

A long-term follow-up study on biochemical and clinical biomarkers of response to interferon beta-1b treatment in relapsing-remitting multiple sclerosis

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Conflict of interest

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Abstract

Background. While interferon beta-1b (IFN-β-1b) is still a commonly used disease-modifying drug in the treatment of multiple sclerosis (MS), therapeutic possibilities are expanding, and treatment failure should be identified early. Markers to predict response to IFN-β-1b, either clinical or biochemical, are therefore urgently needed. Interferon-induced proteins, including viperin, suppressor of cytokine signaling 3 (SOCS3), ubiquitin specific peptidase-18 (USP18), and myxovirus resistance protein A (MxA), are possible markers of IFN-β-1b bioavailability and treatment response.

Objectives. To evaluate viperin, SOCS3, USP18 and MxA as markers of treatment response in Polish IFN-β-1b-treated patients with MS.

Material and methods. In 45 IFN-β-1b-treated Polish patients with MS, serum concentrations of viperin, SOCS3, USP18, and MxA were assessed before and after 24 months of IFN-β-1b treatment. The patients were followed clinically and with magnetic resonance imaging (MRI) for a median of 6.8 years.

Results. Low viperin, USP18 and MxA at baseline and 24 months and high SOCS3 at 24 months correlated with higher disease activity up to the 6th year of observation, but only baseline MxA and USP18 were independently related to outcome, with higher concentrations predicting less disease activity in the first 3 years and after the 1st year, respectively.

Conclusions. We confirm the predictive value of MxA and propose USP18 as a possible new prognostic biomarker in IFN-β-1b-treated MS patients.

Key words: multiple sclerosis, interferon beta, viperin, suppressor of cytokine signaling 3, ubiquitin specific peptidase 18

Background

Multiple sclerosis (MS), a chronic inflammatory and degenerative disorder of the central nervous system (CNS), remains a major cause of disability in young adults.¹ With the introduction of new, increasingly effective disease modifying drugs (DMD) for the relapsing-remitting form of MS (RRMS), the therapeutic paradigm has shifted from decreasing to abolishing any detectable disease activity. The concept of no evidence of disease activity (NEDA)^{2,3} assumes no detectable activity in 3 domains: 1) no clinical relapses, 2) no disability progression (measured with the Expanded Disability Status Scale (EDSS)) and 3) no magnetic resonance imaging (MRI) activity (no new or enlarging T2/FLAIR lesions and no gadolinium enhancing lesions). As MS etiology and its exact pathomechanism are still uncertain, there is no single biomarker that could be used to measure disease activity. Therefore, combined assessment tools, such as NEDA, are often used as surrogates.

The arrival of new, more effective DMDs has been progressively decreasing the use of interferon beta (IFN- β) over the years, although the new agents are costly and often less safe,⁴ and a subset of patients experience an uneventful disease course on IFN- β for many years. A marker capable of identifying the patients likely to respond to treatment with IFN- β would be of great value.

Prognostic tools to predict treatment response may comprise the patient's initial characteristics, such as gender, age and disease course, including current disability, number of relapses before treatment or features of the first relapses. Alternatively, markers of early response to DMD may be used to predict longer-term outcome: composite scores featuring clinical and imaging features over the 1st year of treatment were used, e.g., the Rio⁵ and modified Rio score.⁶ Unfortunately, neither classic baseline risk factors nor early clinical response scores are specific and sensitive enough to guide treatment decisions; furthermore, the response scales require a year of treatment trial. In an attempt at finding earlier and more reliable prognostic tools, numerous biochemical markers were proposed. Among them are markers of inflammation and neuronal damage as well as drug-specific markers, measured either at baseline or as an early response marker. Biomarkers intended specifically for IFN- β response prediction include IFN-inducible proteins, such as viperin and myxovirus resistance protein A (MxA), anti-IFN antibodies,^{7–10} cytokines (tumor necrosis factor α ¹¹, interleukin 17,^{11–13} interleukin 25,¹¹ interleukin 6¹² and interleukin 10¹³) and certain miRNA profiles.^{14,15}

Based on previous research (Table 1), we selected 3 little-explored IFN-inducible particles with distinctive traits and promising reports: viperin (also known as radical s-adenosyl methionine domain-containing protein 2 – RSAD2), suppressor of cytokine signaling 3 (SOCS3) and ubiquitin specific peptidase 18 (USP18) to evaluate and compare with a more acknowledged marker, namely MxA. Specifically,

Table 1. Selected markers of response to IFN- β therapy in patients with MS

Name	Function	Clinical findings	Reference
Viperin	multifunctional antiviral	markedly lower expression in the presence of NABs	10
SOCS3	STAT3 inhibitor	decrease in expression during relapse; expression higher in MS than in healthy controls	26, 27
USP18	protease (ubiquitin-like protein ISG15 residuals deconjugation), negative class I IFN receptor regulator	expression lower in untreated MS than in healthy controls; USP18 polymorphism with lower expression associated with more active MS course	7, 20
MxA	antiviral GTP-ase	MxA mRNA at 1 year of treatment predicts relapses and disability progression; low expression before treatment associated with more relapses and MRI activity during IFN treatment; higher mean MxA mRNA independently predicts lower risk of disability progression	9, 32–35

STAT – signal transducer and activator of transcription; ISG15 – interferon-stimulated gene 15; SOCS3 – suppressor of cytokine signaling 3; USP18 – ubiquitin specific peptidase 18; MxA – myxovirus resistance protein A.

the aim of this study was to compare serum levels of viperin, SOCS3, USP18 and MxA in IFN- β -1b-treated RRMS patients with and without disease activity during a long-term follow-up.

