Cytokine & Growth Factor Reviews 26 (2015) 241–248

Contents lists available at ScienceDirect

Cytokine & Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Mini review

Biological monitoring of IFN-β therapy in Multiple Sclerosis

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ARTICLE INFO

Article history:
Available online 24 December 2014

Keywords:
Multiple Sclerosis
Interferon-β
Biological activity
Nabs
MxA

ABSTRACT

Multiple Sclerosis (MS) is a heterogeneous disease and a variable percentage of patients are non-responders to common treatment. Early diagnosis of non-responders allows change to a more useful therapy for the patient and better allocates a large amount of financial resources. Quantification of Neutralizing antibodies (Nabs) and of biological activity of IFN-β are recognized approaches to identify immuno-pharmacological non-responders. A consistent number of studies have demonstrated that quantification of Myxovirus-induced protein A (MxA) is a valid biomarker to detect immune-pharmacological non responders after one year of treatment. Persistent high titre of Nabs and absence of biological activity predict abolition of IFN-β effects in disease activity measured through MRI, number of relapses and disability. Guidelines and flow-charts including both Nabs and MxA quantification are presented.

1. Introduction

Interferon beta (IFN-β) is the most commonly prescribed disease-modifying therapies (DMT) in relapsing-remitting multiple sclerosis (RRMS). IFN-β therapy reduces the annual relapse rate and the lesion load measured by MRI, brain atrophy and disability progression [1,2]. Unfortunately, most patients are non-responders to treatment [3]. As MS is a heterogeneous disease, all the approved DMTs are only partially active and a variable percentage of patients are non-responders. Non-responsive patients can be divided into two subgroups: pathogenesis-related non-responders and immuno-pharmacological non-responders. Pathogenesis-related non-responders present a pathogenic mechanism of disease that is not hampered by the biological activity induced by the DMT. The immuno-pharmacological non-responders are patients treated with IFN-β, as with all the protein-based DMTs, who develop Neutralizing antibodies (Nabs) that abolish the biological activity normally elicited by IFN-β. This mini-review will focus on the strategies for the early identification of immuno-pharmacological non-responders. Pathogenesis-related non-responders can be recognized only late, after the disease has already caused a permanent damage in the CNS, whereas immuno-pharmacological can be identified very early.

2. IFN-β treatment in MS

IFN-β has been available for MS patients since 1993, as the first drug able to modify the natural history of MS; an editorial in *Neurology* underlined the importance of that event as follow "The natural history of MS has been altered favourably, substantially and, above all, safely. Whether it is also the beginning of the end, time alone will tell. This is, I believe, the end of the beginning" [4]. The biochemical characteristics of IFN-β influence clinical efficacy, adverse events and risk of losing therapeutic efficacy. IFN-β is a recombinant cytokine available in 3 formulations, two as IFNb-1a and one as IFNb-1b. The latter is produced in *Escherichia coli* and it differs from the natural human product by amino-acid modifications and lack of glycosylation.

IFN-β uses the same metabolic pathways as the natural IFN-β including binding to the receptor IFNAR, activation of Janus kinase/signal transducer and activator of transcription (Jak/STAT) signalling pathway [5], and induction or reduction of expression of a

Abbreviations: ARR, annualized relapse rate; Babs, binding antibodies; CIS, clinically isolated syndrome; DMT, disease-modifying therapies; EDSS, expanded disability status scale; EFNS, European Federation of Neurological Societies; ELISA, enzyme-linked immuno-sorbent assay; IFN, interferon; IFNAR, IFN-β receptor; ISG, IFN-stimulated gene; MRI, magnetic resonance imaging; MS, multiple sclerosis; MxA, myxovirus-induced protein A; Nabs, neutralizing antibodies; PBMC, peripheral blood mononuclear cells; TRU, ten-fold reduction unit; WHO, World Health Organization.

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http://dx.doi.org/10.1016/j.cytogfr.2014.12.002
1359-6101/

large number of genes, collectively called Interferon Stimulated Genes (ISGs) [6]. The functional gene products of ISGs mediate the antiviral, growth-inhibitory and immune-regulatory functions attributed to IFN-β. Two companion chapters of this issue of Cytokine and Growth Factor Review are focused on the antiviral action of IFN-β in MS patients (Annibali et al.; Severa et al.).

3. IFN-β biological activity

The biological activity of a drug is defined as the total pharmacological, physiological and biochemical effects determined by the interaction of the molecule with its target receptor. Biological activity is a necessary, but not sufficient, condition for clinical efficacy of a drug, and as a consequence, a drug without biological activity is not clinical effective [7]. The measurement of IFN-β biological activity in every single patient can allow the detection of the subset of patients who are non-responsive to the drug for lack of biological activity.

