ORIGINAL ARTICLE

Different pathological processes for acute white matter lesions in multiple sclerosis

Murad ALTURKUSTANI[1,2,3]*, Basem BAHAKEEM[2,3,4], Qi ZHANG[2,3], Lee-Cyn ANG[2,3]

[1]King Abdulaziz University, Jeddah, Saudi Arabia; [2]London Health Sciences Centre, London, Ontario, Canada; [3]University of Western Ontario, London, Ontario, Canada and [4]Umm Al-Qura University, Makkah, Saudi Arabia

Abstract

Introduction: Multiple sclerosis (MS) has variable clinical presentations, prognoses, pathogeneses, and pathological patterns. We conducted a pathological review of acute MS-associated lesions that focused on the degree of axonal injury, myelin loss, and glial reaction to determine whether the observed demyelination was of the primary or secondary type. *Materials and Methods:* After searching the records for a 15-year period at the London Health Sciences Centre Pathology Department, we identified 8 cases of surgical acute lesion biopsies in which clinical MS diagnoses were made before or after the biopsy. *Results:* The white matter pathologies in these cases could be sorted into 3 morphological patterns. The first pattern, which represents typical demyelinated plaques, was observed in 4 cases and was characterised by nearly complete demyelination accompanied by variable degrees of axon preservation and axonal swelling. The second pattern was observed in 3 cases and was characterised by demyelinating lesions containing variable numbers of myelinated axons mixed with a few demyelinated axons and variable numbers of axonal swellings. The myelinated axons ranged from scattered fibres to bands of variable thickness, and the demyelination was a mixture of primary and secondary demyelination. The third pattern was observed in 1 case and was characterised by well-demarcated areas of reduced myelin staining and numerous apoptotic nuclei. Axonal staining revealed many fragmented axons with reduced myelin staining but no definitely demyelinated axons. *Conclusion:* This report shows that the predominant pathology underlying acute MS-related lesions is not limited to demyelination but can include axonal degeneration alone or in combination with primary demyelination which reflect different pathogenesis for these acute lesions.

Keywords: Multiple sclerosis, demyelination, white matter, axonal degeneration, myelin

INTRODUCTION

Multiple sclerosis (MS) has variable clinical presentations, prognoses, pathogeneses, and pathological patterns. The pathogenesis of demyelination in active MS-related lesions can be divided into 4 immunopathologic patterns. Patterns 1 and 2 involve antibody- and T cell-mediated demyelination, and patterns 3 and 4 involve primary oligodendrocyte injuries,[1] though pattern 3's pathogenesis is more closely related to hypoxic-ischemic injuries.[2] Although these patterns differ between patients, they are similar within a single patient's active lesions.[1]

Demyelination is classified as primary when myelin loss extent exceeds the axonal injury and secondary when its extent is proportional to the axonal injury. The pathological distinction between primary and secondary demyelination is complex, as illustrated by the many debates about the pathological processes underlying various white matter diseases such as adult-onset leukoencephalopathy/leukodystrophy with axonal spheroids.[3,4] Axonal injury associated with active demyelination is well-documented in MS,[5-7] and neurodegeneration is increasingly understood to be an important pathological component of MS that outperforms the degree of demyelination in predicting patient prognosis.[8] As neurodegeneration is mainly described in autopsy cases, it is unclear if it represents a sequela of demyelination or another disease occurring concomitantly with MS. We, therefore,

*Address for correspondence: Murad Alturkustani, Pathology Department, King Abdulaziz University, Abdullah Sulayman, PO Box 80205, Jeddah, Saudi Arabia. Tel: +966500936683. Fax: +966 012-6048433 (hosp). E-mail: *alturkustani.murad@gmail.com*

investigated the pathological processes suggested by the morphological features of acute MS-related lesions.

