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Original Article

Predictive value of number and volume of demyelinating plaques in treatment response in patients with multiple sclerosis treated with INF-B

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Abstract: Background: Multiple Sclerosis (MS) is an autoimmune, inflammatory disease of the central nervous system. Magnetic resonance imaging (MRI) findings are associated with disease clinical activity and response to treatment. This study aimed to evaluate the future value of plaque number and volume in MRI as radiological criteria in determining the treatment response to INF-B in patients with MS. Methods: This is a cross-sectional study performed in 2016-2021 in Iran on patients with the newly diagnosed (less than one year) relapsing-remitting MS. Brain MRI was taken for all patients. The number and volumes of the MS plaques were evaluated from FLAIR images by the two radiologists. Patients were treated with INF-B1a with a dosage of 12 million units equal to 44 micrograms subcutaneously, three times per week. Patients were visited monthly by neurologists to examine their clinical status. After one year, the brain MRI was conducted with the similar characteristics to the beginning of the study, and the number and volume of MS plaques were measured again. Results: The study population consisted of 33 males and 90 females with a mean age of 28.37 ± 6.29 years. The mean Expanded Disability Status Scale (EDSS) of the patients was 3.16 ± 0.23 at the beginning of the study. The specificity for a 50% reduction in the number and volume of plaques as two separate criteria was the same and equal to 100%. The sensitivity of the number and volume of plaques were 65.5% and 90.6%, respectively. In addition, considering 10% as the cut-off point of the number of plaques, the sensitivity of the number of plaques as a criterion was equal to the sensitivity of the plaque volume. Conclusion: The results of this study showed that imaging criteria provide a more objective tool for evaluating the effectiveness of treatment. These findings indicate that the number and volume of plaques could be two reliable MRI imaging criteria for assessing therapy response. The number of plaques was less accurate than the volume of plaques.

Keywords: Multiple sclerosis, relapsing-remitting, interferon β, magnetic resonance imaging, accuracy

Introduction

Multiple Sclerosis (MS) is an autoimmune, inflammatory disease of the central nervous system mostly affecting young adults. The exact mechanism and pathogenesis of MS remain still undiscovered but there have been valuable treatments with different efficacy rates [1, 2]. Different lines of evidence have revealed the important functions of T lymphocytes and inflammatory and anti-inflammatory cytokine balance. Various MS models have been studied to determine the pathology of MS and factors affecting disease course [3-5].

Interferon-beta (INF-B) is currently the first line of treatment for relapsing and remediating multiple sclerosis (MS). It has been shown to reduce the recurrence rate and progression of the disease and has acceptable side effects

[6]. However, not all MS patients respond to this treatment and more invasive treatment is needed to control the disease. Early detection of cases of non-response is essential and early change in treatment may be helpful; because it prevents the progression of the disease [7, 8].

There is currently no reliable way to predict the response to the variety of treatments available; therefore, the response to treatment is judged based on the patient's clinical condition, recurrence and progression of disability and through Expanded Disability Status Scale (EDSS) [9, 10].

The various clinical criteria that have been proposed have a sensitivity of 47 to 77% and a specificity of 84 to 97% to determine the rate of treatment failure. There seems to be a need for more sensitive and specific adjuvant criteria that can be objectively calculated and accurately determine response to treatment prior to treatment [11, 12].

Magnetic resonance imaging (MRI) has revolutionized the diagnostic methods for MS and also provides a glimpse into the natural history of the disease [13, 14]. However, the use of MRI in monitoring patients with MS undergoing various treatments has not been clearly evaluated [15, 16]. Although disease-modifying therapies have had significant effects on MS plaque activity on MRI, there are few reports that MRI findings are associated with disease clinical activity and response to treatment [17-19].

This study aimed to evaluate the future value of plaque number and volume in MRI as radiological criteria in determining the response to INF-B in patients with MS.

Methods and material

Study design

This is a cross-sectional study performed in 2016-2021 in Imam Khomeini hospital, affiliated to Tehran University of Medical Sciences. The current study was conducted on patients with newly diagnosed (less than one year) relapsing-remitting MS using easy sampling. The Research Committee of Tehran University of Medical Sciences has approved this study and the Ethics committee has confirmed it (Ethics code: IR.TUMS.MEDICINE.REC.1392.309).