Material and methods

Patients and treatment

Through years 2008–2013, we consecutively recruited 45 patients (31 women and 14 men), diagnosed with RRMS, who were started on IFN- β -1b treatment in the setting of national MS treatment program at Heliodor Świącicki University Hospital in Poznań, Poland. Retrospective analysis revealed that all patients fulfilled the revised 2010 McDonald criteria¹⁶ at the time of enrollment. Eligibility criteria included: age ≥ 18 years, no prior DMD treatment, qualification for the national MS treatment program (Supplementary Table 1). Exclusion criteria included pregnancy, decompensated liver (aminotransferase levels ≥ 2 upper reference limit) or thyroid disease (no euthyrosis), intractable depressive mood disorder, history of suicidal ideation, or epilepsy. The study protocol was approved by the Ethics Board of Poznan University of Medical Sciences. All subjects gave written informed consent for study participation.

The participants were started on subcutaneous IFN- β -1b (Betaferon, $n = 41$, Extavia, $n = 4$) 250 μ g (8 MIU) every other day.

Follow-up

The study cohort was followed through years 2008–2018. Each patient was assessed monthly by a neurologist. Imaging follow-up consisted of initial and then yearly 1.5 Tesla brain MRI (Siemens Avanto, Erlangen, Germany) using a 12-channel head coil with gadolinium contrast administration, including T1, FLAIR, T2, and PD sequences. Spinal MRI was performed in selected cases based on the physician's judgment. For each patient, every year, we determined: the number of relapses, the EDSS score, the presence of new or enhancing lesions in MR, NEDA-3 status and, if relevant, information about treatment termination.

Definition of NEDA

We used a previously established NEDA (NEDA-3) definition²: for a given period of time, NEDA means the absence of relapses, disability progression and MRI activity. A relapse was defined as the appearance of new or the worsening of past symptoms with focal neurological abnormality applicable to MS that persists for ≥ 24 h, is not accompanied by fever and was preceded by ≥ 30 days of clinical stability. Disability progression was defined as an increase in EDSS by at least 1.5 from the baseline EDSS 0, by 1.0 from baseline 1.0–5.0 and by 0.5 from baseline 5.5 or more, confirmed after 3 months. The activity of MRI denotes new or enlarging T2/FLAIR hyperintense lesions or contrast enhancing lesions.

Laboratory assays

Serum samples were collected at baseline and after 24 months of treatment and stored in -70°C . Serum concentrations of MxA, viperin, USP18, and SOCS3 proteins were measured with the use of viperin, SOCS3 and USP18 ELISA kits (product No. MBS2023571, MBS703435 and MBS9338610, MyBiosource, San Diego, USA) and human MxA ELISA kit (product No. RD 194349200R, BioVendor, Brno, Czech Republic), according to the manufacturer's instructions.

Statistical methods

P-values ≤ 0.05 were considered significant. Normalities of distributions were assessed with d'Agostino-Pearson test. Results were accordingly reported as either means \pm standard deviation (SD) or medians with interquartile range (IQR).

Baseline clinical characteristics (sex, age at first relapse, time from 1st to 2nd relapse, time to treatment initiation,

number of relapses prior to treatment initiation, EDSS score at treatment start) and baseline concentrations of MxA, viperin, USP18, and SOCS3 were compared between subgroups of patients with and without disease activity, including NEDA-3 and its components, in different time ranges.

Then, MxA, viperin, USP18, and SOCS3 were compared at 24 months. We used t-test and paired t-test for normally distributed interval variables, the Mann–Whitney U test for variables with non-normal data distribution and ordinal variables, and Fisher's exact test for nominal variables.

Stepwise logistic regression models were calculated. Variable sets included baseline characteristics as defined above and markers measured at baseline or at 24 months or their change from baseline at 24 months.

Statistical analyses were performed with the use of STATISTICA v. 13¹⁷ (StatSoft, Inc., Tulsa, USA) and MedCalc for Windows, v. 15.8¹⁸ (MedCalc Software, Ostend, Belgium).

Results

Baseline

A total of 45 patients were included. Their baseline characteristics are displayed in Table 2. Women were significantly older than men; otherwise, there were no gender-based differences. Median concentrations of biochemical markers are presented in Table 3. A statistically significant change from baseline to 24 months was noted for SOCS3 (a decrease) and MxA (an increase).