MATERIALS AND METHODS

This study was approved by the University of Western Ontario's research ethics board. We searched the records for a 15-year period (2002-2016) at the London Health Sciences Centre Pathology Department to identify cases of surgical white matter lesion biopsy with a diagnosis of acute MS, tumefactive MS, leukoencephalopathy, or demyelination. We included cases that met the clinical diagnostic criteria for MS before or after the biopsy. We excluded cases with no available follow-up data. Using the medical and imaging data available in electronic medical records, we focused our pathological review on the degree of axonal injury, myelin loss, and glial reaction to determine whether primary or secondary demyelination was the predominant pathological process.

The biopsies had been fixed in 10% buffered formalin. All samples had been submitted for microscopic examination and stained with Luxol fast blue (LFB) with hematoxylin and eosin and periodic acid Schiff (PAS) with and without diastase. They were also immunostained with antibodies against the glial fibrillary acidic protein (GFAP, IR524, RTU; Dako, Carpinteria, CA, Poly R), amyloid precursor protein (MAB348, 1:10000, Chemicon, Temecula, CA, 22C11), phosphorylated neurofilament (SMI-31R, 1:1000 ; Covance, Princeton, NJ, SMI-31R); nonphosphorylated neurofilament (IR607, RTU, Dako, 2F11), CD68 (IR609, RTU, Dako, KP1), and Ki67 (IR626, RTU, Dako, MIB-1).

RESULTS

We retrieved 26 cases, but only 8 met the inclusion criteria. All 8 were diagnosed with acute demyelination, and the clinical histories are summarised in Table 1. These 8 cases exhibited reactive gliosis and variable degrees of oligodendrocyte apoptosis and perivascular lymphocyte presence. Depending on the interval between the episode and the biopsy, the macrophages exhibited variable positivity for LFB and PAS with diastase. The white matter pathologies in these lesions were sortable into 3 morphological pattern categories that reflect different pathological processes.

The first pattern, which represents the typical

acute demyelinated plaques, was observed in 4 cases. It was characterised by an area of complete demyelination (Fig. 1A), relative preservation of axons (Fig. 1B), few axonal spheroids (Fig. 1C), and both gemistocytic (Fig. 1D) and fibrillary astrocytosis (Fig. 1E-F). None of the sampled areas showed necrosis. The pathological process in this pattern was consistent with predominantly primary demyelination.

The second pattern was observed in 3 cases and was characterised by variable numbers of myelinated axons in the demyelinating plaque, ranging from single fibres to bands of variable thickness (Fig. 2A-C). The myelin staining patterns showed many of the same features present in the first pattern but only a few demyelinated axons, which indicated that the demyelination was predominantly secondary to axonal injury. The axonal spheroids ranged from few to numerous, with the numbers being especially high in areas with reduced myelin staining adjacent to normal-appearing white matter (Fig. 2D). Cells with fragmented nuclei (Fig. 2F) were immunonegative for GFAP (Fig. 2G) but immunopositive for the proliferative marker Ki67 (Fig. 2H). Although these cells were present in the first pattern, they were more numerous in the second. The pathological process in this pattern was consistent with a mixture of primary and secondary demyelination, but with secondary demyelination as the major process.

The third pattern was observed in 1 case and was characterised by well-demarcated areas of reduced myelin staining (Fig. 3A) with many apoptotic nuclei. The background of this plaque showed numerous macrophages (Fig. 3B), proportional losses of myelin and axons (Fig. 3D), and numerous axonal swellings that were widespread but small (Fig. 3E). It contained small-calibre myelinated fibres but no definitely demyelinated axons (Fig. 3C). The pathological process in this pattern was consistent with demyelination being predominantly secondary to axonal injury. The third pattern was distinguished from the second by the absence of demyelinated axons or preserved myelinated axons in the affected area under light microscope examination.

