NEUROPSYCHOLOGICAL FUNCTIONS AND ASSOCIATION WITH SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY (SPECT) IN GREEK MULTIPLE SCLEROSIS PATIENTS: EFFICACY OF A COMPUTERIZED COGNITIVE REHABILITATION INTERVENTION

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To my beloved parents and children, Thomas and Evi and partner in life Katerina…

“Education is an admirable thing, but it is well to remember from time to time that nothing that is worth knowing can be taught”

Oscar Wilde

“To the patients and their families living with multiple sclerosis, who have encouraged me to look for answers regarding their cognitive difficulties and provide the quality of cognitive rehabilitation that they deserve”

Lambros Messinis
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Summary

While the prevalence and pattern of cognitive dysfunction in multiple sclerosis (MS) is well documented, cognitive deterioration remains one of the most disabling characteristics of MS, and among the most difficult symptoms to manage. The impact of cognitive dysfunction in this population is significant with negative consequences on activities of daily living, loss of employment and ultimately quality of life. Past and current pharmacological treatments have shown inconsistent findings in alleviating cognitive impairment in MS patients requiring further clarification. This inconsistency regarding the effects of pharmacological interventions on cognition, coupled with the reduced ability to effectively handle everyday tasks, loss of employment and social interaction capacity, prioritizes the need for utilizing potentially more effective non-pharmacological, neurobehavioral interventions to adequately address cognitive dysfunction and everyday functioning abilities. Although the utilization of compensatory strategy training and use of external aids may be used in cognitively impaired MS patients with extensive brain atrophy, when neural plasticity mechanisms might be hampered and cortical reorganization of the brain is limited, restorative or functional cognitive training interventions utilizing either computer assisted or manualized interventions have demonstrated positive pre to post treatment effects, including good adherence, acceptability and safety rates.

In this multicenter, randomized controlled trial we implemented a computer assisted (RehaCom software), functional training cognitive rehabilitation intervention of 10-weeks duration (twice weekly for 60 minutes), on (n =32) (Intervention group; IG), cognitively impaired relapsing remitting multiple sclerosis (RRMS) patients, with low disability status (EDSS = 3.14), and compared them to a demographically and clinically matched group of cognitively impaired RRMS patients (n =26) (Control group; CG), who received only standard clinical care. Our data showed, that this relatively short period of domain specific functional cognitive training (attention, processing speed, executive functions and episodic memory), was helpful in
ameliorating the trained functions, and that effectiveness persisted at 6-months follow up for the attention domain. For the other trained domains, performance did not deteriorate to pretreatment levels after 6-months, implying a possible protective long-term effect of the intervention in terms of cognitive deterioration rate. Our findings are further supported by recent explorative functional neuroimaging studies, which have reported that cognitive rehabilitation interventions, including those that incorporated the RehaCom software, may induce an increase in the brain activation of treated patients and more efficient neural network activity.

Another positive attribute of the rehabilitation intervention, was that the majority of treated MS patients responded positively at post treatment assessment to four verbal questions, related to the benefit gained from the intervention and their everyday functioning capacity.

Moreover, a cohort of cognitively impaired RRMS patients (n=31), that underwent regional cerebral blood flow (rCBF) brain SPECT evaluation at baseline, demonstrated greater hypoperfusion rates in several predefined Brodmann areas and lobes of the brain, relative to demographically matched healthy controls according to an established normative database (NeuroGam). Furthermore, a different pattern of cortical hypoperfusion severity, between patients with more severe (n=19); (failed ≥ 2 cognitive tests on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean) and less severe (n=12); (failed 1 cognitive test on the administered neuropsychological battery with performance ≤ 1.5 SD below normative data mean) cognitive deterioration was established. Another significant contribution of the study was the important associations that were established between results of perfusion decrements and neuropsychological performance. We found moderate associations between a measure of verbal fluency-language expression and reduced blood flow in the left posterior lateral prefrontal cortex, verbal episodic memory and hypoperfusion in the left temporal lobe, and strong relationships between two
measures of executive functions, and severity of hypoperfusion in the left frontal lobes respectively.

Overall, the study demonstrates the potential utility of brain perfusion SPECT in monitoring cognitively deteriorated RRMS patients, and due to its accessibility, relatively low cost, practical ease and provision of objective quantitative information, it may be utilized in order to complement neuropsychological assessment in surveillance of cognitive decline in RRMS patients.
Εκτεταμένη Περίληψη

Ενώ ο επιπλασιασμός και το μοτίβο γνωστικής δυσλειτουργίας στην Σκλήρυνση Κατά Πλάκας (ΣΚΠ), είναι καλά τεκμηριωμένα στην κλινική πράξη και επιστημονική βιβλιογραφία, η γνωστική εξασθένηση αποτελεί ένα από τα ανθεκτικότερα στην χειρισμό συμπτώματα της νόσου προκαλώντας σημαντική αναπαρία, με επιπτώσεις στην καθημερινή λειτουργικότητα, απώλεια της εργασίας και ποιότητα ζωής. Τα νοσοτροποποιητικά φάρμακα που χρησιμοποιούνται στην ΣΚΠ μπορούν δυνητικά να επηρεάσουν θετικά τις γνωστικές λειτουργίες του ασθενή. Ειδικότερα, όλα τα εγκεκριμένα νοσοτροποποιητικά φάρμακα μειώνουν τη συσσωρευμένη μη-αναστρέψιμη νευρολογική βλάβη, όπως διαπιστώνεται από την μείωση του όγκου βλαβών στις Τ1 και Τ2 ακολουθίες της μαγνητικής τομογραφίας εγκεφάλου και μέσω μειωμένης εγκεφαλικής ατροφίας. Επιπροσθέτως, η μείωση της ενεργού φλεγμονής, πιθανώς να συνεισφέρει ευεργετικά στις επιδόσεις των ασθενών στις νευροψυχολογικές δοκιμασίες. Παρόλα αυτά, αν και υπάρχουν ενδείξεις από παλαιότερες και νεότερες φαρμακευτικές μελέτες ότι οι νοσοτροποποιητικές θεραπείες επιβραδύνουν την εξέλιξη της γνωστικής δυσλειτουργίας στην ΣΚΠ, δεν υπάρχουν μέχρι σήμερα αδιάσειστα ευρήματα για κλινικά σημαντική βελτίωση των γνωστικών ελλειμμάτων με γενίκευση στην καθημερινή λειτουργικότητα.

Ωστόσο, πρόσφατες νευροψυχολογικές μελέτες έχουν αναδείξει την σημαντική συνεισφορά νευροσυμπεριφορικών προσεγγίσεων στην αντιμετώπιση των γνωστικών ελλειμμάτων με παρεμβάσεις γνωστικής αποκατάστασης. Οι προσεγγίσεις γνωστικής αποκατάστασης που φαίνεται να έχουν τη σημαντικότερη επίδραση είναι αυτές που χρησιμοποιούν λειτουργικές τεχνικές ενδυνάμωσης και επανεκπαίδευσης των γνωστικών λειτουργιών, ενισχύοντας την ενδογενή ευπλαστότητα του εγκεφάλου και βοηθώντας στην αντιστάθμιση της απώλειας στην φυσιολογική συνδεσιμότητα και κατ’ επέκταση στις γνωστικές διαταραχές. Προϋπόθεση επιτυχίας των παρεμβάσεων ενδυνάμωσης είναι να
προσφέρονται έγκαιρα πριν η εγκεφαλική ατροφία ξεπεράσει ένα όριο. Οι παρεμβάσεις αυτές επιτυγχάνονται με την χρήση έντυπου υλικού και ειδικού λογισμικού σε ηλεκτρονικό υπολογιστή (π.χ., RehaCom).

Περαιτέρω, υπάρχουν ισχυρές ενδείξεις από λειτουργικές απεικονιστικές μελέτες (f-MRI), ότι ασθενείς με ΣΚΠ που συμμετείχαν σε παρεμβάσεις γνωστικής αποκατάστασης, υφίστανται εύπλαστες μεταβολές του εγκεφάλου τους, επιστρατεύοντας εναλλακτικές περιοχές, ενώ οι μεταβολές αυτές παρουσίαζαν αυξημένη συσχέτιση με βελτίωση στις γνωστικές λειτουργίες που είχαν επανεκπαίδευτε. Η λειτουργική νευροαπεικόνιση (f-MRI) αποτελεί πλέον ισχυρό σύμμαχο στην προσπάθεια να κατανοήσουμε καλύτερα τις διορθωτικές «εύπλαστες» διεργασίες του εγκεφάλου και την θεραπευτική αποτελεσματικότητα της γνωστικής αποκατάστασης σε ασθενείς με ΣΚΠ.

Στην παρούσα μελέτη, εφαρμόζοντας ένα πολυκεντρικό (δυο κέντρα) τυχαιοποιημένο ελεγχόμενο ερευνητικό σχεδιασμό, διερευνήθηκε η αποτελεσματικότητα μιας λειτουργικής παρέμβασης ενδυνάμωσης και επανεκπαίδευσης των γνωστικών λειτουργιών, διάρκειας 10 εβδομάδων (2 συνεδρίες την εβδομάδα διάρκειας 60 λεπτών), σε ασθενείς με υποτροπιάζουσα διαλείπουσα ΣΚΠ που παρουσίαζαν γνωστική δυσλειτουργία. Συγκεκριμένα, με την χρήση του λογισμικού προγράμματος γνωστικής αποκατάστασης, γνωστό ως RehaCom, επανεκπαιδεύτηκαν (n=32) ασθενείς με υποτροπιάζουσα διαλείπουσα ΣΚΠ και σχετικά χαμηλό επίπεδο αναπηρίας (EDSS = 3.14), λειτουργώντας ως πειραματική ομάδα (ΠΟ), με εστίαση στις λειτουργίες της επεισοδιακής μνήμης, προσοχής, νοητικής ταχύτητας επεξεργασίας και επιτελικής λειτουργίας, ενώ συνέχιζαν να λαμβάνουν κανονικά τις φαρμακευτικές τους αγωγές και υπόλοιπη κλινική περίθαλψη. Μια δεύτερη ομάδα (n=26) ασθενών με υποτροπιάζουσα διαλείπουσα ΣΚΠ που παρουσίαζαν γνωστική δυσλειτουργία, κλινικά και δημογραφικά εξίσωμενη με την πρώτη, λειτουργώντας ως ομάδα ελέγχου (ΟΕ), συνέχισε την τυπική φαρμακευτική αγωγή και κλινική περίθαλψη για την ίδια περίοδο των 10
εβδομάδων, χωρίς ωστόσο, να δέχεται παρέμβαση γνωστικής αποκατάστασης. Οι δυο ομάδες συγκρίθηκαν πριν την έναρξη και αμέσως μετά την λήξη του προγράμματος γνωστικής αποκατάστασης με περιεκτική συστοιχία νευροψυχολογικών δοκιμασιών, ενώ η πειραματική ομάδα αξιολογήθηκε και 6 μήνες μετά την λήξη της παρέμβασης αποκατάστασης. Τα δεδομένα έδειξαν ότι η σχετικά σύντομη περίοδος των 10 εβδομάδων παρέμβασης, είχε θετική αποτελεσματικότητα στις γνωστικές λειτουργίες που επανεκπαιδεύτηκαν, ενώ παρουσίασε και χρόνια θετική επίδραση, αφού διαστήθηκαν τα θετικά αποτελέσματα για τουλάχιστον 6 μήνες στο πεδίο της προσοχής, ενώ στα υπόλοιπα γνωστικά πεδία η επίδοση στις νευροψυχολογικές δοκιμασίες δεν έπεσε στα επίπεδα που καταγράφηκαν πριν την παρέμβαση, υποδεικνύοντας την πιθανή προστατευτική επίδραση της απώλειας για τουλάχιστον 6 μήνες στο πεδίο της προσοχής, ενώ στα υπόλοιπα γνωστικά πεδία η επίδοση στις νευροψυχολογικές δοκιμασίες δεν έπεσε στα επίπεδα που καταγράφηκαν πριν την παρέμβαση, υποδεικνύοντας την πιθανή προστατευτική επίδραση της, στον ρυθμό γνωστικής εξασθένησης. Δεν διαπιστώθηκαν παρόμοια θετικά αποτελέσματα στην ομάδα ελέγχου.

Τα ευρήματα μας, όπως αναφέρθηκε προηγουμένως, υποστηρίζονται από πρόσφατες μελέτες λειτουργικής νευροαπεικόνισης (fMRI), που αναφέρουν ότι οι παρεμβάσεις γνωστικής αποκατάστασης σε ασθενείς με ΣΚΠ, συμπεριλαμβανομένης αυτές που έκαναν χρήση του λογισμικού RehaCom, οδηγούν στην αναδιοργάνωση του εγκεφαλικού φλοιού, αντισταθμίζοντας την απώλεια της γνωστικής λειτουργίας με αποτέλεσμα την καλύτερη αποδοτικότητα των νευρονικών δικτύων και θαλάμου της γνωστικής λειτουργίας. Η θετική έκβαση του προγράμματος γνωστικής αποκατάστασης, διαπιστώθηκε περαιτέρω από την υποβολή των ασθενών της ομάδας σε 4 λεκτικές ερωτήσεις μετά την ολοκλήρωση της θεραπείας, σχετικά με την αποδοτικότητα του προγράμματος σε προσωπικό επίπεδο. Οι πλειοψηφία των ασθενών της ομάδας αυτής ανέφερε θετική επίδραση στις γνωστικές λειτουργίες που γενικεύτηκε στην καθημερινή τους λειτουργικότητα.

Πολύ ενδιαφέροντα ευρήματα προέκυψαν και από την αξιολόγηση πριν την έναρξη του προγράμματος γνωστικής αποκατάστασης, με τομογραφία εκπομπής μονήρων φωτονίου (SPECT), δηλαδή την μέτρηση/απεικόνιση της περιοχικής αιματικής εγκεφαλικής ροής (tCBF),
σε μια επιμέρους ομάδα (n = 31) ασθενών, από το σύνολο των ασθενών με υποτροπιάζουσα διαλείπουσα ΣΚΠ, που συμμετείχαν στην μελέτη αποκατάστασής. Ειδικότερα, στους ασθενείς αυτούς, διαπιστώθηκε υψηλότερος βαθμός/ρυθμός περιοχικής υποαιμάτωσης σε διάφορες προκαθορισμένες εγκεφαλικές περιοχές, σύμφωνα με την χαρτογράφηση Brodmann, και λοιπόν του εγκεφάλου, συγκριτικά με δημογραφικά εξισωμένη ομάδα ελέγχου που είναι ενσωματωμένη στην βάση δεδομένων αναφοράς φυσιολογικής αιμάτωσης του προγράμματος ανάλυσης της SPECT μελέτης γνωστό ως NeuroGam. Μια άλλη σημαντική συνεισφορά της μελέτης, ήταν οι συσχετίσεις που βρέθηκαν μεταξύ της τοπικής αιματικής ροής (υποαιμάτωσης) και την επίδοση σε ορισμένες νευροψυχολογικές δοκιμασίες. Πιο συγκεκριμένα, διαπιστώθηκαν μέτριες συσχετίσεις μεταξύ μιας δοκιμασίας λεκτικής ευφράδειας - γλωσσικής έκφρασης και μειωμένης τοπικής αιματικής ροής στον αριστερό οπίσθιο πλάγιο προμετωπιακό λοβό και στην δοκιμασία λεκτικής επεισοδιακής μνήμης με την υποαιμάτωση στον αριστερό έσω κροταφικό λοβό. Επιπλέον, βρέθηκαν ισχυρές συσχετίσεις μεταξύ δυο δοκιμασιών που εκτιμούν την επιπλοκή λειτουργίας, αλλά με υψηλή φόρτιση στην γλώσσα και τον βαθμό υποαιμάτωσης στον αριστερό μετωπιακό λοβό αντίστοιχα.