Inclusion and exclusion criteria

The inclusion criteria were age between 18-45 years, diagnosis of relapsing-remitting MS based on McDonald's criteria by expert neurologists, candidates of treatments with INF-B, EDSS between 1 to 5 and signing the written informed consent to participate in this study. The criteria of the MS diagnosis included: two attacks or symptom flare-ups (lasting at least 24 hours with 30 days between episodes), plus two lesions. Two episodes, one lesion, and evidence of dissemination in space (or a different attack in a different part of the nervous system). Patients with the following criteria did not enter the study: receiving corticosteroids in the past three months before the study initiation, any recurrence within the past month, any history of previous neurological or psychiatric disorders, pregnancy and history of other autoimmune diseases.

Sample size calculation

Patients were included according to the mentioned criteria. Based on formula for estimating the sample size and 90% reliability equal to 1.96 and 0.84 for the test power, and the effects size of 0.62S, we considered 125 patients as the study population. The study population was calculated equal to 125 patients using sample size formula. At the beginning of the study, the clinical and demographical information of patients were collected.

Brain MRI assessments

The main aim of this study was to assess the volume and frequencies of MS plaques in the brain MRI before treatments with INF-B1a and compare the results with 1 year after treatments. As a result, patients underwent brain MRI before and 1 year after receiving INF-B1a.

Brain MRI was taken for all patients using 1.5 tesla apparatus (Phillips Gyroscan) Intra 1.5-Tesla MR imaging system. We used Spin-echo axial, sagittal and coronal T-weighted repetition/echo time [TR/TE] 500/20 ms, T2-weighted (TR/TE, 11000/140 ms) Sequences with 8 mm thickness slices containing 1 mm

gap. The imaging was performed without contrast injection. The image matrix was 256*256 with 24 cm field of view.

Study variables

The brain MRI images were evaluated by two expert radiologists that were unaware of the demographic and clinical conditions of the patients. The primary variables in this study were the number and volume of the MS plaques. These were evaluated from FLAIR images by the two radiologists and in cases of disagreement; the third radiologist with at least 15 years of experience in this field assessed the images. The number of plaques was assessed for each patient. The volume of the plaques was evaluated using MRI Chris Rorden MRICRO Version 1.40 build 1 software on FLAIR axial images. The slice with the largest plaque diameter was used for determination of plaque diameter.

Treatments and clinical assessments

Patients were treated with INF-B1a with dosage of 12 million unit equal to 44 microgram subcutaneously, three times per week. Patients were visited monthly by neurologists to examine their clinical status. Extra visits were also conducted in cases of suspicious recurrence. The use of corticosteroids was permitted in cases with recurrence. Increase in disability was considered as an increase in Expanded Disability Status Scale (EDSS) for at least 1 score that lasted at least 6 months during the study which also was equal to lack of response to treatments [20].

EDSS is a clinical assessment tool for MS induced disability. This scale could be widely used by health care providers diagnosing MS and provides a total score on a scale that ranges from 0 to 10. The first levels 1.0 to 4.5 refer to people with a high degree of ambulatory ability and the subsequent levels 5.0 to 9.5 refer to the loss of ambulatory ability. This scale assesses the following domains in patients: pyramidal (motor function), cerebellar, brainstem, sensory, bowel and bladder, visual and cerebral or mental [20].

After 1 year, the brain MRI was conducted with the similar characteristics to the beginning of the study, and the number and volume of MS plaques were measured again.

Further assessments

After assessing plaque number and volumes, we aimed to evaluate the changes in these variables regarding patient responses to the treatments and assess the possible cut-off points to determine patient's progression. The negative predictive value was considered as the ratio of subjects diagnosed as negative to those who had negative test results. The positive predictive value was defined as the ratio of patients truly diagnosed as positive to all those who had positive test results. The sensitivity was considered as the probability of the positive test, conditioned on truly having the condition and the specificity was the ability of the test to identify people without the disease correctly. The cut-off point regarding the treatment response, including a reduction in the number and volume of plaques was conducted using the Receiver operating characteristics (ROC) curve.

Statistical analysis

The obtained data were entered into the Statistical Package for Social Sciences (SPSS) (version 24, SPSS Inc., Chicago, IL). Quantitative data were reported as mean ± standard deviation and qualitative data as frequency distribution (percentage). Independent t-test, Chi-square were used to analyze the data. *P*-value <0.05 was considered as significance threshold.