The third pattern is a novel finding, so we will present a detailed history for this case. A 30-year-old right-hand dominant woman presented with slurred speech and right upper limb weakness. The signs had an acute onset. They progressively worsened and evolved to include decreased sensation on the left side of her

TABLE 1: Clinical information for the cases

N	Age (y)*/ Sex	Biopsy-related episode characteristics Presentation	PP Du	History of MS Du	T	Post-episode follow-up
1	62/F	Seizures and increase in right-side weakness	5 d · 1	15 y	10 y	Controlled for 5 y
2	36/M	Symptoms were difficult to separate from those related to a concurrent brain abscess secondary to impacted wisdom tooth**	7 d · 1	13 y	2 y	Controlled for 11 y
3	59/F	Left facial numbness and left-side weakness	14 d · 1	8 mo	1st episode	Death after 5 mo from sepsis secondary to UTI
4	62/F	Ataxia and cognitive problems	19 d · 1	3 y	2 y	Controlled for 1 year and then lost to follow-up
5	38/F	Confusion and aphasia	11 d · 2	9 y	1 y	Controlled for 8 y
6	60/F	Speech and word-finding difficulty and dysarthria	28 d · 2	2 y	1st episode	Controlled for 2 y
7	34/F	Right-side weakness and speech difficulty	30 d · 2	11 y	9 y	Controlled for 2 y
8	30/F	Slurred speech and weakness in the right upper limb	12 d · 3	2 y	1st episode	See text for details

*age at the time of the episode that necessitated the biopsy

** Brain biopsies from 2 sites: abscess at 1 site and acute demyelination at the other

d: days; Du: duration; F: female; M: male; mo: months; PP: Pathological pattern; T: Timing of biopsy-related episode; UTI: urinary tract infection; y: years

