Endometriosis-associated Skeletal Muscle Regeneration: A Hitherto Undescribed Entity and a Potential Diagnostic Pitfall

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Abstract: Skeletal muscle undergoes regeneration generally after an injury and in some cases it may mimic a malignant process. We observed these aspects in association with abdominal wall endometriosis and as no similar conditions were found in the literature this prompted us to study the main clinicopathologic and immunohistochemical profile of this phenomenon. Thirteen cases of abdominal wall endometriosis were retrieved from the files of our Institute. All original slides were reviewed to reveal the presence of skeletal muscle and 8 cases were enrolled for morphologic and immunohistochemical studies as follows: vimentin, desmin, myoglobin, myogenin, myoD1, CD56, S100, and p21. Histologically, in 4 of the 8 cases in the skeletal muscle adjacent to the endometriotic foci there was a proliferation of round cells with the typical appearance of maturing myoblasts. More peripherally, myotubes and early myocytes were present. This proliferation was florid in 1 case and focal in 3 cases. At immunohistochemical investigation, the less differentiated cells reacted with vimentin, desmin, S100, CD56, myoD1, and myogenin but not with myoglobin or p21. On the contrary, immediately differentiated cells showed a progressive loss of vimentin, CD56, and myoD1 whereas they were positive for desmin, S100, myogenin, myoglobin, and p21. Terminally differentiated cells reacted only with desmin and myoglobin. This peculiar immunohistochemical profile was consistent with the immunophenotype of maturing myoblasts, confirmed the regenerative nature of the phenomenon and allowed differential diagnosis with other proliferations sharing a similar morphology. The expression of early differentiation markers was greatest in the islands of cells nearest to endometriosis, whereas in the more distant areas the markers of late differentiation prevailed. This gradient of expression suggests that muscle cells are stimulated by growth factors or other signals produced by the cycling endometrioid foci. In conclusion, we report a hitherto undescribed entity that may mimic a malignant process, especially when the reaction is florid or when endometriotic glands and stroma are not clearly evident, as during the examination of small biopsies, frozen sections, or cytology samples. Therefore, although the histologic diagnosis of endometriosis is usually straightforward, pathologists should be aware of the concomitant regenerative effects on skeletal muscle, which may represent a possible diagnostic pitfall.

Key Words: endometriosis, skeletal muscle regeneration, diagnostic pitfall

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Skeletal muscle regeneration is an ubiquitous reaction generally after an injury to the skeletal muscle. The process of regeneration is a finely orchestrated set of cellular responses characterized by 2 successive phases, degenerative and regenerative, and is regulated by mechanisms involving cell-cell and cell-matrix interactions and extracellular-secreted factors. Often the morphologic features of the different phases are present simultaneously and, in some clinical conditions, can mimic a malignant process, as recently reported. Histologic diagnosis of endometriosis is usually straightforward, but there are many known diagnostic problems that can potentially confuse the histologic appearance and lead to under diagnosis or misdiagnosis, even such as malignant tumor. For example, some problems can arise due to the absence of glandular or stromal components generally when small biopsies are observed. Other difficulties that may raise the question of a neoplasm include necrotic pseudoxanthomatous nodules, polypoid endometriosis and venous, lymphatic, or perineural invasion. Finally, the histologic diagnosis can be difficult when endometriosis involves an unusual or unexpected site.

This study is based on 4 cases of skeletal muscle regeneration in the abdominal wall harboring endometriosis foci and analyzes the immunohistochemical profile and possible pathogenesis.