The biological activity of IFN-β can therefore be studied by measuring a number of ISGs including MxA on the protein [8] or mRNA level [9–13], β2-microglobulin [14,15], neopterin [16], oligo-adenylate-synthetase [17,18], TNF related apoptosis inducing ligand (TRAIL) [11,15,19], viperin [20], IFI27, CCL2 and CXCL10 [21]. A recent study showed that the determination of phosphorylated STAT1 by phosphor-specific flow-cytometry could represent an excellent biomarker to monitor the biological activity of IFN-β [22]. In fact the degree of phosphorylation of pathway-specific transcription factors, which is directly related to IFNAR activation on the cell surface, could be a more reliable parameter than gene expression. The approach however requires further demonstration.

Among all the tested biomarkers of IFN-β biological activity MxA has proven to be one of the most reliable.

4. mRNA MxA as a biomarker of biological activity and clinical efficacy of IFN-β therapy

mRNA MxA expression in peripheral blood mononuclear cells (PBMC) can be used as a biomarker for IFN-β biological activity and clinical efficacy because of the following characteristics: (1) it is specifically and directly induced by IFN-β; (2) the methods of quantification of mRNA MxA are easy to perform and not expensive; (3) its expression differs in untreated and treated patients; (4) the absence of its induction indicates no residual biological activity; (5) it is involved in the pathogenesis and/or therapeutic action of IFN-β in MS; (6) it correlates with the clinical course of MS (Table 1).

(1) *MxA is specifically and directly induced by IFN-β*. MxA, one of the ISGs, is an antiviral protein that causes resistance to influenza virus. It has a dose-dependent specificity for IFN-α [23] and IFN-β in human mononuclear cells, but not for IFN-γ [24]. In the absence of viral infections it remains at low and constant levels [25]. MxA level is not influenced by relapses.

(2) *The methods of quantification of mRNA MxA are easy to perform and not expensive*. MxA was first measured on the protein level with methods such as chemiluminescence [26], ELISA [8,27] and FACS [28]. Later quantification of mRNA was preferred [9]; in fact as mRNA half-life is shorter than the protein half-life [27], mRNA MxA measurement evaluates the fluctuations of the transcript level associated with each IFN-β administration with greater precision. Another advantage of mRNA is that its levels, unlike the protein's, are not influenced by post-transcriptional modifications. Real-time-PCR has been shown to be the best method for mRNA MxA quantification [29], it is easily available in several laboratories [10,11,17,30–33] and its cost has decreased in recent years.

(3) *MxA expression levels differ between untreated and IFN-β treated patients*. It has been shown that low levels of mRNA MxA are constitutively expressed in untreated MS patient, with values similar to those of a healthy population, although about 5% of the patients show a moderate increase of the basal level of mRNA MxA [10,34]. While injection of INF-β significantly increases mRNA MxA expression within 3 h, peak levels of expression occurred at 12 h, with more than 10-fold increase above baseline [9,10,17,29,34,35]. Such increase is a necessary condition to distinguish IFN-β-treated from non-treated patients. The high level of mRNA MxA after 3 h and the peak at 12 h identify the suitable time interval for blood collection after IFN-β injection; measurement of mRNA MxA at 24 h is less sensitive because some patients display values in the pre-treatment range and could be classified as non-treated [34]. Usually patients self-inject IFNb in the evening and the blood tape scheduled 12 h after injection does not modify their habit and can be performed with routine blood examinations.

The level of MxA is affected by the number of injections per week, as shown by a study on five consecutive days [36]. An evening i.m. injection of IFN-β1-a at once a week induces an increase of mRNA MxA peaking 12 h after the injection and gradually tapering in the three following days. On the fourth day MxA levels are comparable in treated and untreated patients. Conversely IFN-β requiring three injections per week (IFN-β 1-a s.c.) or an injection every other day (IFN-β 1b s.c.) display two peaks of mRNA MxA in the two days following the infusion, with a cumulative effect on biological activity [36].

As MxA is an anti-viral molecule induced by IFN-β and IFN-α, both healthy persons and patients can show an increased level of MxA during symptomatic or asymptomatic viral infections [37].

(4) *The absence of MxA induction indicates no residual biological activity*. As the therapeutic mechanism of IFN-β in MS is complex and not completely defined, it can be argued that, IFN-β may still have a biological activity also in the absence of MxA induction through the induction of other ISGs. Hesse and collaborators [6] showed that low mRNA MxA levels in patients treated with IFN-β reflect a complete loss of drug bioactivity. Twelve patients with high levels of Nabs and no MxA induction did not show increased expression of other 1077 IFN-regulated genes. This study demonstrates that mRNA MxA is a reliable biomarker of the biological response to IFN-β therapy; in fact when it is not induced no other IFN-regulated gene is induced.

(5) *MxA is involved in the pathogenesis and/or therapeutic action of IFN-β in MS*. MxA protein was found by immunohistochemistry in post-mortem brains of MS patients not treated with IFN-β [38]. Positive staining was particular evident in active MS presenting early myelin degradation products and/or perivascular inflammation, while it was less intense in inactive lesions. MxA protein was detectable in infiltrating lymphocytes, in

Table 1
MxA as biomarker of biological activity and clinical efficacy of IFN-β therapy.

