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Review article

What you cannot get from routine MRI of MS patient and why – The growing need for atrophy assessment and seeing beyond the plaque

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ABSTRACT

Multiple sclerosis is a disease that still has not been fully understood and calls for better diagnostic procedures for the improvement of everyday patient care and drug development. Routine magnetic resonance examinations reveal demyelinating focal lesions, but they do not correlate sufficiently with the patients' disability and cognitive impairment. For more than 100 years it has been known that demyelination affects not only white but also grey matter of the brain. Recent research has confirmed the serious consequences of grey matter pathology. Over the last several years, atrophy of the brain and especially of its grey matter has become a most promising marker of the patients' clinical status. The paper discusses the concept and importance of atrophy assessment in relation to the standard magnetic resonance results.

1. Introduction

Multiple sclerosis (MS) affects approximately 2.5 million people worldwide. It is an acquired, chronic inflammatory disease of the central nervous system (CNS) characterised by the presence of inflammatory-demyelinating lesions and progressive loss of brain tissue. The condition was first described over 170 years ago; however, its aetiology is still unclear. Until the 1980s, that is, until partial efficacy of immunosuppressive drugs was demonstrated, MS was considered an incurable disease. Symptoms of MS include progressive impairment of motor and sensory functions, as well as cognitive disorders. The disease is the most common non-traumatic cause of disability in young people [1,2].

Primary lesions in MS are believed to be of inflammatory aetiology. However, due to gradual deterioration of the patients' clinical condition and to lesions found in diagnostic imaging, MS has recently been considered also a degenerative disease. The primary pathological process in MS affects myelin

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but there is also secondary atrophy and degeneration of other brain components [3].

In a routine MRI scan, demyelinating lesions are usually found in the white matter. However, since 1880s it has been known from histopathological examinations that the pathological process also takes place in the grey matter (GM) [4]. This has been confirmed by modern diagnostic imaging techniques, and nowadays the classification of MS as solely a white-matter disease is increasingly regarded as obsolete [5].

2. Magnetic resonance imaging in MS

What can be visualised by routine MRI still remains ''the tip of the iceberg''. The amount of lesions revealed, in most cases in the white matter (WM) only, is quite small in comparison to the known extent of CNS damage which remains beyond the capabilities of the standard imaging sequences. This often results in a discrepancy between the clinical symptoms and the findings in routine MRI, i.e. the so-called clinical-radiological paradox [11]. In the relapsing-remitting (RR) form, MRI reveals 10–20 times more new lesions than the number of known clinical relapses. In some patients, active lesions remain visible in MRI for a very long time, and yet the clinical course of the condition is stable. Half of untreated asymptomatic patients present active plaques in MRI [6].

Neuroradiological markers are constantly being sought that would be more clearly correlated with the patient's condition and would be more useful for effective modification of treatment. For this reason, attention has been paid to the damage of normal-appearing white matter (NAWM) and normal-appearing grey matter (NAGM). It is supposed that these areas could be damaged as a result of Wallerian degeneration of the fibres passing through the plaques visible in the MRI image, or due to an independent pathological process. The progression of damage to the normal-appearing brain tissue (NABT), resulting in increasing atrophy, is associated with the progression of the patient's disability. As there is no direct relationship between the clinical symptoms and the number of plaques, it is the atrophy that is considered to reflect MS pathology, being invisible in conventionally assessed MR imaging [12].

Given that MR imaging went into clinical use in 1980, the publication *The evaluation of multiple sclerosis by magnetic resonance imaging, Val M. Runge et al.* is one of the first to describe the application of this method in MS. The today's standard MRI scan protocol is not much different from that presented in the article. Certainly present routine scans are of better quality and are obtained in a shorter time, but they are still unable to show CNS pathology located beyond demyelination lesions. With the use of modern MRI scanners which allow to modify the sequence in research mode, DIR (double inversion recovery) sequences can be activated. Nevertheless, they remain imperfect, revealing up to 20% of GM lesions [13,14]. Only a few centres are able to track the atrophy of the brain and its components, but it is still not a standard procedure to use this information for the purpose of therapy modification.

