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Drug Efficacy Monitoring in Pharmacotherapy of Multiple Sclerosis With Biological Agents

Marzia Caldano, PharmD, William Raoul, PhD,† Theo Rispens, PhD,‡ and Antonio Bertolotto, MD**

Abstract: Multiple sclerosis is a heterogenous disease. Although several EMA-approved disease-modifying treatments including biopharmaceuticals are available, their efficacy is limited, and a certain percentage of patients are always nonresponsive. Drug efficacy monitoring is an important tool to identify these nonresponsive patients early on. Currently, detection of antidrug antibodies and quantification of biological activity are used as methods of efficacy monitoring for interferon beta and natalizumab therapies. For natalizumab and alemtuzumab treatments, drug level quantification could be an essential component of the overall disease management. Thus, utilization and development of strategies to determine treatment response are vital aspects of multiple sclerosis management given the tremendous clinical and economic promise of this tool.

Key Words: biopharmaceuticals, therapeutic drug monitoring, drug efficacy monitoring, health economics

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INTRODUCTION

Multiple sclerosis (MS) is an autoimmune, inflammatory, and degenerative disease of the central nervous system (CNS) that affects more than 2 million people worldwide. MS is characterized by chronic inflammation leading to CNS damage that results in neurological deterioration along with a multitude of other symptoms.

Depending upon the pattern of the progression of disease, 3 subtypes have been characterized: (1) relapsing remitting MS (RRMS): this is the most common disease course, characterized by the appearance of new or increasing neurological symptoms. These attacks, known as relapses, are followed by periods of partial or complete remissions, during which the symptoms may disappear, or may continue and become permanent. However, there is no continuous progression of the disability. Approximately 85% of all patients with MS are initially diagnosed with RRMS; (2) primary progressive MS (PPMS): this subtype is characterized by the worsening of neurological functions (accumulation of disability) right from the onset of the symptoms, without early relapses or remissions. Approximately 15% of patients are diagnosed with PPMS; (3) secondary progressive MS (SPMS) subtype follows an initial relapsing-remitting course. Most patients diagnosed with RRMS eventually evolve in to a SPMS which is characterized by progressive worsening of neurological functions with accumulation of disability. Here, evidence of disease activity as indicated by relapses or changes on magnetic resonance imaging (MRI) may or may not be present.[1]

Over the past decade, the landscape of care for MS has changed tremendously due to the advent of multiple disease modifying treatments (DMTs). Till date, 15 pharmaceutical formulations have been approved (Table 1) for RRMS. Amongst these, only mitoxantrone and IFNβ1-b are approved for SPMS as well. These DMTs differ with respect to the efficacy, formulation, method and schedule of administration, and possible adverse drug reactions (ADRs) in addition to cost. These latest formulations also include biopharmaceuticals such as different formulations of IFNβ, monoclonal antibodies (MAbs) against α4/β1 and β7 integrin (NAT) and anti-CD52 (alemtuzumab). Many of these drugs are associated with serious ADRs such as cardiac events, opportunistic infections, and secondary autoimmunity.[2] Therefore, the selection of the right drug for the right patient, or personalized treatment, is highly desirable. Consistent progress has been made towards the identification of pharmacogenomic markers of DMT response[3] in MS. However, limited pharmacogenetic or pharmacogenomic tests are available to predict the efficacy of a treatment till date, and as a result, predicting patient response to DMT in advance is very difficult. The general approach is to weigh benefits and risks taking into

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From the *Neuroscience Institute Cavalieri Ottolenghi (NICO) and Neurology Department—Centro Riferimento Regionale Sclerosi Multipla (CReSM), San Luigi University Hospital, Turin, Italy; †CNRS, GICC UMR 7292, Université François-Rabelais de Tours, Tours, France; and ‡Sanquin Research and Landsteiner Laboratory, Department of Immunopathology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands.

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Correspondence: Caldano Marzia, PharmD, Clinical Neurobiology Laboratory, Neuroscience Institute Cavalieri Ottolenghi (NICO), Neurologia—San Luigi Hospital, Regione Gonzole 10, 10043 Orbassano, Turin, Italy (e-mail: neurobiologia@sanluigi.piemonte.it).