body. A physical examination revealed slurred and slow speech, right-side facial droop with an upper motoneuron pattern, the spastic tone in her left limbs, and reduced pinprick sensitivity on the left side of her body. The assessment of her left arm revealed powers of 4/5 for elbow flexion, wrist extension, and finger extension and 3/5 for elbow extension and finger abduction. The assessment of her left leg revealed powers of 4/5 for ankle dorsiflexion and ankle plantar flexion, 3/5 for knee extension, and 2/5 for hip and knee flexion. The assessments of her right limbs revealed normal muscle function. Her toes were bilaterally down-going. Magnetic resonance imaging of the brain, including T2-weighted imaging and fluid-attenuated inversion recovery, revealed bilateral, multifocal, discrete hyperintensities. These hyperintensities were located both supratentorially and infratentorially, with 1 involving the corpus callosum and some featuring incomplete, irregular rims of enhancement. The radiological differential diagnoses that could cause these pathological features included demyelinating diseases such as acute disseminated encephalomyelitis, tumefactive MS, and lymphoma. She was treated with multiple rounds of intravenous immunoglobulins, plasma exchange, and high-dose intravenous steroids. After several cycles of treatment, her radiologic abnormalities stabilized, and she began to recover. Over the following 2 years, she suffered recurrent symptoms and exhibited radiologically detected enhancing lesions in her spinal cord and brain, including the supratentorial and infratentorial regions. These imaging findings fulfilled the Macdonald criteria for an MS diagnosis that require dissemination in space and time. Her physical exams showed little improvement from the initial attack.
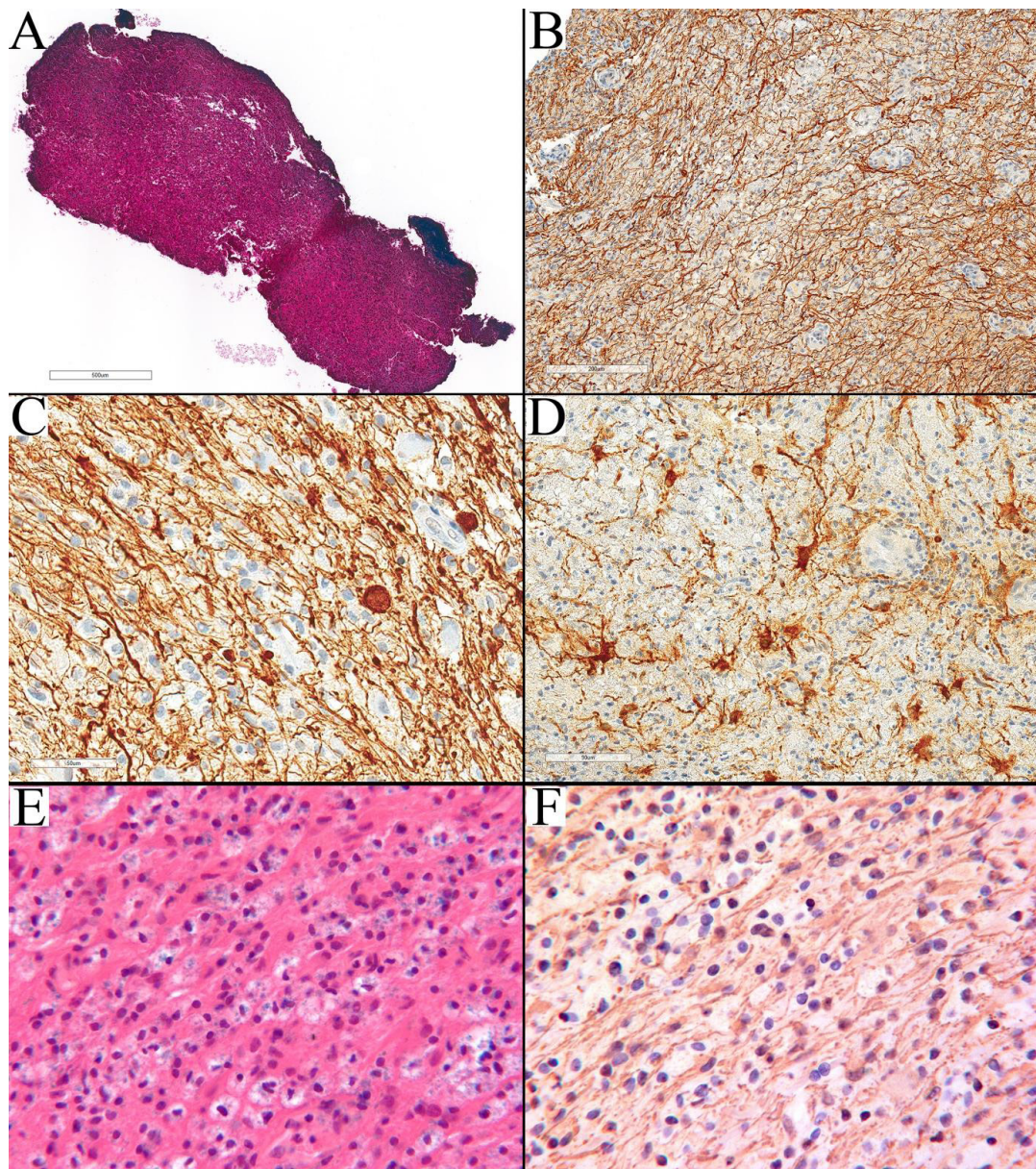
FIG. 1: Typical demyelinating pathology with axonal spheroids. (A) Complete loss of myelin (Case 2, LFB-HE).
(B) Relative preservation of axons (Case 2, SMI31) with (C) Few axonal spheroids (Case 2, SMI31). (D)
Gemistocytic (Case 2, GFAP) astrocytosis. (E, F) Fibrillary astrocytosis (Case 3; E: LFB-HE, original
magnification: 400x; F: GFAP, original magnification: 400x).

