NEW DIRECTION IN THE IMPROVEMENT OF CLINICAL CONDITIONS IN MULTIPLE SCLEROSIS PATIENTS

F. De Silvestri, E. Romani, A. Grasso

Introduction

Multiple sclerosis (MS), in its many and varied clinical forms, is an inflammatory and demyelinating disease of the nervous system characterized by perivascular infiltration of mononucleal inflammatory cells (lymphocytes and plasma cells), resulting in impaired glia, demyelination, loss of neuronal activity and synaptic foci of demyelination have the character to appear in various stages of development, reflecting the trend clinical oscillating in time of illness.

An important aspect is the presence of immune T cells type anti-oligodedrocytes (MOG - oligodendroglial myelin glycoprotein), that testifying in part the course of autoimmune desease. These lymphocytes, attack the myelin structure that surrounds the axon, degrading it gradually and in stages over time, so as to affect the entire operation to the inside and to the outside of the nervous system. In fact, the primary neuronal activity is to transmit and receive electrical signals, said action potentials along long routes, paths and synapses, coated by an insulator which allows all forms of operation: the myelin sheath. In demyelinating disease of the patient's immune system attacks and damages the sheath with autoimmune mode; for this time-axonal neuronal activity is compromised.

The name "Multiple Sclerosis" is derived from the multiple scars (sclerosis, better known as plaques or lesions) that form in the white matter of the spinal cord and brain, which are in various stages of evolution and involution. Although the autoimmune mechanism that causes the disease is well known enough, the initial trigger is still unknown.

The most credible hypothesis as a possible cause of the disease are of genetic, infectious, environmental and food. In the context of infectious diseases has an important role mononucleosis and the respective Epstein-Barr virus. In addition, many diseases or asymptomatic course-like infuenzali or seasonal factors may trigger the autoimmune syndrome; in this regard, it recalls the importance which over time has been given to the exanthematous diseases and in particular to the measles virus. This reference has a significant value because exanthematous diseases can cause subacute demyelinating encephalomyelitis with clinical and histopathological very similar to multiple sclerosis. It is also very important to note that some vaccinations (polio ...) can lead to forms of subacute disseminated encephalomyelitis-like measles very similar to demyelinating diseases, particularly multiple sclerosis.

In addition, the body in its immunocompetent system keeps in the long latent pathogens of various types (capsids, fungi, parasites, viruses, toxic agents such as various types of metals) that can modify the immune system in a direct or indirect or for accumulation, resulting in a reaction at a distance and / or chain is relevant to the inflammatory-demyelinating syndrome and its consequences axonal, synaptic and clinics.

The balance between the immune system which held back in time various risk factors and external pathogens, can be modified in various stages of life but especially between 20 and 50 years, and particularly in women, for many reasons, environmental , hormonal, food.

Finally, we note that there is an important relationship between some forms of demyelinating autoimmune diseases such as progressive multifocal leukoencephalopathy in the genesis and paraneoplastic diseases demelinizzanti primary, the mielinoclasiche subacute encephalomyelitis and multiple sclerosis; does not seem to be any relationship between MS and demyelinating diseases such as genetically determined "Adrenoleukodystrophy", the "MLD", the "disease Shilder" and "disease Balo" (concentric sclerosis).

Although the etiology of multiple sclerosis appears as yet unknown, it is believed that the following items, in general, be associated with autoimmune diseases, may be factors triggering the immune reaction; Therefore, we can assume that the etiologies are multiple, variable and specific to each individual, but always genetically determined.

Some examples of possible pathogenetic can be:

- Epstein Virus presence and / or the tracks (capsids) due to a course of mononucleosis;
- presence of other types of viruses and / or related tracks (capsids) related to a course of exanthematous diseases such as measles;
- the presence of bacteria and / or related traces;

- presence of fungal infections (eg Candida albicans) in the course silent or asymptomatic;
- presence of toxins in it from time;
- presence of toxins derived from external factors of type pollutant;
- presence of toxin-induced prolonged pharmacological treatments;
- toxins derived from recruitment and / or contact with elements "toxic" as some heavy metals.

Multiple sclerosis may present with various clinical and then become evident over time with various forms of physical, psychological and cognitive evaluated with many steps, including the EDSS (Expanded Disabilty Status Scale).