TABLE 1. EMA and FDA Approved DMTs for Multiple Sclerosis

Treatment	Brand Name	Type of MS	Posology and Route of Administration
IFNβ-1a	Avonex	RRMS	30 mcg weekly, IM
IFNβ-1a	Rebif 22 Rebif 44	RRMS	22 or 44 mcg 3 times a week, SC
IFNβ-1b	Betaferon Extavia	RRMS SPMS	250 mcg every other day, SC
PEG-IFNβ-1a	Plegridy	RRMS	125 mcg every 2 wk, SC
Glatiramer Acetate	Copaxone 20 mg Glatopa 20 mg Copaxone 40 mg	RRMS	20 mg once a day or 40 mg 3 times a week, SC
Natalizumab	Tysabri	RRMS	300 mg every 28 d, IV infusion
Fingolimod	Gilenya	RRMS	0.5 mg once a day, PO
Mitoxantrone	Novantrone	RRMS SPMS	12 mg/m^2 every 3 mo, IV infusion with a lifetime cumulative dose of no more than 140 mg/m^2
Teriflunomide	Aubagio	RRMS	7 or 14 mg daily, PO
Dimethyl Fumarate	Tecfidera	RRMS	120 mg twice a day for 7 d, PO; after 7 d, 240 mg twice a day, PO
Alemtuzumab	Lemtrada	RRMS	First course: 12 mg/d on 5 consecutive days, IV infusion Second course after 1 yr: 12 mg/d on 3 consecutive days, IV infusion

PO, orally.

consideration factors such as the aggressiveness of the disease, the efficacy of the drug, and the possibility of ADRs. In addition, several other factors including tolerability, planning of pregnancy, preference and life style of the patient, previous treatments, adherence to treatment, clinical, and MRI examinations along with the cost may play an equally important role in the selection of the right drug. In most cases, the neurologists and patients must rely on a "trial and error" approach. This is inadequate and risky because a treatment failure can cause an irreversible damage of CNS functions. Thus, an approach like drug efficacy monitoring is important to enable the physician to detect nonresponsive patients as early as possible. Monitoring of drug efficiency can essentially include any biochemical, clinical or genetic evaluations that could aid in modulation of drug type, dosage or schedule of administration to optimally benefit the patient and minimize the possibility of ADRs. On the other hand, the concept of therapeutic drug monitoring (TDM) essentially involves measurement of the concentration of the drug in the serum. In the context of MS, TDM alone may not be sufficient to provide enough information regarding drug response to enable the physician to effectively individualize the treatment. Therefore, drug efficacy monitoring in MS must include other components such as the quantification of antidrug antibodies (ADA) (induced by IFNβ or NAT), and evaluation of biological activity in addition to TDM in order to predict the efficacy of biopharmaceuticals. However, the measurement of biological activity can be useful in clinical practice only if a biomarker is specifically up- or down-regulated after the drug administration.[4,5]

In the present review, attempts have been made to explore the available literature with respect to 2 of the most commonly used biopharmaceuticals in MS, namely, IFNβ and NAT, in addition to a newer drug such as alemtuzumab, and delineate the available methods for drug efficacy monitoring in detail.

IFNβ

Mechanism of Action of IFNβ

Natural IFNβ, the type I IFN, is secreted by fibroblasts. It binds to the IFN receptor and activates the JAK/STAT pathway to phosphorylate STAT1 and STAT2.[6] These factors dimerize and associate with IFN regulatory factor-3, and bind to IFN-stimulated response elements in the cell nucleus. This, in turn, activates hundreds of IFN-stimulated genes and leads to the production of antiviral, antiproliferative, and antitumor products.[7] The mechanism of action of IFNβ is complex. It balances the expression of antiinflammatory and proinflammatory cytokines, reduces the trafficking of inflammatory cells across the blood-brain-barrier, and increases the production of nerve growth factor. Moreover, in the peripheral blood, it increases the number of natural killer cells, which are producers of antiinflammatory mediators. In MS, IFNβ acts via decreasing annualized relapse rate (ARR), the risk of sustained disability progression, reducing MRI lesion activity and brain atrophy. It might also delay the onset of clinically definite MS after the first appearance of neurological symptoms.[8]

Drug Level

To evaluate IFNβ serum level an "antibody sandwich" ELISA has been developed, which involves coating the plates with a mouse monoclonal antihuman IFNβ antibody.[9,10] However, drug level has never been used as a parameter to monitor the efficacy of any form of IFNβ, because of relatively short half-lives (range: 5–78 hours).