DISCUSSION

The morphological spectrum of acute MS-related
lesions includes 3 underlying pathological
processes: 1) primary demyelination alone,
2) a mixture of axonal degeneration and
primary demyelination, and 3) primary axonal
degeneration with secondary demyelination. MS
has a very heterogeneous clinical and radiological
definition, but it is always considered a primary
demyelinating disease with a variable degree of
axonal injury. However, recent investigations
into cortical injury and normal-appearing white
matter in MS showed that axonal degeneration
can occur independently of local demyelination
and exhibit a stronger correlation with the
progression of MS.[5,8,9] Our results indicated that
neurodegeneration could be the predominant
or only pathological process underlying acute
MS-related lesions. This finding expands the
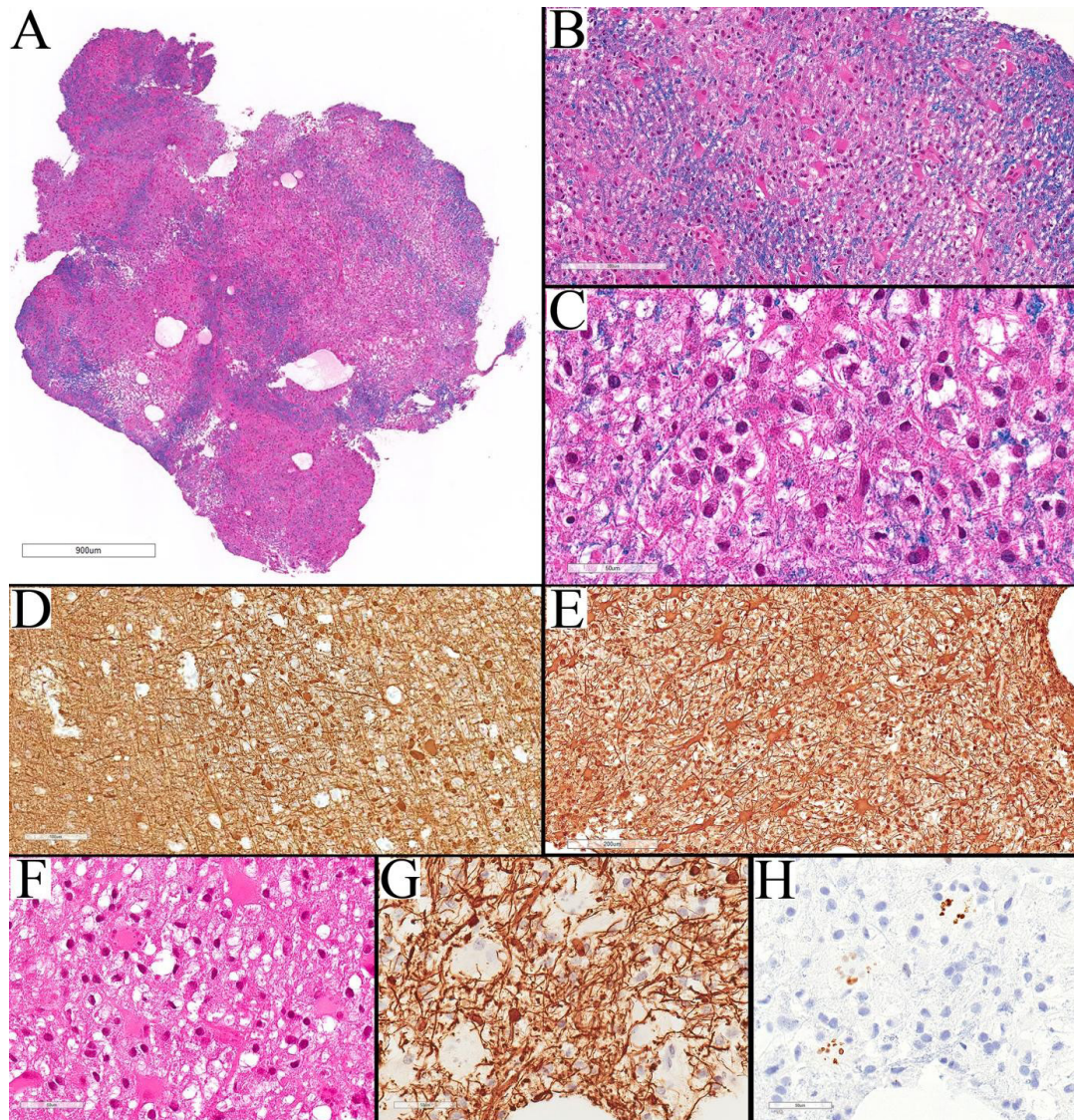morphological spectrum of MS lesions to