Median follow-up lasted 6.5 years (IQR 4.6 to 8.5). Throughout the observation, a gradual loss of NEDA was observed (Fig. 1).

Discontinuation statistics

In 29 patients, treatment was terminated during the follow-up. The national treatment program was initially restricted to 3 years. This was the sole discontinuation reason in 2 patients (6.9%). Three patients (10.3%)

Table 2. Baseline characteristics of the study cohort

Parameter	All	Women	Men
Age at first relapse [years], mean \pm SD	29.7 \pm 7.9	31.4 \pm 8.5*	25.8 \pm 5.0*
Time to second relapse [months], median (IQR)	10 (3.3 to 25.0)	9 (3.8 to 25.0)	10 (3 to 36)
Time to treatment initiation [months], median (IQR)	17 (8.8 to 37.0)	22 (9.8 to 36.8)	15.5 (8 to 60)
Relapses before treatment, median (IQR)	2 (2 to 3)	3 (2 to 3)	2.5 (2 to 4)
EDSS at baseline, median (IQR)	1 (0 to 1.5)	1 (0 to 1)	1 (0 to 2)
OCB in CSF, % positive	89.7%	88.9%	91.7%

*p = 0.008.

Table 3. Serum concentrations of different markers of response to IFN- β -1b in relapsing-remitting patients with MS

Marker	Baseline		24 months		0–24-month change	
	N	median (IQR)	N	median (IQR)	N	median (IQR)
USP18 [ng/mL]	43	1.294 (0.707 to 2.099)	41	1.283 (0.659 to 2.653)	40	0.168 (–0.637 to 1.132)
SOCS3 [pg/mL]	43	5.703 (2.417 to 13.249)	41	0 (0.000 to 1.592)	40	–2.098* (–9.368 to –0.235)
viperin [ng/mL]	43	0.651 (0.385 to 0.943)	41	0.459 (0.243 to 0.722)	40	–0.204 (–0.505 to 0.145)
MxA [ng/mL]	24	2.612 (0.811 to 8.066)	19	4.747 (2.417 to 13.249)	8	1.498** (0.579 to 9.015)

* $p < 0.001$; ** $p = 0.036$; SD – standard deviation; IQR – interquartile range.

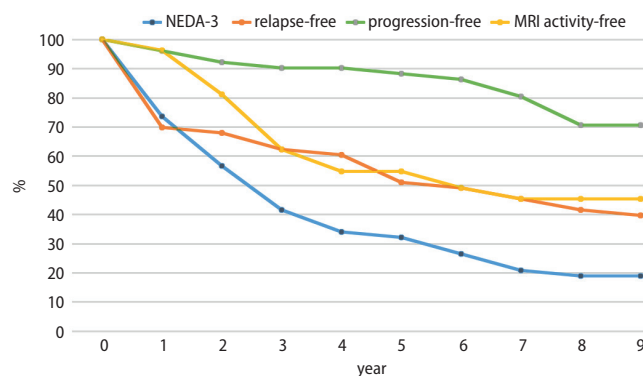


Fig. 1. Proportion of patients with no disease activity (according to NEDA-3 criteria), relapses, progression, and MRI activity over time

became pregnant or were planning pregnancy. Adverse reactions were the indication in 9 cases (31.0%) and treatment failure (2 or more severe relapses, >2 new MRI lesions, >1 enhancing lesion or EDSS increase beyond 4.5) in further 11 patients (37.9%). Four patients (13.8%) resigned for personal reasons. Patients who discontinued treatment did not differ in marker concentrations.

Mutual correlations

There were few correlations between baseline clinical features and biomarkers considered in this study. Among clinical features, there was a weak correlation between older age at 1st relapse and shorter time to 2nd relapse. More pre-treatment relapses were associated with higher pre-treatment EDSS score.

Baseline USP18, SOCS3, viperin, and MxA had no significant correlations. On the other hand, pre-treatment SOCS3 and USP18 correlated positively with USP18 at 24 months. Baseline viperin and MxA correlated positively with MxA at 24 months.

There was little association between our markers and clinical characteristics. For baseline EDSS, there was a positive correlation with baseline SOCS3 and negative with baseline and 24-month MxA, however, not with MxA change. The interval between 1st and 2nd relapse was positively correlated with viperin at 24 months.

Correlations with disease activity

A summary of marker concentration differences in patients with and without disease activity is presented in Table 4. Detailed results concerning USP18 (Supplementary Table 2), SOCS3 (Supplementary Table 3), viperin (Supplementary Table 4), and MxA (Supplementary Table 5) can be found in supplementary materials.

Baseline molecular biomarkers

Numerous statistically significant differences were found for USP. Higher concentrations were associated with less disease activity in the first 5 years. Baseline SOCS3 had no correlations with disease activity. Baseline viperin levels were generally higher in subjects without disease activity, but they were lower in those without progression after the 7th year. Baseline MxA levels were higher in patients with no activity after the 5th year. More importantly, baseline MxA was higher in patients without progression during the entire follow-up.

