GREY MATTER ATROPHY IN PATIENTS SUFFERING FROM MULTIPLE SCLEROSIS

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SZÜRKEÁLLOMÁNYI ATROPHIA SCLEROSIS MULTIPLEXBEN SZENVEDŐ BETEGEK ESETÉBEN
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White matter lesions are defining characteristics of multiple sclerosis (MS), whereas grey matter involvement is a less recognised attribute. Recent investigations using dedicated imaging approaches have made it possible to depict cortical lesions. Additionally, grey matter atrophy may be estimated using various methods. Several studies have suggested that grey matter atrophy closely correlates to clinical disability. In this review we have collected information on grey matter atrophy in MS and the effect of disease modifying therapies upon brain atrophy.

Keywords: *multiple sclerosis; atrophy, gray matter; MRI; voxel based morphometry*

A fehérállományi laesiók a sclerosis multiplex (SM) meghatározó jellemzői, a szürkeállomány elváltozásai kevésbé ismertek. A megfelelő agyi képalkotó eljárásokkal végzett újabb vizsgálatokban láthatóvá tudták tenni a kérgi elváltozásokat. Emellett különféle módszerekkel felmérhető a szürkeállományi atrophia. Számos vizsgálat eredményei szerint a szürkeállomány atrophiája szorosan korrelál a klinikai rokkantsággal. Összefoglaljuk az SM-ben megfigyelhető szürkeállományi atrophiával kapcsolatos információkat és a betegségmódosító terápiák hatását az agyi atrophiára.

Kulcsszavak: *sclerosis multiplex, atrophia, szürkeállomány, MRI, voxelalapú morfometria*

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Multiple sclerosis (MS) is an inflammatory, neurodegenerative disease. The primary feature of MS is the demyelination paralleled by varying degrees of axonal damage[1]. The focal inflammatory demyelinating lesions in the white matter are not only the hallmarks of the disease, but in vivo demonstration of these lesions with MR imaging is also part of the diagnosis[2]. Recently, it has become clear that grey matter is also adversely affected. This review aims to summarize the recent advances made by studies assessing grey matter atrophy in MS patients for both clinical and scientific purposes.

Grey matter lesions

Early post-mortem histological studies established the existence of grey matter lesions in MS patients[3–6]. Grey matter lesions are classified as Types I to IV. Type I lesions, located at the border of the grey and white matter, rarely occur. Type II lesions occur within the cortex. Type III subpial lesions are the most frequent. Type IV lesions cover the full width of the cortex and occur infrequently. While the neuropathological studies[4–9] have provided insight into the cortical pathology, these studies are limited by the inclusion of patients with different disease types and stages, consequently restricting inter-study comparability. Advanced MRI methods may offer an alternative approach to track progression of the grey matter lesions and elucidate their significance in the disease process. With modern MRI sequences, such as the double inversion recovery[10, 11] it is possible to detect many more cortical lesions than conventional T2 weighted sequences. However, the detection rate is still only around 20% when compared to neuropathological investigations[12]. Furthermore, the inter-rater agreement in identifying lesions is rather low[13]. Ultra-

high field imaging may facilitate increased sensitivity and specificity of the grey matter lesion detection[13], although the modality is not widely available.

Grey matter atrophy

Apart from depicting grey matter lesions MRI investigations are also capable of identifying grey matter atrophy. There are several approaches available to evaluate grey matter atrophy. Apart from manual segmentation, gross grey matter volume can be estimated (cortical with or without subcortical) by automatic segmentation of brain tissue types[14–16]. Another approach for detecting atrophy is by measuring cortical thickness. One of the most common approaches for this is the voxel-wise comparison of intensities (or more precisely the probability of tissue types in individual voxels)[15, 17]. The advantage of this method is that it can identify focal atrophy. However, inter-subject registration is the critical limitation of this method.