Characteristic	References
Specifically and directly induced by IFN-β	[23–25,86]
Easy to perform and cheap analysis	[8,9,26–29,87,88]
Significant different expression between treated and not-treated patients	[9,10,17,29,34–37,89]
Absence of expression indicates no residual biological activity	[6]
Involved in the pathogenesis and/or therapeutic action of IFN-β in MS	[38–41]
Correlated with the clinical course of MS; prognostic value	[30,90]

astrocytes and in endothelial cells. These data suggest that MxA can be induced by endogenous biologically active type I IFNs in the site of inflammation in untreated patients, and they indicate a role of MxA in the neuro-inflammatory process.

Viral infections can trigger relapses in MS [39] and MxA could play a direct role in the mechanism of action of IFN-β in MS acting as a powerful antiviral molecule [40]. The role of the IFN-β antiviral activity involved in reducing MS relapses is reviewed by Annibali et al. and Severa et al. in two companion chapters of this issue of Cytokine and Growth Factor Reviews.

A recognized mechanisms of action on IFN-β is the reduction of metallo-proteases, such as MMP9 and MMP2; their level of expression has been correlated with that of mRNA MxA in MS patients treated with IFN-β, indicating that the level of MxA reflects the induction of a therapeutic pathway in MS [41].

(6) *Levels of MxA correlate with the clinical course of MS*. The levels of mRNA MxA in PBMC during the treatment with IFN-β has a prognostic value, as they can predict the treatment efficacy. Malucchi et al. [30] performed a 3-years' study in 137 MS patients to establish whether the level of mRNA MxA at the end of the first year of IFN-β treatment correlates with relapse-free survival and with the time till the first relapse in the following two years. Patients without mRNA MxA increase showed a median time to the first relapse of seven months and only 21% remained relapse-free, whereas 57.5% of those with increased levels of mRNA MxA did not present relapses during the 2-years' follow-up ($p = 0.0001$, hazard ratio (HR) 2.87).

5. Factors influencing IFN-β biological activity

5.1. Therapy adherence

Chronic diseases are characterized by a low level of adherence [42]. A recent review about the adherence to injectable DMTs in MS showed that weighted mean adherence ranged between 58.4% and 69.4% [43]. Forgetfulness or anxiety about the injection, and adverse effect (flu-like symptoms, injection site reaction and fatigue) are common obstacles to adherence [43].

Clearly, in the absence of injection, MxA does not increase; therefore if a patient under treatment with IFN-β does not present MxA increase and we can exclude the presence of Nabs and Babs, not adherence must be thought of (Fig. 2). A previous work [31] singled out 1.8% such patients (3 out of 167).

5.2. Neutralizing antibodies (Nabs) and Binding antibodies (Babs)

IFN-β is a protein inducing antibodies collectively named Binding antibodies (Babs) as they can bind the protein. Some Babs can neutralize the biological activity of IFN-β and they are called neutralizing antibodies (Nabs). The major cause of loss of biological activity is the presence of Nabs against IFN-β, which have high affinity for specific epitopes on the host protein involved in the binding of IFN-β with its receptor IFNAR. Nabs prevent the interaction between IFN-β and IFNAR, which in turn blocks downstream IFN signalling, transcription of ISGs and expression of ISGs products [6,8,10,14]. As a consequence Nabs block the clinical effect of IFN-β [44] and immuno-pharmacological non-responders show a lack of clinical efficacy due to development of Nabs. Nabs positivity peaks after 6–12 months of treatment, while Babs are detectable since the first trimester of therapy [45]. About 50% of Bab-positive patients become Nab-positive [46,47]. Babs development, in particular with high titres, precedes the appearance of Nabs [48,49].

5.2.1. Nabs measurement

The principle of the assays used until now in different laboratories is similar: serum samples are incubated with IFN-β, then added to cells belonging to cultured cell lines responsive to IFN-β; if the samples contain Nabs, the binding IFN-β-IFNAR will be hampered and antiviral proteins will be not induced. Cytopatic effect assay (CPE) [50,51]), MxA Protein assay [52] and MxA gene expression assay [53] are the three main methods used.

CPE is based on IFN-β antiviral activity; after the incubation described above, the cells are infected with a specific virus and the cellular vitality is measured; the presence of Nabs blocks the antiviral activity, preventing cellular protection and leading to cells death. The World Health Organization (WHO) approved the use of CPE to detect Nabs [54] and it recommended the use of human lung carcinoma cell line A549 and encephalomyocarditis murine virus. This assay has also been suggested by the European Guidelines by European Federation of Neurological Societies (EFNS) [55].

