mesenchymal cells are the precursors of the fibroblasts, but in some situations, they might also originate from epithelial cells, endothelial cells or adipocytes [42]. These cells, under the action of TGF- $\beta$  and hypoxia, differentiate into fibroblasts, which under the action of local mediators produced by platelets and inflammatory cells, trigger collagen and ECM proteins synthesis, with the disorganization of normal architecture. Thus, imbalance between excessive degradation and defective production of ECM proteins leads to the main damage of skin and tissues in SSc [42, 43, 45].

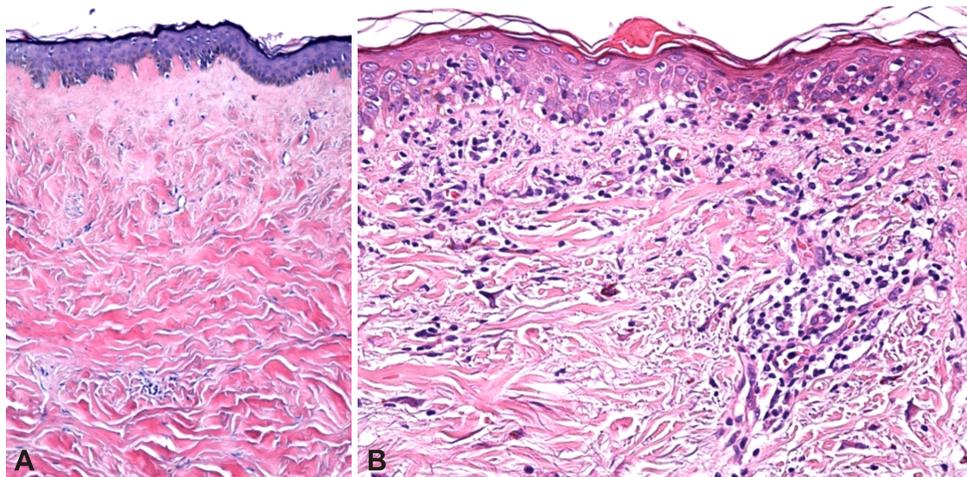
In *early stages of SSc*, HP aspect includes reticular derma infiltrate and collagen fibers gathered in thick bundles, with reduced adipocyte counts; as the disease progresses, subcutaneous cellular tissue is replaced by

inflammatory infiltrate, thin collagen fibers and edema of the vessels walls.

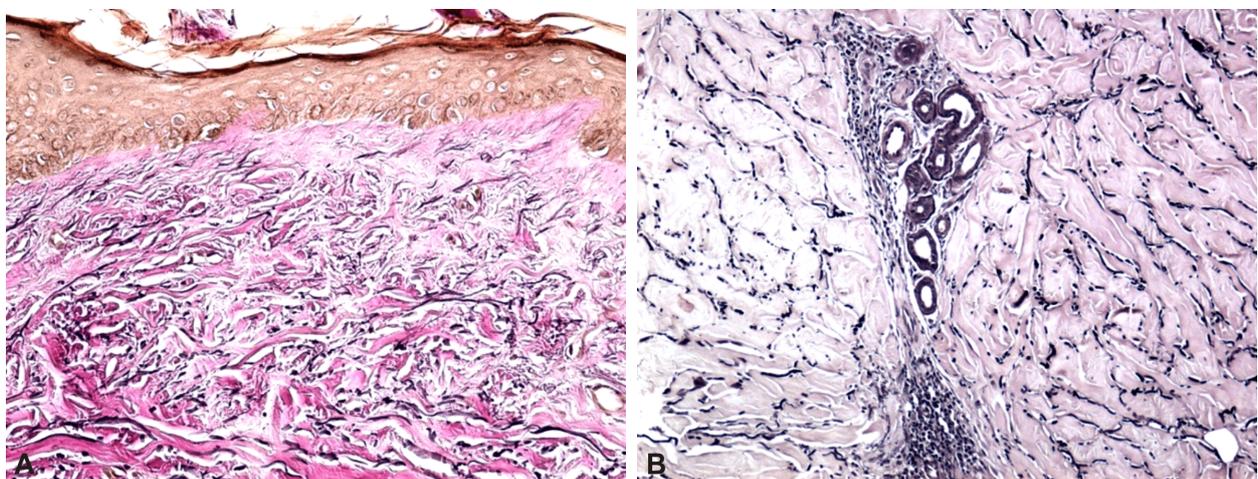
Lymphatic vessels are reduced in numbers and dilated, withal, in reticular and papillary dermis; dilatation compensates the reduction of the number, but this mechanism does not prevent accumulation of fluids and macromolecules in interstitial tissue and subsequently leads to fibrosis, with clinical expression of edematous phase and later fibrotic phase of SSc [46].