MRI studies employing various methodologies agree on the significant grey matter atrophy in MS. The earliest MRI study on gross brain atrophy using manual measurements showed significant brain volume change in MS patients[18], as confirmed by semi-automatic larger dataset studies[19–22]. Likewise, the brain parenchymal fraction (the ratio of the premorbid brain size -measured as intracranial volume- and the brain parenchymal volume) is about 3-5% smaller compared to normal controls[23–25] and considerably smaller in secondary progressive MS[26]. Tissue type segmentation made it possible to identify atrophy in both grey[24, 26–28] and white matter[26, 29].

Voxel-based morphometry (VBM) studies demonstrated that the grey matter atrophy is not uniform over the cortex **(Table 1.)**. These studies revealed that the grey matter pathology exhibits considerable heterogeneity in patients, because a clear pattern of grey matter atrophy cannot always be established. Other studies failed to find atrophy in MS patients when compared to controls subjects. *Audoin* and co-workers found no difference between control subjects and relapsing-remitting MS patients early in their disease course. However over two years, atrophy developed in the bilateral thalami and the right frontal cortex[30]. Similarly *Sepulcre* et al. found no focal grey matter atrophy in 31 primary progressive MS (PPMS) subjects, except in the cerebellum when not correcting for multiple comparisons[31]. Nevertheless, if a specific pattern of grey matter atrophy is to be tentatively established, than the thalamus is invariably affected, as well as the temporal, insular and frontal lobes (often sensory-motor cortex).

The location of white matter lesions in MS or the total lesion load only moderately correlate with clinical symptoms and cognitive impairment (clinic-radiological paradox[32]). Several reports suggested that disability has a closer relationship with grey matter atrophy[33]. However, upon closer inspection of these studies, using VBM approach correlation between focal atrophy and disability was identified infrequently. Audoin found correlation between expanded disability status scale (EDSS[34]) and focal atrophy in the cerebellum[35]. With regard to the cognitive performance, *Morgen* and co-workers found extensive correlation with paced auditory serial addition test (PASAT) scores[36]. *Cerasa* and colleagues, using many cognitive tests, only found correlation with performance on symbol digit modalities (SDM) and controlled oral word association test (COWAT)[37].

Moreover, global grey matter atrophy was found to correlate with the clinical features of the disease as measured via the EDSS[19, 25, 26, 38, 39] and cognitive function tests[39–42].

Pathology and pathophysiology of grey matter injury in multiple sclerosis

The methods for detecting grey matter pathology are especially important, considering the grey matter demyelination in MS. *Gilmore* et al. reported an "overall 28.8% of the grey matter demyelinated as compared with only 15.6% of the white matter"[7]. Most of this demyelination (about 60%) is subpial[6] and is present in the form of ribbon like demyelination often affecting several adjacent gyri. Importantly, based on post-mortem MRI imaging of three MS cases there was no association between the cortical demyelination and the focal or diffuse white matter pathology[43]. The various mechanisms behind the lesions are indicated by the fact that in contradistinction to the white matter lesions, in cortical lesions there is no inflammatory cell infiltration and foamy macrophages, immunoglobulin deposition or complement activation is scarce[44, 45]. Meninges were also shown to be affected in MS[46, 47], B-cell follicle-like structures were described in the inflamed meninges. More recently this meningeal inflammation was hypothesized to be the major contributor to the cortical pathology: the B-cell follicle-like structures were associated with increased subpial demyelination and cortical atrophy[48]. Apart from the demyelination, a gradient of neuronal loss was observed in the affected cortex, which was accompanied by astrocyte loss and opposite to this gradient a microglia activation[48]. These changes were suggested to be consistent with a non-targeted general immunopathological response

Table 1. *Correlation of grey matter atrophy and clinical signs and cognitive impairment*