A pharmacokinetic study carried out in a group of 6 patients with MS receiving 6 MU of non-PEGylated IFNβ-1a intramuscular (IM) once a week, demonstrated that the IFNβ-1a levels become detectable at 4 hours, and peak at 8 hours postinjection. IFNβ-1a levels became undetectable in serum 24 hours postinjection. Peak serum levels range from 92 to 102 IU/mL, with a mean of 94.8 IU/mL.[9] Additionally, other recent studies conducted on a new formulation of PEGylated IFNβ-1a (PEG-IFNβ) have shown that the concentration peak, measured using an ELISA, occurs later in this form of IFN as compared with the non-PEGylated IFNβ-1a (~36 hours).[11] After subcutaneous doses of PEG-IFNβ-1a in patients with MS, the mean Cmax is 280 pg/mL, and the peak of serum concentration occurs between 1 and 1.5 days. The pharmacokinetics (PK) profile of PEGylated form in a study involving 1512 patients with RRMS was consistent with that in healthy subjects. In healthy volunteers, the median area under the curve (AUC) from time 0 to 168 hours postdose [AUC (0, 168 hours)] was reported to be 27.2 ng·mL^{-1}·h,

while in patients with MS the same AUC (0, 168 hours) ranged from 23.5 to 32.0 $ng \cdot mL^{-1} \cdot h$.[12]

A dosing regimen of PEG-IFNβ-1a once every 2 weeks provides 4.5-fold higher cumulative AUC, as compared with non-PEGylated IFNβ-1a administered weekly. Although definitive exposure–efficacy relationships are yet to be established, the increased cumulative exposure potentially explains the maintained efficacy of PEG-IFNβ-1a despite its reduced dosing frequency. However, such pharmacokinetic studies have only helped to define the best route and frequency of administration, and have not been utilized so far in the individualization of the treatment.

Pharmacogenomics: Identification of Biomarkers

Quantification of biological activity of IFNβ allows the early identification of patients who are not responsive to the treatment. The biological activity of IFNβ is investigated by evaluating a number of IFN-stimulated genes, induced by IFNβ injection, including myxovirus-resistance protein A (MxA) at the level of protein or mRNA, b2-microglobulin, oligo-adenylate-synthetase, TRAIL, viperin, IFI27, CCL2, and CXCL10.[13] A strong risk of relapses in the absence of biological activity has been found.[14] The European recommendations suggest the combined evaluation of MxA mRNA and ADA to assess the continuing efficacy of IFNβ therapy.[15]

Anti-IFNβ Antibodies

Several studies have reported the occurrence of binding antibodies (BAbs) and neutralizing antibodies (NAbs) against IFNβ during the treatment.[15] A majority of the patients develop BAbs, however, only NAbs interfere with the biological activity of IFNβ, and they are present in a smaller proportion of patients with ADA. NAbs inhibit the binding between IFNβ and IFN receptors, abolishing its biological activity and, consequently, the therapeutic effect. The development of BAbs occurs during the first months of IFNβ treatment, whereas the occurrence of NAbs requires several months. Most patients become positive for NAbs during the first 18 months of therapy and rarely during the second or third year of treatment as well.

The importance of quantification of the NAbs and of the biological activity of IFNβ in the management of patients with MS is underlined by the European and Italian National Guidelines,[16,17] and by international expert consensus[17] that provide recommendations for timing of measurement and therapeutic consequences of NAbs against IFNβ, and of absence of biological activity (Fig. 1).

ELISA, both with or without a capture antibody, is the most commonly used method for BAbs measurement.[18] For NAbs measurement, 3 methods are used based on the antiviral MxA protein: (1) cytopathic effect assay, considered as "gold standard" and recommended by both the World Health Organization and European Guidelines[18]; (2) MxA protein assay,[19] and (3) MxA gene expression assay.[20] Another type of assay based on the evaluation of luciferase expressed after sera incubation on cells transfected with an IFN-regulated luciferase reporter-gene construct has been proposed.[21]

ADA abolishes the biological activity of IFNβ, but also other factors such as noncompliance and soluble circulating IFNβ receptors could contribute to the lack of biological activity.[13] Many evidences indicate that NAbs reduce or abolish the therapeutic efficacy of IFNβ in preventing relapses, independently of the type of IFN used.[15–18] In fact, MRI, clinical disease activity,[15] and the risk of disability progression are higher in NAbs-positive patients.[22] The risk of development of NAbs varies between <1% and 31% for different IFNβ formulations.[23,24] This immunogenicity difference is intensely