Atrophy assessment predicts disease progression in the years to come. MRI techniques permit the detection of differences in brain volume in a relatively short period of time – even within 6–12 months. That is why we need software that could be used for daily diagnosis of patients, and not only for research [6]. The increasingly available programmes are relatively easy to use and will soon allow to include an assessment of brain atrophy in multiple sclerosis patients in routine MRI reports [15,16].

3. Grey matter pathology in MS

Basing on histopathological examinations, several types of plaques in the cerebral cortex are distinguished: (1) located at the border between the cortex and the white matter, (2) not reaching the surface of the cerebral cortex, (3) located externally, under the arachnoid (the most common ones). Some authors distinguish type 4: lesions involving the entire thickness of the cerebral cortex.

Microscopic examination has also demonstrated that demyelination is often more pronounced in the grey matter than in the white matter. Its presence correlates better with the patient's clinical condition than the WM pathology but routine MRI performed in MS does not show GM plaques well enough. Grey matter damage involves diffuse and local demyelination, and is often secondary to lesions in the white matter. The percentage of inflammatory lesions in the GM is lower and the process involves other inflammatory cells besides WM. Also, partial BBB damage takes place [6].

Degenerative processes in the grey matter are not always associated with demyelination, and the pathologies of the white and grey matter are independent processes, at least to some extent. However, 80% of lesions examined in microscopy are not visible in the modern sequences (including DIR), applied for visualisation of the grey matter pathology in MS. This is particularly relevant for lesions located under the pia mater. The number of demyelinating lesions in the grey matter is clearly associated with the patient's disability progression and the reduction of his/her intellectual abilities. Besides DIR sequence, the use of diffusion tensor imaging (DTI) can also help detect cortex damage. A decrease in fractional anisotropy (FA) is associated with the plaques volume and worse patient's clinical condition [7].

The grey matter represents 65% of the parenchymal brain volume. Therefore, its atrophy clearly affects the volume of the whole brain. GM atrophy correlates better with the patient's clinical presentation than WM atrophy or the total plaque volume [6,8].

The difficulty in observing grey matter pathology is due to the fact that this process cannot be adequately shown in vivo in routine MRI scans. The visualisation of demyelinating lesions and GM atrophy with advanced MRI techniques could not only supplement routine diagnostics but also allow to better correlate imaging with the clinical status of patients. On the other hand, it suggests that GM damage should be considered as a target for modern MS therapies [9–11].

4. Brain atrophy and its assessment

Atrophy of the brain leading to an irreversible loss of its tissues is a well-known phenomenon, directly related to the clinical

