Localized panniculitis secondary to subcutaneous glatiramer acetate injections for the treatment of multiple sclerosis: A clinicopathologic and immunohistochemical study

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Background: Glatiramer acetate has been shown to be effective in reducing the relapse and improving the disability of patients with multiple sclerosis. The most common adverse effects at the injection sites include pain, inflammation, and induration that spontaneously disappear within hours or a few days.

Objective: We sought to characterize the histopathologic findings of localized panniculitis induced by glatiramer acetate at the injection sites.

Methods: Seven patients receiving daily glatiramer acetate injections for treatment of multiple sclerosis developed localized panniculitis at the injection sites. The lesions were histopathologically and immunohistochemically studied.

Results: The lesions consisted of a mostly lobular panniculitis, with lipophagic granuloma, namely histiocytes engulfing the lipids from necrotic adipocytes. In many areas, scattered neutrophils and eosinophils were seen both in the septa and in the fat lobules. Connective tissue septa showed widening and fibrosis in conjunction with many lymphoid follicles, presenting with germinal center formation. Immunohistochemically, the inflammatory infiltrate of the fat lobule consisted of $CD68^+$ histiocytes and suppressor/cytotoxic T lymphocytes. In contrast, the lymphoid follicles in the septa and at the interface between septum and fat lobule were mainly composed of B lymphocytes.

Limitations: Only one biopsy was performed in each patient and, therefore, it was not possible to study the histopathologic evolution of the panniculitic process.

Conclusions: Localized panniculitis at the sites of subcutaneous injections of glatiramer acetate for treatment of multiple sclerosis seems to be a rare, but characteristic side effect of this therapy. The histopathologic pattern of these lesions consists of a mostly lobular panniculitis, with histiocytes and T lymphocytes in the fat lobule and thickened septa with scattered lymphoid follicles, which are mostly composed of B lymphocytes. (J Am Acad Dermatol 2006;55:968-74.)

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Glatiramer acetate consists of the acetate salts of a mixture of synthetic polypeptides, containing 4 naturally occurring amino acids: L-glutamic acid, L-alanine, L-tyrosine, and L-lysine. It simulates the myelin basic protein and is currently used for treatment of multiple sclerosis because it has been shown to be effective in reducing the relapse and improving the disability of patients with relapsing-remitting multiple sclerosis.[1,2] The drug is administered in daily subcutaneous injections of 20 mg. The most common adverse effects, which occur in approximately 20% to 60% of the patients, include pain, inflammation, and induration at the

injection site, all of which spontaneously disappear within hours or a few days. A more rare adverse effect is a frank panniculitis followed by localized lipoatrophy at the injection sites, which has been described only in a few patients receiving treatment with glatiramer acetate injections.[3-7] In those reports, the authors only described "lipoatrophy," in some cases even without histopathologic study of the lesions,[5,7] and in others with vague histopathologic descriptions of "inflammatory infiltrate involving the subcutaneous tissue."[3,4,6]

We report a series of 7 patients who developed localized panniculitis at the sites of subcutaneous injections of glatiramer acetate for treatment of multiple sclerosis. Our goal was to characterize the histopathologic and immunohistochemical features of this drug-induced panniculitis. We also discuss the histopathologic differential diagnosis with other types of panniculitis showing similar histopathologic features.

METHODS

The clinical characteristics of our series are summarized in Table I. Briefly, all 7 patients were female, with age range between 28 and 51 years (median: 38 years). All patients had been instructed in self-injection techniques to assure the safe administration of a daily subcutaneous injection of 20 mg of glatiramer acetate. The commercially available form is a white, sterile, lyophilized powder containing 20 mg of glatiramer acetate and 40 mg of mannitol supplied in refrigerated single-use vials for subcutaneous administration after reconstitution with sterile water.[8] As a regular procedure, prefilled syringe packages from the refrigerator were kept at room temperature for 20 minutes before the injection to allow the solution to warm to room temperature. The patients injected the drug into the subcutaneous fat at the recommended sites (periumbilical skin, upper side aspects of arms, hips, and front of thighs) and they did not use any site more than once each week. The patients denied constitutional symptoms, trauma, or other skin problems, and they were not taking any other medications at the time. Five patients received previous treatment with subcutaneous injections of interferon beta, but this therapy had been withdrawn at least 1 month before treatment with glatiramer acetate injections was initiated.