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General Section

1. Introduction

1.1 A brief historical overview of Multiple Sclerosis

The disease that we now know as multiple sclerosis (MS) has been referred to in the historical medical literature with a variety of terms, including, disseminated sclerosis, insular sclerosis, and sclerose en plaque (Murray, 2000). The first potential case of MS appears to date as far back as 1421, when Jan van Berieren commented on the illness of a patient known as Lidwina van Schiedam (1380–1433). At a relatively young age (about 16), Lidwina was noted to have fallen while ice skating and broke some of her ribs in this event. Later on, she was recorded as having difficulties walking, right arm paralysis, decreased sensation ability and visual difficulties (Kesselring, 2008).

Historically, there are other examples of potential MS cases, including, Sir Augustus d’Esti (grandson of George III) (1794–1848), the poet Heinrich Heine (1797–1857), and William Brown, (early 1800s). These cases were all noted to present with major signs and symptoms of what we today classify as MS (i.e. exacerbation and remission periods, slowly progressive disease, gait and balance difficulties, numbness and other sensory symptoms, sensitivity to heat, pain, and difficulties in bladder function, vision, and mood) (Murray, 2000). Although several individuals (e.g. Charles Ollivier, Edmí Vulpian, Robert Carswell), have provided pathological and clinical descriptions of MS, Jean Martin Charcot (1825–1893), is considered as having provided the first comprehensive description of MS, incorporating both clinical features and pathological disease characteristics (Charcot, 1877. Murray, 2000), (see figure 1.1).
Figure 1.1 Jean Martin Charcot (1825–1893) is considered as having provided the first comprehensive description of Multiple Sclerosis.
Source: Adapted from National Library of Medicine

Historically, a wide range of approaches without scientific evidence have been used in an effort to treat or reduce MS patient’s symptoms. A classical example, are the diaries of d’Esti, where descriptions are provided regarding the range of treatments that were attempted for his symptoms, including spas to bathe in “steel-water”; eating of beef steaks, drinking sherry and wine, alcohol and oil massages, wrapping of the body in hot bandages and even horse riding (Murray, 2000). On the contrary, treatments based on scientific evidence initially emerged over the past half century. A trial published in 1969, which investigated the efficacy of Adrenocorticotropic hormone (ACTH), for treating acute MS, is considered the first valid and efficacious pharmaceutical treatment trial for this population when presenting with acute symptoms (Rolak, 2009). This work laid the foundations for development and use of intravenous corticosteroids; medications still commonly used today for the treatment of acute MS attacks (Burton, O Connor, Hohol, & Beyene, 2012). In 1993, beta interferon β-1b (Betaseron®), the first disease modifying therapy (DMT), i.e. medication that demonstrated scientifically the ability to modify the disease course of MS was FDA approved. In 1996, intramuscular beta interferonβ-1a (Avonex®), and glatiramer acetate (Copaxone®), were approved. Subsequently, and more specifically in 2002, subcutaneous beta interferonβ-1a (Rebif®), received approval for clinical use. However, many more emerging DMTs are been
investigated in order to cover the unmet need for treating neurodegeneration and halting the progression of MS disability (Coclitu, Constantinescu, & Tanasescu, 2016).

As far as rehabilitation is concerned the earliest recorded article in the National Library of Medicine’s database that used both “multiple sclerosis” and “rehabilitation” as key index terms were published in Northwest Medicine in 1948 (Robson, 1948). The article although primarily focusing on pharmacotherapy, did acknowledge the important role of physiotherapy for improvement of gait disturbance by treating spasticity (Robson, 1948).

1.2 Multiple Sclerosis: synopsis

Multiple Sclerosis is considered the most common form of non-traumatic brain disability among young and middle age adults and is often grouped with the degenerative diseases due to its progressive accumulation of neurological deficits and persistent behavioral and cognitive dysfunction later on in its disease course (Adams, Parsons, Culbertson, & Nixon, 1996. Kargiotis, Paschali, Messinis, & Panagiotopoulos, 2010). However, contrary to most other degenerative diseases, it primarily affects young adults, during their prime wage-earning years, with average age range of onset between 20 - 40 years, without necessarily shortening the lifespan of these patients, who reach 90-95% of a normal life expectancy (Lezak, Howieson, Bigler, & Tranel, 2012). This makes MS a substantial social burden and an extremely costly disease for both the individual and society (Kurtzke& Wallin, 2000). Women are more prone then men to develop MS, and outnumber men at a ratio of about 2.5:1 (McDonald et al. 2001).

Neuropathologically MS is considered a progressive inflammatory demyelinating disease affecting the central nervous system (CNS) at multiple and distant sites, resulting in a widely heterogeneous clinical expression. In this respect, patients present with high variability as regards clinical onset, speed and severity of disease progression, and aspects of mental, cognitive and physical manifestation (Demetriou, 2006. Kargiotis et al. 2010). MS symptoms are often erratic as they flare up acutely over several days, have great variability in terms of the
time period they persist, and then remit or partially remit for unpredictable time periods (Keegan & Noseworthy, 2002). Each new presentation of symptoms may involve different sites of the brain or spinal cord white matter, leading to a wide range of different symptoms (Lezak et al. 2012). Most of the common physical symptoms are related to the specific lesion sites (Lezak et al. 2012. Demetriou, 2006). Prominent MS symptoms include stiffness, weakness, gait disturbance, visual impairments, bladder and bowel symptoms of a neurogenic nature (retention or incontinence), sexual disorders, sensory disorders and sensitivity to heat. Cognitive impairment is also very common in MS and approximately 50% of these patients will have clinical problems and experience everyday difficulties owing to cognitive symptoms during the course of the disease (Winkelmann, Engel, Apel, & Zettl, 2007). Currently, there is no cure for MS, although effective disease modifying therapies (DMTs), as mentioned previously in the historical overview have emerged since the 1990s, that have managed to slow down disease progression, making early diagnosis and treatment especially important (Cohen & Rudick, 2003).

1.3 Neuropathological – Pathogenic mechanisms

Although the etiology of MS is unknown, and the precise mechanisms triggering the autoimmune response remain unclear, T lymphocytes do appear to be crucial in the development of MS lesions (Rinker, Naismith, & Cross, 2006). More specifically, MS an autoimmune disease is consistent with the concept of molecular mimicry (Demetriou, 2006), implying that infectious agents such as viruses’ express proteins that are similar in sequence to native human body proteins, which subsequently are targeted by activated T-cells in the immune system.

In MS activated cells cross the blood–brain barrier from the circulatory system and penetrate the CNS. As a result, an inflammatory cascade is initiated whereby, the T-cells encounter the proteins of the myelin and secrete cytokines, which in turn recruit macrophages
and microglia that attack and damage the myelin covering neuronal axons and oligodendrocytes that produce myelin. This results in widespread demyelination of axons and leads to the formation of plaques by astrocytes, which has a significant negative impact on the conduction of nerve impulses by either slowing or blocking this process (O’Connor, 2002). This pattern of immunopathologic demyelination varies considerably among patients, with some experiencing primarily inflammatory responses and relative sparing of oligodendrocytes while in other individuals there is extensive death of oligodendrocytes. This variability seems partly responsible for the noted heterogeneity in neurologic symptom expression and reversibility of acute relapses (Demetriou, 2006). While inflammation and demyelination are hallmarks of MS neuropathology primarily affecting the myelin sheath that covers axons, there is increasing evidence of axonal damage, which appears to take place early on in MS and may ultimately result in permanent disability (Keegan & Noseworthy, 2002), (see figure 1.2). The attenuation of symptoms reflects edema reduction, partial remyelination of axons, and a redistribution of sodium channels along demyelinated axon segments (Noseworthy, Lucchinetti, Rodriguez, & Weinshenker, 2000). However, increased disease progression may lead to irreversible axon damage and exhaustion of myelin production by oligodendrocytes (Noseworthy et al. 2000).
While MS lesion sites are to be found in practically any myelinated area of the CNS, suggesting a random distribution of plaques, this is not absolutely the case. While MS lesions affect primarily white matter, there is a preference for the periventricular white matter, optic nerves, corpus callosum, brainstem, cerebellum, and spinal cord (Lucchinetti & Parisi, 2006). Moreover, there is evidence that MS lesions may be found in the gray–white matter junctions and the involvement of gray matter in the pathognomic process has been confirmed by pathologic studies (Messina & Patti, 2014). Sites preferentially affected are the cortical sulci and the cingulate, temporal, insular, and cerebellar cortices (Rossi et al. 2012). As shown in figure 1.3 periventricular and frontoparietal lesions are very common in MS and appear to be frequently associated with cognitive impairments in this population (Fillipi & Rocca, 2010).

Thus, two primary sources are involved in the pathogenesis of MS. These include acute inflammatory demyelination and axonal degeneration. Although disability associated with acute inflammatory demyelination is dominant primarily during the early stages of the disease
process this is reversible. On the contrary, while axonal injury, which also begins early on in the disease process appears to have minimal impact on disease disability due to the fact that the CNS is able to compensate for the neuronal loss, when a threshold of axonal loss is exceeded, disease disability becomes irreversibly progressive (Noseworthy et al. 2000. Demetriou, 2006).

Figure 1.3 Magnetic Resonance Imaging (MRI) of a 42-year-old female with RRMS disease course, EDSS score of 3.5 and 11 years disease duration without disease modifying therapy. From left to right: T2-weighted, FLAIR and T1-weighted post-gadolinium contrast images. Periventricular hyperintense lesions and frontoparietal lesions are noted on the T2 and FLAIR images; a gadolinium-enhancing lesion is also apparent. Source: Adapted from Thorton & DeFreitas, (2009), p. 281.

1.4 Epidemiological characteristics: geographic latitude and genetic predisposition

It is estimated that worldwide approximately 2,500,000 individuals have been diagnosed with multiple sclerosis. Moreover, reports suggest that MS in women is increasing and that roughly for every man diagnosed with MS there are two to three women who have the condition (National Multiple Sclerosis Society, 2009). The total prevalence rate of MS is estimated at 100-150/100,000, while the yearly incidence of new cases is estimated in the range of 3.5 - 7/100,000 (Compston & Coles, 2008).

Despite increasing evidence that genetic susceptibility may influence who will develop MS, environmental factors and especially geographical latitude, exerts a significant impact, making the distribution of MS uneven around the world (Demetriou, 2006). Moreover, studies on individuals that have emigrated at a relatively young age from countries with high to low MS
prevalence rates show rates related to the country of immigration, whereas immigrants of older ages, present with prevalence rates of the origin country they grew up in until their 15th year of age (Gale & Martyn, 1995).

Prevalence areas of low (between 1 and 4/100.00 inhabitants), medium (between 5 and 25/100.00 inhabitants), and high (≥ 30/100.00 inhabitants), geographical risk in relation to geographical latitude, have been proposed by Kurtzke, (1975). The general consensus is that MS prevalence increases further north or south from the equator. Populations residing between latitudes 40° North and 40° South have a very low risk to develop MS (Rosati, 2001). In general, persons from Western Europe who live in temperate zones have much higher prevalence rates, as do Canada and Scotland. On the contrary, countries that lie on the equator, such as parts of Asia, Africa and America have extremely low prevalence rates of MS (National Multiple Sclerosis Society, 2009. Rosati, 2001).

Epidemiological studies on the prevalence of MS in Europe and the Mediterranean have shown that it is composed of two distinct zones, one for medium and one for high prevalence rates. The medium prevalence zone extends from 32° to 47° latitude including two sites from the west coast of Norway, and the high prevalence zone extends from 44° to 64° north latitude (Kurtzke, 1980). Kurtzke & Wallis, (2000) and Piperidou, Heliopoulos, Maltezos, & Milonas, (2003), placed Greece in the high prevalence zone with estimated rates in the 40s /100,000 inhabitants. A more recent epidemiological report on the prevalence and incidence of multiple sclerosis in western Greece, which was based on a 23-year survey, found that the crude prevalence rate of definite MS increased significantly in the last 23 years from 10.1/100.00 which was recorded in north eastern Greece in 1984, to 119.61/100.000 as recorded on the 31st of December 2006 for the 780 live cases in western Greece. There was also a significant increase from 2.71/100.00 recorded from 1984 to 1989 to 10.73/100.000 for
the period 2002 to 2006 (Papathanasopoulos, Gourzoulidou, Messinis, Georgiou & Leotsinidis, 2008).

As mentioned previously, environmental factors and especially geographical latitude may significantly influence the development of MS. However, genetic susceptibility as is evident from twin studies and familial cases, suggests that MS disease causality is due to a complex interaction between multiple genes and environmental factors, which eventually leads to inflammatory-mediated central nervous system deterioration (Hawkes & Macgregor, 2009). A large series of genomic studies, with specific HLA antigens (HLA-DR2), have confirmed the genetic susceptibility of MS (Dyment, Ebers, & Sadovnik, 2004. Hawkes & Macgregor, 2009).

Advances in technology, including single nucleotide polymorphisms (SNPs) associated with MS have been assessed by two main groups of genotyping analyses: candidate gene approaches and genome-wide association studies (GWAS). Although these methods, combined with novel collaboration models across research centers, have identified polymorphisms in the human leukocyte antigen (HLA) region as the strongest susceptibility loci for MS, confirming older reports, recent investigations have identified a broad spectrum of over 50 non-HLA genes prominently associated with MS. The most significant of these are the alleles of the interleukin-2 receptor α gene (IL2RA), interleukin-7 receptor α gene (IL7RA) and one related to the lymphocyte antigen 3 (LFA-3 or CD58) (Cohen & Rudick, 2011). These studies have significantly expanded the roster of non – HLA genetic risk factors for the development of MS (Hawkes & Macgregor, 2009. Gourraud, Harbo, Hauser, & Baranzini, 2012). However, considering the fact that a large proportion of MS disease heritability remains unaccounted for, current studies are generated towards identification of causal alleles, associated pathways, epigenetic mechanisms, and gene-environment interactions (Gourraud et al. 2012).

Numerous other environmental factors have been evaluated that may be associated with MS, but methodological caveats have casted doubts on their validity. On average, MS
patients contracted common childhood illnesses at later ages than healthy controls (Lezak et al. 2012). A biomarker of Epstein-Barr virus (anti-EBNA IgG seropositivity), infectious mononucleosis, and smoking have shown the strongest consistent evidence of an association. However, additional data and better-designed studies are needed to establish robust evidence (Belbasis, Bellou, Evangelou, Ioannidis, & Tzoulaki, 2015).

1.5 Clinical symptoms and signs

MS is characterized by a wide variety of symptoms and signs, which can arise from one or more lesions in any portion of the CNS white matter, including the spinal cord, brain stem, optic nerves, corpus callosum, cerebellum, subcortical white matter and cortex. This “dissemination in space” of lesion sites in the CNS is a key characteristic of the disease, which explains the great variability and heterogeneity of symptom presentation in these patients (Lucchinetti & Parisi, 2006). Table 1.1 presents a list of the common symptoms that MS patients may present with and which may occur either singly or concurrently in various combinations for different individuals and at different time periods in the disease course. These symptoms have been grouped not according to their frequency of occurrence or clinical significance, but by eight functional systems as described by Kurtzke, and are used widely by neurologists to assess the impact and severity of MS symptoms, and quantify the disability status of these patients during all stages of the disease (Kurtzke & Wallin, 2000). This scale is called the Expanded Disability Status Scale (EDSS) (Kurtzke, 1983).
Table 1.1 Common clinical symptoms in multiple sclerosis

<table>
<thead>
<tr>
<th>Somatosensory symptoms</th>
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<tbody>
<tr>
<td>Tingling and numbness</td>
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<td>Lhermitte sign (electric-like sensation radiating down the spine)</td>
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<td>Pain</td>
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<tr>
<th>Motor (pyramidal syndromes)</th>
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<tr>
<td>Weakness</td>
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<td>Spasticity</td>
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<td>Abnormal reflexes</td>
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<table>
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<tr>
<th>Brainstem syndromes</th>
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<tbody>
<tr>
<td>Nystagmus and other ocular motor symptoms (blurred vision and double vision)</td>
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<tr>
<td>Dysarthria and swallowing difficulties</td>
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<td>Facial paresis and pain</td>
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<td>Auditory disturbances</td>
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<td>Vertigo</td>
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<th>Cerebellar syndromes</th>
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<td>Gait and limb ataxia</td>
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<td>Intention tremor</td>
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<th>Visual pathway syndromes</th>
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<td>Optic neuritis (dimming of vision and eye pain)</td>
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<td>Visual field defects</td>
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<tr>
<th>Symptoms of bowel, bladder, and sexual dysfunction</th>
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<tr>
<th>Neuropsychiatric disturbances</th>
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<tr>
<td>Neuropsychological deficits: mental information processing speed, learning and episodic memory, attention, visuospatial abilities, and executive functioning (Rao, 2004)</td>
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<tr>
<td>Disorders of mood (e.g. depression) and affect (e.g. pathologic laughing, crying)</td>
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<th>Other symptoms</th>
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<tr>
<td>Mental and physical fatigue</td>
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<tr>
<td>Sleep disturbance</td>
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<tr>
<td>Olfactory and gustatory symptoms</td>
</tr>
<tr>
<td>Paroxysmal symptoms (e.g. seizures, dystonic posturing)</td>
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Source: Adapted from Miller, 2001
There was a time when MS was thought to involve mainly gait and sphincter (bowel and bladder) dysfunction, especially urinary urgency or hesitancy (Scheinberg & Smith, 1987). We now know that somatosensory symptoms, particularly numbness in the extremities, is among the most common initial (21-55% of cases) and frequent (up to 70% of cases during the course of the disease) manifestation of the disease (Miller, 2001). In approximately 32-40% of cases, patients will initially present with motor syndromes reflecting involvement of the corticospinal tract and over 50% of cases will present such syndromes over the course of the disease (Lucchinetti & Parisi, 2006). From the perspective of the patient, fatigue, either mental or physical is reported frequently by 75-90% of the patients during their disease course, and 50-60%, will indicate this as one of the most debilitating symptoms that significantly impacts their everyday functioning capacity (Miller, 2001. Braley & Chervin, 2010).

