Application of the Technique of Magnetic Resonance Voxel-based Morphometry in Patients with Multiple Sclerosis before and after using High-dose Immunosuppressive Therapy with Autologous Hematopoietic Stem Cell Transplantation: Preliminary Results

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Abstract: Correlation analysis of magnetic resonance voxel-based morphometric parameters of patients with multiple sclerosis before and after high-dose immunosuppressive therapy with transplantation of autologous hematopoietic stem cells (HDIT + AHSCT). The use of MR-morphometry methods makes it possible to quantitatively and objectively changes of the volume and size of the structures of the brain and cerebellum in patients with MS. We observed 10 patients with MS (3 men, 7 women) who underwent HDIT + AHSCT, and MRI studies were performed before and after transplantation. After postprocessing of MRI data and statistic analyses of morphometry parameters some changes were accrued: some patients showed negative dynamics in white and grey mater volumes and decrease of the absolute volume of the thalamus. Also, study showed positive dynamics in reducing the number of new MS lesions. These results can be associated with the result of treatment, with a local decrease in edema and inflammation. Study showed the need for dynamic MR control of the grey and white matter of the brain and subcortical structures with the help of MR morphometry.

ABBREVIATIONS

MS – multiple sclerosis
VBM – voxel-based morphometry
GM – grey matter
WM – white matter
HDIT – high-dose immunosuppressive therapy
AHSCT – autologous stem cell transplantation.

1 INTRODUCTION

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system characterized by frequent episodes of inflammation with demyelination and neurodegeneration (Lipp, 2018). MS usually begins at a young age (20–40 years) and is more common in women. (Lublin, 2014). MS is characterized by an immune attack on the myelin surrounding the axons of neurons. This inflammation can damage the axons. Evidence of neurodegeneration that extends beyond inflammatory foci is the primary neurodegenerative component of MS followed by secondary inflammation (Lipp, 2018; Trapp, 2008; Stys 2012). Traditionally lesions in multiple sclerosis are the focus of diagnosis, prognosis and evaluation for treatment. More recently, studies in multiple sclerosis have focused on abnormalities in a brain and brain volume loss, not only as predictors, but also as a result of clinical trials evaluating the effectiveness of the treatment (Lipp, 2018).

According to statistics, the prevalence of MS is 50–100 cases per 100 thousand population. There are more than 2 million patients with multiple sclerosis in the world. In addition, the average age of patients

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with this disease has dropped significantly and the disease usually affects the working-age population.

There are different types of MS: a type with repeated exacerbations and periods of remission, the so-called "relapsing-remitting type", which can later develop into a "secondary progressive type" or may also have persistent progression from the onset of the disease with no relapses or remissions - "primary progressive type" (Silva, 2018; Lublin, 2014).

MS is characterized by a progressive course. Current disease-modifying therapy inevitably leads to disability, loss of the ability to provide self-care and loss of cognitive functions.

Today, different neuroimaging techniques such as magnetic resonance spectroscopy - quantitative determination of metabolites in tissues, diffuse tensor MRI (DTI) (Ontaneda, 2017; Das, 2019), magnetic resonance morphometry have the potential to reveal the pathogenic mechanisms of the disease and determine the markers of the neurodegenerative and atrophic process in multiple sclerosis (Bogachev, 2014).

The method of high-dose immunosuppressive therapy with transplantation of autologous hematopoietic stem cells (HDIT + AHSCT) is intended to provide specialized medical care to patients with MS. HDIT + AHSCT is considered by the American Society for Blood and Bone Marrow Transplantation (ASBMT) as the “standard” treatment for MS in the nearest future, because this course of treatment can possibly regenerate the cells of immune system repertoire and enhance the immune tolerance (Cohen 2019; Mancardi, 2017, Gholamzad, 2018). This method of treatment has a long-term immunomodulation effect, as a result of which 85% of patients after AHSCT maintain a stable remission from 5 years and more. This method is relatively safe, but unfortunately, it is expensive and is not included in the standards of care for MS patients.

Atrophy of the grey (GM) and white matter (WM) of the brain and subcortical structures is manifested in the early stages of the disease, and its degree correlates with physical and cognitive impairment. The effect of treatment on cerebral atrophy predicts further disability (Ontaneda, 2017; Righart, 2017; Ghione, 2018). The annual decrease of brain volume occurs 4 times more often in patients with apolipoprotein E-e4 in the genotype, than in patients without it. However, according to other studies, the genotype with the presence of apolipoprotein E-e4 does not affect the degree of atrophy (Fernandez, 2015). Initially, the presence of cerebral atrophy in patients with MS was qualitatively identified like an expansion of the cerebral ventricles and subarachnoid spaces and a decrease in the volume of brain matter. The next step was the automatic quantitative assessment of brain atrophy using voxel magnetic resonance morphometry (Krotenkova, 2014).