In the *sclerotic stage*, in the reticular derma, the inflammatory infiltrate disappears and the collagen fibers arranged in the bundles are thickened; in the papillary dermis, there is atrophy of the eccrine glands, which are surrounded by newly formed collagen fibers, few fibroblasts, but no adipocytes (Figure 7). The blood vessels are sclerotic with narrow lumen and the elastic fibers are thickened and disposed alongside the hypocellular collagen parallel to the epidermis [30, 47].

In the SSc, there is sometimes fragmentation, or even loss of the parallel arranged elastic fibers (by traction of the collagen fibers arranged in bundles), as opposed to morphea, where no changes of the elastic fibers are detected (Figure 8). There is also a loss of CD34+ dendritic cells, which correlates with changes in collagen and elastic fibers, association that could be a diagnostic marker for SSc [48].



**Figure 7 – (A and B) Skin biopsy with mild atrophy of epidermis, thickening and hyalinization of connective tissue of deep dermis and subcutaneous fat, atrophy and reduced density of adnexal structures; increased fibroblasts, thickening and luminal narrowing of small vessels. Mild perivascular and focal interstitial lymphocytic and plasma cell infiltrate in the deep dermis. HE staining: ×100 (A); ×200 (B).**



**Figure 8 – (A) Skin biopsy in SSc patient, with diminished dermal elastic tissue. (B) Skin biopsy in morphea, with diminished dermal elastic tissue. Elastic Van Gieson staining: ×200 (A and B). SSc: Systemic scleroderma.**

The CD34+ dendritic cells in SSc seem to be also correlated with the presence of interstitial lung disease (ILD), as there is a reduction in their number in patients with ILD compared to those without [49].

The labeling of myofibroblasts with 1A anti-alpha-smooth muscle actin ( $\alpha$ -SMA) monoclonal antibody on the skin biopsies showed that the myofibroblasts preceded the formation of hyalinized collagen, assuming a correlation with the degree of dermal fibrosis; thus, quantification of myofibroblasts in the skin could be useful in the SSc evaluation. To support this hypothesis, the treatment with Cyclophosphamide modifies only the number of myofibroblasts and not the already hyalinized collagen [50]. This could suggest that early treatment with Cyclophosphamide, could limit the dermal fibrosis.

CD109, a TGF- $\beta$  co-receptor that inhibits TGF- $\beta$  signaling in keratinocytes, is overexpressed in SSc patients and it increases the production of type I collagen and fibronectin; exposing fibroblasts to recombinant exogenous CD109 might reduce excess ECM production and it could be used as an antifibrotic marker, while the expression of CD109 as a biomarker for active disease [51].

Vascular damage is also important and is considered the primary event in SSc, as the tissue's hypoxia leads to overexpression of proangiogenic growth factors [vascular endothelial growth factor (VEGF)] and thus, to vascular changes [52].

The migration of leukocytes into inflammatory infiltrates implies regulating endothelial cell permeability by adhesion molecules [CD31, vascular cell adhesion molecule-1 (VCAM-1), ICAM-1, CD99, junctional adhesion molecules (JAMs)]. Of these, JAMs belong to the immunoglobulin family and allow leukocyte migration by binding to the lymphocyte function-associated antigen-1 (LFA-1) receptor; this results in faulty angiogenesis and loss of blood vessels in the SSc. There is an increased expression of JAMs in the dermis of patients in early SSc phase, with inflammatory infiltrate present and reduced expression in the late phases [53].

## ☒ Conclusions

In connective tissue disorders, the skin and muscles are affected because of an autoimmune mechanism with the appearance of characteristic signs, but clinical interpretation is not always shallow and biopsy could play an important role both in confirming diagnosis and differentiating between distinct entities. In conclusion, the benefits of skin and muscle biopsy described above provide important support for physicians improving diagnosis and optimizing management connective tissue diseases.

## Conflict of interests

The authors declare that they have no conflict of interests.

## Authorship

Sineta Cristina Firulescu, Ananu Florentin Vreju and Diana Rodica Tudoraşcu contributed equally to this work and thus share main authorship.

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