Results

Study population

In the current study, 125 patients entered and followed for 1 year. Two cases were excluded due to improper follow-up. Data of 123 patients were analyzed. The study population consisted of 33 males and 90 females with the mean age of 28.37 ± 6.29 years. The mean EDSS of the patients was 3.16 ± 0.23 at the beginning of the study. The demographic data of cases are presented in **Table 1**.

Evaluation of MS plaques

During the 1 year follow-up, 27 cases (21.9%) were unresponsive to the treatment based on the mentioned criteria. Our data showed significant decrease in the number (P<0.001) and

Table 1. Evaluation of the demographic information

Variable		Mean ± Sd./Number (%)
Age (year)		28.37 ± 6.29
Gender	Female	90 (73.1%)
	Male	33 (26.9%)
EDSS		3.16 ± 0.23

Table 2. Accuracy of predicting the reduction in number and volumes of plaques

Variable	Plaque volume (%)	Plaque numbers (%)
AUROC	(0.90-1.02)	(0.79-0.99)
Cut-off	45.2	90.6
Sensitivity	90.6	90.6
Specificity	100	77.7
Positive predictive value	100	93.5
Negative predictive value	92.6	87.8

volume (P<0.001) of MS plaques after the follow-up. The average number of license plates increased from 8.7 ± 5.68 at the beginning of the period to 15.14 ± 8.83 at the end of the period.

Cut-off point assessments

Assuming a 50% cut-off point and response to treatment in the form of a decrease in the number and volume of plaques, 62 patients (50.4%) responded to the treatment based on the changes in the number of plaques. 87 patients (70.7%) responded to the treatment based on the changes in the volume of plaques. Sensitivity, specificity, positive predictive value, negative predictive value and accuracy were calculated in the same way, which is shown in **Table 2**. **Table 2** shows that the volume of plaques has a higher sensitivity, negative predictive value, and accuracy than the number of plaques in order to predict the clinical response to treatment. In order to determine the appropriate cut-off point to reduce the number and volume of plaques after treatment, ROC curve analysis was performed (**Figure 1**). There was no significant difference between the area under the ROC curve (AUC) to reduce the number and volume of plaque in predicting response to treatment. Sensitivity, specificity, positive predictive value, and negative predictive value of reducing the number of plaques as well as reducing the volume of plaques to respond to treatment based on the cut-off point are shown in **Table 2**.

Assessing the sensitivity

The specificity for a 50% reduction in the number and volume of plaques as two separate criteria was the same and equal to 100%. The sensitivity of the number and volume of plaques were 65.5% and 90.6%, respectively. In addition, considering 10% as the cut-off point of the number of plaques, the sensitivity of the number of plaques as a criterion was equal to the sensitivity of the plaque volume.

Discussion

This study suggests that both the number and volume of plaques can be reliable imaging criteria for estimating response to treatment in patients with MS, but plaque volume is a more accurate indicator. In addition, if the cut-off point for the number of plaques defined before this 50% is reduced to 10%, the sensitivity of the number of plaques will be used as an imaging criterion for the sensitivity of the volume of plaques. Some reports have shown the usefulness of MRI as an adjunct to treatment response. Patients treated with IFN-β have been shown to have an increased risk of poor tissue response if they develop more than two new lesions in T2 after two years of treatment [21, 22]. In 2021, Sánchez et al. conducted a study to evaluate the use of IFN-β-1a in patients with relapsing-remitting MS. In this study, 31 patients with relapsing-remitting MS between the ages of 10 and 65 were treated with IFN-β-1a three times a week for one year. The treatment was determined to be safe and effective based on the number of adverse events, the disability scale score, and the number of lesions at MRI. In this study, a significant reduction in the number of attacks, disability scale score, and the number of lesions on MRI was observed in patients with relapsing-remitting MS treated with IFN-β-1a [23, 24]. In 2018, Zettl et al. conducted a study on the effect of IFN-β-1a and β-1b in treating patients with MS. According to the findings of this study, IFN-β treatment is well tolerated and has one of the best-identified safety characteristics among all first-line therapies. The overall severity of side effects does not appear to vary with exposure to IFN-β. Also, the