2014 there are still some methods that can restore balance to the immune compromised. Some drug treatments are available to hold new attacks, and reduce disability. The prognosis is difficult to predict and depends on many factors, while the life expectancy is approximately from 5 to 10 years lower than that of the healthy person.

Multiple sclerosis is a common cause of acute and chronic disability in young and middle-aged. It usually occurs for the first time between 20 and 50 years, with a peak incidence in young adults, affecting twice as many women than men. A specific cause is unknown, although genetic factors seem to be decisive in the predisposition to develop the disease.

The patient usually experiences a neurological deficit of various types (double vision, optic neuropathy, hemiparesis, paraparesis, bladder disorders, ataxia, paresthesia migrants syndromes algo-paresthetic ...) and is treated with nonsteroidal anti-inflammatory drugs (oral, bolus or intramuscular). In periods of quiescence between episodes and the other, are generally administered immunosuppressive or immunomodulatory treatments, which depending on the case are suspended during the acute phases.

All treatments proposed to deal with this disease have various side effects of varying severity and cause a deterioration of the general condition and immune recovery without providing an improved and stable, often with severely impaired quality of life.

MS and lipids metabolic syndrome: a new point of view

In developed countries is reported a dramatic increase in obesity, insulin resistance, dyslipidemia, hypertension, low glucose tolerance, diabetes mellitus, endothelial dysfunction, pro-thrombotic and pro-inflammatory conditions.

At cellular and molecular level, we must carefully consider the role of adipocytes, hepatocytes, muscle cells and insulin action, insulin resistance, endothelial dysfunction and the inflammation emerging areas. It follows from the foregoing, we are experiencing an era in which the metabolic syndrome has become a matter of discussion and scientific investigation as necessary as important. Because metabolic syndrome is epidemic in it's spread, it's clear that the way of life whit a well balanced diet and physical

activity, play a primary role in the onset of metabolic syndrome and related conditions and becoming fundamental elements of prevention and therapy.

In numerous studies have highlighted the pathological significance related to alterations in lipids metabolism, shedding new light on metabolic syndrome in various diseases.

It is therefore considered that the main cause of multiple sclerosis can be traced to transcription factors in cell nuclei that control the absorption, distribution and release of lipids (fats and similar compounds) throughout the body. The alteration of protein, known as "peroxisome proliferator-activated receptors (PPARs)", in particular of PPAR-alfa, would cause a dysfunction of peroxisomes concerning the peroxidation metabolism and consequently the production of LDL oxidant, a toxic byproduct commonly known as" bad "cholesterol. LDL forms the atheromatous plaques on the affected tissue, increases ROS such as hydrogen peroxide causing an pH organic increase and the Cu-Zn-SOD activation (in MS copper and zinc level are lower). A similar mechanism is involved in atherosclerosis, in which the PPAR alteration results in the accumulation of plaques as immune response and scars in the coronary arteries. The plaques buildup in blood vessels determines an immune response mediated by CNS macrophages located in microglia, as a result of inflammatory events with alteration of the endothelial wall: the blood-brain barrier (BBE).

Vasculitis reactive provokes the variation of the permeability of the vasal endothelium with further passage in the brain of T lymphocytes directed towards the CNS. T cells, previously sensitized, (sensitization due to

viruses, bacteria, toxic substances) penetrate into the CNS where recognize, perhaps through the help of macrophages microglia, endogenous molecules such as MPB (myelin basic protein) or MOG (myelin oligodendrocyte glycoprotein) and are able to activate them and trigger a further inflammatory event through production of pro-inflammatory cytokines. Th0 activated lymphocytes differentiate into Th1, responsible for inflammation, Th2 lymphocytes, architects inhibition of inflammation, and Th17 cells that recently both in EAE in MS that have proved relevant in inflammatory processes. The Th1 and Th17 penetrated areas of the CNS begin to replicate releasing pro-inflammatory cytokines such as IL-2, IL-12, IL-17, IL-21, IL-22, TNF and INF-γ, damaging oligodendrocytes responsible coating axonal in the CNS and then causing axonal damage. Lymphocytes autoreactive are lymphocytes escaped from negative selection implemented by the thymus. Women have a greater propensity to immune responses:

- thymus more developed
- transplantation allotypic rejection more frequent
- resistance to tolerance induction

Estrogen promote hyperactivity of B cells also have a different lipid metabolism than humans ever-related hormone.

More recent studies indicate that PPARs also play critical roles in controlling immune responses. Authors have previously demonstrated that PPAR-γ agonists modulate the development of experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS).

