Histopathology and serial, multimodal magnetic resonance imaging in a multiple sclerosis variant

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Defining tools in magnetic resonance imaging (MRI) representing specific pathological processes is needed to understand the complex relationship between inflammation, myelin breakdown, axonal injury and clinical symptoms in multiple sclerosis (MS) and its variants. Here, we describe a case of histologically-defined MS, in which the radiological appearance of the lesion and clinical course support the diagnosis of Balo's concentric sclerosis. Serial magnetization transfer, diffusion tensor imaging and ¹H-magnetic resonance spectroscopy, from 14 days to 13 months after biopsy, allow the contextual interpretation of specific pathological changes. In our case, acute inflammation was sensitively traced by fractional anisotropy and increased lactate in spectroscopy. In contrast, magnetization transfer ratio and the apparent diffusion coefficient monitor the sequential loss of tissue in selected rings of the lesion. The delay from the peak of symptoms in a dramatic clinical course to the maximum tissue destruction indicated through MRI suggests that compromise of axonal function may be decisive for the acute clinical situation. This is the first report comparing ¹H-magnetic resonance spectroscopy, magnetization transfer and diffusion tensor imaging with histopathology in a patient with Balo's concentric sclerosis. *Multiple Sclerosis* 2007; 13: 471–482. <http://msj.sagepub.com>

Key words: Balo's concentric sclerosis; diffusion-weighted imaging; histology; magnetic resonance imaging; magnetization transfer; multiple sclerosis

Introduction

Balo's concentric sclerosis, a specific variant of multiple sclerosis (MS), is a rather acute, often monophasic and rapidly progressive, inflammatory demyelinating disease mainly affecting young adults. A single or very few lesions, often large and not readily distinguishable from neoplasms, show alternating bands of demyelination, and relatively intact white matter, resulting from preservation of tissue rather than remyelination [1,2]. Hypoxic preconditioning of oligodendrocytes is now thought to be a major factor contributing to the pathognomonic onion-bulb appearance of the lesions [3].

The pathomechanisms in MS and its variants are poorly understood [4]. Histopathological studies in

humans, although highly valuable, are limited by the lack of longitudinal analysis. Lesions can be detected non-invasively via magnetic resonance imaging (MRI), however, conventional techniques lack pathological specificity. In a study of six biopsied cases neither T₁- nor T₂-intensities nor gadolinium (Gd)-enhancement were demonstrated as specific to either age of the lesion or a predominant pathological process [5]. Modern MRI techniques, such as magnetization transfer imaging (MTI) and diffusion tensor imaging (DTI), promise to better differentiate the underlying pathology. Correlating well with disability in patients with MS, a decreased magnetization transfer ratio (MTR) is considered a measure for tissue destruction [6]. Although not specific for demyelination, as MTR also correlates with axonal density [7] and decreases

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during Wallerian degeneration of axons [8,9], there is some support for the argument that MTR can be used as a marker for myelin destruction. The MTR is greatly reduced in chronic MS lesions, but less so in experimental autoimmune encephalomyelitis, where inflammation with only minor demyelination is the main finding [10]. Most convincingly, in pontine myelinolysis, a condition with 'pure' demyelination without inflammation and axonal damage, MTR is greatly reduced [11]. Furthermore, in patients with optic neuritis MTR is inversely correlated to the latency of visually evoked potentials, an electrophysiological indicator of demyelination [12].

MR diffusion imaging is sensitive to pathological changes in MS lesions [13–15], but the exact histopathological correlate is not clear [7]. Fractional anisotropy, a measure for the orientational dependence of diffusion, should monitor particularly well the integrity of white matter tracts which can be seen as oriented barriers of diffusion. Biochemical properties of tissue can be examined by ^1H magnetic resonance spectroscopy (^1H -MRS). N-acetylaspartate (NAA) is almost exclusively contained in neuronal cell bodies and axons. A reduction of NAA has been demonstrated in the acute and chronic lesions [6,16–19] of MS patients as well as in normal appearing white matter (NAWM) [18,20–22]. Importantly, the degree of NAA reduction seems to correlate with disability [22–25]. Concentrations of free choline (Cho) increase when membrane turnover is accelerated [26]. This occurs during inflammation, demyelination, gliosis and even remyelination. As a marker of acute inflammation, a rise in lactate can be measured within active lesions [16].