MxA protein assay uses an ELISA method for the quantification of the MxA protein, that will be found only if the serum does not contain Nabs [52]. MxA gene expression assay quantifies mRNA MxA through a real-time PCR method [53].

The assays used in the different laboratories vary as to the cell line, the IFN-β preparation and concentration, the incubation period and the virus [55].

Lack of a standardized method led "marketing authorization Holders" to promote a study to validate a common method using a MxA protein assay [56]. The validation obtained on 62 samples tested by three firms producing IFN-β provided consistent results. This allows to consider the method suitable to detect Nabs in patients treated with IFN-β. The method is superior to the CPE because it can be performed automatically, it requires less time and it does not need virus manipulation.

A recent study [57] tested a non-cell-based assay to detect Nabs. The method utilizes chemioluminescence to detect the binding of IFN-β to a recombinant IFNAR receptor that is coated to plate. The presence of Nabs in the serum analyzed prevents the binding of some or all the ruthenium-conjugated molecules of IFN-β to the immobilized receptor and the test signal is reduced. The signal reduction will be directly proportional to the amount of Nabs. The study tested 114 samples and found excellent consistency of the results with those obtained by the classic methods. The advantage of the test lies in the absence of cell lines and of viruses. Further studies are needed to demonstrate the real usefulness of the test as it showed a lower sensitivity than the already available tests [57].

5.2.2. Impact of Nabs in the biological activity expressed as MxA level

In order to evaluate the correlation between MxA expression and Nabs status, mRNA MxA levels were examined in 99 untreated patients, 17 healthy volunteers and 92 INF-β treated patients [10]. Of the IFN-β treated patients 15 (16%) were persistently Nab-positive (Nab+ $\geq$2 consecutive positive samples), 68 (74%) Nab-negative, 9 (10%) isolated Nab-positive (one positive sample). The biological activity was measured after a single dose of each of the three commercial IFN-β formulations (IFN-β 1a i.m.; IFN-β 1b s.c.; IFN-β 1a s.c.). Results demonstrated that MxA expression was greater in Nab-negative and isolated Nab-positive patients than in persistently Nab-positive patients. These data emphasize a strong correlation between the presence of Nabs and the absence of biological activity, in agreement with a previous study [8]. Only three Nabs-negative (3/68, 4.4%) patients showed mRNA MxA levels in the range of un-treated patients. This suggests that mechanisms other than Nabs could abolish biological activity: presence of non-neutralizing binding antibodies [31], diminished IFNAR expression, increased soluble circulating IFNAR [58] or low therapy adherence [31].

Even if the methods to detect Nabs have a sensitivity limit of 5 ten-fold reduction unit (TRU) per ml [59,60], conventionally the titre >20 TRU represents the positivity threshold and the titre 20 TRU is considered of "low" clinical impact [50,51,60]. Many studies demonstrated that a high titre of Nabs abolish the biological activity; however the definition of high titre greatly varies from one laboratory to another, ranging between >45 TRU and >600 TRU [10,32,35,61–63]. The relationship between Nabs and loss of biological activity is not an all/nothing one; on the contrary there is an inverse correlation with the biological activity decreasing with the increasing titres. The phenomenon has been reported by two studies, the first published in 2008 [62] and the second in 2011 [31]. The first study regarded 97 patients with MS under treatment with IFN-β. Nabs were determined by the MxA protein method [52] and the biological activity by quantifying mRNA MxA through Real Time PCR. Nabs titres lower than 150 TRU/ml were associated with the presence of biological activity, titres between 150 and 600 TRU/ml were associated with reduced biological activity, whereas titres higher than 600 TRU/ml caused loss of expression of mRNA MxA. The second study was performed on 167 patients. Nabs were measured by CPE, Babs by ELISA and biological activity by Real-time PCR [31]. Also in that study the biological activity progressively decreased with increasing Nabs. In particular biological activity was absent in 92% of patients (11/12) with a Nabs titre >100 TRU, in 73% of patients (11/15) with values between 20 and 100 TRU and in 30% of patients (6/18) with values lower than 20 TRU. This shows that patients with Nabs titres higher than 100 TRU are very likely to experience loss of biological activity of IFN-β. Patients with titres between 5 and 100 are in a "grey area" and they need to be closely monitored as to biological activity and Nabs titre.

6. Impact of Nabs on disease activity

Many studies and several years have been necessary for most neurologists to accept that patients with persistently high levels of Nabs show abolished clinical effect of INF-β treatment and consequent increase of relapses, disease progression and MRI activity [44,55,64–66].

The role of Nabs has been hard to define for various reasons: different methods of quantification, late clinical and radiological repercussions, different duration of the studies, high number of patients needed to evaluate the disappearance of the moderate effect of IFN-β. Moreover in some patients Nabs disappear continuing the treatment [45]; different formulations carry different risks of inducing Nabs [67,68]; it is impossible to organize a randomized trial [65].