Cognitive difficulties are not frequently reported by patients among the initial symptoms of MS, although there is sufficient evidence that cognitive impairment is present from the early stage of the disease (see for e.g. the study by Schulz, Kopp, Kunkel & Faiss, (2006), which assessed MS patients neuropsychologically, not more than two years after experiencing their first neurological symptoms, and Faiss, (2007), who presents three cases evaluated at different stages of the disease). Moreover, cognitive impairment may be present in the early stages of the disease in patients with relatively low or mild physical disability (see for e.g. the studies by Ruggieri, Palermo, Vitello, Gennuso, Settipani, & Picolli, (2003), and Messinis, Anyfantis, Paschali, & Paphathanasopoulos, (2009), who found cognitive deficits in patients with an EDSS disability score of ≤ 3.5, that had not yet been influenced significantly in their daily functional abilities and employment status).

In an attempt to explain cognitive dysfunction that occurs at the early stages of MS, functional MRI studies have shown that there is a dysfunction of high controlled information processing early on in the MS disease process that is attributed to connectivity disturbances
inside the working memory network, and is associated to the extent of structural diffuse white matter damage (Audoin et al. 2006). The variability seen in terms of which MS patients will develop cognitive deficits may be attributed to the fact that some patients may partially compensate for the connectivity dysfunction by a larger cognitive control, therefore limiting the significant impact of diffuse white matter damage on high controlled information processing (Audoin et al. 2006). A recent anatomofunctional study utilizing diffusion imaging and resting state functional MRI, revealed that disconnection in the default mode network (DMN) and attentional networks (ATT), may deprive the brain of the necessary compensatory mechanisms required to face the widespread structural damage during the early course of MS, providing a possible explanation for the cognitive dysfunction in these early stages of the disease (Louapre et al. 2014).

Some patients may also present with symptoms related to speech production, for e.g., they may develop a cerebellar syndrome, which causes dysarthria that is characterized by a thickened, spasmodically paced, sluggish sounding speech. Almost a third of MS patients will also develop a particularly debilitating swallowing disorder (dysphagia) over the course of the disease, which presents with coughing or choking when eating, as if the food remains lodged in the throat. Dysphagia already develops in patients with mild disability, but becomes prominent in MS patients with moderate to severe disability (De Pauw, Dejaeger, D’hooghe, & Carton, 2002) any may also lead to recurrent lung infections or even pneumonia and unexplained malnutrition (De Pauw et al. 2002. Lezak et al. 2012). Other symptoms include disorders of mood, affect and behavior that will be described in the sections that follow.

On the contrary to the studies mentioned previously, which found deficits in learning, memory, executive and visuospatial functions, Simioni, Ruffieux, Kleeberg, Bruggimann, Annoni & Schluep, (2008), found that decision making ability, an important indicator of the capacity to function independently, was preserved in early multiple sclerosis. Moreover, cortical
signs (e.g. aphasia, agnosia and apraxia) are rarely diagnosed in this population, providing a possible explanation as to why neurologists failed for so many years to recognize and appreciate the prevalence of cognitive dysfunction in these patients (Lezak et al. 2012).

1.6 Disease Course – Classification of MS

Multiple sclerosis is classified according to its clinical disease course, which presents with large variability among patients. In 1996, the Advisory Committee on Clinical Trials of New Agents in Multiple Sclerosis of the National MS society, in collaboration with Lublin & Reingold, proposed standardized definitions for the four most common clinical courses observed in MS patients (Lublin & Reingold, 1996). Disease course classification depends on its manifestations over time and not neuroimaging or biological differences. Table 1.2 provides a brief summary of the elements that define each of these four clinical types based on disease course characteristics.

<table>
<thead>
<tr>
<th>Type of Disease Course</th>
<th>Key Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relapsing –remitting MS (RRMS)</td>
<td>Distinct episodes of acute worsening of neurologic symptoms followed by a variable recovery of function, with periods of stability between attacks</td>
</tr>
<tr>
<td>Secondary-progressive MS (SPMS)</td>
<td>Initial RRMS clinical course followed by progressive worsening of the baseline between attacks, with or without additional attacks</td>
</tr>
<tr>
<td>Primary-progressive MS (PPMS)</td>
<td>A nearly continuous gradual worsening of baseline functions from the onset with minor fluctuations but no distinct relapses</td>
</tr>
<tr>
<td>Progressive-relapsing MS (PRMS)</td>
<td>Progressive deterioration in baseline functions from the onset but with clear acute relapses, with or without return to baseline; periods between relapses characterized by continued gradual deterioration</td>
</tr>
</tbody>
</table>

Source: Adapted from Messinis et al. (2010), pg. 24
From the table noted previously, it becomes evident that MS can follow several distinct clinical courses. What does not appear on table 1.2 is a rare “clinically silent” form of the disease, which presents with findings of MS plaques on autopsy studies in individuals that were asymptomatic during their whole life (Gilbert & Sadler, 1983).

Although lesion and symptom “dissemination in space and time” constitute key features of the disease and contribute significantly to its diagnostic accuracy, as we will see in the next section, the disease course of MS is often initiated with a clinically isolated syndrome (CIS). This clinical entity was not included in the initial MS clinical description, but is now recognized as the first clinical presentation of a disease showing characteristics of inflammatory demyelination, possibly MS, but not yet fulfilling the criteria of dissemination in time required for MS diagnosis (Lublin et al. 2014). This term refers to patients who have experienced a single episode of CNS involvement, such as optic neuritis, transverse myelitis or dysfunction of the spinal cord or brain stem. Although a CIS may often represent the first MS episode, the term is also used to define a monosymptomatic or monophasic inflammatory demyelinating disease of the CNS, which may or may not eventually progress to MS (Panou, Mastorodemos, Papadaki, Simos, & Plaitakis, 2012. Lublin et al. 2014). The risk rate of developing MS after the occurrence of a CIS ranges from between 30-70%, and the presence of lesions on MRI, increases the risk rate to between (56-68%), after a 7-20 year period, compared with a lower risk rate of developing MS between (8-22%), in patients that have normal MRI findings (Miller, Barkhof, Montalban, Thompson, & Filippi, 2005). Other prognostic factors that may contribute to MS conversion after a CIS include, a multisymptomatic clinical attack, development of cerebellar or pyramidal disorders, presence, total number and activity of pathological lesions on MRI, older age and only partial remission of symptoms related to the clinical attack. On laboratory and other ancillary studies, pathological evoked potentials, evidence of inflammation in the cerebrospinal fluid (CSF), such as presence of oligoclonal
bands, elevated immune markers such as IgG synthesis and rate, and myelin basic protein contribute to an increased conversion rate to MS (Tintoré et al. 2003. Tintoré et al. 2006).

Another interesting clinical entity that is not considered a distinct MS phenotype, initially presented in 2009 by Okuda, et al. (2009), is the “radiologically isolated syndrome (RIS). This term is applied to MRI findings of white matter lesions suggestive of MS, demonstrating dissemination in space, in individuals with an otherwise normal neurological examination and without a history of typical MS symptoms and signs (Fontcuberta & Leon, 2016). It should be noted that changes on MRI that are highly suggestive of demyelinating pathology carry a high risk of developing MS clinical symptoms in the future (Lublin et al. 2014). Asymptomatic spinal cord lesions, gadolinium-enhancing lesions, or positive CSF findings significantly increase the likelihood of an MS diagnosis (Okuda et al. 2011). However, RIS consists an incidental MRI finding and its diagnosis depends to a large extent by the frequency of MRI utilization in the diagnostic work up of other medical conditions (e.g., dizziness, headaches) in everyday clinical practice. In general, various reports have noted that approximately 30% of RIS patients will develop clinical symptoms related to the demyelinating event and two-thirds will develop new lesions on MRI (Forslin et al. 2016. Labiano-Fontcuberta & Bonito-Leon, 2016).

Approximately 85% of MS patients initiate with an RRMS clinical course developing symptoms over several days to weeks that gradually resolve over a period of weeks to months, either partially or fully, and show clinical stability between these attacks. Relapses occur on average every one to two years (Fox & Cohen, 2001). A subgroup of these RRMS patients (about 10%), show minimal observable neurological symptoms and signs, with infrequent clinical attacks after a period of about 15 years. This clinically privileged group defined as “benign MS” (BMS) appears to be underrepresented in clinical studies as these patients have no need for follow-up neurological examinations (Lezak et al. 2012). However, this definition
remains controversial, and is based primarily on changes in motor function. Recent studies, however, also report impairment of cognitive function, mental and physical fatigue, pain, and depression, which may negatively impact employment and social activities, despite complete preservation of motor function. Utilization of conventional MRI techniques showed that the lesion load observed in benign MS patients is similar to levels in other disease subtypes; however, newer quantitative MRI techniques report less tissue damage and more significant repair and compensatory efficacy mechanisms. It is therefore assumed that currently accepted criteria for BMS diagnosis may cause overestimation of true prevalence, underscoring the need for routine monitoring of nonmotor symptoms and neuroimaging studies (Correale, Ysrraelit, & Fiol, 2012).

Another interesting issue that has been studied only recently in RRMS patients and is worth referring to at this point, is whether they may present with “isolated cognitive relapses” (ICR). Pardini, Ucelli, Grafman, Yaldizli, Mancardi & Roccatagliata, (2014), reported that of ninety-nine clinically stable relapsing-remitting MS patients that were followed for six months, seventeen presented with ICRs that were not associated with subjective cognitive deficits, fatigue or depression. They based their findings on a meaningful change in cognition defined as a transient reduction of Symbol Digit Modalities Test score of at least four points at t₁ (MRI positive for at least one area of gadolinium enhancement) compared with t₀ (baseline evaluation) and t₂ (two gadolinium enhancement-negative follow-up evaluations after 6 months) and one year (t₃).

There is a general consensus that if RRMS patients are left untreated they will develop SPMS over a course of between 10 -15 years after disease onset (Noseworthy et al. 2000) However, as mentioned previously, after the introduction of DMTs that modulate the immune systems inflammatory mechanism, the rate and time to conversion from RRMS to SPMS is speculative (Demetriou, 2006). As far as the remaining 15% of MS patients are concerned that
does not present with the relapsing type of the disease, they show progressive disability from
disease onset, and especially susceptible for this clinical course are patients with initial onset of
symptoms over the age of 40. About 10% of patients will have gradual progression without
fluctuations (PPMS), whereas the remaining 5% have a clinical course characterized by
progressive disability from disease onset with occasional acute flare ups (Schapiro, 2003.
Lezak et al. 2012).

An important question that often arises in clinical practice is whether there are
modifiable risk factors that may influence time to conversion from remitting relapsing to
secondary progressive MS, and the odds of reaching a high disability status as indicated by an
EDSS score of 6 (requiring a cane for walking). Numerous prospective studies have assessed
such factors, and concluded that lower Vitamin D levels are associated with higher EDSS
disability scores, and a faster progression rate is present in smokers compared to nonsmokers
(Hempel et al. 2015). The use of epidural analgesics during childbirth delivery has been
assessed in 3 studies and none reported a statistically significant association with EDSS
scores. Results for sun exposure, sunscreen use, month of birth, diet, fish consumption,
alcohol consumption, exercise, trauma, oral contraception, and education have been
addressed in more than one study, but the differences in risk factor operationalization’s and
outcome measures did not allow concrete conclusions to be reached (Hempel et al. 2015).

1.7 Assessing disease severity - disability

Assessing disease severity has become increasingly important both in clinical settings and
therapeutic trials in order to measure and assess progression disability levels. Traditionally the
“gold standard” for measuring disability in this population as mentioned previously, is the well-
known Kurtzke Expanded Disability Status Scale (EDSS), initially presented in 1983 (Kurtzke,
1983). The scale owes its origin to the traditional neurological examination, and measures
impairment or activity limitation based on the examination of eight functional systems, including
ambulation, and is ideally administered by an experienced and EDSS-accredited physician. Motor function and walking capacity have the strongest contribution to total EDSS score, although brainstem, sensory, bowel and bladder, and visual functions also contribute. Cerebral function assessment is based mainly on clinical judgment rather than neuropsychological examination, significantly confounding cognitive function and affective state assessment (Lezak et al. 2012) (see Table 1.3 for disability levels associated with the EDSS scale).

Moreover, limitations of the scale have been well documented by several reports. These include, its ordinal psychometric properties, bimodal score distribution, moderate inter- and intra-rater reliability, relative insensitivity to change, especially at the low end of severity levels and decreased reproducibility capacity (van Wijnen et al. 2010). Identification of sustained disability progression is an important outcome measure in therapeutic trials of relapsing-remitting multiple sclerosis (RRMS) patients. In several recent studies, disability progression has been used as the primary endpoint, underscoring the significance placed on progression events rather than relapses. The identification of “sustained disability progression” is based on assessment of repeated EDSS measurements. An increase of 1 point on the EDSS above baseline (or 1.5 EDSS points if the baseline EDSS is 0), subsequently confirmed at repeat assessment either 3 or 6 months later (3 or 6 months confirmed progression) are the most commonly used measures (Gray & Butzkueven, 2008).

In 2005, the Multiple Sclerosis Severity Score (MSSS) was developed in order to measure disease progression by normalizing the Expanded Disability Status Scale (EDSS) score for disease duration, and is a useful tool for assessing cross-sectional disability data, with proven stability over time (Roxburgh et al. 2005. Lezak et al. 2012). Another small battery of tests that is often utilized in clinical practice and research trials to measure disability status is the Multiple Sclerosis Functional Composite (MSFC) (Fischer et al. 2001). It includes a timed walk, pegboard test and the PASAT and has been reported to correlate well with disability
progression owing to gray matter atrophy in contrast to the EDSS (Rudick, Lee, Nakamura, & Fischer, 2009. Lezak et al. 2012)

Table 1.3 Kurtzke Expanded Disability Status Scale (EDSS) disability levels

- **0.0** - Normal neurological exam (all grade 0 in all Functional System (FS) scores*).
- **1.0** - No disability, minimal signs in one FS* (i.e., grade 1).
- **1.5** - No disability, minimal signs in more than one FS* (more than 1 FS grade 1).
- **2.0** - Minimal disability in one FS (one FS grade 2, others 0 or 1).
- **2.5** - Minimal disability in two FS (two FS grade 2, others 0 or 1).
- **3.0** - Moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three or four FS grade 2, others 0 or 1) though fully ambulatory.
- **3.5** - Fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3 (others 0 or 1) or five grade 2 (others 0 or 1).
- **4.0** - Fully ambulatory without aid, self-sufficient, up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combination of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 500 meters.
- **4.5** - Fully ambulatory without aid, up and about much of the day, able to work a full day, may otherwise have some limitation of full activity or require minimal assistance; characterized by relatively severe disability usually consisting of one FS grade 4 (others or 1) or combinations of lesser grades exceeding limits of previous steps; able to walk without aid or rest some 300 meters.
- **5.0** - Ambulatory without aid or rest for about 200 meters; disability severe enough to impair full daily activities (e.g., to work a full day without special provisions); (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.0).
- **5.5** - Ambulatory without aid for about 100 meters; disability severe enough to preclude full daily activities; (Usual FS equivalents are one grade 5 alone, others 0 or 1; or combination of lesser grades usually exceeding those for step 4.0).
- **6.0** - Intermittent or unilateral constant assistance (cane, crutch, brace) required to walk about 100 meters with or without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).
6.5 - Constant bilateral assistance (canes, crutches, braces) required to walk about 20 meters without resting; (Usual FS equivalents are combinations with more than two FS grade 3+).