In order to more clearly define the pathological processes represented by MTR, DTI and ^1H -MRS, direct comparison of these parameters and correlation with histopathology is needed.

We present here a case of Balo's concentric sclerosis with lesion histopathology and serial MRI and MRS up to 13 months after the biopsy, providing, to date, the most detailed magnetic resonance and histology-based description of the evolution of such a lesion.

Methods

Patient

An open brain biopsy was taken from a 26-year-old male, 24 days after the first clinical presentation. Tissue specimens were immediately fixed in formaldehyde and embedded in paraffin.

Histopathology and immunohistochemistry

Paraffin-embedded tissue was sectioned into 4- μm slices, which were stained with haematoxylin and eosin (H&E), Luxol-fast blue/periodic acid-Schiff LFB/PAS for myelin, as well as Bielschowsky's silver impregnation for axons. Immunohistochemical staining was performed with a biotin-avidin or an alkaline phosphatase/anti-alkaline phosphatase technique. Primary antibodies, their source, specificity and dilution, are listed in Table 1. Primary antibodies were omitted in controls. *In situ* hybridization, using digoxigenin-labeled riboprobes specific for proteolipid protein (PLP) mRNA, was carried out as previously described [27].

Lesion staging

The lesion was staged for its demyelinating activity according to recently established criteria [4]. Active demyelinating lesions, characterized by infiltrating macrophages positive for myelin degradation products, can be distinguished from inactive, comple-

Table 1 Primary antibodies

Specificity	Reactivity	Dilution	Source
KiM1P (mo)	Macrophages/microglia [30]	1:20	Professor HJ Radzun, Department of Pathology, Göttingen, Germany
CD3 (mo)	T lymphocytes	1:200	Serotec, Oxford, UK
CD8 (mo)	Cytotoxic T lymphocytes	1:50	Dako A/S, Glostrup, Denmark
GFAP (mo)	Gliosis	1:50	Dako
APP (mo)	Acute axonal damage	1:100	Chemicon International, Temecula, CA, USA
MBP (po)	Myelin basic protein	1:500	Dako
CNPase (mo)	2,3'-Cyclic nucleotide 3'-phosphodiesterase	1:200	Sternberger Monoclonals Inc., Lutherville, USA
MOG (mo)	Myelin oligodendrocyte glycoprotein	1:1000	Professor Linington, Aberdeen, UK
MAG (mo)	Myelin-associated glycoprotein	1:1000	Professor Richter-Landsberg, Oldenburg, Germany
PLP (mo)	Proteolipid protein	1:500	Serotec

mo, Monoclonal; po, polyclonal.

tely demyelinated lesions and remyelinated lesions, in which uniformly thin, irregularly formed myelin sheaths are usually present.

MR imaging and MR spectroscopy

Written informed consent, according to the approval of the local ethics committee, was obtained from the patient before MR examinations.

Post-biopsy MR imaging and ^1H -MRS were performed on a 1.5 T General Electrics Signa Horizon LX neuro-optimized MR tomograph (GE, Milwaukee, WI). A diffusion-sensitized echo planar imaging sequence (b value 1000 s/mm^2 , TE 70 ms) was applied to measure 39 axial slices (slice thickness 3 mm, FOV 28 cm, matrix 128×128) covering the whole cerebrum. Acquisitions without diffusion-weighting and with 12 non-collinear orientations of the diffusion gradient were performed (four averages per orientation), each measured additionally with inverted gradient polarity (another four averages), enabling eddy current correction [28]. After additional correction for head movements, diffusion tensors were computed and apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps calculated.

MTI was performed based on a proton-density (PD)-weighted spin echo sequence (TR 2600 ms, TE 20 ms, one average, 256×256 , 48 slices, FOV 25 cm) with and without MT saturation pulses. After correction for head movements, MTR maps were calculated.

Single-voxel ^1H -MRS used point-resolved spectroscopy (PRESS) with TR 1500 ms, TE 135 ms and 256 averages. In total, three voxels were outlined: (1) in the central lesion area, including the position of tissue extraction; (2) in an area superior to the biopsy, including solely lesioned tissue but excluding the biopsy region itself; and (3) in the NAWM contralateral to the location of the biopsy. During follow-up, exact repositioning of the voxels was achieved by manual alignment relative to anatomical landmarks.