Defining the lowest titre of Nabs leading to a clinically loss of efficacy is very difficult and to date there is no consensus on a threshold. As the presence of biological activity is indispensable for clinico-radiological activity, it is reasonable to use Nabs titres blocking the biological activity as those blocking the therapeutic activity of IFN-β.

Nabs appear about 6–12 months after the beginning of the treatment with IFN-β [61,67–69]. As the drug clinical efficacy is modest (about 30% reduction of the attacks) and Nabs begin to impact on the clinical picture after 18–24 months' treatment, only clinical trials of ≥3 years duration, non-randomized prospective studies and real-world propensity-score studies indicate that Nabs reduce or abolish the therapeutic efficacy of IFN-β on relapses, independently of the type of IFN used [44,55,64–66]. Clinical trials with all the three types of IFN-β, namely IFN-β 1b [70], IFN-β 1a s.c. [71] and IFN-β 1a i.m. [72], demonstrated the negative impact of Nabs on disease activity or on MRI. A recent trial with IFN-β 1b in Clinical Isolated Syndrome (CIS) did not detect any negative clinical effect but only an effect on MRI, because the patients are in an early phase of the disease and have a low risk of clinical attacks and therefore they constitute a population unsuitable to verify the impact of Nabs [73].

In 2003 Sorensen et al. [44] performed the first observational prospective non randomized real-world study on the impact of Nabs on relapse rate, time of the first relapse and disability progression. Five hundred and forty-one patients with MS were recruited, treated with different formulations of IFN-β, tested for Nabs every 12 months and followed for up to 60 months. The study showed higher relapse rate in patients with antibodies (0.64–0.70) than in those without antibodies (0.43–0.46; $p > 0.03$) and a higher risk to develop relapses in the Nab positive period than in the Nab negative one (odds ratios in the range 1.51–1.58 ($p < 0.03$)). The time from the beginning of treatment to the first relapse was 8.1 months (244 days) longer in Nab-negative patients [44].

Although EDSS worsened more in Nab-positive than in Nab-negative patients, the difference was not statistically significant because of the low number of patients followed up for 5 years (52 patients). Later real-world studies confirmed the negative impact of Nabs on the efficacy of IFN-β [30,46,74–80]. The most recent study on the impact of Nabs on clinical outcome has been performed in 567 Italian RRMS patients. In this 5-years observational study, the Nab-positive period was characterized by a significant increase in relapse rate (IRR = 1.39; $p = 0.0076$) and by a shorter time to first relapse (IRR = 1.71; $p = 0.0038$) than the Nab-negative period. Propensity score analysis in a selected cohort of patients demonstrated a negative trend of Nabs on the time to reach the EDSS 4 (IRR = 2.94; $p = 0.0879$) [81].

7. Guidelines and consensus for the quantification of Nabs in IFN-β treated MS patients

Nabs quantification has been the topic of European [55] and American guidelines [64] and of international [65] and Italian consensus [66]. In 2005 a EFNS Task Force on IFN-β antibodies in MS drew guidelines on the use of anti-IFN-β antibody measurements in MS [55]. The guidelines confirmed the usefulness of testing Nabs and suggested to discontinue the treatment with IFN-β in Nab-positive patients. The guidelines of the American Academy of Neurology [64] acknowledge the negative impact of high titres of Nabs on the efficacy of IFN-β, but thought that technical difficulties and discrepancies in the test prevented its implementation in the clinical practice. The decision was strongly criticized [82]. European guidelines and international and Italian consensus define a flow chart for the use of the test in the clinical practice. In particular the European guidelines [55] indicate to dose Nabs 12 months after beginning the treatment and again after 24 if negative. If the test is still negative there is no need to repeat it unless a new attack occurs or disability progresses. If on the contrary Nabs are present at 12 months, the test must be repeated after three months and if still positive with high titre (>100 TRU) IFN-β should be discontinued. The international [65] and Italian [66] consensus take into consideration also clinical course, evaluation of the biological activity through dosage of MxA and Nab positivity with low titre (Fig. 1).

8. Implementation of Nabs and of MxA quantification in the clinical practice for the identification of IFN-β non-responder subsets

The great number of scientific evidences on the value of MxA as a bio-marker of biological activities and on the role of Nabs in blocking activity and efficacy of IFN-β allow us to use them to identify non-responders to the treatment (Fig. 2). The flow chart in Fig. 2 points to dose MxA on the 12th month of treatment. If there is no biological activity, Nabs have to be dosed and a high title identifies the subgroup of immuno-pharmacological non-responders. In those patients IFN-β has to be discontinued and substituted