This review will discuss the cellular and molecular mechanisms by which these agonists are believed to modulate disease. The therapeutic potential of PPAR-γ agonists in the treatment of multiple sclerosis will also be considered.

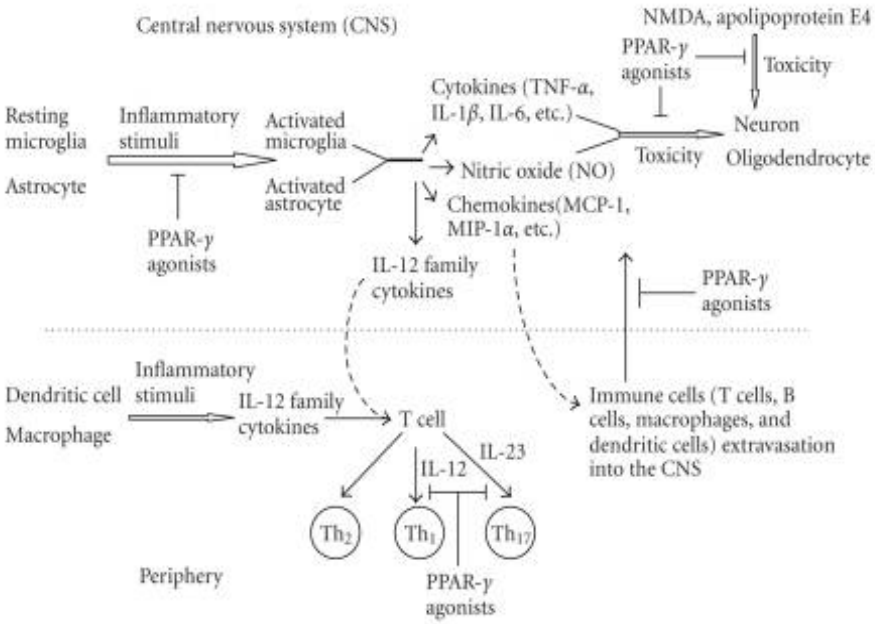

Figure 1: PPARs role in immune response

Foamy macrophages, containing myelin degradation products, are abundantly found in active multiple sclerosis (MS) lesions. Recent studies have described an altered phenotype of macrophages after myelin internalization. However, mechanisms by which myelin affects the phenotype of macrophages and how this phenotype influences lesion progression remain unclear.

Data show that myelin modulates the phenotype of macrophages by PPAR activation, which may subsequently dampen MS lesion progression. Moreover, results suggest that myelin-derived PS mediates PPARβ/δ activation in macrophages after myelin uptake.

Statins and their use in autoimmune diseases, the interaction and synergies whit tetracyclines

Minocycline is a tetracycline which presents discrete solubility in water (5% g / mL) and medium polarity (CLOGP = 0). These physical properties provide it a high oral bioavailability, the possibility of being administered intravenously as hydrochloride form and a great antibacterial activity.

Minocycline is mainly used as an antibiotic, while presenting a spectrum of activity and mechanism of action similar to that of tetracycline. It is more active, because of its characteristics mildly hydrophilic, on many bacterial species such as Staphylococcus Aureus, Streptococci, Neisseria Meningitidis, various Enterobacteriaceae, Acinetobacter, Bacteroides, Haemophilus, Nocardia, Propionibacterium Acnes and some Mycobacteria.

Even if there is a partial cross-resistance, some strains resistant to various tetracyclines remain sensitive to minocycline probably due to a better penetration of the same in the bacterial wall and commonly found application as anti-inflammatory in the treatment of acne in the acute form.

Some studies (Brundula et al. - 2002) show that minocycline reduces the levels of enzymatic activity in the matrix metalloprotease (MMP) -9, that in patients with Multiple Sclerosis presents values of over- activity. The subsequent degradation of the extra-cellular matrix protein of the basal lamina of blood vessels (Yong et al., 2001) makes it possible the contact of leukocytes with the nervous system. In other studies (Yrjanheikki et al., 1998) is analyzed the anti-inflammatory action of minocycline and the modulating inhibition of nitric oxide synthesis in caspase 1-3 (Chen et al.,

2000) and in the process of modulation and in the activation of cellular mitogenesis, through phosphorylation of the kinase 8 (MAPKs) (Du et al., 2001; Lin et al., 2001). Minocycline attenuates the immune activity of microglia and cytotoxicity of glutamic acid (Tikka and Koistinaho, 2001; Wu et al., 2002; Yrjanheikki et al., 1999) and inhibits cells apoptosis by preventing mitochondrial permeability (Zhu et al., 2002). They also documented anti-inflammatory and neuroprotective actions of brain lesions in Huntington's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis (Chen et al., 2000; Du et al., 2001; Wu et al., 2002; Yrjanheikki et al., 1999; Zhu et al., 2002). More generally, recent studies (Maier et al., 2007) show several neuroprotective properties of minocycline in autoimmune diseases at the central nervous system level.

