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REVIEW ARTICLE

What we know so far about Myxovirus Resistance Protein A (MxA) as a Biomarker of Interferon-Beta Therapy in Patients with Multiple Sclerosis: A systematic Review

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Abstract: **Introduction:** Multiple sclerosis (MS) is one of the most common neurological disabling diseases in human societies with no complete cure. IFN-β has been proven to be an important advance in the MS treatment, but early identification of treatment failure is its major concern. Some researches revealed that MxA is an appropriate biomarker for predicting response to IFN-β, so we performed this study to evaluate the relationship between MxA level and response to INF- β treatment. **Methods:** International and internal databases were searched using "MxA", "Myxovirus resistance protein A", "IFN-β", "interferon Beta", "multiple sclerosis" and "MS keywords until October 2019. Inclusion criteria were original studies considering the MxA assays in MS patients under IFN-β therapy. Some reported cut-offs from partially the same settings (7 studies) were pooled using the weighted average. Finally, the overall statements of the included studies were compared and discussed to obtain a comprehensive conclusion about the clinical value of MxA assays in patient monitoring and designing their treatment plan. **Results:** A total of 456 articles were identified. The Screening was led to exclusion of 427 articles. Finally, 28 original studies met the inclusion criteria for this systematic review. Almost all studies have concluded that the MxA is significantly correlated with response to IFN-β therapy and also MxA expression is under the direct effect of Neutralizing antibody (NAb) against IFN-β. Reported cut-offs for MxA ranged from 3.3 to 6.3 NR and the weighted average of them was estimated to be 4.1 NR. **Conclusion:** It could be suggested that in patients under IFN-β therapy with an active disease which doesn't fulfill the criteria for the breakthrough disease, MxA level can help to determine whether to continue the drug and follow up a patient or change the treatment regimen.

Keywords: Interferon-beta; Multiple Sclerosis; MxA; Neutralizing Antibody

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1. Introduction

Multiple Sclerosis (MS) is a disabling disease that may cause weakness, loss of vision, imbalance, urinary incontinence, and sensory symptoms (numbness and paresthesia) by affecting the brain and spinal cord. Destruction of myelin (demyelination) in MS patients, causes a slowing of nerve impulses, or conduction block to produce the common symptoms of this disorder. According to the involvement of 400,000 people in the United States and 2.1 million world-

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wide, MS is one of the most common neurological diseases in human societies [1].

Although there is no drug for the complete cure of MS but treatment and care can help to reduce its attacks and progression. Viral infections have been hypothesized as an environmental susceptibility factor for multiple sclerosis [2]. Interferons are molecules in the body that raise the anti-viral defenses of cells, so they can be used against them as a treatment method. The three main types of known interferon include alpha, beta, and gamma interferons based on antiviral effect, anti-growth, and activation of natural killer cells. IFN-β is a 166-amino-acid glycoprotein with a complex three-dimensional structure consisting of five a-helices, a disulfide bond (between cysteine 31 and 141) and a glycosylation site (at asparagine 80) which is approved by FDA for treating MS and nowadays recombinant interferon-beta (IFN-β) with some differences compared to the natural form is produced [3, 4].

IFN-β can have its effect to reduce the immune response that is directed against myelin in the central nervous system in people with MS [3]. Nevertheless, IFN-β has proven to be an important advance in the MS treatment and its therapeutic possibilities are expanding, but early identification of treatment failure is its major concern, so the determination of optimal markers to predict its clinical effectiveness and also evaluating its bioavailability, seems to be necessary [5].

Bioavailability determinates clinical efficacy by representing the amount of drug that interacts with specific cells which are involved in clinical action. The bioavailability of IFN-β can be evaluated by quantifying drug levels or by measuring IFN induced proteins (e.g., neopterin or myxovirus resistance protein 1[MxA]) released by cells involved in the therapeutic effect [6]. And also by detecting the binding and neutralizing components such as antibodies and soluble receptors. Neutralizing antibodies (NAb) are induced against IFN-β and as a result, they can reduce treatment efficacy in patients with multiple sclerosis [7].

The human MxA by its ability to self-assemble into highly ordered oligomers and forming ring-like structures around liposomes, induces liposome tubulation and acts as a key mediator of the interferon-induced antiviral response against a wide range of viruses. According to the results lots of researches in this field, MxA gene expression evaluation is one of the most appropriate methods for measuring the biologic activity of exogenous IFN-β, so may be useful for predicting whether multiple sclerosis patients will respond or not to interferon-β treatment [6, 8].