RESULTS

The lesions were located at the injection sites, and all patients developed subcutaneous erythematous nodules in several areas (periumbilical skin, upper

during the treatment. The duration of glatiramer acetate treatment before localized panniculitis at the injection sites was 1 to 2 months. When glatiramer acetate injections were withdrawn, the cutaneous lesions disappeared within 2 to 3 months, but in 5 of the 7 patients, subcutaneous erythematous nodules at the injection sites developed again when glatiramer acetate injections were reintroduced. In all patients, residual lesions of lipoatrophy (Fig 1) and hyperpigmentation developed in previously inflamed sites.

Histopathologic studies were performed in all cases. Although the histopathologic findings varied from case to case, there were some common features. These features consisted of a mostly lobular panniculitis, with a papillary and reticular dermal perivascular infiltrate, mainly composed of lymphocytes. In the subcutaneous fat, the so-called lipophagic granuloma was the main histopathologic finding, showing macrophages with large foamy cytoplasm engulfing the lipids from necrotic adipocytes (Fig 2). In addition, small mature lymphocytes had infiltrated the necrotic fat lobules. In many areas, scattered neutrophils and eosinophils were seen both in the septa and the fat lobules. Connective tissue septa showed widening and fibrosis. Many lymphoid follicles, with prominent germinal center formation, were seen both in the septa and at the interface between the septum and the fat lobule (Fig 3). These lymphoid nodules were uniformly composed of small mature lymphocytes at the center and abundant plasma cells at the periphery. In 3 cases, some of the septal blood vessels showed swollen endothelial cells and small lymphocytes involving the vessel walls, suggesting lymphocytic vasculitis, albeit without nuclear dust and fibrinoid necrosis (Figs 4 and 5). Examination of the histologic sections under polarized light failed to disclose refractile foreign bodies.

Immunohistochemical studies were performed in all biopsy specimens of the 7 cases. The antibodies used, their sources, dilutions, and results are summarized in Table II. Briefly, the inflammatory infiltrate of the fat lobule was mainly composed of $CD68^+$ histiocytes (Fig 6), whereas the lobular lymphocytes were mainly suppressor/cytotoxic T lymphocytes, expressing CD45, CD3 (Fig 6), CD8 (Fig 7), and TIA-1 (Fig 8). Only a few lymphocytes involving the fat lobule showed a CD4 immunophenotype (Fig 7). TIA-1 expression was also seen in neutrophils, with coexpression of myeloperoxidase and neutrophilic elastase. In contrast, the lymphoid follicles in the septa and at the interface between septa and the fat lobule were mainly composed of B lymphocytes,

Table I. Clinical characteristics of patients developing localized panniculitis at the sites of glatiramer acetate injections

Case/age, y/sex	1/51/F	2/33/F	3/33/F	4/28/F	5/46/F	6/36/F	7/41/F
RRMS duration, y	6	2	9	5	6	2	3
Glatiramer acetate treatment duration, y	3	1	1	2	1	2	1
Daily dosage, mg	20	20	20	20	20	20	20
TI between first injections and development of panniculitis, mo	1	1.5	2	2	1	2	1.5
Regression of lesions after injections were withdrawn, mo	2	3	2	3	2	3	3
Recurrent lesions with reintroduction of injections	No	Yes	Yes	Yes	Yes	No	Yes
Clinical features	EN	EN	EN	EN	EN	EN	EN
Residual lesions	LA, HP	LA, HP	LA, HP	LA, HP	LA, HP	LA, HP	LA, HP
Administered treatment	TCEs	TCEs	TCEs	TCEs	TCEs	TCEs	TCEs
Previous treatment with interferon beta	6 mo	2 mo	4 y	3 y	1 y	No	No
TI between interferon stopped and glatiramer acetate introduced	2 mo	1 mo	1 mo	I y	I mo		

EN, Erythematous nodules; *F*, female; *HP*, hyperpigmentation; *LA*, lipoatrophy; *RRMS*, relapsing-remitting multiple sclerosis; *TCEs*, topical corticosteroids; *TI*, time interval.