Multiple sclerosis (MS) is the most common autoimune inflammatory demyelinating disease of the central nervous system (CNS). A new approach to improvement in the treatment of multiple sclerosis is to identify suitable combinations of existing drugs or therapies that may well affect the various aspects in disease process. Statins are drugs that inhibit the synthesis of endogenous cholesterol acting on the hydroxymethylglutaryl-CoA reductase, which converts the molecule of 3-hydroxy-3-methylglutaryl-CoA to mevalonate, a precursor of cholesterol. Some statins, such as atorvastatin, have demonstrated anti-inflammatory and neuroprotective properties in murine animal models treatment in which was induced a form of Multiple Sclerosis called Experimental Autoimmune Encephalomyelitis (EAE).

Statins are promising candidates in future treatments of MS. In recent

studies (Neuhaus et al. - 2005) was discussed the role of statins in immune mechanisms and immunomodulators GA and IFN-β context. Statins in correlation with these agents show a synergic action mechanism in the inflammatory cascade of MS. An immunomodulatory action of statins, compared to IFN-β, was documented on T and B lymphocytes in MS patients; the two agents combination brought a decrease in T cell proliferation (Neuhaus et al., 2002). Promising results have been obtained with the combined use with statins such as simvastatin in patients with MS and atorvastatin in patients with rheumatoid arthritis (McCarey et al, 2004 .; Vollmer et al, 2004 .; Neuhaus et al., 2005b). Statins are effective in preventing and reducing the incidence of chronic and relapsing forms of Experimental Autoimmune Encephalomyelitis (EAE) in mice (Stanislaus et al., 1999, 2002; Youssef et al., 2002).

It's seen that the combination of suboptimal doses of GA and atorvastatin (Stuve et al., 2006a) or lovastatin and 5-aminoimidazole-4-carboxa- mide-1-β-D-ribofuranoside (AICAR), an immunomodulating agent (Paintlia et al., 2006), has synergistic effects and improvements that cause remission and / or prevention of Experimental Autoimmune Encephalomyelitis (EAE), so more than the optimal doses of both drugs individually taken. As shown in these researches, agents with various immune modulation and neuroprotective mechanisms can be combined in a synergistic interaction in autoimmune diseases treatment.

In this study, is evaluated the combination of minocycline and atorvastatin would bring a better response than the two drugs taken individually; the application was conducted on mice models induced with Experimental Autoimmune Encephalomyelitis (EAE).

From these studies it appears that the combination of minocycline and atorvastatin decreases the degree of disability in mice with experimental autoimmune encephalomyelitis (EAE). The combined treatment, compared to the use of single agents, bring a significant reduction in severity disease both acute and chronic, and attenuation in demyelination and a better axonal function. Is also detected a significant reduction of anti-oligodendrocytes (MOG), whose presence means local inflammation.

Atorvastatin is a lipophilic statin, in association with minocycline has immunomodulatory effects and promotes Th2 response in leukocyte functional differentiation (Aktas et al., 2003; Lyons et al., 2007; Youssef et al., 2002) through cytokine secretion (interleukin 4), inhibiting T-lymphocytes proliferation and antibodies anti-MOG infiltration. A very strong atorvastatin anti-inflammatory action in the EAE treatment has been reported in other studies (Youssef et al.2002) where is shown that, in addition to its effect on the immune system, atorvastatin promote the neuronal cells recovery. For this reason atorvastatin has an important role as immunomodulator in the acute relapsing-remitting multiple sclerosis and as neuroprotective agent, in the chronic phase of the disease. It's important remembering that statins act on different fronts and in this case atorvastatin, inhibiting HMG-CoA reductase, plays a critical role in the mevalonate pathway that regulates the synthesis of cholesterol, as well as isoprenoids that mediate the associations to the membrane GTPasi (Zhang and Casey, 1996 - Dunn et al., 2006)

The combination of GA and IFN-β-1a and IFN-β and azathioprine-1a has been evaluated in clinical studies, including patients with relapsing-remitting MS (Lublin and Reingold, 2001). Experiments "in vitro" have documented the immunological modulation action of statins comparable to IFN-β (Neuhaus et al., 2005a) and showed a synergy immunomodulatory effect evoked by the combination of atorvastatin and glatiramer acetate; the same effect in EAE (Stuve et al., 2006b).