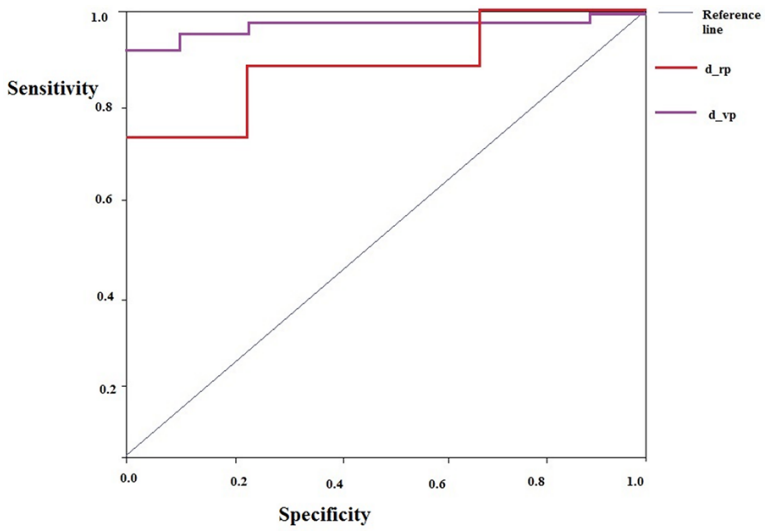

Figure 1. ROC for reducing the number and volumes of plaques.

side effects that patients may experience with IFN-β are mild, reversible, and manageable. According to the study, IFN is one of the best-characterized therapies for MS, with a vast body of clinical and real-world evidence confirming the risk-benefit profile. Furthermore, high-dose/frequency regimens may result in improved long-term outcomes [25]. This study is in line with our study.

In another study, a poor response to treatment was defined as an increase in disability after two years of treatment, and this rate was higher in patients with three or more new active lesions (new or larger in T2 or gadolinium-bearing after one year of treatment) than in patients with two or fewer active lesions [26, 27]. Other studies have shown that the absence of gadolinium contrast or a new lesion in T2 after one year of treatment may be a criterion for a better response to treatment [28, 29]. It has also been shown that those who do not respond to treatment have a higher volume of hyperintense lesions in T2 [30]. These studies demonstrate that the presence and size of lesions examined by MRI may indicate a response to IFN-β. The results of this study were consistent with previous observations in which the extent and presence of MRI lesions were used as an adjunct to treatment response. Another study showed that new lesions in T2 after one year of treatment compared with baseline MRI were associated with a poor response to IFN-β treatment and the risk of responding to poor treatment increased with an increasing number of lesions, which was a tenfold increase for each new lesion [31].

Our results showed that both the number and volume of plaques can be reliable imaging criteria for estimating response to treatment in patients with MS, but plaque volume is a more accurate indicator. These data have high clinical importance and could be used by neurologists. Here we had a evaluation of patient's documents and radiologic images. The limitations of this study were that this study could have unknown potential confounders. We used the data originally collected for these purposes, not all the relevant information, and we had also inferior level of evidence compared with prospective studies. We also had a restricted study population compared to some former studies and therefore, suggest that more studies on larger populations should be performed.

Conclusion

This study showed that imaging criteria provide a more objective tool for evaluating the effectiveness of treatment. In addition, quantitative monitoring of response to imaging therapy may be a suitable complementary method for clinical response in MS patients. These findings show that the number and volume of plaques could be two reliable MRI imaging criteria for assessing therapy response. The number of plaques was less accurate than the volume of plaques. Considering the relative diversity in MS treatment strategies, the critical point is cost-effectiveness evaluation, which is evaluated by quality criteria; different results have been obtained from that point of view and subjective judgment.

Disclosure of conflict of interest

None.

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References

[1] Rafiee Zadeh A, Askari M, Azadani NN, Ataei A, Ghadimi K, Tavoosi N and Falahatian M. Mechanism and adverse effects of multiple sclerosis drugs: a review article. Part 1. Int J Physiol Pathophysiol Pharmacol 2019; 11: 95-104.

[2] Dobson R and Giovannoni G. Multiple sclerosis - a review. Eur J Neurol 2019; 26: 27-40.