7.0 - Unable to walk beyond approximately 5 meters even with aid, essentially restricted to wheelchair; wheels self in standard wheelchair and transfers alone; up and about in wheelchair some 12 hours a day; (Usual FS equivalents are combinations with more than one FS grade 4+; very rarely pyramidal grade 5 alone).

7.5 - Unable to take more than a few steps; restricted to wheelchair; may need aid in transfer; wheels self but cannot carry on in standard wheelchair a full day; May require motorized wheelchair; (Usual FS equivalents are combinations with more than one FS grade 4+).

8.0 - Essentially restricted to bed or chair or perambulated in wheelchair, but may be out of bed itself much of the day; retains many self-care functions; generally has effective use of arms; (Usual FS equivalents are combinations, generally grade 4+ in several systems).

8.5 - Essentially restricted to bed much of day; has some effective use of arm(s); retains some self-care functions; (Usual FS equivalents are combinations, generally 4+ in several systems).

9.0 - Helpless bed patient; can communicate and eat; (Usual FS equivalents are combinations, mostly grade 4+).

9.5 - Totally helpless bed patient; unable to communicate effectively or eat/swallow; (Usual FS equivalents are combinations, almost all grade 4+).

10.0 - Death due to MS.

*Excludes cerebral function grade 1.

Note 1: EDSS steps 1.0 to 4.5 refer to patients who are fully ambulatory and the precise step number is defined by the Functional System score(s). EDSS steps 5.0 to 9.5 are defined by the impairment to ambulation and usual equivalents in Functional Systems scores are provided.

Note 2: EDSS should not change by 1.0 step unless there is a change in the same direction of at least one step in at least one FS.

Source: Adapted from Kurtzke, 1983
1.8 Diagnostic issues

The diagnosis of MS is not always a straightforward issue, especially in its early stages, as there is no single pathognomonic clinical feature, laboratory test, or neuroimaging finding that is diagnostic of MS. Ultimately, a combination of clinical findings, and supportive laboratory and neuroimaging studies are required to reach this diagnosis and differentially exclude other conditions and diagnoses (Noseworthy, 2002). Generally, what is required is a detailed patient clinical history, findings of clinical abnormalities on neurological examination (see symptoms, signs and clinical course as described previously), objective evidence of pathology obtained from MRI, cerebrospinal fluid (CSF) analysis, or auditory, visual and somatosensory evoked potentials (McDonald et al. 2001). In several occasions other, ancillary studies such as spectroscopy, diffusion tensor imaging, etc. may be utilized if deemed necessary (Lezak et al. 2012).

Magnetic Resonance Imaging of the brain and spinal cord is extremely helpful in identifying the presence of CNS lesions and has been found to be highly sensitive in this regard (McDonald et al. 2001). Areas of demyelination appear as bright lesions and can provide the evidence of chronicity needed for a definite diagnosis of MS. MRI can also reveal lesions which occurred previously, but produced no clinical symptoms (Miller, 2001). Typical MRI characteristics include white matter abnormalities in approximately 95% of the patients, and white lesions indicative of fresh inflammation (see figure 1.4). Moreover, brain atrophy with widened lateral ventricles and cortical sulci may be observed (see figure 1.5). Laboratory studies with abnormalities in the Cerebrospinal fluid (CSF) (e.g., an elevated immunoglobulin G [IgG] index, mildly elevated CSF white blood cell count, or the identification of unique oligoclonal (IgG bands), which are present in more than 90% of MS patients) support the presence of an inflammatory process (Compston & Coles, 2002) (see figure 1.6). Visual, auditory and somatosensory evoked potentials (VEP, AEP, SEP), are electrical potentials
recorded following the presentation of stimuli. The brain of an MS patient often responds less actively to stimulation of the visual (optic nerve), auditory, and somatosensory nerves. Decreased activity on either test can reveal demyelination which may be otherwise asymptomatic (Compston & Coles, 2002). Furthermore, findings of delayed but well-preserved VEP waveforms are consistent with the slowed nerve impulses seen in demyelinated axons (Compston & Coles, 2002. McDonald et al. 2001) (see figure 1.7).

Figure 1.4 Typical MRI white lesions in MS indicative of fresh inflammation
Source: Images are courtesy of Prof. Panagiotis Papathanasopoulos, Department of Neurology, University of Patras Medical School

Figure 1.5 Typical MRI in MS showing widened lateral ventricles and cortical sulci
Source: Images are courtesy of Prof. Panagiotis Papathanasopoulos, Department of Neurology, University of Patras Medical School
As mentioned previously, no single clinical feature or diagnostic test is sufficient to diagnose MS, and the diagnosis is mainly a clinical one. Over the years, several sets of criteria have been proposed for the diagnosis of MS, based mainly on the cardinal requisite of *dissemination in space* (DIS) and *dissemination in time* (DIT) of CNS lesions, implying that lesions have developed at different time points or have progressed over time with multiple, discrete areas of CNS white matter involvement and the exclusion of other conditions with similar clinical characteristics. With each revision, new diagnostic criteria modified disease

According to the older Schumacher and Poser criteria, MS can be diagnosed clinically by demonstrating 2 separate attacks (fulfilling DIT criteria) involving at least 2 different areas of the CNS (fulfilling DIS criteria) (Poser et al. 1983). Specifically, the Poser criteria divided MS patients into those with definite MS and those with probable MS, and further subdivided these categories into clinical and laboratory supported. Criteria for clinically definite MS included either (1) two attacks and clinical evidence of two separate lesions, or (2) two attacks, clinical evidence of one lesion, and more objective evidence of a second separate lesion. A diagnosis of laboratory supported definite MS was given in individuals with (1) two attacks, either clinical or objective evidence of one lesion, and CSF IgG or oligoclonal bands, (2) one attack, clinical evidence of two separate lesions, and IgG or oligoclonal bands, or (3) one attack, clinical evidence of one lesion, objective evidence of a second, separate lesion, and CSF IgG or oligoclonal bands. Clinically probable MS was defined as having (1) two attacks and clinical evidence of one lesion, (2) one attack and clinical evidence of two separate lesions, or (3) one attack, clinical evidence of one lesion, and objective evidence of a second, separate lesion. Finally, a diagnosis of laboratory-supported probable MS was given if the patient had two attacks and evidence of IgG or oligoclonal bands (Poser et al. 1983. Thornton & DeFreitas, 2009).

The 2001 McDonald criteria and their 2005 revision incorporated defined MRI criteria for DIS and DIT that provided guidance on how to diagnose MS after CIS (McDonald et al. 2001. Polman, Reingold, & Edan, 2005. Milo & Miller, 2014). According to the McDonald criteria, a diagnosis of MS is typically given if there have been two or more distinct attacks consistent with MS, as well as objective evidence of at least two CNS lesions (i.e. in the cerebral white matter, cerebellum, optic nerves, brain stem, or spinal cord) that are separated
in time and space. However, a diagnosis of MS can also be given when there has been only one attack as long as it is accompanied by objective evidence of at least two CNS lesions that are disseminate in time and space. Moreover, the physician must determine that there is “no better explanation” for the observed abnormalities before giving a diagnosis of MS. Failure to meet the diagnosis of MS results in the classification of either not MS or possible MS (i.e., when a patient clinically presents as having MS but has not yet been diagnostically evaluated, or if the evidence of the diagnostic examination is inconclusive). The Panel also recommended against the use of the terms clinically definite MS and probable MS (McDonald et al. 2001. Thornton & DeFreitas, 2009).

The most recent 2010 McDonald criteria simplify requirements for DIS and DIT and may allow for an earlier diagnosis of MS from a single baseline brain MRI if there are both silent gadolinium-enhancing and non-enhancing lesions (Polman et al. 2011) (see Table 1.4). Despite these important advances in the diagnosis of MS, some questions still remain regarding the application and the implications of the new criteria in the daily clinical practice and in clinical trials. Most importantly, thorough clinical evaluation and judgment along with careful differential diagnosis still remain the basics in the diagnosis of MS (Polman et al. 2011. Thornton & DeFreitas, 2009. Gafson, Giovanni, & Hawkes, 2012).
<table>
<thead>
<tr>
<th>Clinical Presentation</th>
<th>Additional Data Needed for MS Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2 attacks, objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack</td>
<td>None</td>
</tr>
<tr>
<td>≥2 attacks, objective clinical evidence of 1 lesion</td>
<td>Dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or await a further clinical attack implicating a different CNS site</td>
</tr>
<tr>
<td>1 attack; objective clinical evidence of ≥2 lesions</td>
<td>Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or await a second clinical attack</td>
</tr>
<tr>
<td>1 attack; objective clinical evidence of 1 lesion (clinically isolated syndrome)</td>
<td>Dissemination in space and time, demonstrated by: For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or await a second clinical attack implicating a different CNS site; and for DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or await a second clinical attack</td>
</tr>
<tr>
<td>Insidious neurological progression suggestive of MS (PPMS)</td>
<td>1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria: 1. Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIT in the spinal cord based on ≥2 T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)</td>
</tr>
</tbody>
</table>

If the criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is "MS"; if suspicious, but the criteria are not completely met, the diagnosis is "possible MS"; if another diagnosis arises during the evaluation that better explains the clinical presentation, then the diagnosis is "not MS."  

An attack (relapse, exacerbation) is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event. Reports of paroxysmal symptoms (historical or current) should, however, consist of multiple episodes occurring over not less than 24 hours. Before a definite diagnosis of MS can be made, at least 1 attack must be corroborated by findings on neurological examination, visual evoked potential response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms.  

Clinical diagnosis based on objective neurological findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; at least 1 attack, however, must be supported by objective findings.  

No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS.  

Gadolinium-enhancing lesions are not required; symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes.  

MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; DIS = dissemination in space; DIT = dissemination in time; PPMS = primary progressive multiple sclerosis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.  

Source: Adapted from Polman et al. (2011), pg. 297
1.9 Cognition in Multiple Sclerosis

Dating back to the seminal writings on MS, Charcot’s observations of the adverse effects that MS exerts on memory, concept formation, and the intellect (Charcot, 1877), were underestimated for many decades in the neurology literature. It was only with the emergence of the comprehensive care model in the early 1980s, that the nature and significance of cognitive dysfunction in MS became appreciated. The medical community, due to the often-subtle nature of cognitive deficits in MS, and the difficulty in detecting these deficits during routine clinical practice, was initially slow to appreciate them as a core clinical symptom of MS. Instead, they believed that cognitive impairment was a relatively rare entity in MS, occurred only in advanced cases with a high level of physical disability and was associated with subcortical dementia (Messinis et al. 2010. Smestad, Sandvik, Landro, & Celius, 2010).

Cognition is a complex process involving the integrated functions of the human mind, by utilizing cognition individuals are able to process information from the environment and through past experiences form behaviors and adaptive strategies (Lezak et al. 2012). In this sense, a dysfunction of cognition in MS may lead to profound functional limitations, affecting daily functional capacity, including, adaptive functions, vocational activities, socialization, and may also alter behavior or mood, leading to behavioural disturbances such as aggression or impulsivity and depression or apathy. Another important issue to consider is that cognitive dysfunction may negatively impact the rehabilitation of motor symptoms in MS. Specifically, cognitive deficits in this population can affect balance and mobility since impaired attention and distractibility force MS patients to actively think about their walking in order to reduce potential falls. On the other hand, MS individuals with cognitive decline may limit their social interaction activities fearing apparent forgetfulness, slowness in thinking or processing information, and consequently develop depression. Moreover, they may show decreased compliance with their medication regimen by forgetting to take it or by taking it in the wrong way.
1.9.1 Prevalence rates of cognitive dysfunction in MS

The process of estimating the prevalence of cognitive dysfunction in patients with MS might appear simple at first, but it is actually a complicated process. Although it is now commonly accepted that roughly one-half of individuals with MS (Chiaravalloti & DeLuca, 2008. Polychroniadou et al. 2016), will experience clinical deficits over the course of the disease, prevalence rates are highly variable and depend to a large extent on the type of MS population studied, the clinical, demographic and sociodemographic characteristics and the year conducted. In studies conducted before the mid-1990s, in the absence of DMTs, essentially reflecting untreated patients, inclusion of relatively small sample sizes of patients, and great variability in use of cognitive measures, the rates are on average reported as being between 45-65% (Rao, 1986) (see Table 1.5).

Table 1.5 Prevalence studies of cognitive dysfunction prior to the immergeence of disease modifying therapies

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample size</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bertando, Maffei, &amp; Ghezzi, 1983</td>
<td>22</td>
<td>55</td>
</tr>
<tr>
<td>DeSmedt, Swerts, Geutjens, &amp; Medaer, 1984</td>
<td>46</td>
<td>65</td>
</tr>
<tr>
<td>Heaton et al., 1985</td>
<td>100</td>
<td>67</td>
</tr>
<tr>
<td>Lyon-Caen et al., 1986</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>McIntosh-Michaelis et al., 1991</td>
<td>147</td>
<td>46</td>
</tr>
<tr>
<td>Parsons, Stewart, &amp; Arenberg, 1957</td>
<td>17</td>
<td>65</td>
</tr>
<tr>
<td>Peyser et al., 1980</td>
<td>52</td>
<td>54</td>
</tr>
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<td>Rao et al., 1984</td>
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<td>64</td>
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<tr>
<td>Rao et al., 1991</td>
<td>100</td>
<td>43</td>
</tr>
<tr>
<td>Staples, &amp; Lincoln, 1979</td>
<td>64</td>
<td>60</td>
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</tbody>
</table>

Source: Adapted from Chelune, Stott & Pinkston, (2008), pg. 607
Moreover, Rao, Leo, Bernardin, & Unverzagt, (1991), reported that the prevalence rates recorded depended to a large extent and differed considerably according to the definition or criteria of “cognitive impairment” utilized in the study. In the Rao et al., (1991) study, for example, which included 100 community dwelling volunteers diagnosed with MS, 43% of these patients, when compared to demographically adjusted healthy participants had performance below the 5th percentile, equivalent to 1.67 SD, of the healthy group on a comprehensive battery of neuropsychological measures. When prevalence rates were assessed according to the various cognitive domains affected (memory, problem solving abilities, visuospatial skills, attention, language and information processing speed) the study reported the highest rates on measures of information processing (22-25%) and episodic memory (22-31%).