So these studies demonstrate that agents with different mechanisms of immune modulation can be combined in the MS treatment and provide the rationale for testing the combination between atorvastatin and minocycline. Experimentation has shown that the combination of low doses of atorvostatin and minocycline is effective to decrease the severity in clinical experimental autoimmune encephalitis (EAE), promoting neuronal protection in the acute phase and preventing the progression of the disease; for against other groups in treatment with drugs not combined together showed however a good improvement level, albeit lower. Furthermore, the combination atorvastatin - minocycline significantly reduced the inflammatory infiltrates and the demyelination level. These findings were reinforced by immunological and serological analysis. The significant recovery of neuronal cells and the reduction of glial reactivity can be seen as the result of the synergistic combination atorvastatin-minocycline, thus demonstrating this particular role both in recovery and in prevention of MS in its different forms.

In mice immunized with EAE treated separately with minocycline and atorvastatin, the average number of NeuN-positive neurons was

significantly reduced compared to non-immunized mice, while in EAE mice treated with the atorvastatin and minocycline combination the 73% neurons were preserved (treatment duration: 26 days).

The atorvastatin - minocycline combination has markedly decreased MOG-specific autoantibodies compared to animals treated with atorvastatin alone; this is particularly important when you consider that the demyelinating lesions formation depends on the synergy between T cells and MOG-specific autoantibodies responses (Adelmann et al., 1995). The activation of the complement cascade by MOG-specific autoantibodies bound to myelin surface, triggers demyelination and, at the same time, increases local inflammatory response through pro-inflammatory factors production.

In conclusion, our results indicate that the atorvastatin and minocyclin combination facilitates neuroprotection and reduces inflammation. Furthermore, our results demonstrate that atorvastatin and minocycline in combination reduce EAE, both clinically and immunologically. This therapeutic strategy seems to be a promising new approach in multiple sclerosis treatment.

It is noted that minocycline, an antibiotic, favors the Candida Albicans development. This can be suitably eradicated with modulated assumption of antifungal drug as fluconazole. Is reported the hepato-toxic potential of fluconazole, which in this case is combined with that of atorvastatin.

Study of volunteers with multiple sclerosis and evaluation before and after treatment in EDSS score (Expanded Disability Status Scale)

It's been selected a sample of 150 volunteers (75 males - 75 females) aged between 35 and 55 years with the disease course of a minimum of 5 to a maximum of 20 years and different levels of disability scale .