[3] Rafiee Zadeh A, Ghadimi K, Ataei A, Askari M, Sheikhinia N, Tavoosi N and Falahatian M. Mechanism and adverse effects of multiple sclerosis drugs: a review article. Part 2. Int J Physiol Pathophysiol Pharmacol 2019; 11: 105-114.

[4] Glatigny S and Bettelli E. Experimental autoimmune encephalomyelitis (EAE) as animal models of multiple sclerosis (MS). Cold Spring Harb Perspect Med 2018; 8: a028977.

[5] Payghani C, Khani F, Zadeh AR, Reisi P, Alaei H and Rashidi B. The effect of levothyroxine on serum levels of interleukin 10 and interferon-gamma in rat model of multiple sclerosis. Adv Biomed Res 2017; 6: 118.

[6] Chitnis T, Arnold DL, Banwell B, Brück W, Ghezzi A, Giovannoni G, Greenberg B, Krupp L, Rostásy K, Tardieu M, Waubant E, Wolinsky JS, Bar-Or A, Stites T, Chen Y, Putzki N, Merschhemke M and Gärtner J; PARADIGMS Study Group. Trial of fingolimod versus interferon Beta-1a in pediatric multiple sclerosis. N Engl J Med 2018; 379: 1017-1027.

[7] Eichinger P, Schön S, Pongratz V, Wiestler H, Zhang H, Bussas M, Hoshi MM, Kirschke J, Berthele A, Zimmer C, Hemmer B, Mühlau M and Wiestler B. Accuracy of unenhanced mri in the detection of new brain lesions in multiple sclerosis. Radiology 2019; 291: 429-435.

[8] Fartaria MJ, Sati P, Todea A and Radue EW, Rahmanzadeh R, O'Brien K, Reich DS, Bach Cuadra M, Kober T and Granziera C. Automated detection and segmentation of multiple sclerosis lesions using ultra-high-field MP2RAGE. Invest Radiol 2019; 54: 356-364.

[9] Gasperini C, Prosperini L, Tintoré M, Sormani MP, Filippi M, Rio J, Palace J, Rocca MA, Ciccarelli O, Barkhof F, Sastre-Garriga J, Vrenken H, Frederiksen JL, Yousry TA, Enzinger C, Rovira A, Kappos L, Pozzilli C, Montalban X and De Stefano N; The MAGNIMS Study Group. Unraveling treatment response in multiple sclerosis: a clinical and MRI challenge. Neurology 2019; 92: 180-192.

[10] Rafiee Zadeh A, Ghadimi K, Mohammadi B, Hatamian H, Naghibi SN and Danaeiniya A. Effects of estrogen and progesterone on different immune cells related to multiple sclerosis. Casp J Neurol Sci 2018; 4: 83-90.

[11] Oh J, Vidal-Jordana A and Montalban X. Multiple sclerosis: clinical aspects. Curr Opin Neurol 2018; 31: 752-759.

[12] Coret F, Pérez-Miralles FC, Gascón F, Alcalá C, Navarré A, Bernad A, Boscá I, Escutia M, Gil-Perotin S and Casanova B. Onset of secondary progressive multiple sclerosis is not influenced by current relapsing multiple sclerosis therapies. Mult Scler J Exp Transl Clin 2018; 4: 2055217318783347.

[13] Zhang H, Alberts E, Pongratz V, Mühlau M, Zimmer C, Wiestler B and Eichinger P. Predicting conversion from clinically isolated syndrome to multiple sclerosis-an imaging-based machine learning approach. Neuroimage Clin 2019; 21: 101593.

[14] Çinar BP and Özakbaş S. Prediction of conversion from clinically isolated syndrome to multiple sclerosis according to baseline characteristics: a prospective study. Noro Psikiyatr Ars 2018; 55: 15-21.

[15] Tintore M, Vidal-Jordana A and Sastre-Garriga J. Treatment of multiple sclerosis - success from bench to bedside. Nat Rev Neurol 2019; 15: 53-58.

[16] Farrokhi M, Beni AA, Etemadifar M, Rezaei A, Rivard L, Zadeh AR, Sedaghat N and Ghadimi M. Effect of fingolimod on platelet count among multiple sclerosis patients. Int J Prev Med 2015; 6: 125.

[17] Bărcuțean LI, Romaniuc A, Maier S, Bajko Z, Moțățăianu A, Adina H, Simu I, Andone S and Bălaşa R. Clinical and serological biomarkers of treatment's response in multiple sclerosis patients treated continuously with interferonβ-1b for more than a decade. CNS Neurol Disord Drug Targets 2018; 17: 780-792.