The acquired data were analysed using LCModel (Vers. 6.1.0) [29]. Concentrations are given in arbitrary units.

The 'conventional' structural MR images depicted in Figure 1 were obtained for diagnostic purposes on a 1.5 T Siemens Magnetom Vision MR tomograph, and have not been used for any quantitative analysis.

Coregistration and segmentation of regions of interest

All coregistration tasks were processed using SPM2 (Wellcome Department of Cognitive Neurology,

Institute of Neurology, London, UK). Accurate anatomical matching (Figure 4) of ADC, FA and MTR maps across all follow-up examinations was accomplished by linear and non-linear transformations. The T_2 -weighted fast spin echo (FSE) images of the second examination time-point served as template for all coregistrations.

Segmentation of the lesion was motivated by their dissimilar appearance, presumably due to different histopathological processes. Three ROIs were defined on MTR maps: (1) the region of the biopsy channel and tissue extraction itself; (2) a central region that featured relatively high MTR values; (3) a ring-shaped low-MTR region surrounding the region 2. Additionally, a fourth ring-shaped region, inconspicuous on MTR maps, was defined by its hyperintensity on proton-density-weighted maps. Segmentations were performed using DisplImage (Dave Plummer, University College London, UK). Any overlap of the ROIs was avoided.

Results

Case report

A 26-year-old male of Italian descent presented with an 11-day history of fluctuating right-sided hemiparesis. Examination disclosed a discrete right-sided facial weakness and spastic hemiparesis, with a grade 4/5 weakness in the upper, grade 4+/5 in the lower extremity, mild hemihypaesthesia on the right, and some slight difficulty in finding words (Expanded Disability Status Scale; EDSS: 3.0). Cranial MRI revealed a left hemispheric paraventricular oval lesion with Gd-enhancement. An additional small lesion, 2 mm in diameter, was seen in the right frontotemporal region. Lumbar puncture showed 3 cells/ μL , slightly raised protein at 612 mg/L, and normal glucose and lactate. The IgG-Index was not raised (0.5), but oligoclonal bands were positive at this point; a repeat examination after six months was negative.

Additional clinical investigations did not show any evidence for malignancy or systemic autoimmune diseases.

A further cranial MRI, 16 days after onset of symptoms, showed that the lesion had increased to 2 cm in diameter and was still Gd-enhancing (Figure 1A). As the appearance of this lesion could resemble a lymphoma, abscess or autoimmune-inflammatory process, a stereotactic biopsy was performed on day 25 after onset of symptoms. Immediate histopathology showed an inflammatory demyelinating process. A few hours after the biopsy, the patient's clinical condition worsened to hemiplegia, severe

Figure 1 Series of an axial section of T₂- as well as T₁-weighted MR images with and without Gd-DTPA contrast. (A) In the examination before the biopsy an oval lesion can be demonstrated, which is hypointense in T₁ and hyperintense in T₂. The core appears slightly more T₂-hyperintense than the remaining lesion. With Gd contrast, the core and the rim of the lesion are enhanced. (B) At one month after onset of symptoms and seven days after stereotaxic biopsy, the site of biopsy can be distinguished clearly in the core of the lesion, small amounts of blood appear hyperintense on T₁. The T₂-weighted image shows two hyperintense rings. The center of the lesion not covered by blood is clearly hypointense in T₁, the periphery is slightly hypointense. There is no Gd-enhancement. Note that the patient was under treatment with high dose corticosteroids at this time. (C) At two months, T₂ hyperintensity of at least two concentric rings is greatly increased. On T₁, several rings of hypo- and hyperintensity can be distinguished. Gadolinium-contrast shows two simultaneous rings of strong enhancement. (D) At four months, the lesion has shrunk. Concentric rings are not readily distinguishable on T₂. On T₁ the biopsy site is hypointense, the surrounding core area hyperintense and the periphery is severely decreased in intensity. There is no longer gadolinium-enhancement.

hemihypaesthesia, facial weakness, deviation of the tongue to the right, apraxia for motor and language tasks with total inability to speak, as well as a pronounced impairment of speech comprehension (EDSS: 8.5). The patient was treated immediately with high doses of iv methylprednisolone: 1 g/day for six days, then 2 g/day for three days, followed by iv immunoglobulins 30 g/day for five days. Due to unresponsiveness, after a treatment pause of 10 days, the patient received five courses of plasmapheresis within 14 days. The slow but profound clinical improvement (illustrated by the changes in EDSS in Figure 4) could not obviously be related to any immunosuppressive treatment. However, these therapies may have halted a very aggressive disease course.