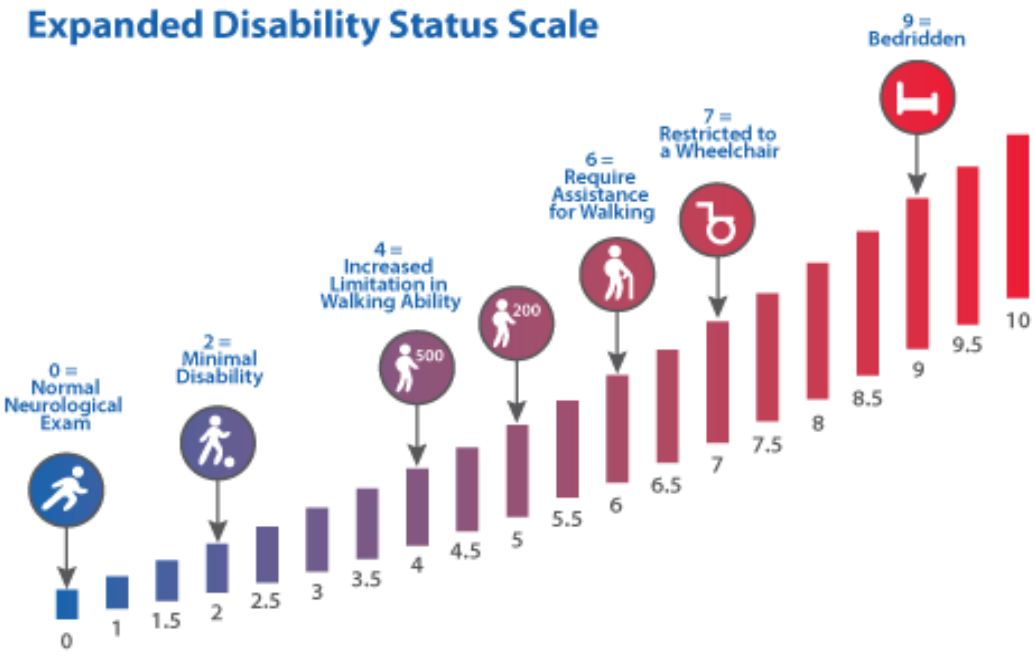

Figure 2: The EDSS (Expanded Disability Status Scale)

The method of analysis chosen was the analysis of movement through:

- the analysis of the mobility with the Barthel Index (disability evaluation) before and after treatment;
- analysis of muscle imbalance Kendall before and after treatment;

All volunteers were eligible to experimental drug treatment does not present abnormalities in liver transaminases, renal overload and prolonged use of psychotropic drugs. The treatment had a total duration of 45 days

with drug administration for 2 times a day and control dietological-food. The current spread in the field of active drug has allowed us to consider acceptable risk taking human factors so as to exclude the application of proven sensitization to one or more active and / or the presence of significant damage to the liver and kidneys, consequences be correlated in time to the action of a progressive poisoning by drugs used in traditional treatments conducted on multple sclerosis (immunomodulatory, immunomodulators, steroids and cortisone).

Another exclusion factor was also assessed by drug addiction: in general it is considered fundamental to evaluate the effectiveness of the drug without any interaction with other pharmaceutical compounds that could alter the effect, in particular inhibiting the action. The treatment had a total duration of 45 days with drug administration for 2 times a day and control dietological-food.

The application of a controlled diet pressed to increase the effectiveness of the experimental drug: in particular was chosen to eliminate milk and fresh cheeses for inhibiting the interaction between lactose and drug, of subjecting the body to a modulation of the physiological pH less stressful as possible by means of a diet without red meats, processed meat, alcohol and refined sugars and focusing on whole foods, white meats, vegetables and fruits except pineapple and grapefruit that have proven interaction with the drug.

ACTIVITY	SCORE
To Eat	0 = no self-supporting 5 = required assistance 10 = self-supporting
To Bathe	0 = no self-supporting 5 = self-supporting
Personal hygiene	0 = no self-supporting 5 = self-supporting
Dress himself	0 = no self-supporting 5 = required assistance 10 = self-supporting
Rectum control	0 = no self-supporting 5 = requires assistance 10 = self-supporting
Bladder control	0 = no self-supporting 5 = requires assistance 10 = self-supporting
To move in bathroom	0 = no self-supporting 5 = requires assistance 10 = self-supporting
To move chair / bed	0 = no self-supporting 5 = required high level assistance 10 = required low level assistance 15= self-supporting
Deambulation	0 = no self-supporting 5 = required high level assistance 10 = required low level assistance 15= self-supporting
Climbing stairs	0 = no self-supporting 5 = requires assistance 10 = self-supporting
	Score (0 – 100)

Table 1: Barthel Scale

The mobility analysis with Barthel Index (disability evaluation) showed an increase in the total value of the scale than the initial test. In particular, the highest increases has been in patients whit the Barthel index lower at pre-treatment (Figure 1). Except patient whit both pre - post treatment highest level, in every others is found a general increment of Barthel index.