Study	Type	EDSS	DD	Comment
[36]	19 RR	1 (0-3.5)*	13m (2-60)*	No correlation with EDSS. Extensive correlation with PASAT.
[30]	21 RR	1 (0-3)*	25.8m (14-45)*	No difference patients vs. controls. Over two years larger change developed in the thalami and left frontal region.
[89]	51 RR	2.6 (1.5-4.5)**	13.1m (4-34)**	Left lateralised group difference. No correlation with EDSS. Right caudate correlate with LL.
[90]	28 CIS	0* (0-1)	0y** (0)	No difference controls vs. CIS. Other groups were not compared to controls. Pairwise comparisons yielded widespread differences. LL correlate with thalamic volume in RRMS. No correlation with EDSS or DD
	26 RR	1 (0-3)	9y (2-22)	
	27 SPMS	6 (4-8)	17.6y (3-41)	
	18 PPMS	5.5 (4-7.5)	8.5y (2-18)	
[91]	46 PPMS	4.5 (1.5-7)*	3.3y (2-5)	No correlation between atrophy and clinical (EDSS, MSFC, NHPT and TWT) or cognitive performance (PASAT). These measures correlated with regional MTR.
[31]	31 PPMS	4.5 (3.5-7)	3y (2-5)	No difference between controls and patients (only in the thalamus, when not corrected for multiple correlations). Anterior thalamic grey matter correlated with lesion load. One-year longitudinal study found reduced GM volume in several basal ganglia structures and in some cortical areas.
[92]	15 RR	2* (0-3.5)	7.3y**± 6.5	Group difference in the left inferior frontal gyrus and lateral occipital cortex.
[37]	26 RR	RRc 2.5* (1-4)	RRc 12.1±8.7	Patients with and without cerebellar;symptoms were examined. Thalamic atrophy, plus putamen and primary motor cortex in the cerebellar group. No correlation with EDSS. SDM and COWAT test showed correlation in the two groups separately. No correlation with other cognitive tests measuring verbal and spatial memory, visuo-spatial processing and;abstract/conceptual reasoning
	12 RRc	RRnc 2 (1.5-4.5)	RRnc 8.8 ± 4.4	
	14 RRnc			
[93]	13 RR	3 (1-6.5)	15±7**	Widespread differences between controls and patients. Cognitive tests on the following domains: Information processing, speed, attention, working memory, verbal memory, Visuo-spatial memory and function, viuso-constructive abilities, executive function, general iteligence. VBM found correlation SDM and CVLT-LDCR.
	5 SPMS			
[94]	26 RR	3* (1-6)	56.3** (6-240)	Study compares normal controls, RRSM and NMO patients. Widespread differences between controls and patients. No correlation with DD and EDSS. LL correlated with GM volume of the right caudate and bilateral thalami.
	26 NMO			
[35]	62 CIS	1* (0-3.5)	4m (0-6)	Mainly subcortical atrophy (thalamus, caudate, nucl. Lenticularis, hipocampus), but some frontal, temporal posterior cingulate and cerebellar cortical atrophy. Thalamic atrophy correlated with LL. Cerebellar atrophy correlated with EDSS.
[95]	34 CIS	1.5* (0-3)	<3m	Thalamic atrophy that disappeared after correction for multiple correlation
[96]	59 RR	1.5* (0-5)	1.8y* (0.1-17)	Diffuse grey matter atrophy. Longitudinal analysis also performed. FSL-VBM and SPM-VBM was used.
[97]	36 PPMS	4.5* (1.5-7)	3.3±0.9	Atrophy in the sensory motor cortex, thalamus, temporal lobe insula.
[98]	PPMS 2	6* (3-7.5)	14.43** ±8.89	No correlation between regional atrophy and age, DD, EDSS or MSFC. No correlation with performace on neurophychological tests (TMTA, TMTB, SDM, DST, RAVLT-DR and DST)
	RR 3			
	SPMS 10			
[99]	21 RR	3.5* (0-85)	10.1** ±5.1	Grey matter atrophy in the thalamus, hypothalamus, caudate and frontal lobe. No correlation with clinical measures and focal atrophy is reported to be investigated.
	11 PPMS			
[100]	26 RR	1.2 ±0.9**	8.5** ±5.9	GM loss in the bilateral thalami and caudate nuclei. No correlation found between GM atrophy and EDSS.