The evolution of the inflammatory lesion was monitored for therapeutic purposes by conventional MRI. At one month, the lesion was edematous and about 4 cm in diameter, extending into the internal capsule, the lateral thalamus and temporal insular cortex. Gd-enhancement at this point was most probably reduced by the treatment with iv steroids (Figure 1B). At two months, the lesion size was only slightly increased on T₂-weighted images, but T₁-weighting showed two hypointense rings alternating with hyperintense rings (Figure 1C). Gd-contrast clearly demonstrated two distinct rings of enhancement. After four months, MRI showed a reduction in lesion size, some enlargement of the left ventricle towards the lesion site, no edema and no Gd-enhancement (Figure 1D).

Neuropathology

Neuropathology revealed an inflammatory demyelinating process (Figure 2). The H&E staining showed a hypercellular lesion with perivascular lymphocytic infiltrates, macrophages and severe edema. Immunocytochemistry identified the infiltrating lymphocytes as CD3- and CD8-positive T cells. There were numerous KiM1P-positive macrophages/microglia present within the lesion (Figure 2D–F). LFB/PAS myelin staining and immunohistochemistry for myelin proteins revealed active demyelination as LFB-positive degradation products (not shown), and all tested myelin proteins could be demonstrated in the macrophage cytoplasm (shown for MBP in Figure 2A) indicating early active, recent demyelination. Oligodendrocytes were significantly reduced in number and there were some MAG- or CNPase-positive oligodendrocytes with condensed nuclei present, indicative of apoptosis (Figure 2B,C). These active demyelinating areas clearly followed an immunopathological pattern III, as previously described [4].

Besides the active demyelinating areas, parts of the lesion were completely demyelinated, with no signs of ongoing myelin breakdown or remyelination. Most strikingly, axonal density was significantly reduced (Figure 2G,H) and massive acute axonal damage was demonstrated by immunoreactivity against APP (Figure 2I).

¹H-MR spectroscopy

To study whether there are differences in pathological processes in the periphery of the large lesion compared to the core, we measured metabolite changes in one voxel containing the biopsied site (biopsy-voxel) and another voxel directly above the core voxel (Figure 3A).

In both voxels (Figure 3B,C) a striking reduction of creatine (Cr) compared to contralateral white matter (34.9 units) was evident. Creatine increased again over the next 13 months, but stayed greatly reduced compared to contralateral white matter. Cho was also diminished compared to contralateral white matter (14.7 units) in the biopsy-voxel and recovered, to some extent, during the observation period. In the voxel above the biopsy, Cho was reduced at three months and otherwise not altered. N-acetylaspartate (NAA) was massively reduced compared to contralateral white matter (68.3 units) and stayed low in the biopsy voxel, but increased slightly in the voxel above the biopsy. An increase of lactate was most pronounced in the voxel above the biopsy and at the initial examination at one month. Levels in both voxels decreased in the following two examinations and returned to not measurable levels, similar to the contralateral side, thereafter.

Magnetization transfer imaging and diffusion tensor imaging

To separately investigate the dynamics of pathological processes in the concentric rings typical for Balo's disease MTR maps were segmented as described (see Methods section and Figure 4A).