The aim of this study was to determine the importance of investigating MxA gene or protein changes in MS patients under interferon therapy and its correlation with other determinants of IFN bioavailability (e.g. NAb), and also the effect of gender, age, and response rate, by reviewing previous studies in this field as described below.

2. Methods

As mentioned the purpose of this study was to figure out all scientific knowledge about the MxA evaluation methods and its value as a biomarker of IFN-β therapy in patients with Ms. Regarding this topic, the study eligibility was defined as all types of studies (cross-sectional, prospective and Cohort studies) reporting the values and methods of MxA assay in MS patients receiving IFN-β. International databases including MedLine, Scopus, Web of Science and ProQuest, and national databases including scientific information databases (SID) from inception until October 2019 were searched using the following keywords: "MxA", "Myxovirus resistance protein A", "IFN-β", "interferon Beta", "multiple sclerosis", and "MS". No language restriction was defined and eligible studies could have been published in either English or Farsi. Inclusion criteria were original studies (including all cross-sectional, longitudinal, Cohort, and prospective studies) considering the MxA gene expression or protein assays in MS patients under IFN-β therapy. Studies about MxA gene polymorphism or promotor as well as studies with no full text available or short commentaries were excluded. The Process of selecting articles was completed by two independent researchers. Extracted data were entered into a table which included bibliographic information of studies (author's name, publication year, and type of study), study design (participants, conducted tests, follow up duration), and main study achievement about the MxA evaluation in MS patients such as significant association with clinical findings, cut-offs, Hazard Ratios, etc. Due to the wide variety of reported data across studies, the meta-analysis was not applicable. Nevertheless, some reported cut-offs from partially the same settings were pooled using the weighted average based on the study's sample size, so an amount from a study with a greater sample size would have a greater effect on the final average. All the quantitative part was performed by Microsoft Excel spreadsheets. Finally, the overall statements of the included studies were compared and discussed to obtain a comprehensive conclusion about the clinical value of MxA assays in patient monitoring and designing their treatment plan.

3. Results

At the initial searching phase, 456 articles were retrieved. The Screening was initially done on study titles which led to exclusion of 321 articles. Additional 8 articles were excluded due to studying polymorphisms of MxA gene and its promotor and also if they were not original studies. Finally, 28 original studies met the inclusion criteria for this systematic review (Figure 1).

As the time-based sorted table 1, reviewing included stud-

ies revealed that the MxA quantification in MS patients was started from the late 90s, early focused on MxA protein detection and then changed to MxA gene expression assays.

Almost all studies have concluded that the MxA is significantly correlated with response to IFN-β therapy and also MxA expression is under the direct effect of neutralizing antibody (NAb) against IFN-β. But binding antibody (BAb) was reported to has either no association or less likely to correlate with MxA and therapeutic responses in MS. Some studies also showed that the baseline MxA level can predict the patient's response to IFN-β. There is a dose-response between MxA and EDSS score, reported by one study. Seven studies have reported a cut-off for MxA gene expression by the unit of Normalized Ratio (NR) which was equal to 2–ΔΔCt, ΔΔCt= (The difference between the ΔCt [Cycle threshold] of the sample and the ΔCt of the calibrator) and ΔCt= (Ct of MxA for each sample minus Ct of GAPDH). Reported cut-offs are ranging from 3.3 to 6.3 NR and the weighted average of them estimated to be 4.1 NR. So it could be concluded that patients with MxA≥4.1 are responsive to IFN-β therapy as they are considered to have acceptable IFN-β bioactivity or MxA induction. Four studies have used a titer of 20 NU/ml (NAb>20 TRU) as a cut-off for NAb positivity. One study has taken 100 TRU and one study has reported 150 TRU as a complete loss of bioavailability of IFN-β. BAb is not as sensitive as NAb and estimated to have no significant clinical value by several studies.

4. Discussion

Since measurement of anti-interferon antibodies can determine the bioavailability of IFN-β and there is a strong association between antibody development and MxA reduction, in some cases the presence of a soluble IFN-β receptor can bind to the injected interferon and lower its bioavailability and we will see the un-responsiveness to treatment and also no MxA induction despite the low level of anti-interferon antibody. Therefore, measuring MxA is more useful than measuring antibodies alone. In fact, the presence of antibodies can predict the poor response to treatment, but its negative results require to be confirmed by MxA measurement.