FIG. 2: Mixed axonal and demyelinating pathology. (A) Alternative myelinated and demyelinated areas (Case 6, LFB-HE). (B) Higher magnification of A. (C) Myelinated axons present in the demyelinated areas. (D) Numerous axonal spheroids in the area with myelin pallor (Case 6, SMI31). (E) Diffuse reactive gliosis (Case 6, GFAP). (F-H) Large eosinophilic cells with multiple small round nuclear materials that are negative for GFAP but stains with Ki-67 (Case 6, F: H&E, G: GFAP, H: Ki67).

include axonal variants of MS, similar to the concept of rare axonal variants of peripheral demyelinating disease (i.e., acute motor axonal neuropathy of acute inflammatory demyelinating polyneuropathy).[10]

The observation of areas of complete myelin loss in the first pattern supports the proposal that activated macrophages cause the demyelination observed in Lucchinetti *et al.* immunopathologic patterns 1 and 2.[1] The diagnosis for our first pattern should be acute demyelination, and the differential diagnosis includes all conditions

known to cause primary demyelination, such as immune-mediated demyelination, infections, metabolic disorders, and others.[11]

In contrast, the second pattern was pathologically characterised by a low proportion of demyelinated axons, which indicated the involvement of significant axonal pathology with secondary demyelination. The presence of preserved myelinated fibres in the affected areas excludes the possibility of macrophage-mediated demyelination. This pattern's pathology resembles the acute demyelination observed in
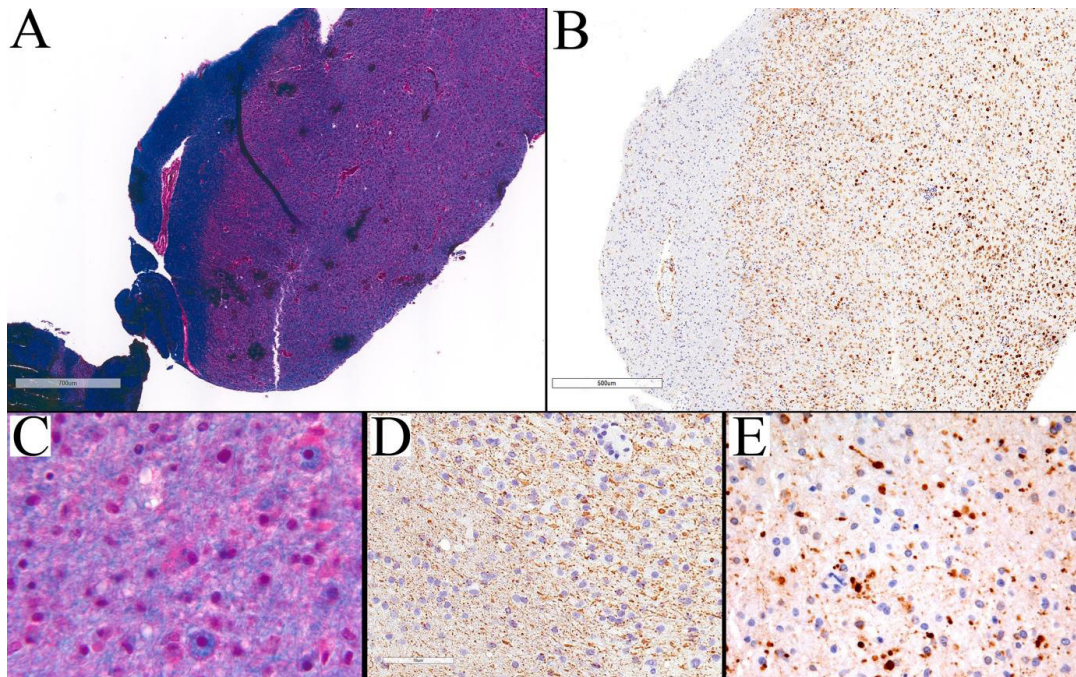
FIG. 3: Myelin pallor with no demyelinating pathology. (A) Well demarcated area of myelin pallor (LFB-HE). (B) Diffuse macrophages in the area with myelin pallor (CD68). (C) Macrophages in these areas contain myelin along with small myelinated fibres (LFB-HE, original magnification: 630x). (D) Fragmented axonal staining by neurofilament protein (SMI31) with small axonal swelling better seen by (E) (APP original magnification: 400x).