Magnetic resonance morphometry is an accurate quantitative technique that allows to study changes in volumetric parameters of brain structures in different diseases, including those with MS. In addition, MR-morphometry can be informative in assessing the treatment and dynamics of the disease development.

2 PURPOSE

To establish significant changes in the structures of the brain of the patients with MS as a result HDIT + AHSCT treatment using magnetic resonance voxel-based morphometry.

3 MATERIALS AND METHODS

3.1 Study Population

An open, single-center, uncontrolled study of the results of magnetic resonance voxel-based morphometry of patients with MS before and after HDIT + AHSCT (at two time points with an interval of about 12 months).

The HDIT + AHSCT is conducted in several stages, which occurred sequentially and included mobilization and procurement of hematopoietic stem cells (HSC), cryopreservation of HSC, immunosuppression state by therapy with doses of cyclophosphamide and rituximab, and transplantation of HSC with subsequent support therapy with post-transplant rehabilitation and assessment of effectiveness.

The mobilization and procurement of HSC was carried out in two stages:

1) Granulocyte colony-stimulating factor (G-CSF) - dose 10 μg / kg / day (4 days)

2) Leukocytapharesis (5th day) when the total number of leukocytes reaches more than 10x10 ^ 9 / The number of CD34 + cells in leukopheresis products should be 2-4x106 / kg of body weight.

Cryopreservation of the HSC was carried out with 10% dimethyl sulfoxide, followed by its storage at -180°C in liquid nitrogen until transplantation.

Conditioning was carried out using high-dose immunosuppressive therapy with cyclophosphamide (50-200 mg/kg) with rituximab (500 mg/m^2).

AHSCT was performed after thawing the frozen graft in a water bath at a temperature of 37°C. The transplantation of AHSC was carried out through a central venous catheter.

Support therapy was aimed at symptomatic treatment and treatment of AHSCT complications.

We observed 10 patients with MS (3 men, 7 women) who underwent HDIT + AHSCT, and MRI studies were performed in 2 time points: before and after transplantation. In this study, the small sample of patients is due to the small number of patients who underwent transplantation. The average age of the patients was 41.6 ± 8.9 years.

The inclusion criteria of the patients for HDIT + AHSCT were:

1. Age 18-65 years; verified diagnosis of MS; EDSS 1.0-6.5;

2. The presence of confirmed progression of the disease against the background of standard therapy;

3. Deterioration in EDSS by 1 point or more at baseline level <5 points, deterioration in EDSS by 0.5 point or more at baseline level> 5 points;

4. The emergence of new (including Gd +) MS lesions;

5. Absence of severe concomitant pathology; no treatment with interferon drugs and immunosuppressants in the last 3 months.

3.2 MR Imaging Protocol

MRI studies were carried out on a high-field magnetic resonance imager "Siemens Magnetom Symphony" with a magnetic field induction of 1.5 T using a head coil.

All patients underwent structural MRI with obtaining T1 and T2 weighted images and FLAIR (Fluid attenuated inversion). Pulse sequence data of a T1-weighted gradient echo (MP-RAGE - Magnetization Prepared Rapid Acquired Gradient Echoes) was collected to combine MRI data of anatomical structures of the brain, (slice thickness - 4.5 mm, number of slices - 29, the number of repetitions - 120, scan time - 6 minutes). This sequence has high resolution with 0.8 mm isotropic voxeles.

3.3 Image Analyses

At present, mathematical models have been developed, with the help of which we can analyse “thin” (not visible on MRI tomograms) morphological changes, including secondary atrophic changes in MS, which can be quantified and presented topographically. One of the new and very promising methods used to quantify GM, WM and lesion volume is voxel-based morphometry (VBM).

The VBM workflow includes three main preprocessing steps:

(1) Tissue classification, which is based on intensity values and mainly serves to segment the brain into grey matter, white matter, cerebrospinal fluid after "removal" of the skull bones and other structures of the head.

(2) Spatial normalization (linear and non-linear) - needed to provide matching across brain voxels, in accordance with the individual brain anatomy of each person.

(3) Spatial smoothing, followed by statistical analysis. Smoothing, since spatial normalization is not ideal and small individual differences in the anatomy of the brain remain. Spatial smoothing takes into account these residual differences in local anatomy. Therefore, after smoothing, each voxel is a sphere, which is similar to the smoothing kernel, or, in other words, the weighted average of the values and values of adjacent voxels (Kurth, 2015; Gaser, 2016).

For morphometric evaluation, T1-WI images were converted from the standard digital imaging and communication (DICOM) format (.dcm) to the Neuroimaging Informatics Technology Initiative (NIfTI) (.nii) format suitable for analysis and post-processing using the SPM12 - CAT extension. Volumetric analysis using CAT allows to accurately assess the structure of the brain and to avoid operator errors when carrying out "manual" segmentations.

3.4 Data Analysis

Longitudinal evaluation of the results of neuroimaging studies of each patient individually, as well as their group totality (analysis of morphometry volumetric data) were carried out.