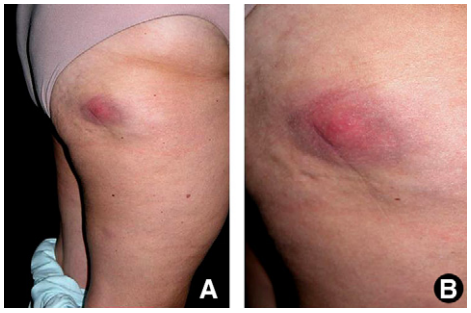

Fig 1. Case 3. Clinical appearance of lesions. **A**, Erythematous subcutaneous nodule on site of injection of glatiramer acetate at hip. **B**, Close-up view.

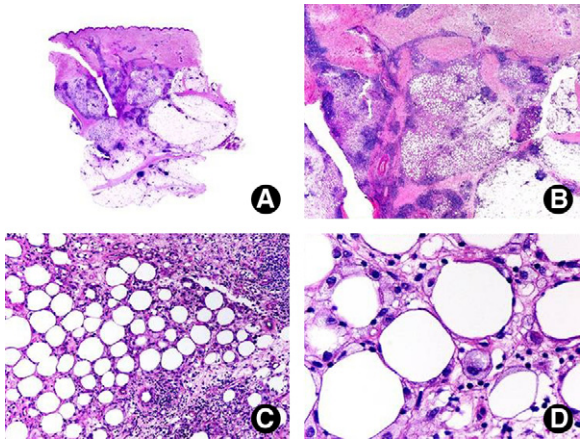

Fig 2. Case 3. Histopathologic features. **A**, Scanning magnification showing involvement of subcutis. **B**, Involvement of subcutaneous tissue consists of mostly lobular panniculitis. **C**, Center of fat lobule shows necrotic adipocytes and inflammatory infiltrate. **D**, Lipophagic granuloma. Most inflammatory cells consist of histiocytes engulfing lipids from necrotic adipocytes. (**A** to **D**, Hematoxylin-eosin stain; original magnifications: **A**, ×5; **B**, ×40; **C** and **D**, ×400.)

scattered lymphocytes showed weak positivity for CD30. The proliferative index was low, with only 5% of the nuclei of the inflammatory infiltrate cells expressing MIB-1 immunoreactivity. The remaining antibody stains were negative.

DISCUSSION

Clinical trials have shown that glatiramer acetate is effective in reducing the relapse rate and improving disability of patients with multiple sclerosis.[1,2] The precise pharmacologic mechanism has not yet been fully elucidated, but it was suggested that glatiramer acetate alters T-cell immune function by inducing

class II major histocompatibility binding,[9] and modifying cytokine profiles.[10] A switch of the immune reaction from a T-helper 1 to a T-helper 2 cell type has been observed during treatment with glatiramer

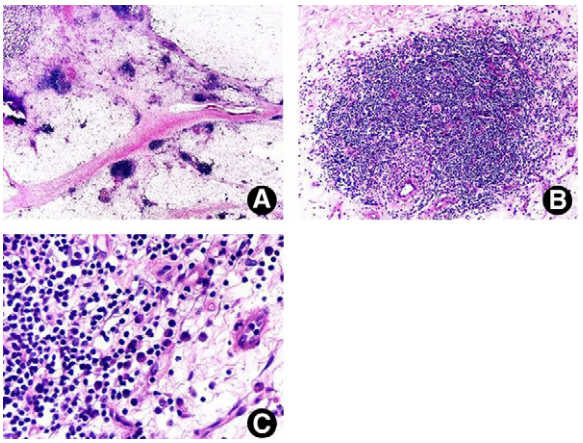

Fig 3. Case 3. Additional histopathologic features of case 3. **A**, Many lymphoid follicles are seen at septa and periphery of fat lobules. **B**, Center of lymphoid follicles is small mature lymphocytes. **C**, Numerous plasma cells are present at periphery of lymphoid follicles. (**A** to **C**, Hematoxylin-eosin stain; original magnifications: **A**, ×40; **B**, ×200; **C**, ×400.)