*median (range), **mean (range or STD), y: year, m: month, RRc: RRSM patients with cerebellar symptoms, RRnc: RRSM patients without cerebellar symptoms, SDM symbol digit modalities, COWAT: Controlled Oral Word Association Test, MSFC: multiple sclerosis functional composite, TMTA and B: Trial making test A and B, DST: Digit span test, RAVLT-DR: Rey's Auditory Verbal Learning Test Delayed Recall, NMO: Neuromyelitis optica

mediated by the CD8+ T cells via cytotoxic tissue damage or indirectly through the activation of microglia. Furthermore, neuronal loss was found in layers III and V in regions where no B-cell follicle-like structures were detected, this suggests that other mechanisms are also involved in the atrophy. Neurodegeneration due to inflammatory insults in downstream white matter tracts, reduced synaptic and glial density may also contribute to the atrophy[49, 50].

Various mechanisms were proposed behind the neurodegeneration in MS. Neuroinflammation can directly induce apoptosis through various cytokines and the oxidative burst arising from the activated microglias may also contribute[51]. As in many neurodegenerative diseases[52], oxidative stress and mitochondrial dysfunction are proposed to be a major mediators of neurodegeneration and axonal loss in MS[53–55]. Another key component in MS neurodegeneration is glutamate excitotoxicity[56] and recently a link between inflammation and excitotoxicity was established[57]. Furthermore, a possible mechanism of neurodegeneration might be an imbalance of neuroprotective and neurotoxic agents, such as the metabolites of the kynurenine pathway a major biochemical pathway in tryptophan metabolism[58–60].

Grey matter atrophy as a biomarker for therapeutic efficacy

It is becoming progressively more evident that grey matter atrophy is a stronger predictor of disability progression in MS than white matter pathology. Moreover, it may also be used as a complementary MRI method for disease progression instead of conventional MRI techniques[25, 39, 61–63]. Thus, making it a promising biomarker for therapeutic response in pharmacological studies.

INTRAMUSCULAR INTERFERON β-1A

A post hoc analysis of the MRI images from 140 patients (72 in the placebo arm, 68 in the treatment arm) participating in a phase III clinical trial of intramuscular interferonβ-1a (INFβ-1a, Avonex) showed a reduced rate of the atrophy (brain parenchymal fraction) in the treatment arm[64]. While there was no difference between the two treatment groups in the first year of treatment, during the second year INFβ-1a reduced the rate of atrophy by 55%. In a subsequent study, treatment over two years with intramuscular, subcutaneous INFβ-1a and glatiramer acetate (GA) resulted in a decreased rate of reduction in brain gray matter fraction[65]. A three-year period open-label study using intramuscular INFβ-1a found a reduced whole-brain and GM atrophy, as well as of T1-hypointense lesion volume accumulation in the treatment group[66]. When compared to year one weekly intramuscular INFβ-1a treatment data, the atrophy rate was reduced in the second and third year of treatment[74]. Subsequent analysis of a 138 patients subgroup having frequent MRI scans, showed that the majority of the atrophy during the first year (approximately 70%) occurred during the first four months[74].

INTERFERON β-1B

Five years post INFβ-1b clinical trials for patients with PPMS, not only were motor and cognitive performance better, but also the decrease of brain parenchymal fraction was similarly smaller[75]. In an earlier randomized study, which measured the parenchymal volumes only in the central slab failed to demonstrate a beneficial effect of INFβ-1b[76]. A smaller open-label study with RRMS patients also failed to demonstrate completely the beneficial effect of INFβ-1b, a reduced rate of atrophy was observed only in the second year, but not in the first or third years of treatment[77].

SUBCUTANEOUS INTERFERON β-1A

In the PRISM trial neither high dose nor low dose (22 and 44 μg three times weekly) INFβ-1a–Rebif showed a beneficial effect against reduction of the whole brain volume[78]. In contrast, in the ETOMS study, subcutaneous INFβ-1a (once weekly 22 μg) given to patients after the first attack, not only delayed the appearance of a second relapse but also reduced the rate of atrophy over two years as measured with SIENA[67]. Furthermore, in a comparative study, glatiramer acetate, intramuscular and subcutaneous INFβ-1a was superior to placebo in reducing brain atrophy at 24 months, while no difference between treatment outcomes was detected[65]. In Gasperini's study there was no difference in brain atrophy using 11μg and 33 μg subcutaneous INFβ-1b treatment (three times weekly)[79].