Quantification in these separate rings (Figure 4B) showed that MTR was most decreased at the initial two weeks after biopsy and five weeks after onset of symptoms in region 1. In this region, MTR continuously increased over the following five months and then stabilized at 70–75% of the value of the contralateral side. MTR in region 2 was much higher than in region 1 at the first time point (69% of the value of the contralateral side), but showed almost no change over time. Region 3 showed the greatest dynamics in MTR. Starting only slightly decreased with 88% of the contralateral

Figure 2 (A) Immunocytochemistry for myelin basic protein (MBP) (magnification $\times 400$): extensive demyelination with sharp border to the periplaque white matter. Within the demyelinated area, foamy macrophages exhibit MBP-positive myelin degradation products (arrow). (B) Detection of few oligodendrocytes (magnification $\times 200$) within the demyelinating lesion stained for PLP mRNA (black cells, arrows) and PLP protein (red); cell nuclei are counter-stained with hematoxylin (blue). (C) Immunocytochemistry for CNPase (magnification $\times 1000$): within the demyelinated area apoptotic oligodendrocytes with condensed nuclei immunopositive for CNPase can be demonstrated (arrow). (D) Immunocytochemistry for CD3 (magnification $\times 200$): diffuse infiltration of CD3-positive T-cells within the lesion. (E) Immunocytochemistry for CD8 (magnification $\times 200$): many of the infiltrating T-cells can be identified as CD8-positive cytotoxic T-lymphocytes. (F) Immunocytochemistry for Ki-M1P (magnification $\times 200$): diffuse and dense infiltration of foamy macrophages within the demyelinated area, as demonstrated with the pan-macrophage-marker Ki-M1P. (G) Bielschowsky's silver impregnation (magnification $\times 400$): extensive loss of axons as well as axonal spheroids (arrow) within the lesion. (H) Bielschowsky's silver impregnation (magnification $\times 200$): in contrast to the lesion, the periplaque white matter displays a homogeneously dense network of axons. (I) Immunocytochemistry for amyloid precursor protein (APP) (magnification $\times 200$): numerous APP-positive axonal spheroids indicate extensive axonal damage within the lesion.

ateral side, it greatly decreased up to 46% three months after onset of symptoms and increased again over the following three months to stabilize at 74%. Region 4 showed an almost normal MTR (95% of contralateral side) with a slight decrease at three months.

The mean ADC showed a mirror image of the changes in MTR over time (Figure 4B), only region 1 differed, showing stably increased mean ADC over the observation period. Quantification of the mean FA adds information to the MTR and ADC measurements (Figure 4B). FA in region 1 was continuously increasing from 48% of the value of the contralateral side one month after onset of symptoms to 86% 13 months later. In region 2, FA showed a slight decrease at three months, but was otherwise stable. Interestingly, in region 3, FA was already greatly decreased in the initial examination at one month, when MTR and ADC still showed values closer to those of the contralateral side. In this region, FA was again lowest at 3 months, increased to 47% of the contralateral side at six months and stabilized thereafter. FA in region 4 showed the same dynamics as MTR and ADC, but was clearly abnormal with values of 70% (one month), 55% (three months) up to 80% (14 months) of the contralateral side.

Discussion

The diagnosis of Balo's concentric sclerosis was supported by the pathognomonic radiological appearance of a single lesion with alternating rings of hypo- and hyperintensity in T₁-weighted images, and at least two rings of simultaneous Gd-enhancement [2,30–32]. Histology revealed active demyelination with oligodendrocyte apoptosis representing immunopathological pattern III[4], which is characteristic for Balo's concentric sclerosis [3]. Other, probably more central lesional areas, showed inactive demyelination with significant axonal loss. Matching the diagnosis of Balo's concentric sclerosis was the young age of the patient, the acute onset and rapid progression as well as the monophasic course of the disease, which is also indicated by the lacking persistence of oligoclonal bands. Balo's disease has been described as progressive and frequently fatal. However, self-limiting cases have been monitored with MRI. Serial imaging in selected cases of Balo's disease showed the centrifugal development of enhancing rings, presumably representing ongoing inflammation. These T₁- and T₂-isointense rings became T₁-hypointense on follow-up, indicating sequential demyelination and potential axon damage [30,31].