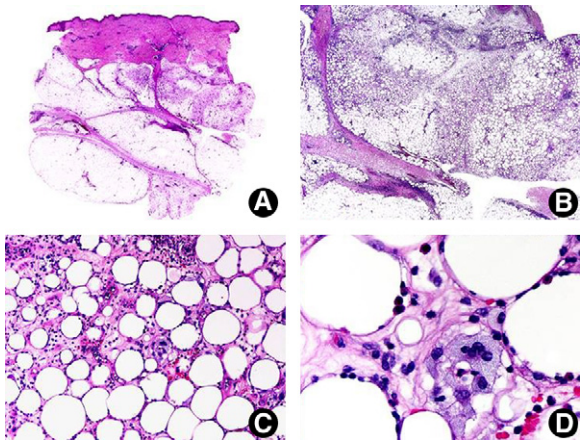

Fig 4. Case 4. Histopathologic features. **A**, Scanning power showing mostly lobular panniculitis. **B**, Septa are thickened, but most infiltrate is within fat lobule. **C**, Necrotic adipocytes and histiocytic infiltrate. **D**, Foamy histiocytes as expression of lipophagic granulomas. (**A** to **D**, Hematoxylin-eosin stain; original magnifications: **A**, ×5; **B**, ×40; **C**, ×200**; D**, ×400.)

mechanism of action.[11] Stimulated glatiramer acetate—reactive T-helper 2 lymphocytes can release anti-inflammatory cytokines, such as interleukin-4 and -10, and neurotropic factor, such as brain-derived neurotrophic factor.[12]

In controlled clinical trials the most commonly observed adverse effects were injection site reactions, vasodilatation, chest pain, asthenia, infection, pain, nausea, arthralgia, anxiety, and hypertonia.[13] Concerning injection-site reactions, erythema (66% of patients), inflammation (49%), pain (73%), and pruritus (40%) are the most commonly described local side effects.[8] In a few cases, lipoatrophy at the sites of injection has also been described.[3-7] Drago et al[3] were the first authors pointing out localized lipoatrophy at the sites of subcutaneous injections in 6 female patients with multiple sclerosis receiving treatment with glatiramer acetate. They reported that the lesions developed without any preceding inflammation and the overlying skin did not exhibit inflammation, sclerosis, or hyperpigmentation. Histopathologic features of those cases were described as: "Normal epidermis and a perivascular infiltrate with lymphocytes, neutrophils, and eosinophils throughout the dermis. There were fibroses of fat septa and occasionally a septal and perivascular inflammatory infiltrate."[3] Mancardi et al[4] reported 3 female patients and one male patient with well-circumscribed areas of skin depression at the injection sites of glatiramer acetate. Histopathologic studies were performed in the 4 cases, with one patient showing erythematous nodules and the others presenting with depressed

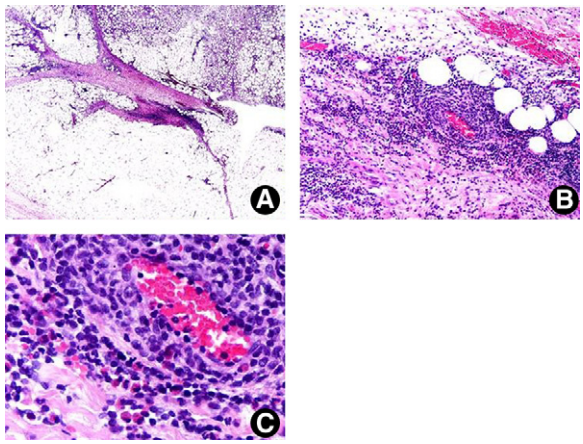

Fig 5. Additional histopathologic features of case 4. **A**, Septa contain dense lypmphocytic infiltrate. **B**, Some septal blood vessels showed swollen endothelial cells and small lymphocytes involving vessel walls, suggesting lymphocytic vasculitis. **C**, Higher magnification demonstrates that nuclear dust and fibrinoid necrosis are absent. Note presence of numerous eosinophils among lymphocytes. (**A** to **C**, Hematoxylin-eosin stain; original magnifications: **A**, ×40; **B**, ×200; **C**, ×400.)

erythematous nodule were described as follows: "Inflammation was present in the subcutis with a septal and perivascular pattern. The morphological appearance of the vessels was normal, and no thrombosis was detected." In contrast, the biopsy specimens from depressed areas of the skin showed "fibrosis of the dermis and subcutis with reduction in the size of the fat lobules and minimal mononuclear infiltrates."[4]

Table II. Immunohistochemical study of 7 cases of localized panniculitis secondary to subcutaneous injections of glatiramer acetate for treatment of multiple sclerosis