MATERIALS AND METHODS

All cases of abdominal wall endometriosis diagnosed between 2000 and 2007 were retrieved from the histologic files of the Institute of Pathological Anatomy and Histology, Perugia University, Italy. Over this period, a total of 13 cases were identified. Clinical data
TABLE 1. Clinical Data of 8 Cases With Abdominal Wall Endometriosis Where Skeletal Muscle Was Present

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (y)</th>
<th>Location</th>
<th>Earlier Surgery (y)</th>
<th>Striated Muscle Fibers Near Endometriosis</th>
<th>Muscle Regeneration</th>
<th>Extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>Left rectus muscle*</td>
<td>Cesarean section (22)</td>
<td>Present</td>
<td>Present</td>
<td>Extensive</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>Not specified</td>
<td>Cesarean section (9)</td>
<td>Present</td>
<td>Present</td>
<td>Foci</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>Right iliac region</td>
<td>—</td>
<td>Absent</td>
<td>Absent</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>Left rectus muscle</td>
<td>Cesarean section (14)</td>
<td>Present</td>
<td>Present</td>
<td>Foci</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>Not specified</td>
<td>—</td>
<td>Absent</td>
<td>Absent</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>47</td>
<td>Right inguinal region</td>
<td>—</td>
<td>Absent</td>
<td>Absent</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>Umbilical and pubic regions‡</td>
<td>Two cesarean sections (15 and 4)</td>
<td>Present</td>
<td>Present</td>
<td>Foci</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>Right inguinal region</td>
<td>—</td>
<td>Absent</td>
<td>Absent</td>
<td>—</td>
</tr>
</tbody>
</table>

*Present from 2 y before operation.
†Present from 2 y before operation.
‡Present from 3 y before operation.

Eight of the 13 cases of abdominal wall endometriosis showed striated muscle fibers in the histologic specimens and were enrolled in the study. Table 1 gives a summary of relevant clinical data of the 8 cases. Patient age ranged from 30 to 47 years, with a mean of 38.9 years. Only in 4 cases a medical history of an earlier surgery was available. These had a history of cesarean section with an interval between earlier procedure and the excision of mass ranging from 4 to 22 years (mean 12.8 y).

Histologically, in all cases endometriotic glands and stroma, hemorrhage, and pigmented histiocytes were present. In 4 cases (cases 1, 2, 4, and 7) there was a proliferation of cells with rhabdoid features in a lobular or infiltrative pattern, predominantly restricted near or around islands of endometriosis (Figs. 1A, B). Cellular proliferation was extensive in case 1, whereas in cases 2, 4, and 7 was present in foci. At high magnification the cells varied in size from small round to large polygonal with a varying amount of cytoplasm (Fig. 1C). The nuclei in the small cells were round and centrally located with single or multiple small nucleoli, whereas in the larger cells they were eccentrically located and sometime binucleated (Fig. 1C). Mixed with these elements and at the periphery of proliferation the cells were significantly larger with many nuclei (giant muscle cells) resembling enlarged Langhans type multinucleated giant cells (Fig. 1D). This cellular polymorphism was suggestive of maturing myoblasts. No mitotic figures or necrotic striated cells were evidenced. In some areas lymphocytic infiltrate was present.

Striated muscle fibers with no regenerative features were observed in the remaining cases (cases 3, 5, 6, and 8). In these they were trapped in the fibrous tissue and distant from endometriosis foci.

The immunohistochemical investigation of the 4 cases with skeletal muscle regeneration revealed that in the small myoblasts the cytoplasm was diffusely positive for vimentin, desmin, CD56, and S100 (Figs. 2A–D) and negative for multicytokeratin, smooth muscle actin, myoglobin, p21, and CD68. Of these cells, some nuclei reacted with myoD1 (Fig. 3A), whereas many nuclei were immunostained with myogenin (Fig. 3B). In immediately differentiated cells the immunostains for desmin, S100, and myogenin were preserved. With increasing maturation vimentin, CD56, and myoD1 were progressively lost, whereas myoglobin and p21 expression increased (Fig. 4). Finally, terminally differentiated and preserved striated muscle cells were negative for vimentin,
S100, CD56, myogenin, myoD1, and p21 and positive for desmin and myoglobin. The investigation with Pax7 antibody evidenced only rare reactivity in nuclei that were morphologically compatible with muscle precursor cells (so-called satellite cells). Ki67 immunoreactive cells were identified only in some fields.