¹H-MRS corresponds to substantial loss of axons and myelin seen in histology and indicates a failure of local repair

The biopsy demonstrated massive tissue destruction, which most likely accounts for the 60–75% reduction of total Cr measured by ¹H-MRS. We, therefore, represent metabolite concentrations as absolute values. Altered Cr levels in lesions [26] and NAWM [33] of MS patients have been described, and a relative increase in Cr has been interpreted as gliosis [33]. In context with other metabolites, the slow increase of Cr over 13 months in our case is likely to also indicate gliosis. The striking finding in the biopsy, an approximately 80% axonal loss, corresponded well to a reduction of NAA by 80–90%. This axonal loss appeared to be largely permanent. Only in the more peripheral voxel above the biopsy, a slight increase in NAA over 10 months could be monitored. This may either represent shrinking of the lesioned tissue and inclusion of unaffected tissue into this voxel or may indicate limited repair. NAA is a well-studied indicator of axonal integrity and was correlated to axonal density in biopsied MS patients [34]. Decreased NAA or NAA/Cr were also found in selected cases of Balo's concentric sclerosis; one of these was confirmed by biopsy [35–37]. Contrary to these previous reports, where increased Cho was thought to represent higher membrane turnover, Cho concentrations were reduced in the lesion centre in our case. This may be explained by the biopsied tissue, which showed substantial loss of membranous structure and apoptosis of oligodendrocytes. Immunological pattern III identified in our case is generally associated with pronounced death of oligodendrocytes via apoptosis and subsequently remyelination is in general very sparse. One may speculate that decreased Cho, which is a marker for membrane turnover, could thus be a negative prognostic factor for repair. Over the next year, Cho levels recovered to some extent, indicating limited tissue reorganization. Interestingly, in the more peripheral voxel, Cho was normal initially, but decreased at three months, thus demonstrating *in vivo* the centrifugal extension of pathogenic processes. Correspondingly, increased lactate, particularly in the periphery of the lesion, persisting for four months indicates ongoing inflammation, as lactate is thought to represent anaerobic glycolytic metabolism in activated leucocytes infiltrating the lesion [38]. It cannot be excluded that the surgical trauma contributed to some of the metabolite changes observed in the core voxel, however, the magnitude of Cr, NAA and Cho reduction cannot be solely explained by lesioning a very small proportion of the voxel volume.

Figure 3 ^1H -MR spectroscopy was carried out in three different voxels (4.8 mL each), which were defined on axial FLAIR images, and are depicted in (A) on T_1 -weighted images from a three-dimensional data set. Metabolite concentrations within a voxel containing the biopsied site (B), as well as a voxel above the biopsied site (C), are shown at different examination time points. Given are the absolute values in arbitrary units. Linear regression curves were fitted for NAA, Cr and Cho. Lactate could only be measured at the first three time points and the decline is not linear. Data are compared with absolute concentrations from the contrary white matter (Voxel 3), which are given in the text. NAA, Cr and Cho are greatly reduced in both voxels containing the lesion. Cr is increasing over time, Cho stays constant. NAA stays constant in the voxel containing the biopsy, but increases steadily in the voxel above the biopsy. Lactate is slightly increased in the voxel containing the biopsy, but massively elevated in the more peripheral voxel at the first examination time point and decreases thereafter. Cr, creatine; Cho, choline; NAA, N-acetylaspartate; Lac, lactate.

Decreased MTR and increased diffusion monitor sequential tissue destruction in selected lesion areas

Whereas the interpretation of spectroscopy results is hampered by the need for relatively large voxels,

thereby averaging pathological changes, a much higher spatial resolution can be achieved in MT and DT imaging. This allows the segmentation of the lesion into rings that are typical for Balo's and to look at pathological processes within these rings separately. Reduced MTR can demonstrate demyelination [12], but more generally represents a decreased amount of macromolecular structure per unit volume [39]. The massive reduction of MTR in the core of the lesion (region 1) in our case therefore agrees with the tissue destruction seen in histology and may also represent some damage caused by the biopsy. It cannot differentiate axonal damage and demyelination, as both are present. In region 2, higher MTR suggests less tissue disintegration. The stability of the moderate reduction over the whole observation period indicates that neither disease nor repair processes were active in this area. Evidence for the hypothesis of centrifugal extension of disease processes in Balo's concentric sclerosis comes from comparison with the MTR dynamics in region 3. Two weeks after the biopsy, which showed active demyelination in more peripheral areas and marked infiltration of macrophages and T lymphocytes, MTR was only slightly reduced. An extensive fall in MTR was only seen two months later. Given the fact that at the time of the first examination, lactate concentrations were high particularly in the periphery, the high MTR in region 3 at this time may not represent almost intact tissue, but rather an area densely filled with inflammatory cells. The second examination may pick up subsequent tissue damage caused by these cells. Between three and six months after onset of symptoms, MTR increased steadily, representing reorganization of the tissue. In region 4, a slight reduction of MTR only at three months would suggest the predominance of edema without substantial demyelination and axonal loss [11].