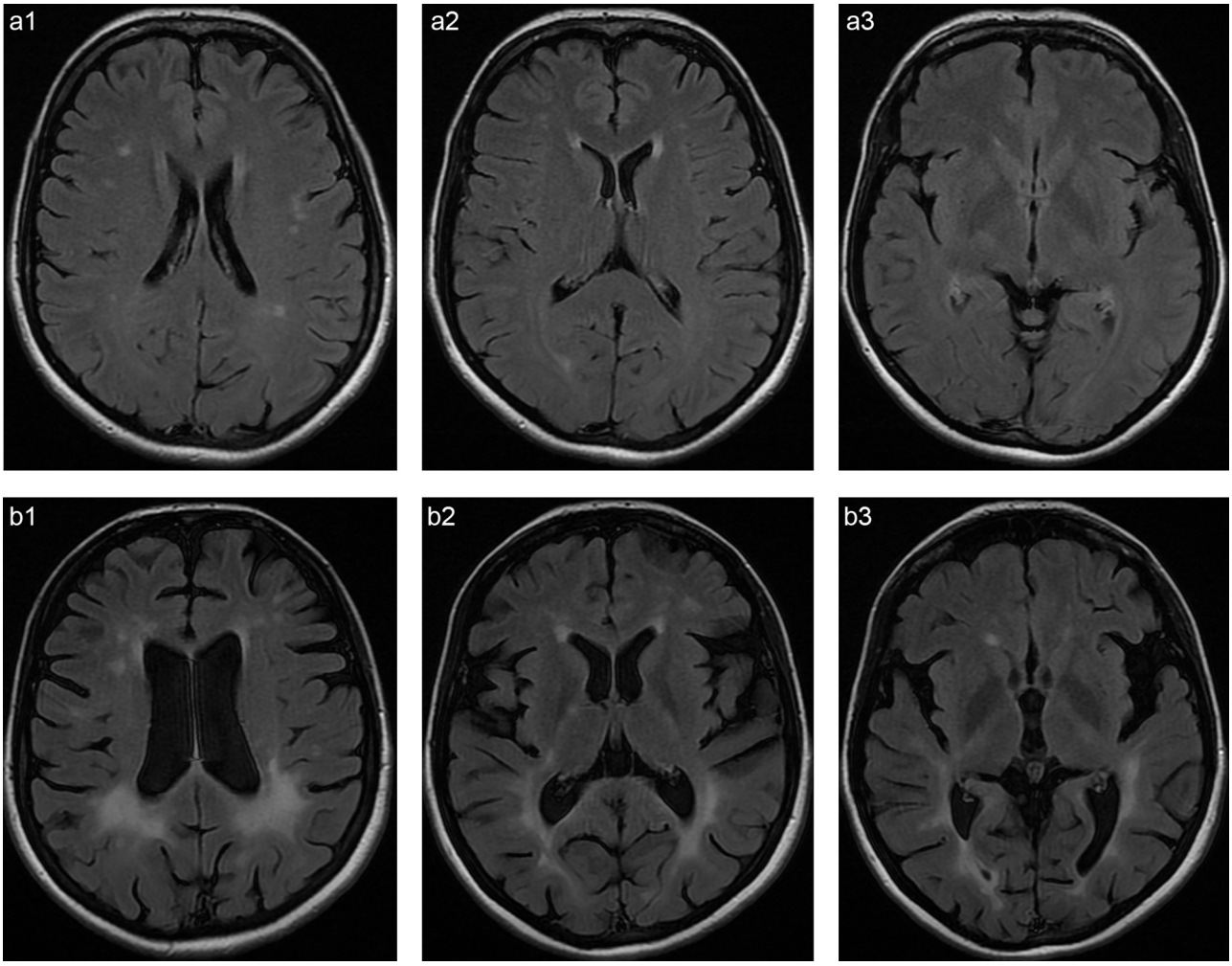

Fig. 1 – (a and b) Series of FLAIR images obtained from two 41-year-old female patients with RRMS: (a) EDSS score 2.0; (b) EDSS score 4.0 with much more pronounced brain atrophy, dilated ventricles, widened grooves and fissures of the cerebral cortex.

status of MS patients (Fig. 1). As atrophy progresses, MRI shows gradual widening of the paracerebral spaces, sulci, fissures, cisterns and ventricles of the brain. These changes occur much faster in MS patients than in healthy subjects who present physiological atrophy progressing with age. Loss of brain per year in MS is up to 1.4% vs. 0.3% loss of brain in healthy subjects; according to other authors, this percentage is 0.5–1.0% vs. 0.1–0.3% in the normal population [17,18].

Compared with healthy subjects, MS patients have lower white matter volume, and when assessed in a long term, the grey matter is lost at a higher rate. At the beginning of this century, thanks to immunohistochemical techniques showing myelin, it was demonstrated that more than 66% of the GM is involved in the disease process, and the pathology affects mainly the basal ganglia [19,20].

Atrophy progresses throughout the continuum of the disease, at any stage of MS and at a similar rate in various forms of MS. Atrophy results not only from demyelination but also from the loss of neurons. This process is secondary, among other mechanisms, to the inflammatory process and demyelination as well as Wallerian degeneration and impaired remyelination [17,18,21]. GM atrophy progresses at a clearly higher rate when the disease has advanced into the secondary progressive form. Slow atrophy rate may suggest a benign form of MS [22].