Molecular biomarkers at 24 months

Higher USP concentrations at 24 months were associated with less disease activity in the first 5 years. Suppressor of cytokine signaling 3 at 24 months was lower in patients with no disease activity, including NEDA in first 4 years and all its individual components in the 2nd year. At 24 months, compared to baseline, SOCS3 decreased in subjects with no MRI activity in the first 2 years, while in active cases, it had a tendency to remain unchanged or increase. Viperin levels at 24 months were higher in patients with no relapses after the 4th year. Regarding viperin change at 24 months, a decrease was noted in patients with no activity in earlier years, while an increase correlated with no disease activity in late follow-up. A notable exception was the increase in patients with no MRI activity in the first 5 years.

Two-year MxA levels were higher in patients without progression during the entire follow-up. In contrast, MxA at 24 months was lower in patients with no MRI activity until the 6th year.

Table 4. Marker concentration in relation to disease activity

Observation	p-value
At baseline	
USP18	
higher in pts with NEDA in the first 5 years	0.018
higher in pts without relapses in the first 5 years	0.041
higher in pts without MRI activity in the first 6 years	0.034
higher in pts without MRI activity during the entire follow-up	0.037
SOCS3	
None	
Viperin	
higher in pts with NEDA in year 2	0.031
higher in pts without relapses in year 2	0.010
higher in pts without disability progression in the first 7 years	0.041
lower in pts without disability progression after year 7	0.044
MxA	
higher in pts with NEDA after year 5	0.027
higher in pts without disability progression during the entire follow-up	0.007
Clinical	
Delay to treatment	
longer in pts with relapses after year 1	0.045
shorter in pts with NEDA after year 1	0.016
Baseline EDSS	
higher in pts with progression anytime during the follow-up	0.027
Pre-treatment relapses	
more in pts with progression within 6 years	0.010
Age at first relapse	
older: progression after year 2	0.036
younger: MRI activity within 7 years	0.014
Age at treatment initiation	
older: pts with NEDA after year 5	0.009
Time to second relapse	
shorter in pts with MRI activity in year 1	0.010
At 24 months	
USP18 at 24 months	
higher in pts with NEDA in the first 5 years	0.016
USP18 change	
none	
SOCS3 at 24 months	
lower in pts with NEDA in the first 4 years	0.042
lower in pts without relapses in year 2	0.008
lower in pts without disability progression in year 2	0.021
lower in pts without MRI activity in the first 2 years	0.039
SOCS3 change	
decrease in pts with no MRI activity in the first 2 years	0.016
Viperin at 24 months	
higher in pts without relapses after year 4	0.024
Viperin change	
decrease in pts with NEDA in the first 2 years	0.005
decrease in pts without relapses in year 2	0.008
decrease in pts without disability progression in the first 4 years	0.048
increase in pts with NEDA after year 7	0.010
increase in pts without relapses after year 4	0.018
increase in pts without disability progression after year 7	0.010
increase in pts without MRI activity in the first 5 years	0.033
MxA at 24 months	
lower in pts with no MRI activity until year 2	0.050
MxA change	
none	

pts – patients.

Table 5. ROC curve analysis results

Outcome	Criterion	ROC p-value	LR (95% CI)
NEDA in first 3 years	baseline USP >1.44	0.045	3.3 (1.4 to 7.7)
NEDA after year 1	baseline MxA >9.07	0.032	7.5 (1.8 to 31.4)
NEDA in first 4 years	delay to treatment onset	0.848	N/A
Relapse in first 5, 6 or 7 years	age at treatment onset	0.182	N/A

LR – likelihood ratio; CI – confidence interval; N/A – not available.

Logistic regression and ROC analysis

Overall, the robust associations were not confirmed by stepwise regression. The only statistically significant models were for three-year NEDA, where baseline USP18 remained as the only contributor ($p = 0.017$), NEDA after the 1st year with baseline MxA ($p = 0.034$), four-year NEDA with the delay to treatment onset ($p = 0.001$), and relapses in the first 7 years, which were inversely correlated to the age at treatment onset ($p = 0.002$). Receiver operating characteristic (ROC) analysis allowed us to select cutoff values for USP and MxA as shown in Table 5.

Discussion

This work is a prospective long-term follow-up study on novel biochemical biomarkers of response to IFN- β 1b treatment in RRMS patients.

Overall, patients with no disease activity during the follow-up had a tendency to exhibit higher levels of USP18, viperin and MxA levels at baseline and at 24 months of treatment. Conversely, there was no difference in baseline SOCS3, while higher levels at 24 months were observed in patients with active disease.