Another important variable that needs to be considered when estimating prevalence rates of cognitive dysfunction is whether the patients were recruited from clinical settings or were community dwelling volunteers. In general, studies that have utilized volunteer community samples, which usually have milder disease and disability status, report lower prevalence rates of cognitive deficits (see Rao et al. 1991). On the contrary, clinical samples with a more progressive disease course and larger neurological disability show higher prevalence rates, at about 60% (Benedict et al. 2006). A more recent study that included RRMS and SPMS patients attending an outpatient neurology clinic reported an overall cognitive dysfunction prevalence rate of 53.7% (Papathanasiou, Messinis, Georgiou & Papatheanasopoulos, 2014). Moreover, Polychroniadou et al. (2016), reported that 47% of their MS patients recruited from an outpatient clinical setting, diagnosed with the revised McDonald criteria (Polman et al. 2011), the majority with RRMS and mean duration of illness at 9.6 years, assessed with a brief cognitive measure (BICAMS), performed below the 1SD cutoff set for impairment on at least one of the three tests that comprise this brief neuropsychological battery.
In an interesting cross-sectional study that evaluated the patterns of cognitive impairment in patients with disease duration of up to 30 years, 20.9% performed below the 1SD cutoff for impairment by the fifth year from disease onset, by 10 years this had reached 29.3%. By utilizing regression modeling the authors suggested that cognitive impairment may precede MS onset by 1.2 years (Achiron et al. 2013). In a longitudinal 18 years follow up study of cognitive impairment in MS patients, 41% were impaired at study entry, whereas, 59% were cognitively impaired at follow up implying that cognitive decline progresses over time. In addition, only one measure of a comprehensive neuropsychological battery, the SDMT identified deficits over time in patients that were intact at baseline (Strober, Rao, Chi-Lee, Fischer & Rudich, 2014).

In general, the cognitive deficits noted in MS patients are more specific and vary considerably from mild to moderate severity (Guimaraes & Jose, 2012. Lovera & Konver, 2012). Moreover, as noted previously several factors influence the prevalence of cognitive deficits, including amongst others already mentioned, MS disease subtype and cognitive domains assessed, which will be discussed in the sections that follow. Although severe cognitive dysfunction and overt dementia are rare in MS, some patients may reach a threshold for diagnosis of dementia, and roughly 10% of clinical samples reach this threshold (Benedict & Bobholz, 2007. Fischer, 2001. Chiaravalloti & DeLuca, 2008).

Further, the relationship between cognitive function and MS disease duration, stage, type or disability status is unclear as current data are conflicting (Patti, 2009). Generally, cognitive deficits appear to increase with worsening physical disability, disease duration and with onset of a progressive disease course (Guimares & Jose Sa, 2012. Messinis et al. 2010).

1.9.2 Cognitive domains impaired in MS

The heterogeneous nature of MS generally precludes manifestation of a typical cognitive impairment pattern. Moreover, cognitive dysfunction is characterized by a varying combination
of domain-specific deficits rather than a uniform general cognitive decline (Panou, Simos, Mastorodemos, Fassarakis & Plaitakis, 2008. Calabrese, 2006. Calabrese & Penner, 2007). Indeed, the variability noted in deficits is striking even in persons with RRMS and minimal disability (Ryan, Clark, Klonoff, Li, & Paty, 1996). For example, based upon the 5th percentile impairment cutoff, 35% of a sample of 177 persons with RRMS showed no impairments. In contrast, 33% of the sample exhibited impairment on three or more tests, with 5% of the patients showing relative global impairment (Ryan et al. 1996).

However, the dissemination of lesions in cerebral white matter including their affinity for periventricular regions provides the basis for some cognitive dysfunction commonalities (Fischer, 2001). In this respect, some cognitive domains appear to be more commonly compromised than others. Information processing efficiency, episodic memory, attention, and executive functioning are the domains found predominantly to be detrimentally affected in MS (Rao et al. 1991. Chiaravalloti & DeLuca, 2008. Guimaraes & Jose Sa, 2012). Among these domains the most common pattern involves circumscribed deficits as a combination of one or two of the above-mentioned domains (e.g., attention/processing speed, learning/memory, and or executive functions (Chiaravalloti & DeLuca, 2008. Messinis et al. 2010. Lezak et al. 2012). (Table 1.6 presents frequency of impairment rates by cognitive domain affected in MS).

In contrast to the significant impact that MS has on the previously mentioned cognitive domains, visuoconstructive and visuospatial abilities are only occasionally affected and often with a subtle severity (Winkelmann, Engel, Apel, & Zettl, 2007). General intelligence as measured by contemporary IQ measures derived from the Wechsler Adult Intelligence Scales is not significantly affected; however, MS patients tend to exhibit greater deficits in Performance Intelligence (PIQ) than Verbal Intelligence (VIQ) (Lezak et al. 2012). Nonetheless, approximately 10% of a large sample of individuals with RRMS suffering minimal neurological disability were significantly impaired in fluid intelligence (Raven Progressive Matrices; RPM),
whereas, approximately 20% of individuals with a variety of courses and relatively severe disability exhibit fairly broad intellectual impairments (Thornton & Defreitas, 2009).

**Table 1.6 Frequency of impairment rates by cognitive domains affected in MS**

![Bar chart showing frequency of impairment rates by cognitive domains affected in MS](image)

Source: Adapted from Chiaravalloti & DeLuca, (2008)

**1.9.2.1 Memory and learning**

Memory is one of the most common self-reported difficulties in MS patients, especially in terms of "short term memory", implying that their difficulty is mainly in remembering details of recent conversations and events, but that they can still recall events from the distant past in quite some detail (Chiaravalloti & DeLuca, 2008; Messinis et al. 2010). This is actually also the finding of several studies in that semantic memory, (i.e. the retrieval and use of lexical/semantic information stored in long term memory), is often well preserved in MS and especially those patients with the RRMS subtype (Zakzanis, 2000; Panou et al. 2008). It should be noted however, that occasionally MS patients may present with deficits in remotely learned facts or
on autobiographical memory measures related to personal events (Rao et al. 1991. Lezak et al. 2012). Implicit memory also appears to be intact in most MS patients and this fact is based mainly on studies that used word stem (lexical) and semantic priming and perceptual motor skill measures (Guimares & Jose Sa, 2012).

On the contrary, explicit memory dysfunction is considered amongst the most prominent cognitive difficulties in MS, and this has been confirmed by several studies (see meta-analysis by Prakash, et al. 2008). These deficits are often observed even at the earlier stages of the disease (Winkelman et al. 2007). As regards the nature of explicit memory dysfunction, early reports suggested that the main difficulty was in retrieval from long term storage and relative preservation of encoding and storage processes, and was based predominantly on findings of preserved recognition ability (see for e.g. Peyser et al. 1990). However, more recent studies suggest that the primary memory deficit is in the initial encoding and learning/ acquisition of the information and the inability to bind contextual information during the encoding process (Calabrese, 2006. Chiaravalloti & DeLuca, 2008. Guimares & Jose Sa, 2012). Characteristic of these encoding and acquisition deficits is the significant difficulty of MS patients to learn lists of presented words on the first trials, which places an overwhelming challenge to their information storing processing abilities. As the word lists are repeated on subsequent learning trials, they manage to encode the information via slow accretion and learn the information, although they require more learning trials and acquire fewer words than controls. This difference between MS patients and controls is not usually sustained during the recognition memory trials, implying that initial learning/encoding difficulties are responsible for the differences in episodic memory performance (Calabrese, 2006. Winkelmann et al. 2007). Having said the above, one of the most typical patterns of MS episodic memory dysfunction that a clinician can expect is deficient recall on the first learning trial, mildly inconsistent recall across the remaining learning trials, mildly deficient delayed
recall and normal recognition (Messinis et al. 2010). Of course, there are exceptions to this pattern and approximately one third of patients included in clinical trials will perform like healthy controls, whereas about one fifth will present with a significantly flattened learning curve, poor delayed recall and several intrusion errors (Lezak et al. 2012).

Other factors that may contribute to deficient encoding on the first trial/s of multi trial learning tasks and on paired associate learning with weak cue-target associations is the inability of these patients to activate novel strategies (e.g. semantic clustering, visual imagery (Calabrese, 2006). They also have greater difficulties in recalling word lists than prose passages, due to the fact that the passages provide a meaningful semantic context, allowing them to better process this kind of material (Chiaravalloti & DeLuca, 2008). Working memory and mental processing speed may significantly influence new learning, especially with material that is delivered rapidly, complex, long and with interference stimuli (e.g., noisy environment, music in the background) (Denney, Lynch, Parmenter, & Horne, 2004. DeLuca, Chelune, Tulsky, Lengenfelder & Chiaravalloti, 2004). Considering this fact, it becomes quite clear that in everyday life situations where working memory and mental processing speed load is high; MS patients will often fail to grasp initial information that is not repeated to them. In this respect, they not only have difficulties encoding and learning new information, but may also have difficulties in remembering to do things, owing to the primary learning difficulty and not prospective memory deficits per se (Lezak et al. 2012).

1.9.2.2 Attention and information processing

When MS patients are tested on tasks of attention, deficits may be observed depending on the applied test system. Complex attention tasks like selected, alternating and divided attention are frequently impaired (Winkelmann et al. 2007). Moreover, the majority of MS patients report “mental slowness” implying that they exert tremendous effort to think rapidly or even to keep up with the normal pace of conversations (Bruce, Bruce, & Arnett, 2007). Simple auditory span
and visuospatial span are normal in the majority of MS patients (Rao et al. 1991). Many patients also have normal performance on self-paced measures with the printed material in front of them (e.g. letter or symbol cancellation tasks), tests with minimum stimuli or response choices (e.g. auditory consonant trigrams) and numerous choice reaction-time tasks (Rao et al. 1991. Lovera & Konver, 2012). When attention deficits are observed they are more apparent on tests utilizing auditory verbal stimuli compared to those with visual stimuli and tasks with greater stimulus load or complexity level, including supraspan tasks, sequence reversal tests and measures that require inhibition of a previously correct response. Such measures include the Stroop interference condition and the PASAT (Lezak et al. 2012). However, Lynch, Dickerson, and Denney, (2010), suggest that deficits on the PASAT are mostly due to slow mental processing difficulties rather than attention or working memory impairment. Shifting attention tasks (for e.g. Trail Making Test Part B and alphanumeric sequences) are also very difficult for MS patients to perform (Grigsby, Kaye, & Busenbark, 1994).

The mental slowness reported by MS patients is also evident on objective measures of information processing. Studies have shown that deficient information processing in MS is mostly related to reduced speed capacity, and if provided with sufficient time these patients are able to perform information processing tasks equally well to healthy controls (De Luca et al. 2008. Denney et al. 2004). These include visuomotor scanning tasks, like the Digit Symbol subtest (DSS) of the WAIS scales or the Symbol Digit Modalities Test (SDMT). Deficits in processing speed are associated with an effect size of approximately one standard deviation on these measures (Thorton & Defreitas, 2009). Notably, impairments on the Trail-Making Test (TMT) appear to be relatively attenuated compared with other processing speed measures. Moreover, the TMT, DSS, and SDMT load very highly on visual search and visuomotor speed. It is worth noting, however, that the latter two tests also place great demands on memory processes (Lezak et al. 2012; Strauss, Sherman & Spreen, 2006). Considering the reciprocal
relationship between attention, memory, and executive functions, reduced speed can also significantly impact everyday functional tasks requiring working memory and higher mental functions, such as planning skills, organization abilities and reasoning (Chiaravalloti & DeLuca, 2008).

1.9.2.3 Language and Verbal fluency

Communication impairments associated with MS are almost exclusively related to speech intelligibility and dysarthria (Murdoch & Theodoros, 2000). Language impairments in MS are less frequently identified, although some patients can even be identified as having a specific type of aphasia (Demirkiran, Ozeren, Sonmezler, and Bozdemir, 2006). A rare pure alexia was recently reported in a Greek MS patient (Potagas, Kasselimis, Peppas, Alexandri, & Dellatolas, 2017), and alexias have been observed in other cases, as have other syndromes associated with cortical lesions (Filley, 2001. Lezak et al. 2012). These syndromes typically present with an acute relapse, on occasion even as the presenting symptom, but most resolve with corticosteroid treatment (Lezak et al. 2012). When language is found to be impaired, the most common deficits are observed on word retrieval and verbal fluency tasks (phonemic and semantic fluency). However, due to the fact that these tasks rely heavily on executive function resources, such as inhibition and set-shifting, this may imply that the deficits observed on word retrieval and fluency tasks are actually part of a dysexecutive syndrome and not language deficits per se. Moreover, impairment in mental speed, search strategies, and/or access to verbal storage may disrupt verbal fluency (Renauld, Mohamed-Said, & Macoir, 2016). Although clinically, phonemic tasks appear to be more impaired than semantic tasks, Henry and Beatty, (2006) conducted a review of 35 studies examining verbal fluency performance in MS. They found that MS patients were substantially impaired on this measure, and that they presented with equal impairment on semantic and phonemic verbal fluency. They further suggested that verbal fluency is one of the most sensitive measures of cognitive impairment in MS, along with
the Symbol Digit Modalities Test of psychomotor speed. They also concluded that SPMS patients presented with more severe impairments in comparison to RRMS patients. In another study examining the use of strategies for maximizing word production, Tröster et al. (1998) found that patients with MS produced more words than healthy controls; more interesting, however, was their compromised ability to switch between semantic or phonemic subcategories, despite producing an average number of words within each subcategory. In a more recent study, Messinis et al. (2013), failed to find differences between RRMS or SPMS patients, on semantic or phonemic fluency word production measures. Moreover, no differences were noted on the strategies used to maximize semantic fluency between the two patient groups. In contrast, they found that the number of switches differed significantly in the phonological fluency task between the SPMS and RRMS subtypes. Generally, confrontation naming is better preserved than fluency, especially in patients with RRMS (Prakash et al., 2008). On some occasions, comprehension of concept meanings and attributes, deciphering complex or ambiguous grammatical structures, and the production of fewer complete and grammatically correct sentences (Wallace & Holmes, 1993).

One of the main limitations of language studies in MS is the absence of sensitive linguistic specific measures such as those assessing syntax or discourse, which may have resulted in different outcomes (Renauld et al. 2016). In a recent MS language study, the authors utilized a sensitive linguistic measure in order to explore whether individuals with RRMS show word class naming deficits favoring either nouns or verbs, and if verb accuracy is affected by semantic and phonological verb type. They reported that verbs were significantly more difficult to retrieve than nouns for the RRMS group on production tasks compared to a demographic and intelligence matched healthy control group. Moreover, they noted a significant difference between instrumental and non-instrumental verb production with instrumental verbs more difficult to retrieve within the MS group. They concluded that the
naming deficit was probably in the connection between the semantic lexicon and the phonological lexicon and that in the case of verbs, the magnitude of the difficulty was larger because of the effects of word frequency on verb retrieval. However, their opinion was that poor performance on verb and noun naming in RRMS patients may be a marker of incipient cognitive decline, and that typical cognitive-linguistic testing is not sensitive or specific enough to capture this phenomenon (kambanaros, Messinis, Nasios, Nousia & Papathanasopoulos, 2017).

1.9.2.4 Executive functions

Executive functions are mental operations needed to independently perform complex, non-routine, goal-oriented tasks (Lezak et al. 2012). Although deficits in executive functions occur less frequently than memory or processing speed, they tend to have a significant impact on MS patients’ ability to generate strategies, think divergently, solve and estimate problems, and reason in abstract terms (Rao et al. 1991. Strauss et al. 2006). Moreover, impaired executive function may have behavioural implications, including reduced cognitive flexibility, such as utilization of feedback, behavior monitoring, perseveration errors with difficulties in eliminating irrelevant hypotheses and affect awareness (Benedict, Carone, & Bakshi, 2004). Reduced awareness in this population, however, is not consistent across different dimensions of cognitive functioning, as they may show better awareness of more concrete declines (e.g., forgetting) and be less aware of abstract declines (e.g. difficulties in problem solving) (Goverover, Chiaravalotti, & DeLuca, 2005). These deficits tend to affect everyday functions significantly, and are often more apparent to family and friends than to the patient with MS (Winkelmann et al. 2007). To others these symptoms may appear as changes or disturbance in personality characteristics, and include stubbornness or disorganization leading to interpersonal and household tensions (Lezak et al. 2012). Moreover, impaired problem-solving strategies, utilization of feedback, and concept formation, can contribute to deficiencies on tests
of memory and visuoconstruction. Specifically, there is a moderate correlation between executive deficits, spontaneous use of systematic learning strategies and application of imagery based mnemonic techniques in rehabilitation (Canellopoulou & Richardson, 1998).