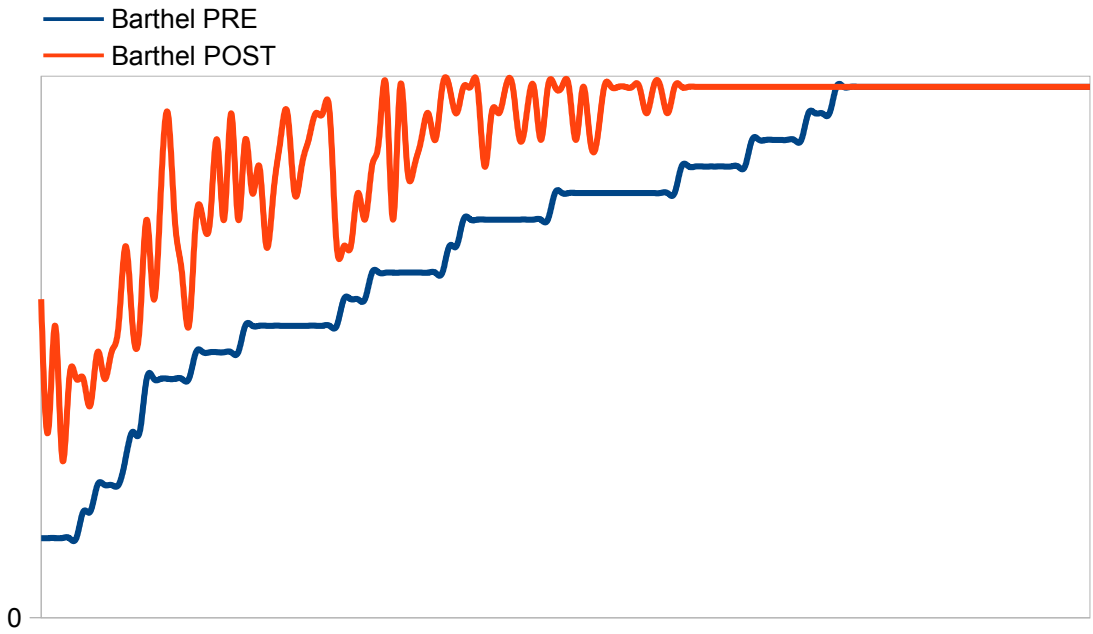

Figure 3: Overall change in Barthel Index pre and post treatment, data are ordinated from lowest to highest score for patient pre-treatment

The Kendall Analysis muscle strength showed an increase in the total value of muscle strength for each subjects. In general, the smaller increase occurred in subjects with high index of Kendall at the first examination and patients with motor deficits pronounced.

KENDALL TEST: muscles

Hip flexors	Gluteus maximus
Hip adductors	Gluteus medius
Hip Lateral rotators	Gluteus minimus
Quadriceps	Tensor fascia lata
Tibialis anterior	Hip Medial rotator
Tibialis posterior	Hamstrings
Flexor digitorum longus	Soleo
Flexor digitorum brevis	Gastrocnemius and soleus
Lobricali and interosseous	Peroneus longus and short
Flexor hallucis longus	Third peroneal
Flexor hallucis brevis	Fingers Extensors
-	Big toe extensor

maximum score: 5 – no muscle disability
minimum score: 0 – max muscle disabiliy
Range: +/- 0,25

Table 2: Kendall Test - muscles table

The calculation of the overall value of the Kendall test has been done considering the maximum value for each muscle equal to 5 (no motor impairment) and giving the label of "more than half of the movement" and "less than half of the movement" a deviation of + / - 0.25. The sum of the values of each subject than the sum of the maximum value (115 = no disability) provided an index rate of mobility (no disability = 100%). The difference (delta %) before and after treatment has always provided values greater than zero, indicating an improvement in mobility for each subjects treated.