As shown in one included study women older than 30 years up to 40 years may be more susceptible to lose their MxA induction that might be due to different NAb response as a result of sex hormone and the difference in immune response in men and women [10]. Also, the type of INF-β used in the treatment regimen is effective in antibody response and it is demonstrated that using IFN-β1b is associated with more antibody formation comparing with IFN-β1a which might be a result of mild receptor desensitization [28]. Pachner in 2009 stated that the antibody formation against IFN-β is transient and the bioavailability can be restored when NAb declined and the European Union task force on NAbs says that all patients treated with interferon should be checked for NAb in the first 24 months after treatment [24].

As mentioned in the results, if neutralizing antibody against injected IFN-β increased to 20 TRU or more these patients are showing some sort of un-responsiveness to IFN-β therapy and should undergo closer monitoring. But NAb more than 100 or 150 TRU mean that these patients have completely lost the INF-β bioavailability and the treatment escalation may be considered for them.

Garcia-Montojo M., et al. in 2010 discussed MxA role in the evaluation of MS treatment efficiency and their results showed no significant correlation with NAb and clinical parameters (relapse, progression, and response)[19]. Their results were close to Deisenhammer F., et al., achievement in 1999 witch confirm no significant difference of MxA (cut-off=1.59 ng MxA/105 Leukocytes) between NAb-positive (≥20 IU/ml) samples and control subjects and BAb however, do not appear to influence the bioavailability of IFN-β1b [35]. In contrast, Gilli F., et al., in 2006 highlighted the important role of MxA as the most sensitive gene to detect decreased bioavailability due to NAbs [29] and Hesse's study concluded that the inability of IFN-β to induce MxA expression (cut-off =5 NR) in NAb-positive patients (TRU>20) equals a completely blocked biologic response [23].

According to Bertolotto A., et al., the MxA gene expression assay is superior to MxA protein assessments because that the MxA protein levels could be affected by different interferon doses, but MxA mRNA is less affected [33].

Finally, an important use of MxA assay in clinical practice may be in making a decision on treatment in MS patients with evidence of clinical and/or radiological disease activity not fulfilling criteria for breakthrough disease especially those with minor relapses or minimal disability. The Breakthrough disease in MS is defined as patients with clinically active disease [≥1 relapse and/or disability progression] and ≥ 2 active MRI lesions [Gadolinium (Gd) Enhancing and/or T2 Weighted], or in patients with severe relapses or ≥3 active lesions) which their treatment needs to be escalated to more potent drugs [36]. In these type of patients (with some evidence of disease activity but not fulfilling the criteria of the breakthrough diseases) MxA<4.1 shows that they are not about to have a good response to IFN-β and may need early escalation. But MxA≥4.1 means that these patients are taking advantage form INF-β therapy and should undergo close monitoring.

5. Conclusion

MxA level (both the gene expression level and the protein) is significantly correlated with treatment response to IFN-β therapy. It could be suggested that in patients under IFN-β

therapy with an active disease which doesn't fulfill the criteria for the breakthrough disease (i.e. those with minor relapses or minimal disability and <2 active MRI lesion or patients with <3 active lesions in MRI) MxA assay (with the priority of gene expression assays) may give a determining checkpoint to make a decision about their treatment plan with more confidence to determine whether to continue the drug and follow up the patient or change the treatment regimen.

6. Appendix

6.1. Acknowledgements

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6.2. Funding Support

This study was a part of an ongoing project, with no independent financial support.

6.3. Conflict of Interest

None.

6.4. Author's contribution

The Idea was conceived by E.Sh and drafting was performed by E.Sh and M.M, under final edit and supervision of M.O and SJ.H; Neurological expert revision was conducted by M.Gh.