Axons represent 46%, and myelin 24% of the WM volume. Even if remyelination occurs, the secondarily produced myelin sheath has a smaller thickness/volume, and axonal loss is not reversed. For this reason, atrophy is determined not so much by the loss of myelin as by the loss of axons. Demyelination in plaques constitutes 1% of total brain atrophy and even if a plaque enlarges by approximately 10% per year, this represents only 0.1% of the total atrophy. It is believed that atrophy means loss of axons and myelin not only in the demyelination lesions but mainly in the normal-appearing brain tissue (NABT).

Brain atrophy correlates with the patients' progressive cognitive and physical impairment much better than an evaluation of changes in T2-weighed images. Atrophy has been found to be related to the number of depression episodes, fatigue, and deterioration in the patients' quality of life [6]. An assessment of atrophy in the first two years of the disease predicted the occurrence of cognitive impairment as early as seven years after the diagnosis [23].

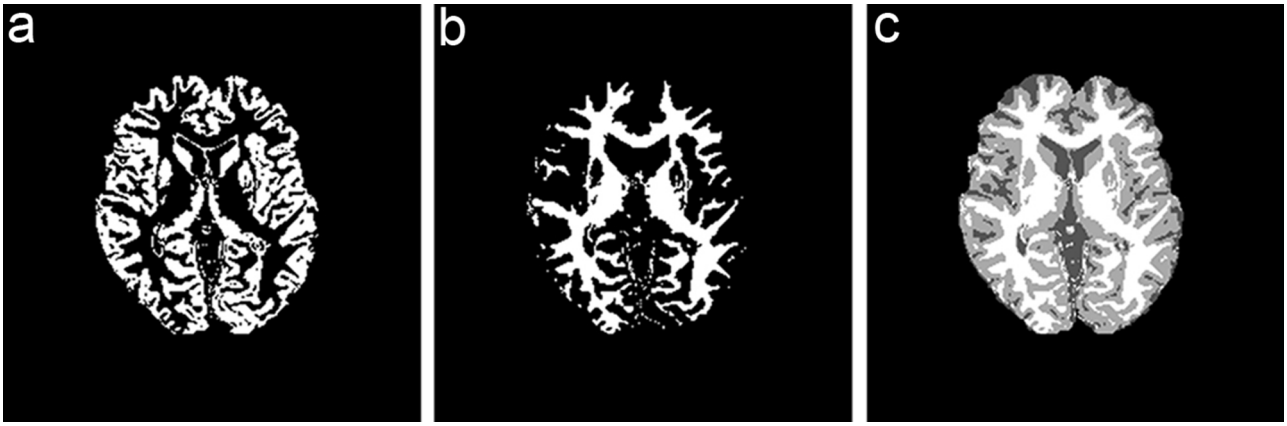

Fig. 2 – (a and c) 3D-T1 image postprocessing performed with SIENAX at the level of basal ganglia: (a) segmented grey matter, (b) segmented white matter, (c) combined image (segmented grey + white matter).

An analysis of subregional grey matter atrophy correlates significantly with the symptoms presented by MS patients – for example, atrophy of the thalamus has an apparent relation to cognitive impairment. Other authors emphasise the fact that atrophy of the subcortical GM and the cerebral cortex is a better marker than the observation of atrophy of the white matter or the total brain volume, and it is visible from the very onset of the disease.

The first scientific papers on the issues of atrophy in MS were published more than 15 years ago [22,24]. From the very beginning, the authors were interested in the changes in brain ventricular volume as indirect indications of the loss of brain tissue. Changes in ventricular volume are mainly due to the loss of subcortical grey matter – an enlargement of the lateral ventricles does not correlate with the loss of the white matter [25,26]. The enlargement of the ventricular system, i.e. central atrophy, has a predictive value in the so-called mean follow-up time (5 years from the baseline assessment). The correlation with clinical symptoms is stronger than in the case of the total plaque volume or total brain atrophy [27].