Other impairments observed in MS are related to the disparities between patient’s cognitive complaints and their objective performance on neuropsychological measures. These disparities are associated with metamemory and metacognition abilities and refer to the knowledge an individual has about their cognitive capabilities as well as the control over cognitive processes (Thorton & Defreitas, 2009). Awareness of one’s abilities and their limitations and the implementation of relevant cognitive processes may improve performance when cognitive demands are extensive. It is important to note, that degradation in these oversight and control aspects of cognition in MS patients possibly contributes to the losses observed in other abilities. Increased depression often emerges as a significant associate of perceived compared with objective cognitive dysfunction (Guimares & Jose, 2012). Moreover, associations between metamemory and executive function, and metamemory and depression, are mediated through depressive attitudes, suggesting that depression and depressive attitudes further intensifies memory complaints (Hoffman, Tittgemeyer, & Yves von Cramon, 2007. Thorton & Defreitas, 2009).

1.9.3. Assessment of Cognition in MS
The identification of cognitive impairment in MS remains challenging as comprehensive neuropsychological assessments are not routine in most neurology clinics (Benedict, 2005. Messinis, Kosmidis, Lyros & Papathanasopoulos, 2010). Moreover, cognitive dysfunction may reflect damage to brain regions that do not affect physical functioning, and therefore may not be detected by the neurologist. In a very interesting study published thirty-seven years ago, Peyser, Edwards, Poser & Filskov, (1980), reported that a neurologist who suspects an MS patient as having cognitive dysfunction based on identification of cerebral involvement is in
most cases correct, but in contrast, when the neurologist does not suspect cognitive impairment, he/she is wrong in about 50% of the cases, underlying the necessity of using standardized neuropsychological instruments. In a more recent study, Romero, Shammi & Feinstein, (2015), evaluated the accuracy of the neurological examination in identifying cognitive impairment in MS patients. Based on a retrospective chart review of 97 MS patients referred by neurologists for neuropsychological assessment due to suspected cognitive impairment, the authors concluded that the clinical interview and standard neurological examination are not sufficiently sensitive to detect cognitive impairment in MS, and suggest the need for a brief, accurate cognitive screen to complement routine clinical evaluation. While significant others and care-givers may also provide important information regarding the cognitive status of MS patients, these self-reports are generally not consistent with objective evaluation (Benedict et al. 2003. O’Brien, Gaudino-Goering, Shawaryn, Komaroff, Moore, & DeLuca, 2007).

However, the unavailability of clinical neuropsychologists who are well-trained in the assessment of MS, in addition to the high cost and lengthy time required for an extensive neuropsychological assessment, may impede routine neuropsychological evaluations in many neurology clinics. Consequently, cognitive deficits in this population, which in some cases are very subtle and may be missed during routine neurological evaluation, often go undetected (Benedict, 2005. Peyser et al. 1980. Romero et al. 2015). Patients in whom cognitive deficits have been addressed inadequately remain vulnerable to the negative effects of cognitive decline on activities of daily living, social skills and employment (Sartori & Edan, 2006), as well as overall quality of life (Patti, 2009).

In contrast to motor and sensory deficits, the detection of cognitive deficits in the clinical setting is both time-consuming (possibly also provoking fatigue and the patient’s refusal to complete the cognitive test battery) and expensive. Consequently, as mentioned previously,
neuropsychological testing may not be applied routinely in neurological settings, and in some settings not at all. Furthermore, this issue is complicated by the scarce literature on risk factors for cognitive impairment in this population, making identification of at-risk patients difficult (Messinis et al. 2010). Nevertheless, some risk factors have been identified including advanced age, low premorbid level of intelligence or educational attainment (Randolph, Arnett, & Higginson, 2001) comorbid depression (Haase, Tinnefeld, Lienemann, Ganz, & Faustmann, 2003) and fatigue (Bruce et al. 2010). Specifically, regarding the influence of age on cognitive functions in MS, a recent report noted that 71% of their MS patients with cognitive deficits were 40 years or older versus 27% that were cognitively intact. The same study also reported that 79% of their patients with cognitive deficits had an educational level of nine years or less versus 23% without such deficits (Sartori & Edan, 2006). The combination of these two factors (low education and advanced age) potentially represents a high-risk factor for cognitive dysfunction and possibly reflects a lower cognitive reserve in these patients, independently of disease status. Moreover, employment status appears to be associated with cognitive deficits in MS patients, as only 21% of these patients retained their professional activity when evaluated versus 73% who were unemployed (Sartori & Edan, 2006. Messinis et al. 2010).

Another important issue is establishing the right time point for cognitively assessing MS patients. As relapses and high-dose corticosteroid treatments may cause deficits in attention and memory processes (see for e.g. Oliveri et al. 1998), it is preferable to delay neuropsychological evaluation for at least eight weeks after a relapse or steroid treatment, and consider all possible co-morbidities (Messinis et al. 2010).

The multidimensional nature of cognitive dysfunction in MS necessitates an assessment of numerous cognitive domains. The challenge until recently was to find the optimal combination of cognitive tests that would provide an accurate picture of the deficits whilst avoiding the use of unnecessary and time-consuming measures (Messinis et al. 2010).
In order to overcome some of the previously mentioned limitations in assessing cognition in MS, and considering the fact that not all neuropsychological measures are appropriate for the MS population, a number of neuropsychological assessment tools (brief screening batteries and comprehensive neuropsychological batteries), have been utilized specifically for this population in routine clinical care and for research purposes. Table 1.7 provides a summary of the most important neuropsychological tools utilized in MS patients.

**Table 1.7 Neuropsychological batteries utilized in MS patients**

<table>
<thead>
<tr>
<th>Cognitive Domain</th>
<th>Rao Brief Repeatable Neuropsychological Battery (BRB)</th>
<th>Minimal Assessment of Cognitive Function in MS (MACFIMS)</th>
<th>NINDS Common Data Elements</th>
<th>Brief Assessment of Multiple Sclerosis (BICAMS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive Processing Speed</td>
<td>SDMT</td>
<td>SDMT</td>
<td>SDMT</td>
<td>SDMT</td>
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<tr>
<td>Language</td>
<td>PASAT</td>
<td>PASAT</td>
<td>PASAT</td>
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<tr>
<td>Visual/Spatial Memory</td>
<td>COWAT</td>
<td>COWAT</td>
<td>COWAT</td>
<td></td>
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<tr>
<td>Memory</td>
<td>SRT</td>
<td>CVLT2</td>
<td>CVLT2</td>
<td>CVLT2</td>
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<tr>
<td>10/36 Spatial Recall Test</td>
<td>BVMTR</td>
<td>BVMTR</td>
<td>BVMTR</td>
<td></td>
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<tr>
<td>Executive function</td>
<td>D-KEFS</td>
<td>D-KEFS</td>
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</tbody>
</table>

BRB; Rao et al., 1991; MACFIMS; Benedict et al., 2002; National Institute of Neurological Disorders and Stroke, 2017; BICAMS; Langdon et al., 2012; SDMT: Symbol Digits Modalities Test; PASAT: Paced Auditory Serial Addition Test; COWAT: Controlled Oral Word Association Test; CVLT2: California Verbal Learning Test 2nd edition; BVMTR: Brief Visuospatial Memory Test Revised; DKEFS: Delis Kaplan Executive Function System Sorting Test.

The Rao Brief Repeatable Neuropsychological Battery (BRB) (Rao et al. 1991) was the most widely used and validated battery in the MS population for many years. In 2001, following a consensus meeting a comprehensive neuropsychological battery known as the Minimal Assessment of Cognitive Function in MS (MACFIMS) was developed (Benedict et al. 2002). These batteries use similar measures to assess cognitive processing speed and language.
fluency, but differ in testing time (the MACFIMS is longer in duration and requires 90 minutes to complete compared to the BRB that requires 45 minutes) and the tests utilized for visual/spatial abilities, memory and executive function. Both these batteries have good psychometric properties including discriminant validity, test/retest reliability, ecological validity, and regression-based norms are available for the MACFIMS interpretation (Benedict et al. 2006. Parmenter, Testa, Schretlen, Weinstock-Guttman, & Benedict, 2010).

Despite the reliable psychometric properties of both these batteries, they are time consuming and not applicable to routine clinical practice, especially in non-specialized MS clinics (they require specialized training or access to clinical neuropsychologists for administration and interpretation). Moreover, they may fail to reflect cognitive function in everyday life situations, in which tasks are more complex requiring multiple abilities and can be influenced by psychoeducational interventions that patients have received to improve everyday abilities (Becker et al. 2012). These limitations led to the development of a brief and practical 15-minute battery known as the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) (Langdon et al. 2012).

The BICAMS was developed in order to be administered by appropriately trained health care professionals who are not specialists in cognition (making it applicable to routine clinical practice). Moreover, it has been translated and validated internationally (see for e.g. Polychroniadou, Bakirtzis & Langdon, (2016), for the Greek validation study). Goal of the authors is to provide an international validation protocol which can be specified and implemented in any country, and guidelines can be agreed upon for test-retest timing and clinically significant change. The three measures comprising the battery are given in the following sequence: SDMT for information processing speed; CVLT2 (the first five recall trials) for verbal memory (immediate recall); and BVMTR (the first three recall trials) for visual memory (immediate recall) (Langdon et al. 2012). As with most neuropsychological measures,
performance on the BICAMS is influenced by demographic variables, physical disorders (e.g. dysarthria, pain, and impaired vision), fatigue, and severe depression (Korakas & Tsolaki, 2016).

Regarding the comparative performance of MS patients between the BICAMS and BRB, Niccolai et al. (2015) in a multicenter study recently reported that all measures included in the batteries distinguished healthy controls from MS patients. Cohen’s d effect size for each of the tests included in BICAMS respectively, were 0.83 for the SDMT, 0.61 for the CVLT2, and 0.60 for the BVMTR (Niccolai et al. 2015).

Whether cognitive impairment on the MACFIMS battery is associated with findings on neuroimaging was investigated by Mike et al. (2011). The study reported significant correlations between performance on the CVLT2 and cortical lesion number, whereas the BVMTR correlated with cortical lesion volume and white matter lesion volume.

More recently, Goverover, Chiaravalloti, & DeLuca, (2016) evaluated whether the BICAMS can predict performance of activities of daily living in persons with MS. The authors report that better performance on the BICAMS was related with more independent actual reality task performance (i.e. accessing the internet to purchase an airplane ticket or cookies). Furthermore, the authors noted that self-reports were not associated with either performance of actual reality tasks or BICAMS performance (Goverover et al. 2016).

Another recent study conducted in Germany evaluated the use of BICAMS in the daily clinical practice of neurologists in private practices (Filser, 2016). The study, which utilized data from 1,547 patients and 59 practices, supported the feasibility of using BICAMS in routine clinical practice in Germany, however the authors note the importance of careful training of staff involved in the performance and scoring of the used neuropsychological measures.

An alternative brief neuropsychological battery that has been used mainly for assessing cognition in neurodegenerative disorders in older patients is the Repeatable Battery
for the Assessment of Neuropsychological Status (RBANS; Randolph, 1998). This battery was evaluated recently in MS patients (Davis, Williams, Gupta, Finch & Randolph, 2015) and multivariate analysis of variance indicated significant differences between the patients and a demographically matched healthy control group. However, disability status as measured by EDSS, predicted neurocognitive performance only in patients with advanced disability, and the authors suggest caution when using this measure with patients having low disability (EDSS) scores.

Recently, Papathanasiou et al. (2014), reported the efficacy of a computerized cognitive screening battery, the Central Nervous System Vital Signs (CNSVS; Gualiteri & Johnson, 2006) in discriminating between healthy controls and MS patients and between relapsing remitting and secondary progressive multiple sclerosis patients.

An important issue that arises with neuropsychological assessments in MS, as with any other clinical outcome measure, is that statistically significant change as an endpoint may not correlate with clinically meaningful change. For neuropsychological testing, determining clinically meaningful change is particularly challenging when the outcomes are not directly associated to everyday functioning activities (Benedict & Walton, 2012).

In this respect, Benedict et al. (2014) evaluated neuropsychological performance in MS patients before, during, and after a relapse and compared them with individually-matched stable MS patients. The authors reported that SDMT scores of the two groups were well matched at baseline, but SDMT raw score dropped significantly by 3.5 points in the MS group following the relapse, in contrast to the MS stable group that showed a small increase in the raw SDMT score. When the two groups were assessed three months after the relapse, SDMT scores in the relapse group had returned close to baseline values. In line with the 3.5-point score decline on the SDMT reported in the Benedict et al. (2014) study, another earlier report
by Morrow, Jurgensen, Forrestal, Munchauer, & Benedict, (2011), proposed a 3-4-point change in the raw SDMT score as a threshold for clinically meaningful change (Morrow, et al. 2011).

1.9.4. Cognitive impairment in multiple sclerosis across disease subtypes


More recently, Planche, Gibelin, Cregut, Pereira, & Clavelou, (2016), investigated the pattern and frequency of cognitive dysfunction between SPMS, PPMS and late relapsing-remitting MS (LRR, disease duration > 10 years), subtypes. By including the LRR group, they controlled for age and disease duration as confounding factors in investigating differences in cognition due to actual phenotype specific variables. After additionally controlling for gender, EDSS status, and education level, they reported that SPMS patients were “2-fold more” frequently impaired than LRR patients in the domains of verbal episodic memory, information processing speed, executive functions, verbal fluency, working memory and visuospatial construction abilities. PPMS individuals had more frequent deficits in verbal fluency, compared to the LRR group. The two progressive groups (SPMS and PPMS) differed only on visuospatial construction.

Comparative studies between progressive subtypes have been inconsistent, with most studies concluding that SPMS patients have more frequent and severe deficits (Huijbregts,

Some studies however, have indicated that their PPMS patients presented with more pronounced and frequent cognitive impairments (Wachowius, Talley, Silver, Jochen Heinze & Sailer, 2005. Rosti-Otajarvi, Ruutiainen, Huhtala, & Hamalainen, 2014. Ruano et al. 2016).

1.9.5 MS disease characteristics and cognitive impairment

1.9.5.1 Disease duration and neurological disability

Clinical disease characteristics generally correlate weakly with cognitive impairment, including disease duration and neurological disability status as assessed by the EDSS scale (Rao et al. 1991. Smestad et al. 2010). An exception to this weak correlation may be found with impaired processing speed and working memory (Lezak et al. 2012). Moreover, some studies have identified more significant associations between cognitive dysfunction and physical disability (Lynch, Parmenter, & Denney, 2005). More recently, and in contrast to most reports in the literature, Borghi et al. (2013), in a relatively large sample of patients identified disease duration, EDSS score and vocabulary performance on the Wechsler Adult Intelligence Scale (WAIS), as significant determinants of cognitive impairment in RRMS patients, a finding that was not observed in a smaller group of progressive MS patients.

A possible explanation of why several studies report a weak association between disease characteristics and cognitive dysfunction is that cerebral atrophy may occur early in the disease process when patients are still physically stable (Amato et al. 2010). Furthermore, patients that present with predominantly spinal cord lesions usually have significant physical
disability which results in high EDSS disability scores, but with minimum cognitive dysfunction (Benedict & Bobholz, 2007. Lezak et al. 2012).

Cognitive functioning in MS may provide important information associated to disease progression, and this is clearly evident in patients with CIS, whose conversion to MS was largely predicted by cognitive function (Zipoli, Goretti, Hakiki, Siracusa, & Sorbi, 2010). Moreover, cognitive dysfunction at the time of MS diagnosis may have prognostic value for the course of physical disability (Deloire, Ruet, Hamel, Bonnet, & Brochet, 2010).

1.9.5.2 Influence of depression and anxiety on cognitive impairment

Psychiatric symptoms are a common manifestation of MS, with studies reporting two main factors. On the one end symptoms are characterized by euphoria and disinhibition and on the other by apathy and depression (Figved et al. 2008). Charcot was the first to recognize the high prevalence of depression in his early characterization of MS (Lyketsos, Kozauer & Rabins, 2007). Several reports have noted high prevalence rates of depression in large community samples in Sweden, Canada and the USA and clinical depression is experienced by approximately one in two patients over the course of the disease (Ghaffar & Feinstein, 2007. Figved et al. 2008). The prevalence of major depression is between 50 and 60%, a rate significantly higher than the general population and other neurological disorders (Feinstein, 2006. Chelune et al. 2008). Depression has been reported to be more common in cerebral than spinal MS, but only modest associations have been noted between cerebral atrophy, axonal loss, MS lesion load on T2 MRI and depression (Bakshi et al. 2000. Lezak et al. 2012).