[18] Tommasin S, Giannì C, De Giglio L and Pantano P. Neuroimaging techniques to assess inflammation in multiple sclerosis. Neuroscience 2019; 403: 4-16.

[19] Rafiee Zadeh A, Falahatian M and Alsahebfosoul F. Serum levels of histamine and diamine oxidase in multiple sclerosis. Am J Clin Exp Immunol 2018; 7: 100-105.

[20] Ashtari F, Esmaeil N, Mansourian M, Poursafa P, Mirmosayyeb O, Barzegar M and Pourgheisari H. An 8-year study of people with multiple sclerosis in Isfahan, Iran: association between environmental air pollutants and severity of disease. J Neuroimmunol 2018; 319: 106-111.

[21] Río J, Rovira À, Tintoré M, Otero-Romero S, Comabella M, Vidal-Jordana Á, Galán I, Castilló J, Arrambide G, Nos C, Tur C, Pujal B, Auger C, Sastre-Garriga J and Montalban X. Disability progression markers over 6-12 years in interferon-β-treated multiple sclerosis patients. Mult Scler 2018; 24: 322-330.

[22] Fahim M, Rafiee Zadeh A, Shoureshi P, Ghadimi K, Cheshmavar M, Sheikhinia N and Afzali M. Alcohol and multiple sclerosis: an immune system-based review. Int J Physiol Pathophysiol Pharmacol 2020; 12: 58-69.
[23] Sánchez RC, de la Fe AD, Suarez AP, Grass D, Vega TM, Canal AS, Siniscalco D and de Los Angeles Robinson Agramonte M. Interferon beta 1a (Rebif®) in relapsing remitting multiple sclerosis. Drug Dev Res 2021; 82: 707-715.
[24] Mazdeh M, Taheri M, Sayad A, Bahram S, Omrani MD, Movafagh A, Inoko H, Akbari MT, Noroozi R, Hajilooi M and Solgi G. HLA genes as modifiers of response to IFN-β-1a therapy in relapsing-remitting multiple sclerosis. Pharmacogenomics 2016; 17: 489-98.
[25] Zettl UK, Hecker M, Aktas O, Wagner T and Rommer PS. Interferon β-1a and β-1b for patients with multiple sclerosis: updates to current knowledge. Expert Rev Clin Immunol 2018; 14: 137-153.
[26] Río J, Rovira A, Tintoré M, Huerga E, Nos C, Tellez N, Tur C, Comabella M and Montalban X. Relationship between MRI lesion activity and response to IFN-beta in relapsing-remitting multiple sclerosis patients. Mult Scler 2008; 14: 479-84.
[27] Payghani C, Khani F, Rafieezadeh A, Reisi P, Alaei H and Rashidi B. Effects of levothyroxine on visual evoked potential impairment following local injections of lysolecithin into the rat optic chiasm. Int J Prev Med 2018; 9: 18.
[28] Pozzilli C, Prosperini L, Sbardella E, De Giglio L, Onesti E and Tomassini V. Post-marketing survey on clinical response to interferon beta in relapsing multiple sclerosis: the Roman experience. Neurol Sci 2005; 26 Suppl 4: S174-8.
[29] Tomassini V, Paolillo A, Russo P, Giugni E, Prosperini L, Gasperini C, Antonelli G, Bastianello S and Pozzilli C. Predictors of long-term clinical response to interferon beta therapy in relapsing multiple sclerosis. J Neurol 2006; 253: 287-93.
[30] Río J, Nos C, Tintoré M, Téllez N, Galán I, Pelayo R, Comabella M and Montalban X. Defining the response to interferon-beta in relapsing-remitting multiple sclerosis patients. Ann Neurol 2006; 59: 344-52.
[31] Gauthier SA, Glanz BI, Mandel M, Tsagkaropoulos A, Neema M, Stankiewicz J, Arora A, Duan Y, Liptak Z, Egorova S, Buckle GJ, Bakshi R, Guttmann CR, Khoury SJ and Weiner HL. Incidence and factors associated with treatment failure in the CLIMB multiple sclerosis cohort study. J Neurol Sci 2009; 284: 116-9.