ultrasound, mostly showing a hypo-anechoic appearance; five of six lesions presented with worrisome features at magnetic resonance imaging (MRI). At cytological examination, most cases showed steatonecrotic debris (87.9%), variably associated to macrophages (63.6%), adipocytes (48.5%), multinucleated giant cells (30.3%), and lipophages (24.2%) (Fig. 1A). Atypical epithelial cells were described in four of 33 cases (12.1%) (Fig. 1B,C), but only one diagnosis of cancer was confirmed on histology (Fig. 1D). The mean follow-up period was 109 ± 56.9 months and no false negatives were found in the observed patients.

Since lipofilling is becoming a common procedure in breast reconstructive surgery, cytopathologists are expected to face this specific diagnostic issue in their routine practice in the near future. Liponecrosis can develop in the breast as a consequence of several conditions, such as trauma, infection, irradiation (i.e., post-operative radiotherapy) etc.[9] Liponecrosis may occur after lipofilling too, especially when large amounts of adipose tissue are injected.[9,10] In patients who received pre- or post-operative radiotherapy, establishing whether liponecrosis was due to irradiation or lipofilling is challenging. However, since in our series liponecrosis also occurred in patients who had not undergone radiotherapy treatment, lipofilling could explain the aetiology of liponecrosis.

Awareness of patients' clinical history and radiological features, albeit valuable, may not suffice to prevent diagnostic errors. Moreover, in case of scanty material, ancillary techniques such as immunohistochemistry may not always be feasible and/or helpful. Our study highlights how good morphological criteria can reduce the number of patients sent to surgery with radiological suspicious lesions after breast lipofilling. For 87.8% of our patients, a diagnosis of cancer recurrence was ruled out by classical cytology. By contrast, if breast cancer recurrence is suspected after lipofilling but cytological findings are not conclusive, caution should be taken to avoid unnecessary surgery and a careful multidisciplinary evaluation would be required.

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A novel ultrastructural finding in statin-exposed patients with inflammatory myositis

Sir,

The clinical spectrum of muscle disorders associated with HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase (HMGCR) inhibitors (statins) includes asymptomatic hyper-CKaemia, myalgia +/− hyper-CKaemia, myalgia with hyper-CKaemia with recovery after cessation of statins, acute and subacute painful proximal myopathies, rhabdomyolysis and progressive myopathies often unresponsive to statin withdrawal. The muscle biopsy changes described in this progressive group includes polymyositis (PM), dermatomyositis (DM) and necrotising myopathy (NM). Among patients with NM, a subset has an immune-mediated NM, known as IMNM or necrotising autoimmune myopathy (NAM) on the basis of positive MHC1 immunoexpression.

We sought to review and compare the clinical and pathological features of patients with histologically-confirmed idiopathic inflammatory myositis (IIM) exposed to statins with those who were statin-naive.

The establishment of the South Australian Myositis Database for patients with histologically-confirmed IIM has been approved by the Research Ethics Committees of all teaching hospitals in South Australia (SA).

All adult muscle biopsies in SA are assessed and reported in a single laboratory at SA Pathology. Routine immunohistochemical inflammatory screen performed included antibodies to CD45, CD68, MHC1, MHC2, neonatal myosin, MAC, caveolin 3 and dysferlin. Ultrastructural examination of all muscle specimens was performed with electron microscopy (EM). Muscle biopsy specimens were fixed with 2.5% glutaraldehyde and postfixed in 2% osmium tetroxide, dehydrated in graduated alcohols and embedded in epoxy resin. Semi-thin sections (1–2 μm) were stained with toluidine blue for light microscopic examination. Ultra-thin sections were stained with uranyl acetate and lead acetate and were examined using a transmission electron microscope.

Generally accepted histopathological criteria are employed for a diagnosis of IIM[1] and biopsies are subjected to peer

review. A diagnosis of IIM includes all primary inflammatory myopathies including PM, DM, inclusion body myositis (IBM), myositis not otherwise specified (MNOS), and NM. Dermatomyositis is characterised by vasculopathy, perifascicular atrophy and variable interstitial inflammation and vascular membrane attack complex (MAC) positivity on immunostaining. Polymyositis is characterised by polyfocal polyphasic muscle fibre necrosis with inflammatory cell (lymphocyte and macrophage) infiltration including lymphocyte infiltration of non-necrotic muscle fibres, regeneration and MHC-1 expression in >50% of myofibres. Inclusion body myositis is characterised by rimmed vacuoles and polyfocal polyphasic muscle fibre necrosis with variable inflammatory cell (lymphocyte and macrophage) infiltration including lymphocyte infiltration of non-necrotic muscle fibres, regeneration and strong MHC1 expression and tubulofilamentous inclusions on EM. Necrotising myopathy is defined by necrotic muscle fibres with minimal inflammation and on the basis of MHC1 expression is further subdivided into necrotising autoimmune myopathy (NAM) (MHC1 positive) and toxic NM (MHC1 negative).