Ubiquitin specific peptidase-18 is a class-I IFN induced enzyme that opposes IFN activity by enzymatic and non-enzymatic mechanisms.¹⁹ Expression of USP18 is lower in untreated MS patients than in healthy controls, which indicates the possible involvement in disease pathomechanism.⁷ In a cross-sectional study comparing USP18 polymorphism prevalence in MS patients and healthy controls, patients with a haplotype associated with lower USP18 expression experienced more active disease.²⁰ More recent research is scarce. A small (20 cases) retrospective study failed to show an association between baseline USP18 and response to IFN- β in MS.²¹

Our results support the association of higher USP18 levels with lower disease activity in MS. Both baseline and 24-month levels were lower in patients with disease activity in subsequent years, indicating a mechanism independent from possible interference with IFN- β treatment. In fact, the change in USP18 after 2 years of therapy did not differ between patients with good and poor response. Further

supporting our observation, baseline USP18 remained the only independent variable in stepwise regression model for NEDA in the first 3 years.

The family of suppressors of cytokine signaling (SOCS) contains regulators of intracellular signaling pathways of various cytokines induced predominately by the JAK-STAT cascade.

Suppressor of cytokine signaling 3 is induced by STAT3 and acts as an inhibitor of STAT3, with a complex impact on the immune system. It attenuates responses to class I and II IFNs, inhibits maturation of Th17 cells and directs macrophage polarization towards the M2 phenotype.²² At the same time, SOCS3 may play a role in neuroprotection, as SOCS3 overexpression promotes neural differentiation and survival *in vitro*.²³

Animal studies indicate both protective and harmful effects on the CNS in experimental autoimmune encephalitis (EAE).²⁴ Experimental autoimmune encephalitis in SOCS3-transfected mice develops later, but is more severe.²⁵

Little research has been done on SOCS3 in human MS subjects. During relapse, a decrease in leukocyte SOCS3 expression was observed.²⁶ On the other hand, in a small American study,²⁷ SOCS3 expression was significantly higher in MS patients than in the healthy controls, with a trend towards more disability in patients with higher expression. This observation was not confirmed in a more recent study.²⁸

In our study, baseline SOCS3 levels were not different in patients with and without disease activity. However, the levels rose and were higher at 24 months in those with activity in the 2nd year, possibly reflecting a maintained, but unbeneficial drug effect. However, the observation was lost when controlled for other variables.

Viperin is an anti-viral protein induced by class I interferons²⁹ and a specific marker of IFN- β activity.⁷ In the INSIGHT trial, viperin and MxA expression were compared among patients with and without anti-IFN antibodies.¹⁰ In patients with NABs, the expression levels of all 3 proteins were lower or absent at NAb titres ≥ 100 TRU. The study was not designed to assess correlations between marker expression and disease activity.

In our cohort, baseline viperin concentrations were higher in patients with no disease activity. However, the baseline values were lower in those with no progression later on. At 24 months, viperin levels were higher in those who did not experience relapses in the subsequent years.

While the absolute values were higher in cases with better outcome, viperin change between baseline and 24 months showed a general trend to be more negative in patients with no disease activity in earlier years (up to the 4th) and more positive in those stable in later follow-up. This was apparent for NEDA, relapses and progression. For MRI activity, which is considered to be the earliest indicator of disease activity among NEDA components, there was a concordant rise in viperin in stable patients in both earlier and later years, with a similar trend for the entire follow-up.

Overall, no independent association with prognosis was found in regression models. Myxovirus resistance protein A (MxA), a class I IFN-induced GTP-ase with antiviral properties,²⁹ is considered one of the most reliable markers of IFN- β bioavailability.³⁰ The absence of MxA induction signifies total loss of IFN activity, as no other IFN-induced proteins are detected in this setting.³¹ Unsurprisingly, numerous studies reported an association between low MxA mRNA and poor response to IFN. MxA mRNA measured at 1 year of treatment was a better predictor of relapses than NAb titers.^{9,32} In another study, MxA induction at 1 year predicted a longer time to a relapse or EDSS progression.³³ More intriguing is that low MxA expression before treatment was also associated with a higher risk of relapses or EDSS worsening while on IFN.³⁴ Higher mean MxA mRNA, measured over the period from 6 months to 2 years of treatment, predicted a lower risk of disability progression in the first 3 years, independently from relapse activity.³⁵ On the other hand, in 2 more recent studies^{21,36} MxA mRNA levels failed to predict treatment response to IFN- β in a two-year observation.

Our results replicated the association of higher MxA levels, both during IFN therapy and in treatment-naïve patients, with less disease activity. What is more, baseline MxA was the only independent predictor of disease activity past year 1, with the highest levels >9.07 ng/mL associated with a 7.5 likelihood ratio for no disease activity.

The heterogeneity of immunologic profiles in MS, as well as their associations with treatment response, has been addressed in several recent works.^{11–13,37} Our study lends support to the notion that certain alterations convey a higher risk of poor outcome. The study yielded multiple findings. However, only several have potential clinical utility. In logistic regression, higher baseline USP18 remained the only independent prognostic factor for NEDA, and only in the first 3 years of treatment. High baseline MxA, on the other hand, was independently associated with NEDA in the observation after the 1st year.

