Clinicopathological features of inflammatory demyelinating diseases in biopsy

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Inflammatory demyelinating diseases (IDD) of the central nervous system (CNS) occur throughout the world and are the leading cause of nontraumatic neurological disability in young adults.[1] Early diagnosis of IDD allows prompt immunotherapies that minimize relapse, disabilities and mortality.[2] Multiple sclerosis (MS) is the most frequent IDD in adults. The histological characteristics of chronic MS lesions are well known to neuropathologists; however, diagnosing an inflammatory demyelinating process in biopsy may be challenging since the specimens are frequently small and often represent only parts of a larger lesion.[3] Furthermore, the histopathological hallmarks of acute MS differ fundamentally from the well-known characteristics of chronic demyelinated plaques observed in autopsies.[3] Kuhlmann *et al*[4] recently proposed a new histological classification system for MS lesions. Whether this new classification system corresponds to clinical manifestations and thus reflects the temporal multiplicity of MS and whether it can be applied to all IDD cases remain to be further studied.

Here, we retrospectively analyzed the clinical and histopathological features of 23 IDD cases diagnosed in the Department of Pathology at Xuanwu Hospital Capital Medical University between 2009 and 2017 and performed Luxol fast blue (LFB) staining and immunohistochemical (IHC) staining to explore how to make a pathological diagnosis of IDD in biopsy. In addition, five consecutive cases of ischemic infarction diagnosed from 2009 to 2016 were selected as the control group. The study was approved by the ethics committees of the Xuanwu Hospital Capital Medical University, and informed consent was obtained from all patients. Data analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis test was used for comparison of the course of the disease among three stage groups. A P value < 0.05 was considered statistically significant.

Among the 23 IDD patients, 11 were males, and 12 were females. The patients' age at the time of diagnosis ranged from 10.0 to 68.0 years, with a median age of 37.0 years (interquartile range [IQR]: 25.0–44.0 years). These patients were younger than those with ischemic infarction (three males and two females with a median age of 50.0 years, IQR: 42.5–59.5 years).

Complete medical history data were obtained from 17 of the 23 IDD patients, and the clinical symptoms, such as headache and dizziness ($n = 9$), limb weakness and movement disorder ($n = 9$), limb numbness ($n = 2$), nausea and vomiting ($n = 4$), blurred vision and vision hemianopia ($n = 6$), slurred speech ($n = 2$), and mouth angle deviation ($n = 1$), varied according to the location of lesions. Only six patients had a single symptom, with most patients manifesting multiple symptoms at the same time; six patients had two symptoms and five patients had three symptoms. The time from the onset of symptoms to biopsy ranged from 7 days to 5 months, with a median of 30 days (IQR: 17.5-55.0 days). It is worth mentioning that one of the patients developed IDD 11 months after hematopoietic stem cell transplantation. Zhang *et al*[5] found that the cumulative incidence of all cases of IDD at 6 years post-transplantation was 3.6%, indicating that IDD is an uncommon but serious complication of allogeneic bone marrow stem cell transplantation, especially in patients with a primary diagnosis of acute lymphatic leukemia and mismatched transplants.

Magnetic resonance imaging (MRI) showed that there were nine cases of IDD with a single focus, four cases involving two regions and four cases involving three or more regions. These findings confirmed the prior impression of the spatial
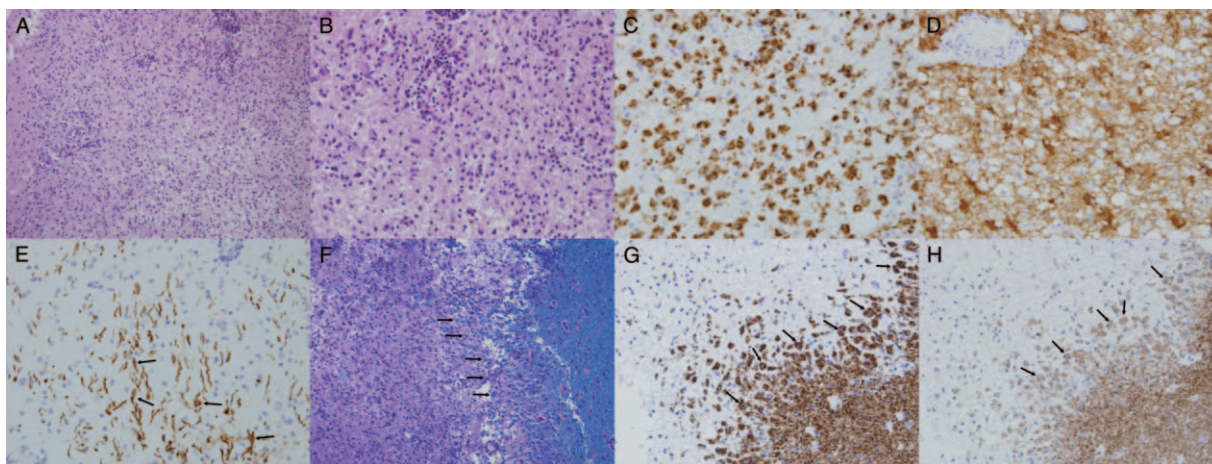
Figure 1: Histological characteristics of IDD. (A) H&E staining gave an overview of the lesion. (B) High power image of A. (C) IHC staining for CD68 showed the microphages in IDD lesion. (D) IHC staining for GFAP showed the reactive astrocytes. (E) IHC staining for NF showed that axons were relatively preserved, with some axons becoming swollen (arrows). (F) LFB+H&E staining, (G) IHC staining for PLP and (H) IHC staining for MOG showed demyelination and myelin degradation products appearing in the cytoplasm of the macrophages (arrows). (A and F: original magnification ×200; B–E, G, H: original magnification ×400). IDD: Inflammatory demyelinating diseases; H&E: Hematoxylin and eosin; IHC: Immunohistochemical; GFAP: Glial fibrillary acidic protein; NF: Neurofilament; LFB: Luxol fast blue; PLP: Proteolipid protein; MOG: Myelin oligodendrocyte glycoprotein.