GLATIRAMER ACETATE

In the open-label extension of the US GA trial, the patients who were in the placebo arm originally had more brain volume loss as compared to subjects receiving glatiramer acetate (GA) treatment[80]. *Kahn* and co-workers showed a significantly reduced loss

Table 2. *The effect of treatment on grey matter atrophy*

	First year	Second year	First two years
INFβ-1a i.m.	✗[64]	✓[64]	✓[64–66†††]
INFβ-1b	–	–	–
INFβ-1a s.c.	–	–	✓[65, 67]
GA	✗[68, 69]	✗††[68, 69]	✓[65, 70]
Natalizumab	✗[71†]	✓[71]	✗[71]
Fingolimod	✓[72, 73††††]	✓[73]	✓[73]

†pseudoatrophy, †18 months, †††three years, ††††at six months also

of brain volume in patients receiving disease-modifying therapy[81] and having only minor progression in their disability (EDSS). Ge and colleagues found reduced rates of brain tissue loss over 24 months of GA treatment in comparison to placebo treated subjects[70]. Conversely, a nine months placebo controlled study showed no significant benefit of GA on brain atrophy[68]. Importantly, in a comparative study GA reduced the rate of atrophy more than low and high doses of INFβ-1a[81], while low dose INFβ-1a was more effective than high dose[81]. However, Calabrese found the use of GA, intramuscular INFβ-1a or subcutaneous INFβ-1a similarly effective in reducing grey matter atrophy[65].

NATALIZUMAB

While natalizumab is effective in reducing MRI-measured disease activity (T2 lesion load and enhancing lesions) the results on brain atrophy are controversial[71]. In the first year of natalizumab treatment a pseudoatrophy appears, which over the next two years converts to a significant reduction of brain volume reduction[71, 82]. This phenomenon was related to the cessation of inflammatory activity.

In comparative studies natalizumab was more effective in reducing grey matter atrophy. The analysis of the secondary and the tertiary MRI measures in the SENTINEL study showed that among beneficial effect of combined intramuscular INFβ-1a and natalizumab (T2 lesion volume, T1 hypointense lesions, enhancing lesions) treatment the atrophy rate was reduced when compared to the group receiving INFβ-1a alone[83]. In RRMS patients enrolled in a non-randomised pilot study, significantly lower changes in brain parenchymal volume were recorded in those treated with natazilumab (n=12) in comparison to those receiving INFβ (n=14)[84]. In a two-year-prospective study natazilumab was also superior to the immunmodulatory agents GA and INFβ in reducing the accumulation of cortical lesions as well as cortical atrophy[85].

FINGOMILOD

Fingolimod is a sphingosine-1-phosphate-receptor modulator that prevents lymphocyte egress from lymph nodes: it has shown clinical efficacy in phase II[86] and III clinical trials[72]. The efficacy of the drug was also proved with paraclinical measures. New T2 lesions as well as the number of gadolinium enhancing lesions were reduced in the group receiving fingolimod when compared to patients receiving intramuscular INFβ[72, 86]. While in a six-month phase II study, there was no improvement in the rate of brain atrophy as compared to placebo arm[86]. However, the 12 months phase III study (TRANSFORMS) revealed significant improvement in the brain atrophy rate in comparison to intramuscular INFβ treatment[72], this effect was evident in the subgroup analysis also[87]. Phase III (FREEDOMS) study showed significant reduction of brain atrophy rate in fingolimod treated patients at 6, 12 and 24 months[73]. Interestingly, this effect was unrelated to the T2 lesion load or the number of enhancing lesions **(Table 2.)**[88].

Conclusions

Paraclinical examinations in the diagnosis of MS such as MRI have a central role. However it is important that conventional MRI modalities cannot identify the full range of abnormalities. Sophisticated analysis approaches have to complement the expert eye of physician and advanced neuroimaging have to complement traditional neuroradiology in order to further elucidate the disease pathomechanism and tailor treatment efficacy.

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