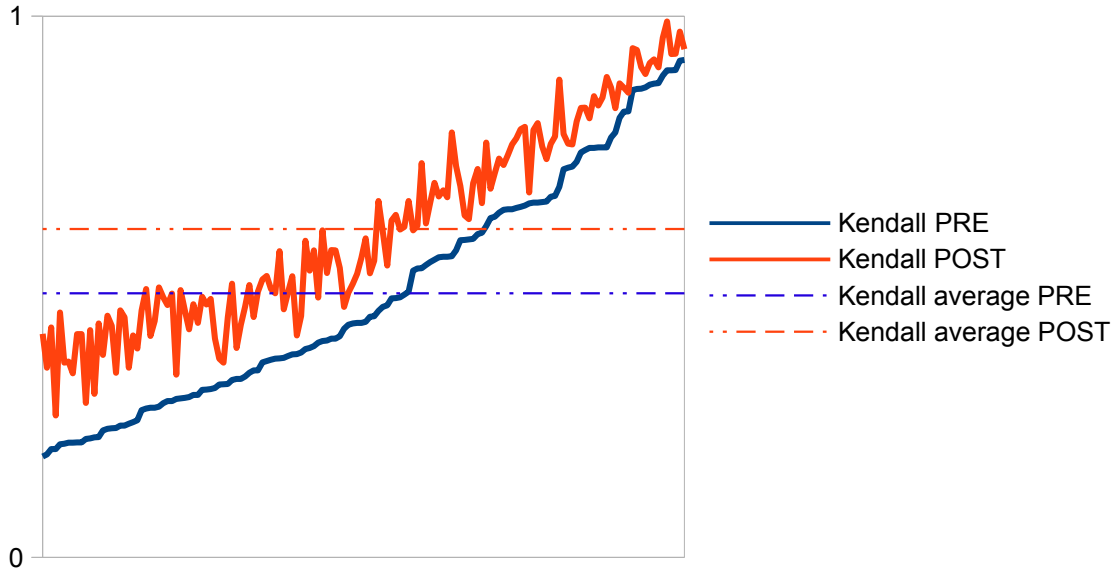

Figure 4: Kendall index delta pre-post treatment are positive in all subjects, data are ordinated from lowest to highest score for patient pre-treatment

Conclusion

The measured values in terms of percentage differences (increase / decrease percentage of the pre-treatment value) show in all test values greater or equal to zero. It then highlights the improvement of quality of life and the consequent reduction in the EDSS value (Expanded Disability Status Scale). Also from a neuro-psychological point of view are presented appreciable clinical improvements regarding the mood, the social and affective skills.

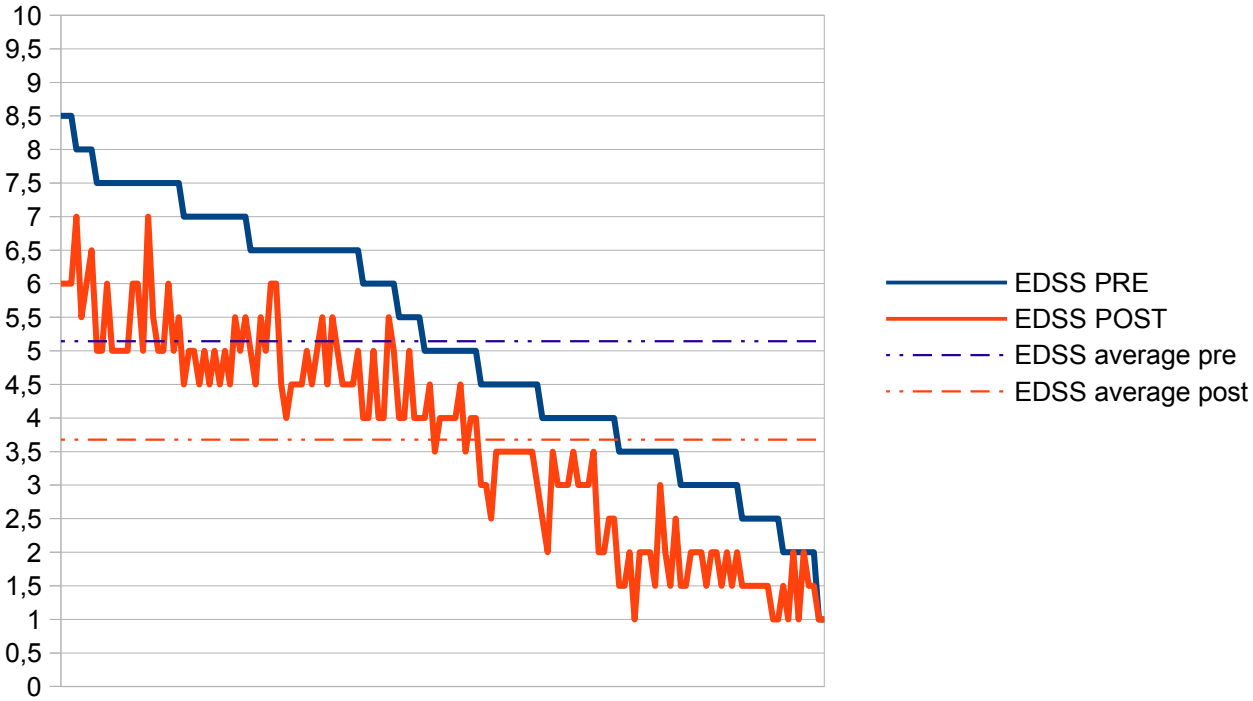

figure 5: EDSS score reduction, data are ordinated from highest to lowest score for patient pre-treatment