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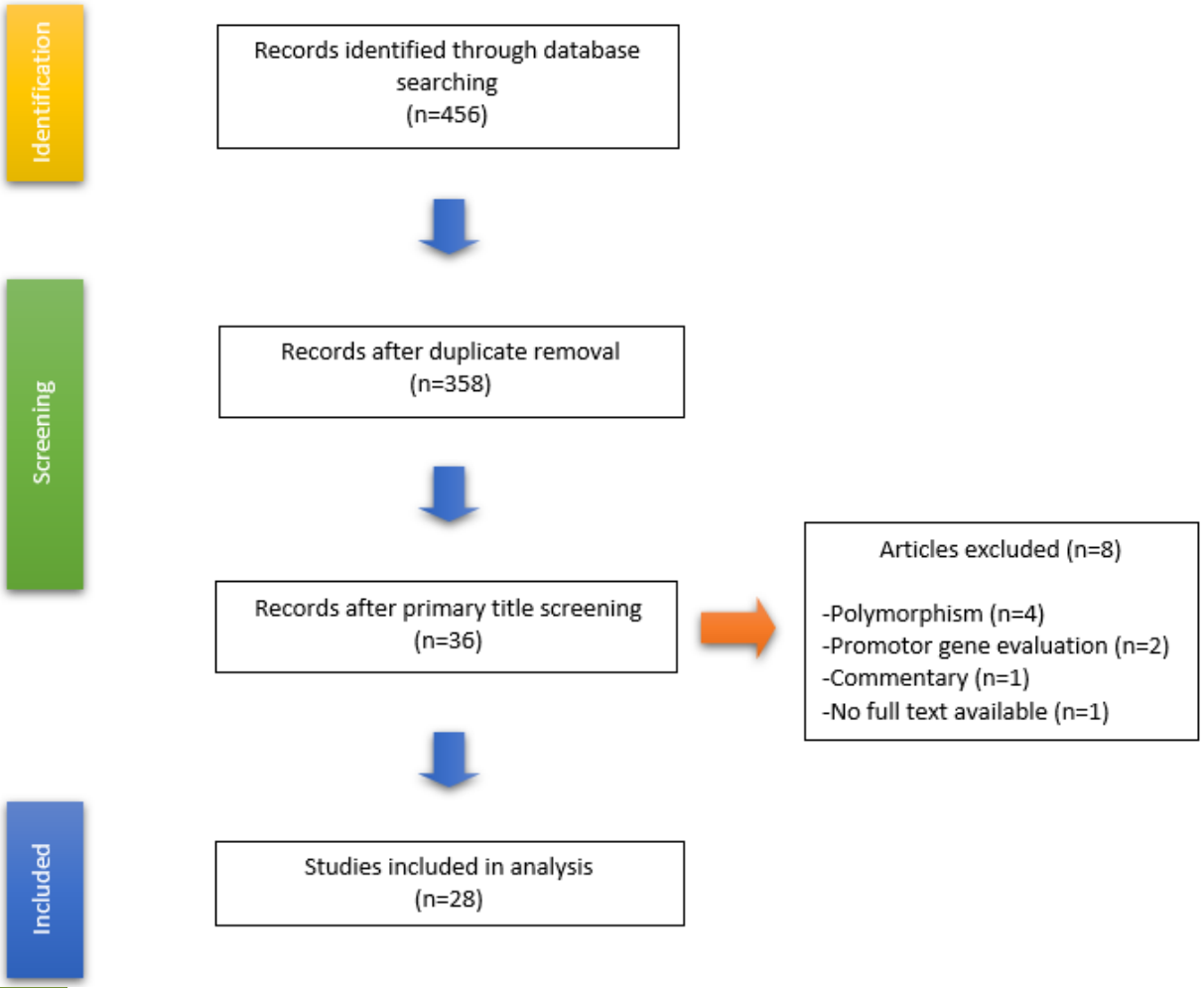

Figure 1: Flow diagram of studies on MxA assay in MS patients undergoing IFN-β therapy.

Table 1: Summary of included studies about MxA evaluation in patients under IFN-β therapy.(Continuous)