MNOS is diagnosed by the presence of an inflammatory myopathy not fulfilling diagnostic criteria for PM, DM, IBM or NM.

All adult muscle biopsies performed in SA between January 2012 and August 2013 inclusive were reviewed. For patients diagnosed with IIM, the demographic, clinical, serological and histopathological details were determined from the South Australian Myositis Database. For patients without a diagnosis of IIM, such details were obtained from standardised muscle biopsy request forms which included clinical data and recent and previous drug exposure. One author (ZR) blinded to biopsy results, reviewed these data and contacted patients and physicians to determine missing information.

The patients from all biopsies reviewed were divided into two groups according to their statin exposure, and comparisons between statin-exposed and statin-naive groups were made using the chi square test with two-tailed p-values.

Between January 2012 and August 2013, there were 186 biopsies assessed in our laboratory. Of these, 78 of 186 (42%) were diagnosed with IIM; the remaining 108/186 'non-myositis' controls included a range of alternative diagnoses (Table 1).

Of all biopsies reviewed, 60 of 186 patients had a history of past or current statin use. Among patients with IIM, 36 of 78 (46%) had a history of statin exposure, compared with 24 of 108 (22%) of the non-myositis cases, $p = 0.0008$ (Table 1). The majority of statin-naive patients (84/126) were in the non-myositis category ($p = 0.0001$). Notably, none of the ten patients with DM had been exposed to statins. There was no difference in MHC1 ($p = 0.83$) or MHCII ($p = 1.00$) expression between the statin-exposed and non-statin exposed patients with IIM.

Routine EM assessment showed a spectrum of ultrastructural abnormalities associated with IIM such as chronic inflammatory cells, myofibres in varying stages of degeneration, degenerate mitochondria, myeloid bodies and other cytoplasmic degradation products. It is our intention herein to draw attention to a striking ultrastructural abnormality which may be a marker of statin exposure.

In nine of 78 biopsies diagnosed with IIM (5 PM, 1 IBM, 3 NM), distinctive electron dense granules (0.2−0.5 μm) within single membrane-bound vacuoles were noted, consistent with type 2 autophagic vacuoles (AV-2) (Fig. 1). Importantly, these AV-2 were present in nine of 36 (25%) IIM patients exposed to statins. The vacuoles were seen to contain single or multiple small dense cores resulting in a characteristic distinctive appearance. The vacuoles varied in size and some contained granular and fibrillar material.

These distinctive structures were identified between myofibrils and at the sarcolemmal surface and extracellular space suggestive of exocytosis (Fig. 2). These AV-2 were present in relatively preserved muscle fibres and fibres in various stages of degeneration including loss and breakdown of myofibrils, degenerate mitochondria and sacrotubular elements, myeloid bodies and other cytoplasmic degradation products.

Table 1 Presence of autophagic vacuoles (AV-2) and distribution of muscle pathology found in statin-exposed and statin-naïve patients with either immune-mediated or non-immune mediated muscle pathology

	Statin-naïve	AV-2s	Statin-exposed	AV-2s	Total
Immune mediated myopathy					
PM	6		11	5	17
IBM	8		8	1	16
DM	10		0	0	10
MNOS	13		8	0	21
NM	5		9	3	14
Subtotal (IIM+NM)	42	0	36	9	78
Non-immune mediated myopathy					
Normal	3		0		3
Non-specific myopathic changes	39		16		55
Mitochondrial cytopathy	3		0		3
Denervation	31		7		38
Dystrophy	7		1		8
Other	1		0		1
Subtotal	84	0	24	0	108
Total	126	0	60	9	186

DM, dermatomyositis; IBM, inclusion body myositis; IIM, inflammatory myositis; MNOS, myositis not otherwise specified; NM, necrotising myopathy; PM, polymyositis.

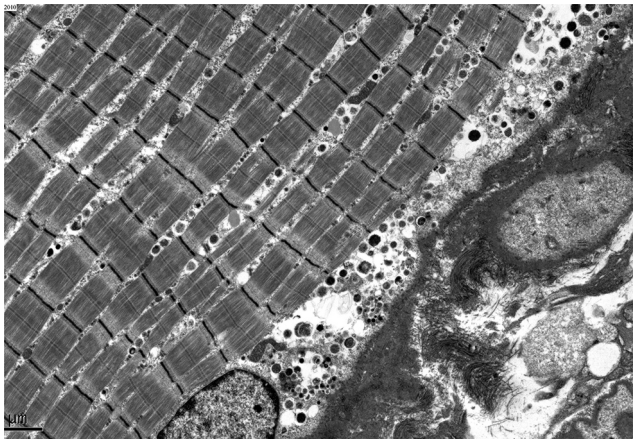

Fig. 1 Numerous electron dense granules within single membrane bound vacuoles consistent with type 2 autophagic vacuoles. They vary in size and central electron density and are present between myofibrils and in the subsarcolemmal space in association with lipofucsin-like material (×50,000).