An objective method for measuring atrophy is relatively easily accessible. The option of volumetric imaging using 3D-T1 sequence is available in almost every MRI scanner. This sequence is the basis which should be acquired in the best possible resolution and image contrast.

The following techniques are used in the objective atrophy assessment:

1. Manual methods like linear measurements and manual outlining (width of the brain, corpus callosum, ventricles, bicaudate ratio, volume calculated from outlining of the brain on every slice or cerebrospinal fluid (CSF) volumes. A disadvantage of these methods is low repeatability and precision of measurements; moreover, they are time-consuming.
2. Semi-automated methods are faster and offer better reproducibility – segmentation algorithms like seed growing, edge detection or contouring.
3. Automated segmentation – still less time-consuming and more reproducible. Segmentation is possible thanks to the differences in signal intensity between the brain parenchyma and CSF, GM and WM, as well as brain parenchyma and MS lesions (brain parenchymal fraction – BPF, SIENAX, SPM, FreeSurfer) (Fig. 2).
4. Registration and boundary-based methods, which reveal the volume change by calculating the difference between the scans of normal controls and those of the patients. Comparison of subjects with different head size is possible thanks to the use of volume normalisation and relative values (SIENA, voxel-based morphometry – VBM) [21,28,29].

In a review article, Radü et al. classify all the above techniques except VBM as whole brain volume methods. More advanced techniques – VBM together with cortical thickness (CT) – belong to the regional volume group [30].

VBM. 3D-T1 data can also be postprocessed with the use of voxel-based morphometry (VBM). It is an automated technique detecting the subtle changes in brain tissue density at a voxel level. Recently it has been increasingly used for predominantly GM changes in MS. VBM provides statistical analysis of the group differences thanks to spatially normalised images. The result is a statistical parametric map showing regions where GM concentration differs significantly between groups [31,32].

Cortical thickness assesses the cortical damage in MS by measuring focal cortical thinning in addition to the analysis of cortical volume. Cerebral cortex thinning is an earlier pathological finding than tissue loss. It correlates with fatigue and cognitive deficits, which have recently been gaining importance as clinical markers [33,34].

BPF is one of the most popular quantifying methods for brain volume loss assessment used in clinical trials. The estimation of brain parenchymal fraction, widely applied since 1980s, is defined as the ratio of brain parenchymal volume to the total brain volume (a sum of brain and ventricle CSF volume). This technique is highly reproducible and takes into account individual variability in head size between subjects [35–38].

The described calculation methods can use data of 1.5–3 T field MRI. Thanks to the technology development a number of

MS researchers have gained access to ultra-high field scanners like 7–9.4 T. High signal-to-noise ratio and high-resolution images permit improved visualisation of demyelination lesions within GM. Better resolution also contributes to more accurate assessment of the cortical volume and cortical thickness [39–41].

Objective measurements can be incorporated into clinical assessment, although this has not been included in the MS treatment criteria. The accuracy of measurements performed with automatic methods is very similar to that of semi-automatic methods [47]. However, other authors have demonstrated advantages of registration techniques in comparison to segmentation techniques. It is crucial that the follow-up of atrophy in a given patient should take place invariably in the same centre, with the same MRI scanner and the same sequences and planes [48].

Since the loss of white matter depends, inter alia, on the volume and activity of plaques, there are differences in assessing atrophy of the grey and white matter. Active plaques appear more frequently in the WM, and WM volume is affected by gliosis and inflammation. This relationship is less marked with atrophy of the grey matter. Therefore, GM assessment is believed to be more reliable for the evaluation of brain atrophy because it is less prone to errors [6].

In their study of 2012, Shiee et al. found no loss of white matter volume in the initial stages of the disease. This component of the brain is more susceptible to inflammation than the grey matter, which affects, among other parameters, the interference in WM volume measurements [6,49]. Horakova et al. demonstrated analogous differences manifested in less distinct loss of white matter as compared to grey matter over a five-year follow-up period [50]. Similarly, according to Raz et al., the damage to the WM could indeed be detected early and have affected most of its fibres. However, it did not deteriorate during the first year after the onset of symptoms. There was a clear decrease in the volume of the cerebral cortex and the subcortical GM in the first year of the follow-up [51]. In 2006, Simon argued that in long-term observation there was also an obvious loss of the white matter, although it was not as marked as in the grey matter [17].