Antibody	Clone	Source	m/p	HIER	Dilution	Specificity	Results
CD15	Leu-M1	BD	m	+	1:400	Mature neutrophils, monocytes, myeloid cells	−/+ (neutrophils)
CD34	HPCA1/My10	BD	m	+	1:100	Hematopoietic precursor/ stem cells	−
CD43	DF-T1	DG	m	+	1:100	Myeloid cells, macrophages	−
CD45 (LCA)	PD7/26	DG	m	+	1:400	Granulocytes, monocytes, macrophages, all hematolymphoid cells	++ (lymphocytes and histiocytes)
CD45RO	UCHL1	DG	m	+	1:400	T lymphocytes	++ (lymphocytes)
CD68	PGM-1	DG	m	+	1:200	Monocytes and macrophages	+++ (histiocytes)
Myeloperoxidase	MPO-7	DG	p	+	1:2000	Myeloid cells, granulocytes and their precursors	+ (neutrophils)
Neutrophilic elastase	NP57	DG	m	−	1:100	Myeloid cells, granulocytes and their precursors	+ (neutrophils)
CD3	F7.2.38	DG	p	+	1:200	Pan T-cell marker	++ (lymphocytes)
CD4	1F6	DG	m	+	1:10	T-helper/inducer cells	+ (lymphocytes)
CD8	DK25	DG	m	+	1:50	T-suppressor/cytotoxic cells	++ (lymphocytes)
TIA-1	2G9	IK	m	+	1:600	T-suppressor/cytotoxic cells, neutrophils	++ (granulocytes)
CD20	L26	DG	m	+	1:500	Pan B-cell marker	++ (lymphoid nodules)
CD79a	JCB117	DG	m	+	1:50	Pan B-cell marker, including plasma cells	++ (lymphoid nodules)
CD30	Ber-H2	DG	m	+	1:10	Ki-1 marker: activated T and B cells, Reed-Sternberg cells	−/+ (lymphocytes)
MIB-1	Ki-67	DG	m	+	1:40	Proliferation marker	−/+ (lymphocytes)

BD, Becton Dickinson, San Jose, Calif; *DG*, Dako, Glostrup, Denmark; *HIER*, heat-induced epitope retrieval; *IK*, Immunotech, Krefeld, Germany; *m*, monoclonal; *p*, polyclonal.
−, Negative; −/+, single scattered cells; +, 15 % positive cells; ++, 15% to 50% positive cells; +++, more than 50% positive cells.

with symmetric soft-tissue depressions on the periumbilical skin, upper back aspect of arms, side of hips, and front of thighs that correlated exactly to the injection sites of glatiramer acetate. Unfortunately these lesions were not histopathologically studied. Soós et al[6] described an additional female patient developing localized panniculitis and subsequent lipoatrophy at the sites of subcutaneous glatiramer acetate injections for the treatment of multiple sclerosis. The patient had several areas of circumscribed cutaneous atrophy on the skin of the abdominal wall and thighs. Histopathologic study of these lesions demonstrated "normal epidermis and dermis. A marked lymphohistiocytic infiltrate was seen as well as swollen vessels with monocytic cells both subcutaneously and in the fatty tissue septa." In spite of the absence of inflammatory clinical features on the overlying skin, Soós et al[6] were the first authors who recognized a panniculitic stage previous to the lipoatrophy induced by the glatiramer acetate injections. Finally, Edgar et al[7] described 5 female patients with lipoatrophy at the sites of glatiramer acetate injections and histopathologic study of three of them. These authors described "normal immunofluorescence and no inflammatory infiltrate" in one patient, whereas in the other one the "skin punch biopsy was normal." The panniculitis secondary to glatiramer acetate injections seems to be much more frequent in female than in male patients. In our literature review we have found that only 1 of the 17 described patients was male and our 7 patients were also women. After reviewing the literature it also becomes clear that a thorough histopathologic study of localized panniculitis secondary to glatiramer acetate injections has yet to be done.

Lipoatrophy secondary to subcutaneous injections has been described in conjunction with several drugs, including insulin, corticosteroids, vasopressin, antibiotics, human growth hormone, iron dextran, diphtheria-pertussis-tetanus immunization serum, and antihistamines.[14] Although different pathogenic mechanisms have been proposed for each of these drugs, lipoatrophy most probably is the common late or residual stage of a previous drug-induced localized panniculitis. In the described cases of localized lipoatrophy at the sites of subcutaneous glatiramer

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