Our study has several limitations. The choice of testing material requires commentary. Previous studies concerning their expression utilized either mRNA (MxA, viperin, USP18, SOCS3) or protein measurement (MxA) in peripheral blood cells. Quantitative real-time polymerase chain reaction (qRT-PCR) for mRNA is more sensitive, but protein detection methods are cheaper; furthermore, the short half-life of mRNA forces strict timing of sample collection in relation to drug administration: For MxA, mRNA levels begin to increase at 3 and peak at 12 h post-injection, decreasing two-fold by 24 h³⁸; MxA protein levels remain more stable,³⁹ allowing more flexible sampling. It is also worth noting that, on the one hand, mRNA detection reflects the earliest expression stage most closely related to gene induction by IFN and, on the other, protein measurement accounts for all post-transcription and post-translation regulatory processes and may, therefore, more accurately correlate with actual biological activity. For MxA, a dose effect of IFN is apparent at protein level, but not at mRNA level.⁴⁰

All the assessed proteins are intracellular. Due to technical limitations, no cellular material was available for this study. We, therefore, performed a number of preliminary measurements on healthy donors (data not published) and found that the aforementioned particles are detectable at various levels in sera. This may reflect either protein release from fragmented cells or the protein contained in nanoparticles.

By detecting associations with disease activity, this study confirms that serum is a valid material for the measurement of USP18, SOCS3, viperin, and MxA. What is more, we provide evidence that the differences in IFN-inducible gene products expression are apparent at the protein stage, not only at mRNA, as shown in previous studies.

A considerable proportion of cases was lost during observation, and other cases were recruited late in the study, resulting in a median follow-up of almost 7 years. The lower number of cases in years 6–9 may have contributed to the fact that most of the significant associations were not present beyond the 5th year. Knowing that the baseline marker levels kept their associations until then, the true prognostic value of measurements at 24 months may be underappreciated in our study.

A number of peculiarities concerning treatment decisions in our cohort need to be addressed. The considerable delay between diagnosis and treatment initiation is caused by procedural barriers, including limited access to imaging facilities and long waiting queues at MS treatment centers in Poland. Due to treatment program rules and lack of registered second-line therapies, many patients with ongoing disease activity continued IFN treatment. In addition, 2 patients were excluded from further treatment due to treatment program duration restriction that was in force until 2012.

Unanswered questions leave implications for future research. It would be informative to assess USP18, viperin, SOCS3, and MxA levels in patients on other DMDs and without treatment, in order to separate their natural variance in SM from drug effects and to assess whether they are predictive of response to any particular drug. In addition, it might be prudent to focus on early dynamics of the markers included in this study.

Conclusions

Low levels of plasma USP-18, viperin and MxA at baseline and at 24 months of treatment and high levels of SOCS3 at 24 months are associated with more disease activity and worse outcome in IFN- β -1b-treated MS patients.

However, only baseline USP18 and MxA were independent predictors of disease activity: Baseline USP18 > 1.44 ng/mL was associated with NEDA in the first 3 years with LR of 3.3, while baseline MxA > 9.07 ng/mL was associated with NEDA in the entire observation beyond year 1 with LR of 7.5, identifying infrequent patients with very mild disease and sustained good response to IFN- β -1b.

Suppressor of cytokine signaling 3 was the only marker with higher instead of lower concentrations in the cases with more active MS. Moreover, its concentration decreased during two-year IFN treatment and at that time point, higher values were observed in patients currently experiencing relapses, MRI activity or progression. While SOCS3 failed to predict later course, this association appears to reflect an important qualitative variability of response to IFN- β -1b.

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Supplementary material

Supplementary Table 1. Polish National MS treatment program eligibility criteria in the years 2008–2013

Criteria	Before 2012	From 2012
Diagnosis	2005 McDonald criteria and contrast-enhanced head MRI consistent with MS	
Disease activity	at least 2 relapses within 2 last years	not specified
Required score	21	15
Scoring system	Age: 16–40 years – 6 pt 40–60 years – 3 pt over 60 years – 1 pt Disease duration: 0–3 years – 6 pt 3–6 years – 3 pt 6–10 years – 2 pt over 10 years – 1 pt RRMS with no deficit – 5 pt Number of relapses in the last year: 3–4 – 5 pt 1–2 – 4 pt 6–7 – 2 pt none (less than 1/year) – 1 pt over 7 – 0 pt EDSS score: 0–2 – 6 pt 2.5–4 – 3 pt 4.5–5 – 2 pt over 5 – 1 pt	Disease duration: 0–3 years – 6 pt 3–6 years – 4 pt 6–10 years – 2 pt over 10 years – 1 pt RRMS with no deficit – 5 pt Number of relapses in the last year: 3 and more – 5 pt 1–2 – 4 pt none – 1 pt EDSS score: 0–2 – 6 pt 2.5–4 – 5 pt 4.5–5 – 2 pt over 5 – 1 pt
Exclusion criteria	1. hypersensitivity to IFN- β ; 2. primarily or secondarily progressive MS; 3. pregnancy; 4. decompensated liver disease (aminotransferase levels ≥ 2 upper reference limit); 5. thyroid disease (no euthyrosis); 6. intractable depressive mood disorder or history of suicidal ideation; 7. epilepsy.	