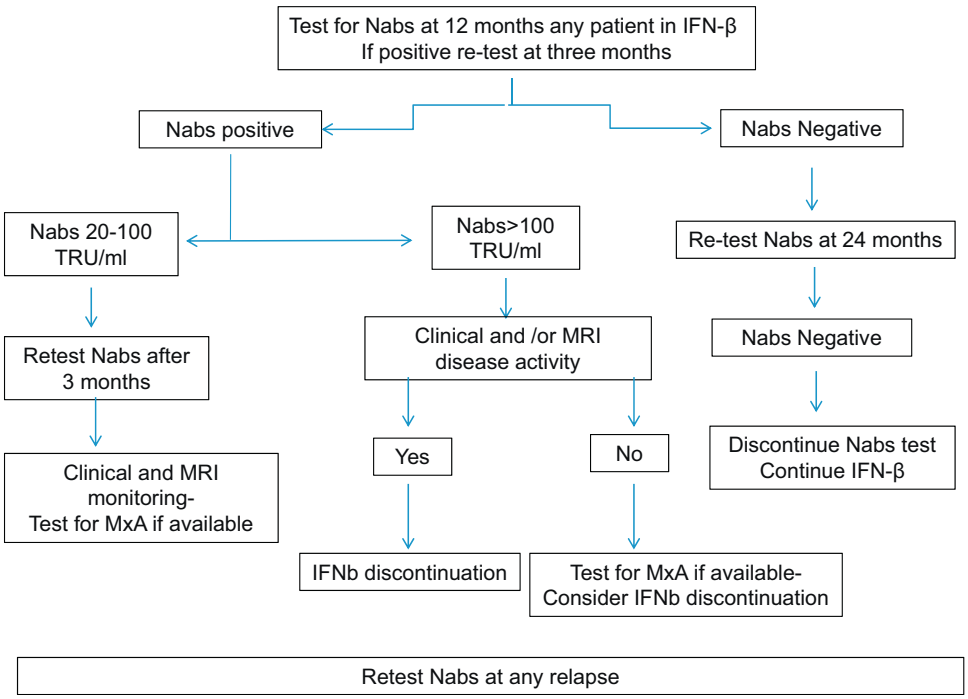

Fig. 1. Flow-chart for Nabs quantification in patients treated with IFN-β.

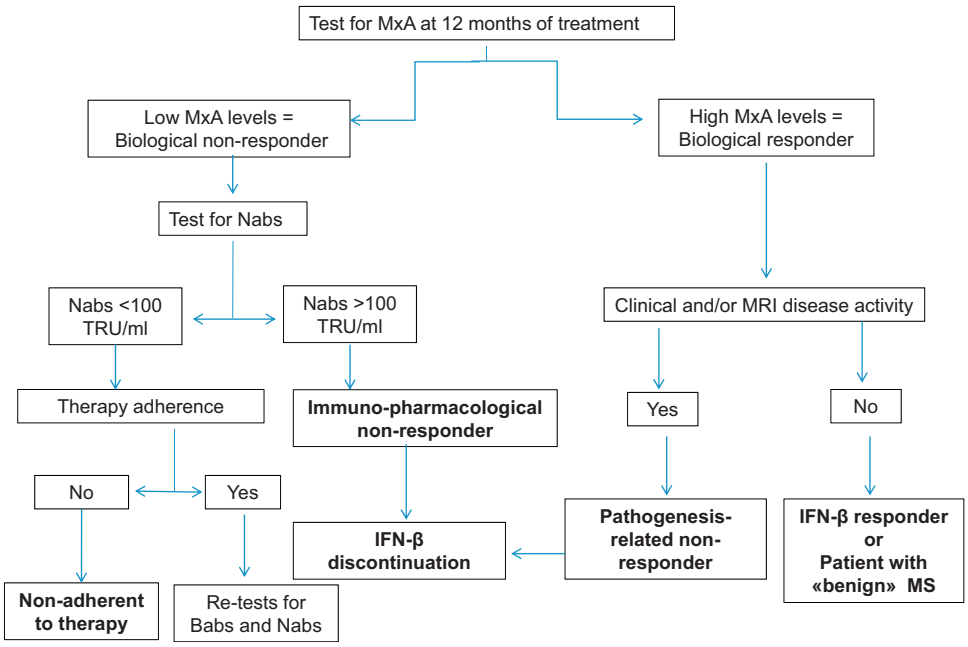

Fig. 2. Clinical and biological flow-chart for identification of subsets of IFN-β non-responders.

by another DMT. On the contrary if the absence or low values of biological activity are associated with negative or low title of Nabs, it is necessary to check adherence to treatment and presence of Babs and to monitor the patient by repeating the evaluation of biological activity. If there is biological activity, it is necessary to evaluate clinical and MRI activity. The presence of active disease identifies patients whose disease has a pathogenic mechanism non responding to IFN-β (pathogenesis-related non-responders). Conversely patients with no or low disease activity are classified as IFN-β responders. This category cannot be distinguished from the so-called "benign" patients whose disease is not active not because of the treatment but because of no disease activity during the observation period. The flow chart has to be applied once a year and in case of disease activity or disability progression.