First Author [Ref.]	Study setting	Overall Statement about MxA in IFN therapy of MS	Study type
Fattahi M.,et al.,2019 [9]	RT-PCR for MxA in 35 responders and 35 non-responders RRMS patients after 1 y IFN-β therapy	MxA Significantly increased by 8 fold in the responders (No increase in the EDSS and no relapse)	Cross sectional
Taheri M., et al., 2017[10]	Quantitative RT-PCR for MxA in 50 RRMS patient and 50 healthy controls	MxA Significantly decreased in females > 30 up to 40 y (3 fold) compared with healthy controls. There is a gender bias in the response to IFN-β therapy	Cross sectional
Matas E., et al., 2016[11]	RT-PCR for MxA in 104 RRMS patients at baseline and after 1 y IFN- β therapy	Next relapse was significantly longer in the MxA>5 fold group (2.8 y vs. 1.3 y)	Cohort
Matas E., et al., 2016[12]	RT-PCR for MxA in 104 RRMS patients at baseline and after 3 m and 12 m IFN- β therapy	High baseline MxA (RE>1.096), and low MxA induction (<5 fold increase) have a higher probability of non-responding to IFN- β	Cohort
Juntunen E., et al., 2016[13]	LFIA and ELISA for MxA in 36 samples from patients receiving IFN-β-therapy for MS	LFIA Detects 96% of the IFN-β responders with 89% specificity (cut-off=100 μg/L) and is more rapidly than ELISA	Cross sectional
Matas E., et al., 2014[14]	RT-PCR for MxA in 104 RRMS patients at baseline and follow up them for 2 y	Baseline MxA expression was significantly lower in responders (RE: 1.07 vs 1.95), (Cut-off = 1.096 RE)	Cohort
Cakal B., et al., 2014[15]	RT-PCR for MxA & ELISA for BAb in 128 MS patients under >3.6 y IFN-β therapy	MxA bioactivity lost (NR≤4) in 82.5% of patients having >500 BTU BAb "sc IFN-β1b" caused the highest seropositivity ratio)70.4%(vs (28.6% in "sc IFN-β1a") and (21.4% in "im IFN-β1a")	Cross sectional
Serana F., et al., 2014[16]	RT-PCR for MxA & RIPA for BAb & CPE assay for NAb in 118 RRMS patients under 3 y IFN-β therapy	Each 1-unit increase in the "average" log2MxA levels predicts a reduction of 47% in the risk of 1-point EDSS increase BAb level was higher in patients receiving "sc IFN-β1b" in comparison to IFN-β1a	prospective longitudinal observational
Hermanrud C., et al., 2014[17]	RT-PCR for MxA and iLiteTM (type I IFN responsive reporter gene cell assay) for NAb in 44 MS patients	MxA expression was greatly reduced or blocked in patients with NAb titer above 150 TRU/mL	Prospective
Malucchi S., et al., 2011[18]	RT-PCR for MxA & ELISA for BAb & CPE assay for NAb in 167 MS patients under 1 y IFN-β therapy	MxA Significantly decreased in patients with NAb >100 TRU/ml and BAb>8 U	Cross sectional
Garcia-Montojo M., et al., 2010[19]	Quantitative PCR for MxA & CPE assay for NAbs in 50 RRMS patients during 2 y IFN-β therapy	MxA had No significant correlation with NAb and clinical parameter (relapse, progression, and response)	Prospective
Zanotti C., et al., 2010[20]	Quantitative RT-PCR for MxA in 500 MS patient over a 4 y	MxA assay (cut-off=3.83 NR) is reproducible and serves as an alternative to NAb determinations for use in routine clinical practice	Prospective

Table 1: Summary of included studies about MxA evaluation in patients under IFN-β therapy. (Continuous)

First Author [Ref.]	Study setting	Overall Statement about MxA in IFN therapy of MS	Study type
Van der Voort L. F., et al., 2010[21]	RT-PCR for MxA and MRI in 116 RRMS at baseline and 2 y follow up	higher baseline MxA (cut-off=0.075 RE) is significantly associated with longer time to first relapse (HR=0.59), lower relapse, and lower enhancing lesion in MRI	Prospective cohort
Van der Voort L. F., et al., 2009[22]	RT-PCR for MxA in 126 RRMS under >6 m IFN-β therapy, re-test after 3 m	Non-responders (MxA< 0.2 NR and <3 fold increase at re-test) showed a higher relapse rate significantly	Prospective
Hesse D., et al., 2009[23]	Screening for IFN-β-regulated genes in 12 MxA+/NAb- and 12 MxA- /NAb+ MS patients	The Inability of IFN-β to induce MxA expression (cut-off =5 NR) in NAb+ patients (TRU>20) equals a completely blocked biologic response	Cross sectional
Pachner A.R, et al., 2009[24]	RT-PCR for MxA & ELISA for BAb & CPE assay for NAb & MRI for lesion occurrence in 36 RRMS patients under IFN-β therapy	In the preserved bioactivity group (NAb≥20 TRU and MxA> 6.3 NR) "enhancing lesion/scan ratio" decreased 66% in the post-treatment period.	Prospective
Millonig A., et al., 2008[25]	Serial ELISA for MxA and NAb in 20 PPMS under IFN-β1b therapy in 1 y	The mean area under the curve of log MxA levels during treatment was significantly higher in stable patients than in progressing patients	Prospective
Vallittu A.M., et al., 2008[26]	EIA and flow cytometric for MxA in 51 RRMS under IFN-β therapy	EIA (cut-off =100 µg / l) was favorable and more sensitive compared with the flow cytometric	Cross sectional
Malucchi S., et al., 2008[27]	RT-PCR for MxA & ELISA for BAb & CPE assay for NAb in 137 MS patients after 1 y IFN-β therapy	MxA- (<87 RE) or NAb+ (>20 TRU) patients showed poorer RFS (HR=2.87, HR=2.49), BAb was not significant. MxA had a slightly stronger prognostic significance	Prospective 3 y follow-up
Capra R., et al., 2007[28]	RT-PCR for MxA & RIPA for BAb & CPE assay for NAb in 99 MS patients after >2 y IFN-β therapy	Anti-IFNβ antibodies well correlated with MxA induction loss (Cut-off=3.82 NR) Mean MxA was significantly lower in patients under "sc IFN-β1b" in comparison to "im IFN-β1a"	Cross sectional
Gilli F., et al., 2006[29]	Quantitative-PCR for Mxa, TRIAL, XAF-1, and CPE for NAb im 73 RRMS patients	MxA was the most sensitive gene to detect decreased bioavailability due to NAbs	Cross sectional
Pachner A. R., et al., 2005[30]	RT-PCR for MxA & ELISA for BAb & CPE assay for NAb in 64 RRMS patients under IFN-β therapy	-IFN-β1a caused the lowest ADB -ADB is a reversible condition MxA assay (Cut-off =6.3NR) is a valuable adjunct for monitoring, especially when the antibody is substantial	Prospective
Bertolotto A., et al., 2004[31]	5 days RT-PCR for MxA (every 3 m) & CPE assay for NAb in 62 RRMS patients under different IFN-β regimen	Higher biological responses (MxA≥ 0.132 RE) and higher risk for NAb (≥20 TRU) were seen in patients treated three times a week (Betaferon and Rebif) instead of once (Avonex)	Prospective