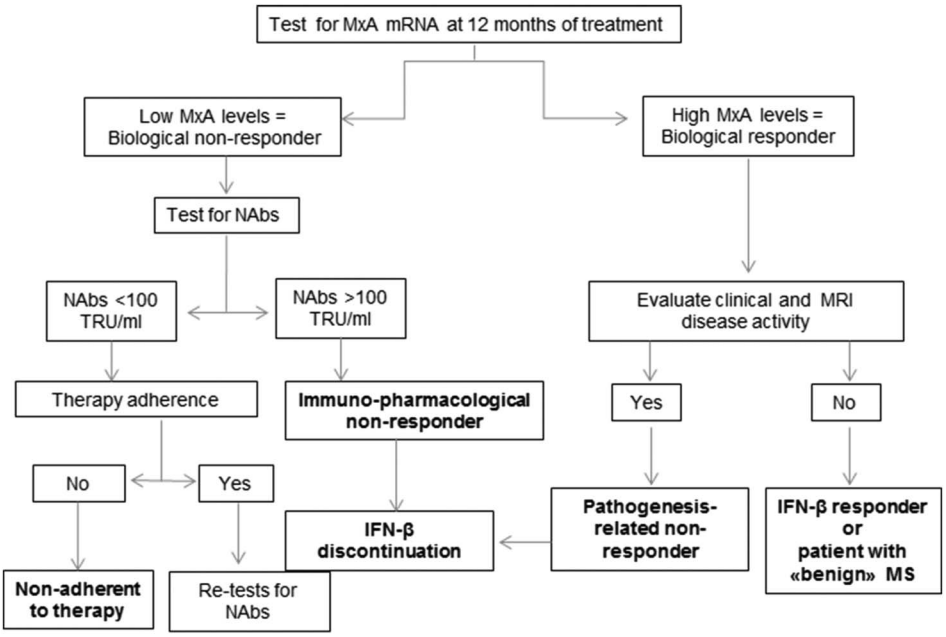

FIGURE 1. Clinical and biological flow chart for identification of subsets of IFN-β responder and non-responder patients using pharmacogenomics and anti-IFNβ ADAs quantification.[48]

influenced by excipients, route, and timing of administration and drug composition that differ among the various formulations.

Neurologists face 2 options during management of MS in patients. Multiple weekly injections offer more clinical efficacy than once a week injection. However, in this approach, many more patients are at risk of becoming NAbs positive than patients treated once a week. As a result, they will lose the clinical benefits of IFNβ. Moreover, they must be switched to another category of DMT, as NAbs are cross-reactive against all types of IFNβ.[25]

NATALIZUMAB

Mechanism of Action of Natalizumab

NAT is a humanized MAb that binds to the α4-subunit of β1 integrin, also called CD49d antigen, which is highly expressed on all leukocytes, except neutrophils. Specifically, after binding to the α4β1 integrin, NAT blocks the interaction of this integrin with its receptor, vascular cell adhesion molecule-1 (VCAM-1), and other ligands. Disruption of these interactions avoids transmigration of leukocytes across the blood-brain-barrier, and recruitment of activated T lymphocytes into inflamed tissue, and may suppress inflammation in the CNS. Normally, VCAM-1 is not expressed in the brain. However, in the presence of proinflammatory cytokines, it is upregulated in endothelial cells and possibly in glial cells close to the sites of inflammation.

A phase III placebo-controlled study[26] showed the efficacy of NAT in reducing ARR and preventing disability progression which might be higher or comparable with IFNβ. These findings were confirmed by another independent trial that compared NAT plus IFNβ-1a against IFNβ-1a alone.[27]