Factors that may contribute to increased prevalence of depression include, being diagnosed at a younger age, lower educational levels, and lack of social support (Lezak et al. 2012). Moreover, the diagnosis of depression is not an easy issue as many symptoms such as fatigue, sleep disorders, difficulties concentrating and apathy may overlap with the primary disease characteristics, requiring careful clinical assessment for diagnosis (Lyketsos et al.
In order to assist in overcoming this potential overlap, rating scales such as the Beck Depression Inventory Fast Screen (BDI-FS) (Beck, Steer, & Brown, 2000), consisting of only seven questions, have been validated in this population (Benedict, Fishman, McClellan, Bakshi, & Guttman, 2003).

The pathological mechanism of depression in MS is relatively complex; however, neuroimaging studies have shed some light in this respect. In one such earlier study, Pujol et al. (1997), noted that MRI hyperintense lesions which were localized to the arcuate fasciculus distinguished patients with moderately severe depression (Beck Depression Inventory score of higher than 17), from other MS patients. Another study reported greater hypointense lesion volume and cerebral atrophy in parietal and discrete frontal regions on MRI (Bakshi et al. 2000). In another study, Feinstein et al. (2004), integrated previous MRI findings reporting that extensive hyperintense lesion volume in the left medial inferior prefrontal region combined with atrophy of the dominant anterior temporal lobe were highly correlated to major depression.

It is widely accepted that depression contributes negatively to cognitive function in patients with neurological disorders (Chelune et al. 2008). Despite this widely accepted contribution, the association between cognitive function and depression in MS is not completely refined (Arnett, Barwick, & Beeney, 2008. Feinstein, 2006). While some studies have reported relationships between cognitive function and depression (Arnett, 2005), others have failed to find this relationship (Chiaravalotti et al. 2008). In a recent review, the authors conclude that when studies include a large and representative sample of MS patients, the possibility of finding associations between depression and cognitive function is relatively high (Arnett et al. 2008). Moreover, depression appears to negatively influence several cognitive domains, including, processing speed, working memory, episodic memory and executive functions (Chiaravalotti, et al. 2008. Feinstein, 2007). There is now evidence that the core depressive symptoms may reduce cognitive and attentional capacity, exerting a significant adverse effect
on the executive component of working memory (Feinstein, 2006). In a recent study, Portaccio, (2016), reported that depression may alter attentional capacity by affecting working memory and specifically through deficits in the executive control. The author notes however, that MS patients without depression may be cognitively impaired and is of the opinion that depression does not cause cognitive impairment, but may exacerbate existing cognitive dysfunction. On the contrary, he stipulates that a dysexecutive syndrome secondary to MS may precipitate depression.

Depression and anxiety are conditions with significant comorbidity, and anxiety in patients with MS ranges from 30-50% (Korostil & Feinstein, 2007). Although the literature on the association between cognitive impairment and anxiety on MS is limited, a recent report by Simioni, Ruffieux, Bruggimann, Annoni, & Schluep, (2007), found that MS patients who were cognitively impaired had higher anxiety scores than the cognitively preserved MS patients. In a more recent study, the contribution of depression and anxiety to cognitive function in MS was assessed. The study found that both conditions (depression and anxiety) independently predicted performance on an executive function measure, and after controlling for depression anxiety remained a significant contributor to cognitive impairment (Julian & Arnett, 2009).

An important clinical question that has been raised is whether successful treatment of depression would improve cognitive function. Demaree, Gaudino & DeLuca, (2003), report that treatment of depression may attenuate cognitive dysfunction. However, Feinstein, (2006), reports that there is insufficient data to address and provide an answer to this issue, although he does suggest that patients with Traumatic Brain Injury (TBI), who have been treated for depression show concomitant cognitive improvement.

1.9.5.3 Contribution of fatigue to cognitive impairment
Fatigue is considered one of the most debilitating and frequently reported symptoms in MS and contributes significantly to unemployment, difficulties in everyday functioning abilities and
poorer quality of life (Dettmers & DeLuca, 2015). Over 75% of patients with MS report levels of severe fatigue (Krupp, 2003), and fatigue has been considered even more disabling than restrictions in movement or pain (Vucic, Burke, & Kiernan, 2010). Fatigue may be induced directly through physical changes due to MS, including axonal loss, reorganization and increased brain recruitment, immunological and neuroendocrine variables. On the contrary, secondary mechanisms that may cause fatigue include depression, pharmacological side effects, reduced physical activity and sleeping difficulties (Tiffany, Braley, Ronald, & Chervin, 2010. Wendebourg, Heesen, Finlayson, Meyer, PoÊttgen, & KoÊpke, 2017).

Fatigue is composed of a physical and cognitive/mental component, but evaluation of cognitive fatigue is not always an easy issue. Measurement of fatigue is currently achieved by utilizing self-report scales owing to its subjective experience. These scales measure the intensity and characteristics of experienced fatigue and/or the impact of fatigue on certain physical or cognitive activities that the patient may or may not complete. However, the association between these measurements may not always be significant, as perceived intensities may not have the same impact on different patients' life (Wendebourg, et al. 2017).

Although numerous scales have been developed over the past years for assessing fatigue (Penner, 2016), one such 21- item scale, the Modified Fatigue Impact Scale (MFIS), (Multiple Sclerosis Council for Clinical Practice Guidelines, 1998), is able to differentiate between cognitive and physical/motor fatigue. A more recently developed scale which measures both physical and cognitive fatigue is the Fatigue Scale for Motor and Cognitive Functions (FSMC) (Penner, Raselli, Stöcklin, Opwin, Kappos, & Calabrese, 2009). It consists of 20 items of which 10 assess cognitive fatigue and the remaining 10 physical/ motor fatigue. The FSMC is able to differentiate between the two fatigue domains, but additionally provides cut-off scores for further graduation of fatigue severity
The association between fatigue and cognitive impairment has not been fully established (Krupp, Alvarez, LaRocca, & Scheinberg, 1988. Bol, Duits, Hupperts, Vlaeyen, & Verhey, 2009). However, several studies have attempted to evaluate this association. More specifically, Krupp & Elkins, (2000), conducted a study in which they used an extended 4-hour battery of neuropsychological tests (baseline and follow up) in order to establish whether cognitive fatigue could be identified in this population. Their findings indicated that persons with MS showed a decline on tests of verbal memory and conceptual planning at the end of the testing period, compared with control group participants who had improved cognitive scores owing to a practice effect.

Another study by Bruce, Bruce, & Arnett, (2010), which evaluated the relationship between self-reported cognitive fatigue and response time variability, showed that patients with MS differed significantly from healthy controls on fatigue and reaction time scores and moreover, there were significant correlations between increased response variability and higher fatigue scores in the MS group.

Various studies have shown that response time variability is related to dysfunction of the frontal lobes, disruptions of the thalamic and inferior parietal circuits and reduced white matter volume (Bruce et al. 2010). Moreover, location rather than number of lesions is a greater predictor of fatigue in MS (Dettmers & DeLuca, 2015. Penner, 2016). In a similar manner to what we know about cognitive dysfunction in MS, it is assumed that fatigue is a result of network disruption, and more specifically of the frontoparietal pathways (Penner, 2016).

In a recent study, Pokryszko-Dragan, Zagrajek, Slotwinski, Bliinska, Gruszka, & Podemski, (2016) evaluated the association between event-related potentials and cognitive dysfunction in MS patients, as they relate to fatigue levels (low, moderate, and severe). Participants with severe fatigue scored significantly lower than those with lower fatigue levels on measures of attention, memory, and visuomotor ability.
From the above, it is evident that symptoms like cognitive and physical fatigue, which are often accompanied by depression and anxiety, may negatively influence cognition in MS patients. This is especially true when extended periods are required to complete certain activities, and appear more relevant for the patient’s daily life than what may be assumed by many physicians treating MS patients.

1.9.5.4 Brain reserve, cognitive reserve and cognitive impairment in MS

Although cognitive impairment is highly prevalent among MS patients, some have a tendency to withstand severe disease burden (e.g., white matter lesions and cerebral atrophy), and present with overall lower levels of cognitive decline. One possible explanation for this protective mechanism is the brain reserve hypothesis and the cognitive reserve theory (Sumowski & Leavitt, 2013).

Recently, it has been verified that highly significant protection for cognitive impairment is provided by brain reserve, defined as the maximal lifetime brain growth (MLBG), and estimated with intracranial volume or head circumference. Larger MLBG a proxy for neuronal and synaptic count has been linked to lower risk for cognitive impairment in MS (Sumowski et al. 2014). This larger MLBG appears to be associated with more robust neural networks resistant to disease-related disruption and also provides more potential degrees of freedom for the brain to plastically reorganize in the face of MS disease related challenges.

Cognitive reserve (CR) or intellectual enrichment evoked by cognitively stimulating lifetime activities, such as reading, creating, and higher occupational and educational attainment, appears to protect against cognitive dysfunction in MS patients independently of MLBG (Sumowski, Chiaravalloti & DeLuca, 2009. Sumowski et al. 2014). A cross-sectional study which explored the cognitive reserve hypothesis in MS, reported that education and working activity levels were the strongest cognitive reserve subscore predictors of better cognitive performance in this population (Nunnari, De Cola, Costa, Rifici, Bramanti & Marino,
That higher level of education may have an independent protective effect on cognitive impairment in MS was also verified (D’Hooghe, Haentjens, Van Remoortel, De Keyser, & Nagels, 2016). In a very interesting study, Sumowski et al. (2016), showed that larger MLBG was associated with lower risk for disability progression in MS over a period of 5 years, extending the brain reserve hypothesis to physical disability in this population.

![Figure 1.8 Protective effects of cognitive enrichment (reserve) against cognitive impairment in Multiple Sclerosis](source: Adapted from Sumowski, 2015)

**1.9.6 Magnetic Resonance Imaging (MRI) correlates of cognitive impairment**

Magnetic resonance imaging (MRI) is the most utilized paraclinical tool to investigate in vivo the pathobiological mechanisms of MS and has contributed significantly to the diagnosis of MS since 2001, with the presentation of the McDonald diagnostic criteria (McDonald et al. 2001). Moreover, it provides valuable information for monitoring MS disease activity and progression, while also providing quantitative information on inflammatory activity and lesion load.
MRI relaxation rates or sequences most commonly utilized in clinical practice include T1-weighted, T2-weighted, fluid–attenuated inversion recovery (FLAIR), and diffusion–weighted types (Fillipi et al. 2010). T1-weighted or spin–lattice relaxation time sequences are able to differentiate white and gray matter in the brain. Gray matter is darker than white matter and in MS the fat is stripped away and replaced by water, making tissues appear darker than usual. MRI images are often enhanced by use of contrast material, usually gadolinium (Gd), administered intravenously. This contrast improves visualization of blood vessels. During the acute inflammatory phase of a new MS lesion there is almost always a local breakdown of the blood brain barrier, which can be detected by T1-weighted Gd enhancement (Chelune et al. 2008). In this respect Gd enhancing lesions are indicators of active areas of new inflammation, and serial Gd enhancing MRIs have shown that MS patients may have numerous active inflammatory events even during periods considered silent between clinical relapses (Chelune et al. 2008) (see figure 1.9). Gd enhanced lesions gradually resolve within periods of 4-6 weeks and leave behind T2 hyperintense lesions, thus providing evidence for dissemination in time (Fillipi et al. 2010. McDonald et al. 2001)
T2-weighted or spin-spin relaxation time imaging is highly sensitive to MS plaques, the majority of which are clinically silent. These plaques are asymmetrically distributed in the periventricular and subcortical white matter, corpus callosum, cerebellar peduncles, midbrain and spinal cord. T2-weighted hyperintensities are useful indicators of overall disease activity reflecting both acute and chronic lesions i.e. cumulative disease burden. Flair sequences on the other hand are used to suppress cerebrospinal fluid in order to highlight hyperintense lesions in MS. Diffusion weighted MRI (dMRI) is particularly useful in studying the connectivity of axons in white matter (Dolan, 2008) (Table 1.8 provides a summary of MRI sequences).
Table 1.8 Summary of Magnetic Resonance Imaging (MRI) sequences

<table>
<thead>
<tr>
<th></th>
<th>T1</th>
<th>T2</th>
<th>FLAIR</th>
<th>dMRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
<td>Detailed anatomical image</td>
<td>Sensitive to pathology</td>
<td>Sensitive to pathology</td>
<td>Detects infarcts within minutes</td>
</tr>
<tr>
<td>CSF appearance</td>
<td>Dark</td>
<td>Bright</td>
<td>Dark (CSF nulled)</td>
<td>Dark</td>
</tr>
<tr>
<td>Gray Matter</td>
<td>Darker</td>
<td>Brighter</td>
<td>Brighter</td>
<td>Brighter</td>
</tr>
<tr>
<td>White Matter</td>
<td>Brighter</td>
<td>Darker</td>
<td>Darker</td>
<td>Darker</td>
</tr>
</tbody>
</table>

Source: Adapted from Abou Khalil, 2017

1.9.6.1 Brain atrophy and cognitive impairment

Brain atrophy is present in all disease stages and develops gradually with the presence of inflammatory sites making it one of the most sensitive indexes of neurodegeneration in MS (Miller, Barkhof, Frank, Parker, & Thompson, 2002). Numerous studies have found strong associations between cognitive decline and different brain atrophy parameters. In this respect, atrophy is considered one of the strongest correlates for cognitive decline in MS, even at the early disease stages (Calabrese et al. 2009, Fillipi et al. 2010). Previous studies have focused primarily on global central brain atrophy, which is not associated with cognitive status exclusively, but which might even be able to predict the longitudinal development of cognitive function (Deloire et al. 2011).

Recent studies have outlined the significance of thalamic volume in relation to cognitive impairment in MS (for an overview see Minagar et al. 2013). One such report by Schoonheim et al. (2015) found lower thalamic volumes in MS patients compared to healthy participants, with the lowest volumes found in severely cognitive impaired patients. Moreover, thalamic volume in contrast to lesion and whole-brain volumes was found to be an independent predictor of cognitive dysfunction. In an interesting study Papathanasiou et al. (2015) provide
evidence that thalamic atrophy was a predictor of cognitive dysfunction in RRMS patients and was also highly associated with activities of daily living and employment status.

Thus, it appears that irreversible tissue loss, as measured by brain atrophy of the white and gray matter, is strongly associated to cognitive function in the MS population. While white matter atrophy has also been reported to contribute significantly to impairment in mental processing speed and working memory, gray matter atrophy was highly predictive for verbal memory status, but additionally predicted neuropsychiatric symptoms such as disinhibition and euphoria (Lanz, Hahn, & Hildebrandt, 2007).

1.9.6.2 White matter and cognitive impairment

While several studies have reported a relationship between lesion burden and performance on cognitive tests, others note only a modest association between T2 lesions of the whole brain or specific sites in white matter and neuropsychological performance. However, it is not the number of lesions that determines outcome, but rather their location. It has been shown that particularly the corpus callosum appears to be an important predilection site for cognitive performance as lesions have been found twice as often in cognitively impaired than in cognitively preserved MS patients (Rossi et al. 2012). This discrepancy in results of white matter lesions and cognitive performance may partly be due to differences in patient populations, sample sizes, types of cognitive tests used, criteria used to define cognitive impairment, methods utilized for MRI quantification of brain lesions, and statistical criteria (Rocca, et al. 2015. Fillipi et al. 2010).