Table 1: Summary of included studies about MxA evaluation in patients under IFN-β therapy.

First Author [Ref.]	Study setting	Overall Statement about MxA in IFN therapy of MS	Study type
Pachner A. R., et al., 2003[5]	RT-PCR for MxA & ELISA for BAb & CPE assay for NAb in 68 RRMS patients under IFN-β therapy	First report of RT-PCR for MxA as IFN-β biomarker (non-responder<3.3 NR) The Best strategy is to monitor with BAb and then RT-PCR for MxA in BAb+ individuals	Prospective
Vallittu A. M., et al., 2002[32]	Flow cytometric for MxA & ELISA for BAb & CPE assay for NAb in 20 RRMS patients under 1 y IFN-β1a therapy	The Presence of NAb does not necessarily inhibit the biologic effects of IFN-β, MxA protein in lymphocytes is promising as an additional marker	Prospective
Bertolotto A., et al., 2001[33]	qc-PCR for MxA & CPE assay for NAb in 48 RRMS patients before and after IFN-β	Bioavailability of the three available types of IFN-β can be evaluated by MxA qc-PCR	Cross sectional
Kracke A., et al., 2000[34]	ELISA for MxA in 52 RRMS patients under IFN-β1b therapy	MxA levels were significantly lower during relapse (Median: 11.2 mU/1,000 Leukocytes) than during stable phases (20.5 mU/1,000 Leukocytes) and within the first month after relapse, MxA levels increased significantly (25.5 mU/1,000 Leukocytes)	Prospective
Deisenhammer F., et al., 1999[35]	ELISA for MxA and BAb & MxA induction assay for NAb in 134 MS patients under IFN-β1b therapy and 54 control	- There was no significant difference of MxA (cut-off=1.59 ng MxA/105 Leukocytes) between NAb+ (≥20 IU/ml) samples and control subjects - BAb, however, do not appear to influence the bioavailability of IFN-β1b	Cross sectional

RT-PCR: Reverse Transcriptase-Polymerase Chain Reaction, PPMS: Primary progressive multiple sclerosis,
RRMS: Relapsing-Remitting Multiple Sclerosis, EDSS: Expanded Disability Status Scale, RFS: Relapse-free survival,
ADB: Antibody-mediated Decreased Bioactivity, Activity, NR: Normalized Ratio, BTU: Bühlmann Titer Units, TRU: Tenfold Reduction Units, BAb: Anti IFN- β Binding Antibody, NAb: Anti IFN- β Neutralizing Antibody, RIPA: Radio immunoprecipitation Assay,
SC: subcutaneous, IM: intramuscular, ELISA: Enzyme-Linked Immunosorbent Assay, CPE: Cytopathic Effect,
EIA: Enzyme immune assay, LFIA: Lateral Flow Immunoassay, RE: Relative Expression (Expression levels relative to GAPDH expression levels), Y: year, m: month, MRI: magnetic resonance imaging.