Drug Level

Population-based modeling of the relationship between dose, concentration, and effects, that is, PK and pharmacokinetics-pharmacodynamics (PK-PD) of NAT, could help to precisely quantify individual sources of variability based on dynamic biomarkers and considering the onset of adverse events. Readers are encouraged to see Chapter 6 of this TDM special issue and the article by Ternant et al[28] for the rationale of developing PK-PD modeling of monoclonal antibodies in TDM of inflammatory diseases. From an initial phase I study,[29] it was concluded that doses from 0.03 to 3 mg/kg were safe, despite minor side effects. The approved 300 mg dose every 4 weeks leads to a mean half-life of 16 ± 4 days and a mean clearance of 13.1 ± 5 mL/h (file EMA/H/C/000603), depending on weight and anti-NAT antibodies. This dose was chosen to achieve 70% of α4-integrin saturation throughout the 28-day dosing interval. In the MS221 study from Biogen (reported in FDA clinical pharmacology and biopharmaceutics review, application number 125104), cytometry analysis of receptor occupancy was nearly saturated at all tested doses ranging from 1 to 6 mg/kg, however, the duration of saturation increased with increasing dose levels. As the noncompartmental analysis performed in this study does not adequately describe the nonlinear elimination of PK and, therefore, receptor saturation, it could be relevant to describe PK profile by compartmental approach, using, for example, a Michaelis–Menten-type elimination to address this problem. However, PML was found to be a major safety concern. Khatri et al[30] developed a plasma-exchange strategy to swiftly reduce concentrations of circulating NAT to restore immune surveillance in the brain; VLA-4 desaturation appears to take place below 1 mcg/mL of circulating NAT. However, the rate of wash-out may vary considerably between patients, which suggests that measurement of NAT concentrations may be helpful to guide plasma exchange strategy.[31] Evaluation of serum NAT concentrations is complicated since NAT can exchange Fab arms with endogenous human IgG4.[32] Several immunoassays were developed to quantify serum NAT concentrations accurately[33,34] without interference by Fab arm exchange nor IgG4 Fc interactions. Interestingly it has been shown that both low NAT concentration, below 1 mcg/mL, and high antibody titers, are associated with a lack of therapeutic efficacy.[35]

Utilizing paired CSF and serum samples, a recent study shows that it would be helpful to measure free and cell-bound NAT to determine the optimal individual NAT dosing regimen for patients.[36] DELIVER study[37] suggests that NAT will probably lead to similar efficacy whatever the administration route (intravenous (IV), subcutaneous (SC) or IM). PK profiles were quite similar with variations in Cmax: SC and IM were about 40% lower than IV, and mean bioavailability relative to IV was about 50% with SC or IM administration. Mean trough serum concentrations were lower with IM administration.

Pharmacodynamics of Natalizumab

Apart from ADA, current data available in the literature do not allow clinicians to design a personalized dosing regimen. However, Defer et al[38] found a 55% decrease of CD49d expression on circulating T and B lymphocytes after NAT infusion. This low level remained stable for the entire period of treatment, except for patients who are ADA positive, in whom CD49d levels reverted to pretreatment levels. Thus, this antigen expression could be used to monitor the effectiveness of NAT. Millonig et al[39] confirmed this finding, suggesting that CD49d is decreased on T cells, but also on B cells and NK cells. Moreover, they showed a significant decrease of serum sVCAM-1 concentration in ADA-negative patients. sVCAM-1 concentration reverts to pretreatment levels in case of ADA development. CD49d and sVCAM-1 could be useful in establishing a personalized timing of NAT administration.

Anti-Natalizumab Antibodies

Clinical trials with NAT have demonstrated the possibility of ADA generation with this treatment.[40] ADAs induce a loss of efficacy with a higher risk of adverse events.[26,35,40] The proposed mechanism of loss of clinical outcomes is the formation of NAT-ADAs immune complexes that lead to enhanced clearance and decreased functional serum concentration of NAT.[35] As per current data, 9%–12% of NAT-treated patients develop ADA, out of which 6% remain persistently positive and 3%–6% are transiently positive for ADA.[40] The treatment is discontinued if the measures reveal persistent ADAs. Patients with infusion reactions, or with disease activity should be tested for ADAs. The assay

currently used to evaluate the presence of anti-NAT antibodies is a standardized bridging ELISA method developed by Biogen Idec (Cambridge, MA); protocol "Assay procedure to determine Natalizumab (Tysabri) immunogenicity (CST02-180AP-R.2)."[40] The combined measurements of ADA, NAT serum level, and CD49d could be utilized to tailor a personalized infusion regimen. These measurements could also be useful to determine the withdrawal of NAT in patients with persistently high levels of ADA.