MRI – magnetic resonance imaging; MS – multiple sclerosis; RRMS – relapsing-remitting multiple sclerosis; pt – points; EDSS – Expanded Disability Status Score.

Supplementary Table 2. Statistically significant differences in USP18 (all values in ng/mL) between patients with and without disease activity

Comparison	Non-active			Active		p-value
	N	mean ±SD or median (IQR)		N	mean ±SD or median (IQR)	
Baseline USP18 vs NEDA						
until 3 years	16	2.142 ±1.5828	>	24	1.195 ±0.6954	0.036
until 4 years	12	2.517 ±1.6506	>	28	1.170 ±0.6694	0.018
until 5 years	12	2.517 ±1.6506	>	28	1.170 ±0.6694	0.018
Baseline USP18 vs relapses						
until 5 years	18	2.037 ±1.5173	>	21	1.188 ±0.7462	0.041
Baseline USP18 vs MRI						
until 3 years	25	1.621 (0.947 to 2.335)	>	12	0.897 (0.484 to 1.217)	0.003
until 4 years	18	2.124 ±1.4866	>	17	0.958 ±0.5297	0.005
until 5 years	18	2.124 ±1.4866	>	17	0.958 ±0.5297	0.005
until 6 years	13	2.253 ±1.7165	>	20	1.092 ±0.5982	0.034
after year 1	19	2.030 ±1.5449	>	22	1.134 ±0.5854	0.026
after year 2	17	2.026 ±1.6095	>	20	1.155 ±0.5947	0.048
entire follow-up	20	1.946 ±1.5496	>	22	1.134 ±0.5854	0.037
USP18 at 24 month vs NEDA						
until 4 years	10	2.586 (1.496 to 4.970)	>	28	0.939 (0.583 to 2.074)	0.040
until 5 years	10	2.462 (1.353 to 3.834)	>	28	0.939 (0.583 to 2.074)	0.016

N – number of cases; SD – standard deviation; IQR – interquartile range; USP18 – ubiquitin specific protease 18; *small groups.

Supplementary Table 3. Statistically significant differences in SOCS3 (all values in pg/mL) between patients with and without disease activity

Comparison	Non-active			Active		p-value
	N	mean ±SD or median (IQR)		N	mean ±SD or median (IQR)	
Baseline SOCS3 vs NEDA, relapses, MRI or progression: no significant differences						
SOCS3 at 24 months vs NEDA						
in year 2	29	0.000 (0.000 to 0.473)	<	11	5.422 (0.101 to 14.834)	0.011
until 4 years	10	0.000 (0.000 to 1.338)	<	28	0.000 (0.000 to 3.889)	0.042
SOCS3 at 24 months vs relapses						
in year 2	31	0.000 (0.000 to 0.534)	<	9	12.086 (0.303 to 44.016)	0.008
SOCS3 at 24 months vs progression						
in year 2*	37	0.000 (0.000 to 0.726)	<	3	12.086 (7.088 to 100.121)	0.021
SOCS3 at 24 months vs MRI						
in year 2	34	0.000 (0.000 to 0.565)	<	5	12.737 (1.766 to 44.016)	0.039
until 2 years	34	0.000 (0.000 to 0.565)	<	5	12.737 (1.766 to 44.016)	0.039
SOCS3 change at 24 months vs MRI						
until 2 years	33	−3.337 (−10.616 to −0.483)	<	5	0.000 (−1.621 to 31.013)	0.016
in year 2	33	−3.337 (−10.616 to −0.483)	<	5	0.000 (−1.621 to 31.013)	0.016

N – number of cases; SD – standard deviation; IQR – interquartile range; SOCS3 – suppressor of cytokine signaling 3.

Supplementary Table 4. Statistically significant differences in viperin (all values in ng/mL) between patients with and without disease activity