ADC measurements mirror the changes of MT ratios for the most part. This overlap in information is expected, as MTR is sensitive to the density of macromolecules, which also form barriers to free diffusion of water.

Diffusion tensor imaging allows the distinction of early and late changes from normal white matter

Importantly, while tissue destruction in MS lesions is well monitored by decreased MTR and increased diffusivity [13,15,40], acute and chronic pathogenic changes are not [15]. This discrepancy is caused by the additive effects of different pathogenic changes: edema, demyelination and axonal loss lead to an increase [15,41–43], high cellular density either due to proinflammatory cells or gliosis, decreases

Figure 4 (A) Representative axial sections of the MTR and proton density maps where regions of interest were defined according to the schematic picture (see also Materials and methods); ADC and FA maps which these regions were transferred on. (B) Magnetization transfer ratio (MTR), apparent diffusion coefficient (ADC) and fractional anisotropy (FA) of specific regions of interest within the lesion for different examination time points. Given are the means of all slices containing these regions. For comparison with the clinical course, EDSS scores for the respective time points are given. Large arrows indicate the onset of symptoms and time points for MRI examinations, small arrow indicate the time of biopsy.

diffusivity [13,44]. Fractional anisotropy, a measure of directionality of tissue, is also decreased in disintegrated parenchyma with increased diffusivity. However, our case demonstrates that FA is more sensitive than MTR and ADC to early inflammatory changes. FA most clearly depicts pathological changes in the periphery (region 4), where accord-

ing to MRS and by extrapolating the biopsy findings, inflammation is ongoing. Indeed, Werring *et al.*, already demonstrated that FA is more sensitive than ADC to the acuity of the lesion [15]. Furthermore, in region 3, FA shows clearly decreased values from the first examination when MTR and ADC are still close to normal. The

comparison of FA with MTR strongly supports our earlier conclusion, that at this time point region 3 is not unaffected, but rather filled with inflammatory cells. While increasing MTR and decreasing diffusivity at later examinations indicate reorganization, a consistently lowered FA demonstrates that directionality is not re-established. This clearly indicates gliosis as the cause of increased tissue density. The weak correlation between ADC and FA in MS lesions [15,45] and the lack thereof between FA and MTR in corpus callosum of MS patients [45] clearly demonstrates that complementary information can be gained from combining MTI and DTI.

Recovery despite the lack of local repair

Rapid development of the lesion prior to biopsy and massive tissue destruction shown histopathologically demonstrate an aggressive inflammatory process typical for Balo's disease. Although the dramatic worsening of symptoms soon after biopsy suggests an either direct or indirect effect of the stereotactic procedure, it is unlikely that the relatively small trauma from biopsying the already severely demyelinated area with minimal axonal preservation caused significant additional damage. We rather think that the observed rapid enlargement of the lesion into the internal capsule and subcortical areas disrupting efferents of Broca's area, possibly triggered by blood-brain barrier damage following biopsy, caused this extensive pyramidal tract damage and aphasia. Indeed, there is evidence from experimental studies that neuropathological damage is augmented in immunized animals after trauma [46]. Acute inflammatory processes may also better explain that the peak of clinical symptoms preceded the peak of tissue destruction. Mediators, such as nitric oxide or small peptides, may have caused extensive conduction blocks [47–49] and edema may have further compromised axonal function. Therefore, recovery may have resulted from resolving inflammation in the periphery of the lesion, whereas the functionally less important centre failed to repair. Additionally or alternatively, neuronal plasticity may have allowed new circuits to form in this young patient.

Conclusion

A multimodal MRI approach enables us to follow tissue changes in a demyelinating inflammatory lesion. Specifically, we can demonstrate areas with apparently normal MTR showing pathological changes in DTI. The combination with DTI and MRS together with the temporal pattern of lesion development serve to contextually interpret MTR

values. While the initial observations appear encouraging, the applicability of these parameters combined to monitor the acuity and the fate of an evolving MS lesion, requires further evaluation.

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