ALEMTUZUMAB

Mechanism of Action of Alemtuzumab

Alemtuzumab is a MAb of the IgG1 subclass that selectively binds to the CD52 protein, present in large amounts on the surface of T and B cells, and to a lesser extent on other cells. The treatment with this drug induces the depletion of circulating T and B cells, followed by repopulation. The repopulation phenomenon is faster for B cells and slower for T lymphocytes. Alemtuzumab action in MS is therefore attributable not only to the destruction of T and B cells, but also to the way in which the repopulation occurs. This treatment has minimal impact on other immune cells, ensuring the protection of the innate immune system. Clinical studies[41,42] comparing alemtuzumab and IFNβ SC 3 times a week, demonstrated that the former reduces both ARR and disability progression more efficiently than IFNβ.

Drug Level

From the EMA approval of alemtuzumab for leukemia in 2001 till its approval for MS in 2014, all pharmacokinetic studies have been carried out only in patients with leukemia. ELISA and FACS have been the assays used in these studies for the assessment of the alemtuzumab serum concentration.[43] In MS, the approved treatment strategy is 12 mg IV daily for 5 consecutive days, and 12 mg IV daily for 3 consecutive days administered 12 months after the first treatment course. This treatment regimen results in a mean Cmax of 3014 ng/mL on day 5 of the initial treatment course, and 2276 ng/mL on day 3 of the second treatment course. The half-life of this drug is approximately 4–5 days, and is comparable between courses. The serum concentration of alemtuzumab reaches low or undetectable levels within approximately 30 days following each treatment course (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR-Product_Information/human/003718/WC500150521.pdf). In addition, attempts have been made in patients with chronic lymphocytic leukemia to delineate the pharmacokinetics of alemtuzumab. A two-compartment model with nonlinear elimination has been proposed by Mould et al. In this study performed in 2007, they demonstrate that the maximal trough concentrations range from 3.6 to 21.0 mg/mL with a mean of 10.2 mg/mL in responders, and below the limit of quantification to 26.8 mg/mL with a mean of 5.9 mg/mL in nonresponders. Additionally, a direct relationship between maximal trough concentrations and clinical outcomes was also described, with increasing alemtuzumab exposure resulting in a greater probability of positive tumor response.[44] Data from any such studies in patients with MS are so far unavailable. Therefore, it would be interesting to design future prospective studies in MS to model dose-concentration-effects relationships of alemtuzumab, and investigate if indeed it is similar to that observed in chronic lymphocytic leukemia. Such studies of alemtuzumab pharmacokinetics in patients with MS would also aid in the implementation of TDM strategies and further individualization of treatment with this drug.

Anti-Alemtuzumab Antibodies

Alemtuzumab-binding antibodies have been shown to be present in 29% of patients immediately before the second course of treatment, and in 86% of patients 1 month after the second course of treatment.[41] The percentage of patients whose test results were considered positive for antibodies to alemtuzumab using an ELISA and confirmed by a competitive binding assay. The presence and concentration of antialemtuzumab antibodies do not seem to influence either the efficacy or the safety of the MAb[41] nor the pharmacodynamics at the beginning of treatment course. However, their impact after many doses remains to be established.

It has been shown that, during the first 5 years of treatment, almost one-third of the patients develop a secondary autoimmunity, in particular thyroid autoimmunity (30%), and idiopathic thrombocytopenic purpura (2%). Some studies have suggested that the pretreatment evaluation of IL-21 serum level could predict the development of posttreatment autoimmunity. However, currently available ELISA kits to evaluate IL-21 level seem to fail as predictive tests to evaluate this potential biomarker of secondary autoimmunity.[45]

Economic Impact of Drug Efficacy Monitoring

Very few studies have investigated the economic impact of drug efficacy monitoring in MS, and all of them have so far focused only on IFNβ. An Austrian study showed that testing for ADA against IFNβ, according to the European guidelines, is cost effective because it reduces total direct costs by approximately 34 million € in 5 years. Translated to the whole of Europe, the reduction of total direct costs would amount to approximately 594 million €.[46]

An Italian study has estimated the annual cost of managing patients with RRMS, with and without NAbs. The results have shown an increase of 3100 € per patient-year as the consequence of the onset of NAbs. Considering the patients with MS treated with IFNβ in Italy and the percentage of NAbs development, the evaluation of ADA could allow a better allocation of approximately 10 million €/year.[47]

For the other DMTs, no study related to the drug efficacy monitoring exists to date, although considering their cost, relapses and disability progression in young patients, it would be surprising if drug efficacy monitoring strategies would not be more cost effective.

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