Comparison	Non-active				Active		p-value
	N	mean ±SD or median (IQR)	N		mean ±SD or median (IQR)		
Baseline viperin vs NEDA							
in year 2	31	0.760 ±0.4035	>	11	0.458 ±0.3156	0.031	
Baseline viperin vs relapses							
in year 2	33	0.763 ±0.3915	>	9	0.381 ±0.2922	0.010	
Baseline viperin vs progression							
until 2 years	38	0.670 (0.447 to 0.970)	>	4	0.193 (0.0474 to 0.385)	0.005	
until 3 years	34	0.670 (0.447 to 0.947)	>	5	0.292 (0.0710 to 0.348)	0.003	
until 4 years	29	0.776 (0.480 to 0.991)	>	5	0.292 (0.0710 to 0.348)	0.002	
until 5 years	28	0.780 (0.469 to 1.012)	>	6	0.298 (0.0947 to 0.478)	0.005	
until 6 years	25	0.687 (0.428 to 0.991)	>	7	0.305 (0.144 to 0.510)	0.023	
until 7 years	20	0.765 ±0.4301	>	10	0.440 ±0.2991	0.041	
after year 7	14	0.503 (0.312 to 0.653)	<	5	1.054 (0.839 to 1.124)	0.044	
Baseline viperin vs MRI: no significant differences							
Viperin at 24 months vs relapses							
after year 4	17	0.570 (0.418 to 0.786)	>	12	0.263 (0.0749 to 0.532)	0.024	
after year 5	19	0.570 (0.360 to 0.734)	>	8	0.241 (0.0749 to 0.412)	0.022	
after year 6	19	0.570 (0.360 to 0.734)	>	7	0.325 (0.152 to 0.426)	0.048	
Viperin change at 24 months vs NEDA							
in year 8	13	0.0133 (−0.188 to 0.187)	>	6	−0.431 (−0.546 to −0.347)	0.005	
until 2 years	21	−0.364 (−0.630 to 0.119)	<	18	−0.124 (−0.326 to 0.355)	0.005	
after year 7	12	0.0280 (−0.234 to 0.212)	>	7	−0.364 (−0.534 to −0.271)	0.010	
Viperin change at 24 months vs relapses							
in year 2	30	−0.337 (−0.525 to 0.117)	<	9	0.0834 (−0.116 to 0.650)	0.008	
in year 5	23	−0.107 (−0.360 to 0.236)	>	6	−0.529 (−1.089 to −0.371)	0.023	
after year 3	15	0.0427 (−0.306 to 0.332)	>	14	−0.367 (−0.546 to −0.107)	0.046	
after year 4	17	0.0427 (−0.361 to 0.383)	>	12	−0.367 (−0.758 to −0.124)	0.018	
Viperin change at 24 months vs progression							
in year 8	14	0.0109 (−0.326 to 0.163)	>	5	−0.497 (−0.652 to −0.322)	0.010	
until 2 years	35	−0.246 (−0.509 to 0.109)	<	4	0.438 (0.107 to 0.661)	0.031	
until 3 years	31	−0.246 (−0.509 to 0.109)	<	5	0.355 (−0.0556 to 0.591)	0.047	
until 4 years	26	−0.286 (−0.512 to 0.117)	<	5	0.355 (−0.0556 to 0.591)	0.048	
after year 7	14	0.0109 (−0.326 to 0.163)	>	5	−0.497 (−0.652 to −0.322)	0.010	
Viperin change at 24 months vs MRI							
until 4 years	15	0.0427 (−0.384 to 0.332)	>	17	−0.364 (−0.530 to −0.202)	0.033	
until 5 years	15	0.0427 (−0.384 to 0.332)	>	17	−0.364 (−0.530 to −0.202)	0.033	

N – number of cases; SD – standard deviation; IQR – interquartile range; *small groups.

Supplementary Table 5. Statistically significant differences in MxA (all values in ng/mL) between patients with and without disease activity

Comparison	Non-active			Active		p-value
	N	mean ±SD or median (IQR)		N	mean ±SD or median (IQR)	
Baseline MxA vs NEDA						
after year 5	8	8.852 (2.570 to 26.005)	>	9	1.404 (0.294 to 2.543)	0.027
Baseline MxA vs relapses: no significant differences						
Baseline MxA vs progression						
after year 1	16	3.844 (1.853 to 12.141)	>	8	0.983 (0.196 to 2.052)	0.007
after year 2	17	3.829 (1.405 to 11.274)	>	7	1.404 (0.435 to 2.078)	0.040
after year 5	11	3.860 (2.086 to 19.293)	>	6	0.983 (0.393 to 2.001)	0.020
entire follow-up	16	3.844 (1.853 to 12.141)	>	8	0.983 (0.196 to 2.052)	0.007
MxA at 24 months vs NEDA: no significant differences						
MxA at 24 months vs relapses: no significant differences						
MxA at 24 months vs progression						
after year 1	12	8.127 (3.118 to 15.994)	>	7	2.536 (0.000 to 3.404)	0.022
after year 2	12	8.127 (3.118 to 15.994)	>	6	2.536 (0.000 to 3.404)	0.025
after year 3	10	11.269 (3.228 to 17.155)	>	5	2.774 (1.723 to 4.216)	0.040
entire follow-up	13	7.718 (3.173 to 15.414)	>	6	2.536 (0.000 to 3.404)	0.022
MxA at 24 months vs MRI						
until year 6	6	2.891 (0.393 to 3.228)	<	9	7.718 (3.127 to 15.414)	0.050
MxA change at 1 month vs relapses: no significant differences						

N – number of cases; SD – standard deviation; IQR – interquartile range; MxA – myxovirus resistance protein A.