Of note, the immunohistochemical profile, consistent with myoblasts in differentiation, was characterized by the expression of early differentiation markers in cells near endometriosis islands and late markers in areas more distant from them.

**DISCUSSION**

In this study, we describe 4 cases of skeletal muscle regeneration in the abdominal wall harboring endometriotic foci. In the first case (case 1), the skeletal muscle regeneration was particularly florid and a malignant lesion was initially suspected. As we found no previous report of endometriosis-associated skeletal muscle regeneration in the English literature, therefore to understand the link between the 2 lesions we decided to study this process retrieving from our histologic archive other cases of abdominal wall endometriosis with aspects of skeletal muscle regeneration. In 8 of the 13 cases found, striated muscle fibers were present in surgical samples but only in 4 cases was skeletal muscle regeneration present. In these cases, the analysis of clinical data evidenced, as expected, that the 4 lesions occurred in surgical scars of former cesarean sections. The great interval between the first surgery and the second excision excluded the hypothesis that the skeletal muscle regeneration was the normal expression of a surgical trauma. Histologic analysis evidenced a proliferation of round cells with the typical appearance of maturing myoblasts. More peripherally, myotubes and early myocytes were present.

Skeletal muscle undergoes regeneration in response to injury, after direct traumas (eg. intense physical activities, lacerations) or results from indirect causes such as neurologic dysfunction or innate genetic defects.1,2,5

In response to injury, skeletal muscle undergoes a sequential progression of regenerative events including degeneration of the damaged myofibers, infiltration of leukocytes, and myogenesis. The initial event of muscle degeneration is necrosis of the muscle fibers and is generally triggered by the disruption of the myofiber sarcolemma resulting in increased myofiber permeability. This phase of muscle injury is usually accompanied by the activation of mononucleated cells, principally inflammatory cells and muscle precursor cells, also called “satellite cells.” Macrophages penetrate the endomysium tube and remove myofibril debris, whereas the muscle precursor cells proliferate, differentiate, and fuse together to form multinucleated myotubes that mature into myofibers. Histologically, on muscle cross-sections the fundamental morphologic characteristics are newly formed small caliber myofibers with centrally located myonuclei. On muscle longitudinal sections and in isolated single-muscle fibers, central myonuclei are observed in discrete portions of regenerating fibers or along the entire new fiber, suggesting that cell fusion is not diffuse during regeneration but rather focal to the site of injury. Once fusion of myogenic cells is complete, newly formed myofibers increase in size, and myonuclei move to the periphery of the muscle fiber.1,2,5

Significant research into the biology of satellite cells has elucidated the cellular and molecular mechanisms during muscle regeneration.2,6,9 Quiescent satellite cells express the paired box transcription factor family member pax7,11,13 When these cells are activated, there is a rapid upregulation of 2 of the myogenic regulatory factor family, myoD (myf-3), and myf-5. Most activated satellite cells then proliferate, downregulate pax7 and differentiate, whereas other proliferating cells maintain pax7 but lose myoD and withdraw from both cell cycle and immediate myogenic differentiation, returning to a state resembling quiescence. The exact role of these genes is still not entirely clear, but some evidence suggests that myf5 promotes satellite cell self-renewal, whereas myoD promotes satellite cell progression to terminal differentiation. The differentiation phase is characterized by the expression of myogenin (myf-4) in cells beginning their terminal differentiation program. This is followed by the activation of the cell cycle arrest protein p21 and permanent exit from the cell cycle. The differentiation program is then completed with the activation of muscle-specific proteins, such as myosin heavy chain, and the fusion of muscle precursor cells to form syncytial muscle fibers.2,6,8,9,11

In our study to confirm the morphologic aspects of regeneration we used a large panel of antibodies. Vimentin, desmin, S100, and multicytokeratin are certainly some of the antibodies used in the first approach to the mesenchymal proliferation. In skeletal muscle regeneration, vimentin stains the less differentiated myoblasts, S100, on the contrary, stains the less and the intermediate differentiated cells, whereas desmin stains the myoblasts regardless of the degree of differentiation. However, this last antibody has high sensitivity, but is not skeletal muscle-specific. For this reason, despite the high amount of background stain that may sometime complicate the interpretation, myoglobin could be useful to confirm skeletal differentiation as it stains both intermediate and terminally differentiated cells. Other markers of muscle differentiation such as myoD1 and myogenin may be useful because they are expressed much earlier in