Of course, the absence of WM atrophy in the early stages of MS may result from the coexistence of both damage and repair processes [49]. Volumetric measurements of the brain in MS patients are affected by many factors, such as the loss of nerve cells, inflammation, microglia volume, fluid shifts, the physiological process of ageing, remyelination, gliosis [17]. Interference may result from changes associated with plaque (oedema/acute phase), introduction of steroid therapy, dialysis, medication effects, rapid dehydration or rehydration, eating disorders, alcohol abuse. These factors should be taken into account in the analysis of atrophy in MS patients but in long-term follow-up their importance decreases [6,52]. Most commonly, low hydration and effects of therapy (anti-inflammatory or immunomodulatory drugs) decrease brain volume in reversible manner due to the loss of water during dehydration and reduction of oedema/inflammation. This phenomenon, called pseudoatrophy, represents loss of water without actual brain damage. GM volume is less affected by pseudoatrophy than the whole brain or WM volume [17,42–44].

The reduction of cerebral cortex thickness, which is already visible at the onset of MS symptoms, was one of the first measurements in the research of GM atrophy [53,54]. Many studies have consistently proved that grey matter atrophy dominates from the earliest periods of the disease in various forms of MS, and it is evident even with few plaques in the GM. According to a newer hypothesis, cortical pathology is present from the early stages of MS and is independent from the ongoing pathological process in the white matter [1,13].

Many authors have emphasised the early occurrence of GM and cerebral cortex atrophy. Zivadinov – the first five years of the disease, Shiee – one-year follow-up in patients with a mean time of eight years from MS diagnosis, and Geurts – the earliest signs of atrophy in MS appearing in the thalamus [49,55,56].

According to Popescu et al., measurements of brain atrophy between the baseline assessment and the follow-up after 1–2 years have a predictive value for disability. They correlate with a decline in the patient's EDSS scores after 10 years. Atrophy is associated with short- and medium-term progression of the clinical status, and the parameters of the whole brain atrophy and central atrophy displayed the highest correlation in the entire study population [57].

With technological advances in the software used for measuring atrophy, it has become possible to determine the volume of ever smaller substructures of the brain. According to Zivadinov, the earliest damage in patients with CIS in the grey matter occurs in the thalamus, and the progression of atrophy in this structure allows to predict the transition to clinically definite MS (CDMS) [37].

Atrophy of the GM, cortex, thalamus and subcortical GM is visible at a very early stage of MS. The first two components evolve over 10 years, correlating with EDSS scores; they have a predictive potential for the patient's symptoms [58,59]. These results were confirmed by Bertrand – atrophy of the thalamus, and not the cortical structures, was significant in patients with the RR form over the first 4 years of the disease. According to Sicotte, hippocampal atrophy correlates with neuropsychological cognitive tests and lexical memory [60,61]. Differences in the corpus callosum atrophy are also characteristic of subtypes of MS, but there is no such relationship in the other parts of the brain [62]. In addition, Kalincik reported being able to predict the evolution of CDMS in patients with a higher degree of atrophy, increased local inflammatory activity and atrophy of the corpus callosum in a short six-month analysis of studies [63].

Predictive value was also discovered in patients with the PPMS form: grey matter atrophy in the early stages of the disease correlated with MS progression observed as early as after 5 years [64]. Furthermore, the atrophy of the substructures has been increasingly clearly associated with more precise clinical data, such as the results of neuropsychological tests [65,66].

For these reasons, it seems that in the near future the development of techniques used for practical measuring of atrophy in daily radiological practice should offer wider opportunities than the calculation of the total brain volume. Atrophy of the brain's main components, such as cerebral cortex, GM or WM, as well as the substructures of the grey or white matter such as the thalamus or the corpus callosum seem to be the desired targets.