9. Conclusion

IFN-β is a milestone in MS treatment, with a moderate therapeutic efficacy, but with a well definite safety profile evidenced by more than 20 years of utilization. The integration of biological, clinical and MRI follow-up can allow early identification of non-responders [83,84], a strategy not available for any of the other DMTs. Safety, early identification of non-responders and new IFN-β pegylated molecule [85] will offer a reliable treatment option to person with MS also for the next years.

Conflict of interest

Dr. Antonio Bertolotto received honoraria for serving in the scientific advisory boards of Almirall, Bayer, BiogenIdec, Genzyme, and received speaker honoraria from BiogenIdec, Genzyme, Novartis, TEVA with approval by the Director of AOU San Luigi University Hospital; his institution has received grant support from Bayer, BiogenIdec, Merck, Novartis, TEVA from the Italian Multiple Sclerosis Society, Associazione Ricerca Biomedica ONLUS and San Luigi ONLUS.

Acknowledgements

A.B. is supported by Associazione Ricerca Biomedica Onlus and San Luigi Onlus.

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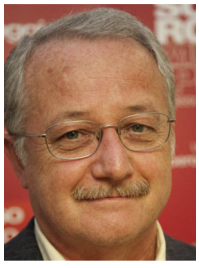

Antonio Bertolotto, graduated in Medicine and specialized in Neurology at the University of Turin. He worked as Clinical Neurologist in the University of Turin till 1992, then he moved to the new Second Medical School of the University of Turin at S. Luigi Hospital, Orbassano (Turin), where he created the Multiple Sclerosis Clinic (CRESM = Centro Riferimento Regionale Sclerosi Multipla) for Piedmont including Clinical Neurobiology Laboratory, Experimental Neurophysiology Laboratory and Bio-bank. Dr. Bertolotto is involved in both clinical activity and research in MS. The Clinical Neurobiology Laboratory serves as the Italy centre for the detection of antibodies neutralizing Interferon beta and Natalizumab. The research at CRESM is focused on the identification of non-responders to Disease Modifying Therapy, on the evaluation of the effects of pregnancy on gene-expression, on anti-central nervous system auto-antibodies, on haematopoietic stem cell transplantation and several other clinical trials. Dr. Bertolotto is a member of the scientific board of the Italian Association for MS and of the "Therapeutics and Technology Assessment subcommittee of the American Academy of Neurology" for Interferon beta Neutralizing antibodies.

Letizia Granieri graduated in Biological Science in March 2006 and specialized in Clinical Pathology in July 2012 at the University of Turin, Italy. From October 2003 to March 2006 she worked at the Neuroendocrinology Laboratory, Department of Anatomy, Pharmacology and Forensic Medicine University of Turin on a research intership and she studied cutaneous nerve fibres regeneration in skin grafts after burn injury. Since May 2006 she has been carrying out diagnostic and research activities (research fellowship) at the Clinical Neurobiology Unit of the Piedmont Regional Referring Multiple Sclerosis Center (CRESM) of San Luigi Gonzaga University Hospital (Orbassano, Turin, Italy). She has gained experience on cerebrospinal fluid examination, drug immunogenity evaluation and central nervous system autoimmunity. Her principal research fields are monitoring biological response to different treatments in multiple sclerosis patients and the study of anti-AQP4 antibodies in Neuromyelitis Optica (NMO) and related pathologies.

Fabiana Marnetto graduated in Medical Biotechnology in July 2004 and got a Medical Specialization in Clinical Biochemistry in July 2008 at the University of Turin, She has been working in the Clinical Neurobiology Unit of the Piedmont Regional Referring Multiple Sclerosis Center (CRESM), at San Luigi Gonzaga Hospital (Orbassano, Turin, Italy) since 2004. In 2007 she was awarded a 4-year fellowship from the University of Turin for a project on fatigue in multiple sclerosis to be carried on in the laboratory of Clinical Neurobiology CRESM at San Luigi University Hospital in Turin. Since 2012 she has had a Project Contract Position with the Cavalieri Ottolenghi Foundation, and she is currently working in the Clinical Neurobiology Unit of CRESM at the Neuroscience Institute Cavalieri Ottolenghi (NICO), San Luigi Gonzaga Hospital. Her research activity includes: (1) Antibodies in autoimmune diseases (anti potassium channel KIR4.1 antibodies in MS and anti-Aquaporin 4 antibodies in neuromyelitis optica (NMO): detection assays, clinical correlations, pathogenic role. (2) Evaluation of clinical/biological response to different therapies in MS and NMO (Interferon beta, Natalizumab, Rituximab). (3) Study of Epstein Barr virus (EBV) involvement in the pathogenesis of MS. She is a member of the Society of Italian Biotechnologists (ANBI).