**FIGURE 1.** A, Case 1. Lobular proliferation of round cells with the appearance of myoblasts near an island of endometriosis (arrow). B, Case 4. Foci of myoblasts were closely adjacent to the endometriotic stroma (upper half). C, Case 1. Small-sized myoblasts located near the endometriotic foci compared with the periphery of the lesions (D) where giant multinucleated myoblasts (arrow) were present (hematoxylin and eosin, A: ×40; B: ×100; C and D: ×400).
A, Case 4. Small myoblasts showed cytoplasmic reactivity for vimentin, whereas in giant muscle cells there was a decrease of immunostain (arrow). B, Case 7. Strong cytoplasmic immunostain for desmin. C, Case 4. Myoblast (arrowhead) and less differentiated myotubes (small arrow) reacted with CD56; in more mature myotubes there was a lack of reactivity (big arrow). D, Case 1. Strong cytoplasmic immunostain for S100 (A: vimentin, ×200; B: desmin, ×200; C: CD56, ×200; D: S100, ×400).
the normal skeletal muscle differentiation program. In our cases, myoD1 and myogenin were observed in less differentiated cells, whereas the intermediately mature cells reacted for myogenin and not myoD1. On the contrary, in our cases Pax7 investigation was not useful for showing muscle differentiation because it only identified the satellite cells. This result was owing to its expression only in quiescent, activated, and proliferating satellite cells but not in cells in differentiation. Other markers that can be used to study this phenomenon are p21 and CD56. P21 shows the final exit from the cell cycle and is positive only in intermediately differentiated cells. CD56 (NCAM) is one of a group of membrane-bound cell adhesion molecules that mediate adhesion between neurons and between neurons and myotubes. In particular, it is expressed during certain stages of muscle maturation, regeneration, or denervation. In fact, in our cases small cells reacted with CD56 antibody, whereas in intermediately differentiated cells it was progressively lost.

Based on these findings skeletal muscle regeneration showed a peculiar dual immunohistochemical profile: the less differentiated cells reacted with vimentin, desmin, S100, CD56, myoD1, and myogenin, whereas with increasing maturation the cells preserved the desmin stain, progressively lost vimentin, S100, CD56, myoD1, and myogenin, and reacted with myoglobin and p21.

The differential diagnosis of skeletal muscle regeneration includes adult-type rhabdomyoma, rhabdomyosarcoma, and other tumors with rhabdoid features. Adult rhabdomyoma is mainly observed in the head and neck region of males. It consists of a well-defined mass composed of cells with finely granular cytoplasm admixed with vacuolated cells. These clinicopathologic features are quite straightforward. Rhabdomyosarcoma, characteristically is composed of poorly differentiated and pleomorphic round or spindle-shaped cells associated with varying numbers of rhabdomyoblasts. Of the 3 main histologic groups, the embryonal type mainly enters morphologic differential diagnosis because it recapitulates the phenotypic feature of embryonic skeletal muscle with cells in various stages of myogenesis. However, the age of incidence (children) and sites of involvement (head and neck) can help to exclude this subtype. The differential diagnosis with the other subtypes depends on the amount of more differentiated rhabdomyoblasts. In fact, in the alveolar subtype the histologic feature is quite typical (monomorphic cells in alveolar or solid pattern) and is in contrast with the morphologic variegated appearance of skeletal muscle regeneration. The pleomorphic subtype is easily recognized because of its appearance-high-grade sarcoma. Finally, in all subtypes immunohistochemical investigation is useful for showing the dual differentiation

FIGURE 3. Case 1. The immunohistochemical investigation of myogenic markers of differentiation showed some nuclei positive for myoD1 (A) and more nuclear positivity for myogenin (B) (A, myoD1 × 400; B, myogenin, × 400).
lineage of the cellular population involved in skeletal muscle regeneration.