Balo's concentric sclerosis, which corresponds with immunopathologic pattern 3[1] and is thought to represent hypoxic-ischemic injury.[2] Whether acute hypoxic-ischemic injury could cause acute demyelination is debatable, as the pathological process underlying severe acute hypoxic-ischemic injury, such as a cardiac arrest, mainly involves axonal degeneration with minimal demyelination.[12] However, animal models show that primary demyelination can result from mild to moderate hypoperfusion.[13,14] This may explain the mixture of both processes in this pattern, which may depend on the degree and the duration of the hypoperfusion in the affected area. The diagnosis in this pattern will be a mixture of acute demyelination and axonal degeneration. The differential diagnoses will be broad, encompassing the differential diagnoses of the first pattern and other destructive lesions that may be associated with demyelination, such as neuromyelitis optica, steroid-treated primary central nervous system lymphoma,[15] changes adjacent to glioma, and other conditions. One unresolved mystery in the first 2 patterns was the nature of the multinucleated cells or large cells with fragmented chromatin, also known as Creutzfeldt astrocytes.[16] These are thought to

be different from granular mitotic cells,[16] but in these cases they were immunonegative for GFAP and immunopositive for the proliferative marker Ki67, indicating that these were mitotically active cells.

The most interesting and novel pattern in acute MS lesions was the third pattern, which involved axonal degeneration with no evidence of primary demyelination. Discussions of white matter lesions have asserted that this pattern does not represent demyelination[11, 17] and that it should be diagnosed as axonal degeneration with secondary demyelination. The differential diagnosis includes causes of axonal injury such as hypoxic-ischemic leukoencephalopathy, toxic leukoencephalopathy, metabolic disorders, neurodegenerative diseases, and other conditions. However, this case fulfilled the diagnostic criteria for MS during follow-up, and no better differential diagnosis was available, so MS should be included in the list of differential diagnoses for this pattern. The pathogenesis of axonal degeneration is complex and has many pathways with 3 main convergence points: impaired axonal transport, mitochondrial failure, and increased intra-axonal calcium.[18] Of these, mitochondrial failure appears to be the most likely pathway for the third pattern.

Axonal degeneration in MS can take many forms depending on location, onset, and the presence of demyelinating plaque. It can include 1) areas of active demyelination, as described in the first pattern, that are thought to be related to the lesional activity; 2) areas of chronic demyelination; 3) diffuse axonal injuries that are related not to the demyelinated plaques but rather to the degree of inflammation and oxidative stresss;[6,7] and 4) Wallerian degeneration that represents proximal axonal injury affecting the distal downstream tract. These forms are different from the third pattern in this series as this pattern predominantly involved focal axonal injury and was not associated with demyelinating plaques. One factor that makes Wallerian degeneration particularly unlikely to represent this pattern is that the biopsy was sampled from the initial active injury, an identification that was pathologically confirmed by the presence of diffuse axonal swelling, which ruled out secondary degeneration of dissected upstream tract axons. This pattern's pathology is consistent with oxidative stress but different from severe acute hypoxic-ischemic injury, as reactive astrocytes were present.[12] Toxic leukoencephalopathy can also be excluded due to the absence of complete necrosis.[19]

Our study has several limitations. As in most biopsy studies, we assumed that each sample was representative of the patient's lesions. This assumption is potentially unreliable, but previous immunopathologic studies have indicated that heterogeneous patterns are homogenous within individual patients.[1] Furthermore, the small number of cases precludes the observation of meaningful prognostic differences between the patterns. However, it is established that the degree of axonal injury outperforms demyelination in correlating with the clinical manifestation and progression of MS.[9] Another explanation for these patterns is that they may represent different diseases rather than a morphological spectrum of the same condition. MS, as currently defined, has variable clinical presentations, prognoses, pathogeneses, and pathological patterns. Since the clinical diagnosis in the follow-up period was consistent with MS and no alternative diagnosis was suggested, we recommend separating MS lesions based on their pathological features and processes and examining whether these correlate with prognoses and responses to therapy. Further studies along these lines are necessary.