Correlations between results of MR-morphometry in 2 time points (before and after stem cell transplantation) were established. Statistical processing of the received data were carried out using the package Statistica by StatSoft, (Mann- Whitney test). Differences were considered significant at $p < 0.005$. Correlations were calculated using Spearman's test.

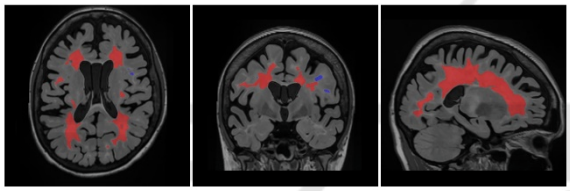
3.5 Results

When assessing the dynamics of morphometric indicators of the same patients at two time points (before and after HDIT + AHSCT), 70% of patients showed negative dynamics in white matter atrophy,

while other 20% showed positive dynamics (increased white matter volume), 10% showed no change). Besides, 70% of patients have had negative dynamics in grey matter atrophy (other 30% ща patients showed positive dynamics in grey matter atrophy).

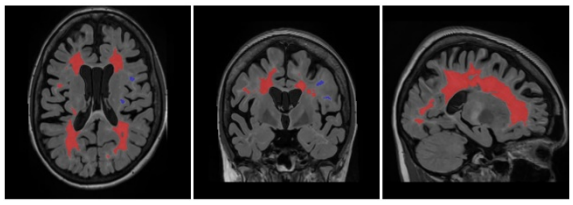
	Average GM volume (cm^3/%)	**Average WM volume (cm^3/%)**
Before HDIT+ AHSCT	735,150 ±72,379	487,896 ±91,716
After HDIT+ AHSCT	720,432 ±54,347	459,019 ±90,089

In addition, 62.5% of patients showed positive dynamics in reducing the number of new MS lesions, which also can be associated with the result of treatment, with a local decrease in edema and inflammation.



a b c

Figure 1: (a - axial, b - coronal, c - sagittal coronal). The results of MRI morphometry of the lesions of 42 y.o. female patient before HDIT + AHSCT. Red colour indicates MS lesions.



a b c

Figure 2: (a - axial, b - coronal, c - sagittal coronal).The results of MRI morphometry of the lesions of 42 y.o. female patient after HDIT + AHSCT. Red colour indicates MS lesions.

In 84% of the cases, there is a decrease of the absolute volume of the thalamus (in cm ^ 3/%), while in 50% of the cases the decrease was below the average age norm. All these patients had a secondary progressive type of MS, while statistically the decrease of the volume of thalamus was not associated with the duration of the disease or EDSS score.

When discussing central nervous system atrophy and MRI morphometry data in MS, it should be mentioned that, in addition to the gradually increasing loss of brain matter in MS, short-term brain volume fluctuations may also occur. Inflammation and edema as a result of the formation of new lesions lead to a temporary increase of the brain volume, and vice versa, for example, taking corticosteroids leads to a short-term decrease of it - pseudoatrophy. The mechanism of this process is not entirely clear, but it is believed that this occurs as a result of a decrease in inflammation in the central nervous system and associated edema (Krotenkova, 2014). As a rconsequence of the ongoing treatment of HDIT+AHSCT and the anticipated results of this treatment, it is expected that the decrease in the absolute volumes of GM and WM is associated with a decrease of edema and inflammation of the brain. However, further dynamic observation is necessary, due to the likelihood of an incorrect interpretation of the results, since a decrease of brain volumes may be associated with the ongoing process of neurodegeneration. It is well known, that cerebral atrophy occurs at all stages of MS, since at the preclinical stages of the disease, and progresses throughout the disease at a faster rate than during the normal aging process (Inglese, 2018; Fox, 2016).

A. Cifelli et al. It was shown that, compared with the control group, of patients with a secondary progressive type of the disease, the volume of the thalamus decreased by 17%, and the transverse size of the third ventricle doubled, and a clear inverse relationship between their volumes was revealed. The above MRI data are confirmed by histological studies: a decrease of the number of neurons in the medial posterior thalamic nucleus and a decrease of the total volume of the thalamus by 22% was revealed (Cifelli, 2002). It has also been proven that the reduce of thalamus volume annually increases by -0.71% per year (Azevedo, 2018).

4 CONCLUSIONS

The use of MR-morphometry methods makes it possible to quantitatively and objectively detect changes of the volume and size of the structures of the brain and cerebellum in patients with MS. The most significant results were obtained for the amount of WM atrophy in patients with MS. The changes identified in our study correspond to the data of some other studies: the process of neurodegeneration can

last up to 1-2 years after the start of therapy with HSC. This indicates the need for dynamic MR control of the grey and white matter of the brain and subcortical structures using MR morphometry to assess the effectiveness of treatment and the patient's life prognosis. Besides, these results can be used for assessing the prediction of the further course of multiple sclerosis for patients who underwent HDIT+AHSCT.

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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