The differential diagnosis with other tumors with rhabdoid features mainly includes a possibility of a metastatic melanoma, further suggested by S100 and vimentin positivity. However, the positivity of muscle markers and the absence of melanoma-associated markers such as MART-1/Melan-A and HMB-45 exclude the possibility of melanoma.

Of minor clinical importance is the differentiation with pseudosarcomatous lesions such as proliferative myositis and myositis ossificans. Proliferative myositis, another reparative phenomenon that may coexist with skeletal muscle regeneration and which can be confused with sarcoma, is characterized by cellular proliferation rich in fibroblasts that proliferate in the surrounding individual fibers rather than being confined within the endomysium. The hallmark of these lesions is the presence of cells with very large basophilic cytoplasm, vesicular nuclei, and very prominent nucleoli, resembling ganglion cells. Finally, the immunohistochemical profile suggests a myofibroblastic nature. Myositis ossificans is easily distinguished from skeletal muscle regeneration by its triphasic pattern and the characteristic presence of osteoid and mature lamellar bone.

In recent years, other authors have reported skeletal muscle regeneration as a condition that may simulate a malignant process and which may be a potential diagnostic pitfall. Guillou et al reported 3 cases of skeletal muscle regeneration in some clinical conditions, 2 of which simulated small round tumors such as alveolar rhabdomyosarcoma. Stefanato et al described 1 case masquerading as a residual, previously excised, squamous cell carcinoma. Pankaj et al reported 4 cases in which regenerating muscle led to a false-positive diagnosis of malignant round cell tumor in fine-needle aspiration cytology and frozen sections. Finally, Ghosn et al described 1 case of skeletal muscle regeneration presenting clinically as a rapidly growing cutaneous nodule on the upper lip after the blunt trauma.

In this study, we report a hitherto undescribed condition of an endometriosis-associated skeletal muscle regeneration and due to some observations we hypothesize that the skeletal muscle regeneration was induced by endometriosis. First, where skeletal muscle regeneration was present it was mainly localized around endometriosis islands, whereas in cases without skeletal muscle regeneration, normal skeletal muscle fibers were distant from endometriosis islands. Second, immunohistochemical investigation evidenced a greater reaction for vimentin, myogenin, and myoD1 in muscle cells around endometriosis islands whereas the more morphologically mature cells and those distant from endometriosis foci reacted with myoglobin and p21 and not vimentin and myoD1. Finally, endometriosis could stimulate skeletal muscle as occurs in the smooth muscle cells. In fact, endometriosis involving indigenous smooth muscle, such as the pelvic ligaments and the walls of the bowel and bladder, often stimulates the smooth muscle cells to proliferate, such as myometrial smooth muscle proliferation around adenomyosis foci. We may, therefore, speculate that muscle cells are stimulated by growth factors or other signals produced by the cycling endometrioid foci. For these reasons, we believe that in the cases observed the phenomenon of skeletal muscle regeneration was triggered by surgery and subsequently maintained by the cycling endometrium implanted in the muscle tissue of the abdominal wall.

In conclusion, our study describes 4 cases of skeletal muscle regeneration in a pathologic condition, never before reported in literature, which could mimic a malignant process, especially when the reaction is florid or when endometriotic glands and stroma are not present in the specimen as during the examination of small biopsies, frozen sections, or cytology samples. Therefore, although the histologic diagnosis of endometriosis is usually straightforward, the presence of skeletal muscle regeneration may represent a potential diagnostic pitfall and pathologists should be aware of these reactive changes in skeletal muscle.

Our observations give interesting biologic insight into the mechanisms controlling skeletal muscle regenerations and, in view of the possible implications in the field of myopathies, further studies could help to clarify the relationships resulting from the direct contact between
a tissue with rapid cellular turnover (endometrium) and a tissue with low cellular turnover (skeletal muscle).

REFERENCES
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