In conclusion, we described different pathological patterns in biopsied acute lesions from patients with clinically confirmed MS. We showed the primary pathology of such lesions is not limited to demyelinating plaque but can also include axonal degeneration either alone or in combination with primary demyelination. Our limited sample precluded the observation of meaningful prognostic differences between the patterns, so we recommend that additional studies with larger samples be performed.

Conflict of interest: The authors declare they have no conflict of interest.

REFERENCES

1. Lucchinetti C, Bruck W, Parisi J, Scheithauer B, Rodriguez M, Lassmann H. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol. 2000; 47: 707-17.
2. Stadelmann C, Ludwin S, Tabira T, *et al*. Tissue preconditioning may explain concentric lesions in Balo's type of multiple sclerosis. Brain. 2005; 128: 979-87.
3. Alturkustani M, Keith J, Hazrati LN, Rademakers R, Ang LC. Pathologic staging of white matter lesions in adult-onset leukoencephalopathy/leukodystrophy with axonal spheroids. J Neuropathol Exp Neurol. 2015; 74: 233-40.
4. Alturkustani M, Sharma M, Hammond R, Ang LC. Adult-onset leukodystrophy: review of 3 clinicopathologic phenotypes and a proposed classification. J Neuropathol Exp Neurol. 2013; 72: 1090-103.
5. Popescu BF, Lucchinetti CF. Pathology of demyelinating diseases. Annu Rev Pathol. 2012; 7: 185-217.
6. Haines JD, Inglese M, Casaccia P. Axonal damage in multiple sclerosis. Mt Sinai J Med. 2011; 78: 231-43.
7. Lassmann H. Axonal and neuronal pathology in multiple sclerosis: what have we learnt from animal models. Exp Neurol. 2010; 225: 2-8.
8. Trapp BD and Nave KA. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci. 2008; 31: 247-69.
9. Kutzelnigg A, Lucchinetti CF, Stadelmann C, *et al*. Cortical demyelination and diffuse white matter injury in multiple sclerosis. Brain. 2005; 128: 2705-12.
10. Schmidt RE, Bilbao JM. Diseases of Peripheral Nerves. In: Love S, Perry A, Ironside JW and Budka H, (eds.). Greenfield's neuropathology. 9th ed.: CRC Press. 2015, p. 1453-7.
11. Moore GRW and Esiri MM. The pathology of multiple sclerosis and related disorders. Diagnostic Histopathology. 2011; 17: 225-31.

12. Alturkustani M, Ang LC. Acute hypoxic-ischemia in cardiac arrest encephalopathy causes only minimal demyelination. Neuropathology. 2016; 36: 413-20.

13. Nukada H, McMorran PD. Perivascular demyelination and intramyelinic oedema in reperfusion nerve injury. J Anat. 1994; 185 (Pt 2): 259-66.

14. Nukada H, Powell HC, Myers RR. Spatial distribution of nerve injury after occlusion of individual major vessels in rat sciatic nerves. J Neuropathol Exp Neurol. 1993; 52: 452-9.

15. Barrantes-Freer A, Engel AS, Rodriguez-Villagra OA, *et al*. Diagnostic red flags: steroid-treated malignant CNS lymphoma mimicking autoimmune inflammatory demyelination. Brain Pathol. 2017.

16. Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. Acta Neuropathol. 2010; 119: 7-35.

17. Love S. Demyelinating diseases. J Clin Pathol. 2006; 59: 1151-9.

18. Coleman M. Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci. 2005; 6: 889-98.

19. Alturkustani M, Ang LC, Ramsay D. Pathology of toxic leucoencephalopathy in drug abuse supports hypoxic-ischemic pathophysiology/etiology. Neuropathology. 2017; 36: 413-20.