Paola Valentino obtained her Master of Science Degree in Medical Biotechnology in 2005 from the University of Turin where she also earned a Board Certification in Clinical Biochemistry in 2009. Since 2005 she has been working as a research fellow in the Clinical Neurobiology Laboratory, University Hospital San Luigi Gonzaga of Orbassano (Turin). She has gained experience in gene expression analysis and evaluation of drug immunogenicity; in particular she collaborated in the study of biological markers for the response to IFN beta therapy, of mechanisms of anti-IFN beta neutralizing activity, of gene expression changes in pregnancy in MS patients. She also participated in the evaluation of a real time PCR method to monitor patients treated with Rituximab. At present she is involved in the study of anti-KIR4.1 autoimmune response in multiple sclerosis patients. She is a member of the Italian Association of Neuroimmunology (AINI).

Arianna Sala graduated in Biological Science in 2000 and then specialized in Clinical Pathology in 2006 at the University of Turin. Since 2000 she has been working at the Clinical Neurobiology Laboratory of the Regional MS Centre at San Luigi Hospital at Orbassano. She is principally involved in the diagnostic process of inflammatory diseases of the nervous system and in the development of novel laboratory procedures for the advancement of diagnostic technologies. She is in charge of the MS Biobank at the same institution.

Marco Capobianco graduated in Medicine in 1997 and then specialized in neurology in 2002 at the University of Turin with a dissertation on Autologous Haematopoietic Stem Cells transplantation on Multiple Sclerosis. He was coordinator of the Young Neurological Association of the Italian Neurology Society (SIN) between 1998 and 2002. He began to work at the Regional MS Centre of San Luigi Gonzaga Hospital in Orbassano in 2002 and he deals with both clinical and research activities. He is mostly working in outpatient and inpatient clinics: in particular he is responsible of the management of the outpatient clinic for monitoring strategies of MS treated patients. He is also involved in several clinical trials and translational researches on Multiple Sclerosis (MS) and Neuromyelitis Optica spectrum disorders (NMOSD) and he is co-author of several papers on both topics. He is co-responsible of the Italian NMO Database Initiative (INMODI) of the MS Scientific Group of the Società Italiana di Neurologia (SIN). He has attended numerous scientific meeting about clinical neurology and neuroimmunology.

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Alessia Di Sapio graduated in Medicine at the University of Turin in 1991 and specialized in Neurology in the same University in 1995. She has been involved in clinical activity and research about multiple sclerosis since 1998, when she began to work at the MS Centre of the Emergency Neurology Department at San Giovanni Battista Hospital. In the meantime she started to work as a neurophysiologist. In 2001 she moved to the Neurology Department of San Luigi Gonzaga Hospital, where in 2003 she joined the Regional Referral Multiple Sclerosis Centre under the direction of Doctor Antonio Bertolotto. Her daily activity include clinical follow-up of in- and out-patient, coordination of clinical trials, diagnosis and research in clinical neurophysiology. At present she is involved in research programmes focused on the optimization of neurophysiological tests to measure disease progression in multiple sclerosis patients. She is a member of the Italian Society of Neurology (SIN) and of the Italian Society of Clinical Neurophysiology (SINC).

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Manuela Matta obtained a Doctor of Medicine degree at the University of Cagliari in 2000 and a Specialization degree in Neurology at the University of Sassari in 2005 (Graduated magna cum laude) and a Specialization degree in Psychotherapy in 2009 at the School of Psychotherapy, School of Clinical Psychology SSPC-IFREP, Ateneo Salesiano, Rome. She obtained a PhD in Neuroscience in 2009 at the University of Sassari. Her research activity was focused on multiple sclerosis and neuro-ophthalmology: eye movement evaluation; tensilon tests; eye movement recording and its analysis (Labview and Matlab). After her PhD she undertook a training in neuro-ophthalmology at "The Daroff-Dell'Osso Ocular Motility Laboratory (OMLAB)", Louis Stokes Cleveland DVA Medical Center, Case Western Reserve University, Cleveland, OH, USA. Since 2012 she has been working at the Centre of Multiple Sclerosis–CReSM, San Luigi Gonzaga Hospital Orbassano (Turin). Her work consists of (1) clinical management of patients with multiple sclerosis (MS), (2) participation in clinical trials in patients with MS, (3) neuro-ophthalmologic evaluation in MS and in other neurological diseases.

Marzia Caldano graduated in Pharmacy in 2003 and specialized in Clinical Biochemistry in 2008 at the University of Turin. From 2003 to 2013 she was granted a research fellowship at the Clinical Neurobiology Laboratory of Regional Referral Multiple Sclerosis Centre (CReSM), San Luigi Hospital; since 2014 she is a research fellow at Fondazione Cavalieri Ottolenghi (NICO). During her activities, Dr. Caldano gained relevant experience in drug immunogenicity, cell cultures, gene expression analysis and cerebrospinal fluid analysis. She is in charge of an Italian Service for the detection of anti-Interferon and anti-Natalizumab antibodies in multiple sclerosis patients. Currently her studies are focused on personalization of therapy and identification of new biomarkers to establish the efficacy of treatment.