Besides the methods which can use 3D-T1 sequence data for atrophy measurements, there are also other MRI techniques. However, they are less practical for including into everyday MRI protocol. They reflect the damage of the NABT, which correlates with the clinical condition of MS patients as well as the brain atrophy. Recently they have also found application, with particular reference to GM.

Magnetisation transfer (MT) concept is based on the mechanism of magnetisation exchange between freely mobile protons (interstitial fluid water) interacting with a pool of restricted protons, bounded with macromolecules (like myelin and brain tissue lipids). The differences in proton mobility create different reaction to the radio wave of MRI scanner. After saturating pulse macromolecular protons transfer part of the saturation onto the free water protons. The transfer can be quantified as a calculation of magnetisation transfer ratio (MTR). During the course of the disease after demyelination and axonal damage the amount of restricted proton is reduced. Because of that the possibility of magnetisation transfer is reduced (low MTR) as compared to normal controls (high MTR). MTR reduction is related to the deterioration of the patient's clinical condition.

Diffusion weighted imaging (DWI) and diffusion tensor imaging (DTI). DWI enables the measurement of water molecule mobility, which is reduced in the brain by tissue and cellular structures. Due to pathological processes the mobility can increase or decrease, which can be characterised and quantified by the apparent diffusion coefficient (ADC). DWI reflects isotropic diffusion – without taking into consideration the spatial orientation of tissue barriers. Since water molecule mobility is not the same in all directions, a more advanced technique (DTI) is used to characterise anisotropic diffusion (including the factor of tissue barrier orientation). DTI reveals the preferred water diffusion along nerve fibres. In the damaged NAWM of MS patients ADC rises. DTI is characterised by fractional anisotropy (FA) and mean diffusivity (MD); FA is typically low and MD is high in brain damage in MS as compared to normal controls.

Magnetic resonance spectroscopy (MRS) determines the relative concentration of brain metabolites, whose levels alter in the course of the disease. The main metabolites whose levels can change in MS, are:

(1) Choline (Cho), which is the marker of axonal loss and neuronal integrity, rising during inflammation and demyelination, (2) N-acetyloaspartate (NAA) – decreased since the first stages of MS, correlating with axonal damage, (3) lactates (Lac) – increasing in the case of an energetic process breakdown and reflecting myelin damage and necrosis, (4) mioinositol (mI), which increases as a gliosis and inflammation marker. Recent studies have demonstrated that mI elevation precedes brain volume loss and NAA decreases in early stages of MS [7,15,45,46].

5. Effects of already approved therapies on brain atrophy

Each modern drug trial in MS includes 3D-T1 sequences in MRI protocol. Atrophy is a marker of neurodegeneration and many new drugs are thoroughly examined as to their effect on the slowing of brain volume decrease. In a meta-analysis by Sormani et al., 13 trials comprising more than 13,500 patients were assessed. The results describe a correlation between atrophy and 2-year disability progression as factors influenced by the treatment. Additionally, an analysis of atrophy decrease adds a lot when assessed together with treatment effects on active MRI lesions. When these factors are analysed in combination, they explain better the reduction of disability progression after initiating treatment. The authors conclude that brain atrophy assessment is a highly valuable marker for disability progression, both alone and in combination with inflammatory markers [35].

6. Conclusions

It must be concluded that today we are not able to translate directly the results of brain atrophy assessment into decisions on the management of patients. However, this is the trend of the latest publications and this parameter is assessed in clinical trials in MS.

A universal access to the possibility of calculating the volume parameters in routine MRI scan will become reality in the near future. An assessment of brain atrophy with the use of 3D-T1 sequences may be implemented into daily practice.

Let us hope that the results of research on brain atrophy in multiple sclerosis will contribute to progress in therapy, and an assessment of atrophy by radiologists will be used effectively in neurological practice.

Conflict of interest

None declared.

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Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; Uniform Requirements for manuscripts submitted to Biomedical journals.

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