multiplicity of IDD. Nine patients exhibited only one lesion, possibly because the disease was at an early stage. The IDD lesions involved almost all areas of the CNS, including the frontal lobe ($n = 9$), temporal lobe ($n = 4$), parietal lobe ($n = 8$), insular lobe ($n = 1$), periventricular area ($n = 1$), basal ganglia ($n = 1$), corona radiata and semioval center ($n = 1$), cerebellum ($n = 2$), and spinal cord ($n = 1$). Most of the MRI signals in IDD lesions were long T1 and long T2 with enhancement and were diagnosed as space-occupying lesions, which are difficult to distinguish from tumors; thus, biopsy was performed.

Hematoxylin-eosin (H&E) staining gave an overview of the lesions [Figure 1A–1B]. All IDD lesions from the 23 patients were hypercellular and characterized by diffuse and dense infiltration of the complete lesion area with foamy macrophages, which were CD68-positive [Figure 1C]. T cell-dominated lymphocytes were localized around small blood vessels and scattered between foam cells. It should be noted that in one case, there were many eosinophils scattered in the lesion, especially around blood vessels. In addition, reactive astrocytes proliferated in all lesions and became plump-shaped (gemistocytes) with homogeneous eosinophilic cytoplasms, and numerous fibrillary processes were shown by glial fibrillary acidic protein (GFAP) staining [Figure 1D]. Mitotic astrocytes and astrocytes with fragmented nuclear inclusions (granular mitoses and Creutzfeldt-Peters cells) were found in two cases. IHC for neurofilament (NF) showed that axons were relatively preserved, with some axons becoming swollen and forming axonal spheroids [Figure 1E].

LFB staining showed myelin sheath loss in all IDD cases studied. IHC staining for proteolipid protein (PLP; rabbit, clone EPR12675(2)(B), Abcam, Cambridge, MA, USA, 1:1000 dilution) and myelin oligodendrocyte glycoprotein (MOG; rabbit, clone EP4281, Abcam, Cambridge, MA, USA, 1:2000 dilution) showed the same results, but staining for these proteins was clearer than LFB staining. In some cases ($n = 21$), LFB-positive myelin sheath fragments were found in the cytoplasm of macrophages [Figure 1F].

In this regard, the PLP staining results were highly consistent with LFB staining and clearly showed degradation products of myelin sheaths in macrophages ($n = 21$) [Figure 1G]. However, the staining results of MOG were different from those of LFB and PLP. In only 15 cases, MOG-positive granules were found in the cytoplasm of macrophages [Figure 1H]. This is probably because the degradation of minor myelin proteins (e.g., MOG) occurs rapidly, and the digestion of major myelin proteins (e.g., PLP) is slower.

IDD lesions were classified based on lesion activity.[4] Of the 23 patients examined, 15 exhibited lesions that were classified as active and early demyelinating lesions, six exhibited lesions that were classified as active and late demyelinating lesions, and two exhibited lesions that were classified as active and post-demyelinating lesions. Thus, the lesions in the small biopsies in this study were all active lesions, and no chronic inactive demyelinating lesions, which are commonly observed in autopsy specimens, were found. If this result is confirmed by a large sample, IDD diagnosis may become relatively simple.

There was no statistical significance in the time from the appearance of symptoms to biopsy among the three groups ($\chi^2 = 5.118$, $P = 0.077$), indicating that disease stage did not correspond to the course of the disease; this may have been because biopsy represents only one lesion, while the symptoms result from a combination of multiple lesions, precisely reflecting the spatial multiplicity of IDD lesions.

In the control group, four of the five ischemic cerebral infarction lesions showed demyelination, and PLP- and MOG-positive granules were found in macrophages in two cases. This is because phagocytes also ingested and degraded necrotic myelin fragments after cerebral infarction. However, axons were also necrotic in ischemic cerebral infarction. Therefore, we can differentiate cerebral infarction and IDD by combining clinical manifestations with IHC staining for NF since axons in cerebral infarction

lesions are absent while axons in IDD are relatively preserved.

In conclusion, we demonstrated that to make a pathological diagnosis of IDD in biopsy, the clinical manifestations, MRI and pathological morphology should be combined for comprehensive analysis. IHC staining of PLP, MOG, NF, CD68 and GFAP is helpful for the pathological diagnosis and staging of IDD in biopsy and can be routinely used in general neuropathological analyses.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Conflicts of interest

None.

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