Studies that have applied magnetization transfer (MT) imaging, in order to get more detailed information on diffuse white matter abnormalities, have shown that microstructural changes in the normal appearing white matter (NAWM) are not only predictive for overall cognitive status (Pinter et al. 2015), but are also predictive for the evolution of cognitive functionality over a period of 7 years (Deloire et al. 2011). However, structural evaluation of
only white matter lesions is not sufficient to explain cognitive impairment in patients with MS as damage to cortical areas, deep grey matter structures and normal appearing white matter may contribute significantly to cognitive dysfunction in MS (Rovaris, Comi, & Filippi, 2006. Wallin, Wilken, & Kane, 2006. Fillipi et al. 2010). The hypothesis of a “multiple disconnection syndrome” to explain the heterogeneity of cognitive deficits observed in MS was first explained by neuropsychological data (Calabrese & Penner, 2007). It was further supported by imaging studies showing that the location of lesions in strategic areas of white matter contributes to this kind of disconnection (Roca et al. 2015). Recently, Rossi et al., (2012) found that the area’s most relevant to impaired cognition in MS lie mostly in the commissural fiber tracts, supporting the notion of a functional multiple disconnection between gray matter structures, secondary to the damage located in specific white matter areas.

1.9.6.3 Gray matter and cognitive impairment

The role of gray matter in the pathogenesis of MS has gained wide acceptance over the last decade. Kutzelnigg & Lassmann, (2006), provided one of the first descriptions that cognitive deficits were a result of cortical demyelination from a post-mortem study. This study showed that as the disease progresses, white matter damage from focal periventricular becomes more diffuse and is further associated with extended demyelination of gray matter. Sensitive immunocytochemical analyses led to the conclusion that the spatial distribution of cortical lesions might be associated with specific cognitive deficits such as information processing speed. In 2009, this assumption was verified by a first application of the double inversion recovery MRI sequence to study distribution of cortical lesions in vivo and to relate these findings to the cognitive profile of the patients (Calabrese, Agosto, & Rinaldi, 2009). Moreover, these histopathological findings have been confirmed by studies showing that although gray matter atrophy may be present from the early stages of the disease process, it becomes more evident in the progressive types of the disease (Fisniku et al. 2008).
Although number of cortical lesions and volume has been shown to be larger in cognitively impaired patients, and comparable to findings in the white matter, it is not the number but rather the location of lesions which appears to be associated with cognitive impairment. Furthermore, T1 hypointense cortical lesions seem to be predictive for decreased memory performance, mental processing speed and verbal fluency (Bagnato et al. 2010). Besides focal lesions, diffuse gray matter damage as measured by magnetization transfer MRI and diffusion tensor MRI contributes not only to overall cognitive impairment, but also to decline in specific cognitive subdomains (Geurts, Calabrese, Fisher, & Rudick, 2012). Despite these findings it is not yet clear whether cortical demyelination is the sole contributor of gray matter atrophy or whether other mechanisms exist (Messina & Patti, 2014).

In the section that follows advanced MRI techniques will be presented which have contributed significantly to the study of how cortical demyelinating lesions may lead to the development of gray matter atrophy.

1.9.7 The contribution of advanced Magnetic Resonance Imaging Techniques to cognitive dysfunction in MS

Advanced MRI techniques are now available to evaluate and better understand the heterogeneity of cognitive dysfunction in MS patients and the associations between structural and functional brain abnormalities and cognitive impairment. In Table 1.9 a summary of advanced MRI techniques used for the MS patient population are presented and advantages as well as disadvantages of each method are briefly discussed. These methods include Double Inversion Recovery (DIR), Magnetization Transfer Ratio (MTR), High-resolution 3D T1-weighted Sequences, Diffusion Tensor Imaging (DTI), Proton Spectroscopy (1H-MRS) and functional Magnetic Resonance Imaging (f-MRI). Following these brief descriptions listed in table 1.9, findings of several studies related to these advanced techniques will be presented.
Table 1.9 Summary of Advanced Magnetic Resonance Imaging (MRI) techniques used in MS patients

<table>
<thead>
<tr>
<th>Sequence</th>
<th>What is Measured</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Setting of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double Inversion Recovery (DIR) [suppression of WM and CSF by two inversion times]</td>
<td>Grey matter lesions quantification and topographic classification (leucocortical, intracortical)</td>
<td>Superior contrast between GM/CSF and GM/WM than conventional sequences (FLAIR, PD/T2, and T1)</td>
<td>Not useful for detection of subpial cortical lesions; limited detection of intracortical lesions; prone to artifacts; low signal; not yet standardized</td>
<td>Research</td>
</tr>
<tr>
<td>Magnetization Transfer Ratio (MTR) [gradient-echo or spin-echo sequences with and without an off-resonance saturation pulse]</td>
<td>Quantitative index derived from magnetization transfer imaging: efficiency of the magnetization exchange between protons in tissue water (relatively free) and those bound to the macromolecules; sensitivity and specificity to myelin</td>
<td>Superior to conventional MRI for the detection and quantification of microscopic tissue abnormalities in the WM and GM</td>
<td>Inter-subject and inter-scanner variability; strong effect from water content; intercenter variability</td>
<td>Research, clinical trials</td>
</tr>
<tr>
<td>High-resolution 3D T1-weighted Sequences</td>
<td>Quantification of degree and topography of atrophy (grey matter and white matter separately)</td>
<td>Highly reproducible; sensitive to longitudinal changes; availability of robust post-processing methods</td>
<td>&quot;Late&quot; biomarker sensitive to irreversible tissue loss</td>
<td>Research, clinical trials</td>
</tr>
<tr>
<td>Diffusion Tensor Imaging (DTI)</td>
<td>Quantitative measurements (fractional anisotropy, mean diffusivity, axial diffusivity, and radial diffusivity) of brain tissue microstructure obtained through exploitation of the properties of water diffusion</td>
<td>Sensitive to WM microstructural damage</td>
<td>Inter-subject and inter-scanner variability; additional investigations are warranted to elucidate the correlates with pathological damage</td>
<td>Research</td>
</tr>
<tr>
<td>Proton Spectroscopy ('H-MRS)</td>
<td>Quantification of different metabolites in voxels of interest</td>
<td>High specificity (N-acetyl aspartate: axonal damage; choline and lactate: myelin damage and inflammation)</td>
<td>Technically challenging; acquisition protocol and post-processing analysis need to be standardized across centers</td>
<td>Research, clinical trials</td>
</tr>
<tr>
<td>Functional MRI (fMRI) [T2* gradient echo or T2 spin echo sequence]</td>
<td>Blood oxygen level dependent (BOLD) signal</td>
<td>Evaluation of cortical reorganization</td>
<td>Inter-subject and inter-session variability; analysis needs appropriate statistical methods</td>
<td>Research, feasible also in multicentric study</td>
</tr>
</tbody>
</table>

Source: Adapted from Fillipi et al., 2013
In a study by Deloire et al. (2005), the pathological mechanism underlying cognitive dysfunction in patients at the early stages of the disease was evaluated by magnetization transfer ratio (MTR) imaging. Relapsing remitting patients who had been diagnosed not more than 6 months previously were assessed neuropsychologically and compared to a demographically matched healthy group. Their findings revealed that MS patients performed significantly poorer on measures of mental processing speed, verbal and spatial memory, attention and executive function (inhibition). Further regression analyses showed that mental processing speed and attention correlated significantly with Normal appearing white matter (NAWM) MTR and lesion load, providing evidence that cognitive decline at this early stage may be attributed to axonal degeneration within the intercortical networks.

More recently, a study by Schoonheim et al. (2014) evaluated the association between cognitive impairment and white matter damage 6 years after their initial diagnosis. By utilizing Diffusion Tensor Imaging (DTI), and applying a multivariate model they found an association between white matter changes, and especially involving the thalamus and performance on neuropsychological measures. Another interesting finding of this study was that male MS patients had more severe and extensive changes in the white matter compared to females, which were not related to disease duration, White matter lesion volume or disability status.

Regarding the association of gray matter damage to cognitive dysfunction in MS, cross-sectional and longitudinal studies have provided such evidence. In one such study, by utilizing Double Inversion Recovery (DIR), Roosendaal et al. (2009) found an increase in the number of cortical lesions over a period of three years. Moreover, patients with a progressive disease course (SPMS) had a higher number of cortical lesions compared to RRMS patients, and these were significantly correlated to cognitive dysfunction.

In a cross-sectional study, Benedict, Ramasamy, Munschauer, Weinstock-Guttman, & Zivadinov, (2009), found significant associations between performance on tests of episodic
memory and lower regional volume of the medial temporal lobe (hippocampus and amygdala) and deep gray matter (thalamus and caudate). The strongest associations were noted between deep gray matter volumes and performance on new learning or free recall trials. Another study by Batista et al. (2012) reported an association between deep gray matter volumes and one of the most sensitive cognitive measures in MS, the Symbol Digits Modalities Test (SDMT) that primarily assesses mental process speed, after controlling for neocortical volume. The strongest associations with the SDMT were noted with the putamen and thalamus.

Functional Magnetic Resonance Imaging (f-MRI) refers to an application of standard MRI technology that produces images of metabolic function. f-MRI utilizes oxygen blood level dependent (BOLD), a physiological contrast used to indirectly map neural activity in the brain and spinal cord. This technique is based on the different magnetic properties of oxyhemoglobin (oxygen – rich blood) and deoxyhemoglobin (oxygen poor blood) (Khalil, 2017). Specifically, deoxyhemoglobins T2-weighted MRI signal is slightly decreased compared to oxyhemoglobin. When a region of the brain is utilized for a specific task (cognitive or motor), the neurons in that region require higher levels of oxygen, thereby increasing blood flow to that region. A change in the ratio between oxyhemoglobin and deoxyhemoglobin takes place and more specifically, deoxyhemoglobin decreases while oxyhemoglobin increases, producing an increase in the T2-weighted MRI signal (Brown, Perthen, Liu, & Buxton, 2007).

Basic paradigms utilized in f-MRI include the active state and inactive (control state). Brain images are acquired in both states and are compared in order to establish the cerebral locations where the images differ significantly between the two states. These regions are said to be “activated” during the assessed task (Fillipi & Roca, 2009. Khalil, 2017). Furthermore, the synchronization between regions may be evaluated, a process known as functional connectivity. Effective connectivity is defined as the process of how activity between different regions is modulated by administering a task or the complexity of a task (Brown et al. 2007).
Several studies have provided evidence using the active or task-based fMRI approach in understanding the cerebral resources involved in completing a specific cognitive task in the MS population. As mentioned previously, this approach provides the opportunity to examine levels of activation during task performance in specific brain regions. Such altered cerebral activation patterns implying functional organization of the brain have been reported in MS patients during tasks involving episodic memory (Bobholz et al., 2006), attention (Nebel et al. 2007), working memory (Chiaravalotti et al. 2005), and mental processing speed (Genova, Hillary, Wylie, Rypma, & DeLuca, 2009). Even more interesting are the findings that early stage MS patients activate additional regions during cognitive task performance, even prior to cognitive decline being detectable on formal neuropsychological evaluations (Forn et al. 2012). This additional activation has been proposed to provide a compensatory mechanism for delaying the onset of cognitive decline, although in patients with more severe impairments the data appear less consistent (Forn et al. 2007). In one such study, Audoin et al. (2003) demonstrated that cognitively intact MS patients show an early abnormality within the detected cognitive network, characterized by an increased and more bilateral recruitment of the network of interest. Through studies that have shown an increased or more bilateral pattern of cortical recruitment owing to the underlying structural damage in cognitively preserved patients, has led to the hypothesis that what can be assessed with these techniques is adaptive for these patients countering the presence of widespread structural damage. On the contrary, empirical evidence exists to support that what sometimes is evaluated with fMRI is maladaptive and related to poor cognitive function. An example of such empirical evidence is provided by Penner et al. (2003), who found that severely cognitively impaired patients had a poor or exhausted pattern of cortical recruitment within different cognitive networks compared with mild cognitively impaired MS patients.
Therefore, an important issue regarding how much can you trust the results is raised when applying an active fMRI task to MS patients who might be cognitively impaired, and how these results may be influenced or biased by the competence of patients to do a particular task compared with healthy participants. Confounding factors such as the one previously mentioned may be minimized by evaluating connectivity between or within the main functionally relevant brain networks in the resting state condition. This approach has shown that even by analyzing functional connectivity at rest, early abnormalities of functional connectivity may be detected in patients with CIS who are cognitively intact. These functional connectivity abnormalities appear to involve most of the resting state networks, including the cognitively-related networks, compared to healthy participants or patients with relapsing remitting MS (Roosendal et al. 2010).

Cortical reorganization is commonly manifested in MS patients independently of disease duration and clinical disease course. Variable patterns of cortical rewiring occur in MS patients providing the potential to limit the functional consequences of tissue damage. In this respect, clinical manifestations reflect mostly the balance between structural damage and cortical reorganization, rather than pure tissue disruption. In combination, adaptive and maladaptive plasticity can occur in the brain networks of MS patients, contributing to the progress of disability and cognitive dysfunction. Functional restoration can be promoted by therapeutic interventions that modify neuroplasticity through inducing adaptive changes or by predisposing functional systems to adaptive plasticity.

Considering the above it is evident that f-MRI technology provides a significant means of understanding functional reorganization and neural plasticity in the MS disease process. Moreover, f-MRI has been used to observe neural plasticity following effective cognitive rehabilitation (see e.g. Fillipi et al. 2012). By utilizing neuropsychological measures in combination with functional neuroimaging to study the outcomes of cognitive rehabilitation
interventions, behavioral and functional improvements on cognitive measures can be detected, but moreover, changes in the functional cerebral structure underlying the efficacy of such interventions on cognitive status may be observed. Therefore, taking advantage of this combined source of knowledge that may be obtained, several recent studies have examined neurofunctional, neuroanatomical and neurocognitive outcomes associated with neuropsychological rehabilitation in MS, an issue that will be elaborated on in the sections that follow. The majority of these studies have revealed brain neuroplasticity effects in MS through the documentation of pre-to post intervention changes at the level of the cerebral substrate following non-pharmacological, non-invasive, neurobehavioral treatments for the noted cognitive deficits (see for e.g. Cerasa et al. 2012. Stuifbergen, Becker, Perez, Morison, Kullberg, & Todd, 2012).

1.9.8 Single-Photon Emission Computed Tomography (SPECT) correlates of cognitive impairment in MS

Single-Photon Emission Computed Tomography (SPECT) is a method that utilizes gamma-ray-emitting radioactive isotopes (i.e lipophilic complexes; examethazine- HMPAO, ethylcysteine dimer or iophet – IMP), labeled with a radioactive tracer similar to positron emission tomography (PET). In contrast to PET, however, the production of SPECT radiotracers does not require a cyclotron and they emit single-photons. The radiopharmaceutical / radioactive tracer that is administered intravenously passes the blood brain barrier and is taken up rapidly by the brain cortex at rates reflecting the regional cortical blood flow (rCBF) in various brain regions. Uptake of the SPECT radiotracer is rapid and the whole process is completed within about one minute after intravenous (IV) injection. The tracer remains trapped in the cortex for several hours. Following IV administration, patients are scanned in a rotating (tomographic) gamma camera and multiple three-dimensional slices are reconstructed, to depict tracer distribution that reflects the r CBF in each cortical region. Advantages of SPECT are its wide
availability, time efficiency (completed in about an hour, and cost effectiveness. On the contrast images may be blurred and the method has limited spatial resolution (Asenbaum et al. 1998. Khalil, 2017).

Recently, statistical software for the automated analysis of brain perfusion SPECT images has been developed. One of these software tools, known as NeuroGam (GE Medical System, Segami Corp., Columbia, MD, USA), provides elaborated reconstructed images of the acquired data. It uses an affine anatomical co-registration by blocks of data defined in the Talairach space. It can be used to investigate rCBF objectively and easily in the cerebral lobes of the left and right hemispheres, and especially the predefined Brodmann functional areas (Br) (see Paschali et al., 2009. Paschali et al. 2010. Valotassiou et al. 2015).

Brain perfusion SPECT is able to detect reduced blood flow or changes in rCBF at injured or abnormal sites, thus providing early markers of functional impairment. This nuclear medicine method has been utilized widely in epilepsy and pre-surgical assessment of medically uncontrolled seizures, (Fu et al. 2015. Desai et al. 2013), dementia type differentiation (Valotassiou et. al 2014), early diagnosis of Alzheimer’s disease (Valotassiou et al. 2015), pre-surgical Parkinson Disease (PD) patients’ candidates for deep brain stimulation (Paschali et al. 2009), PD patients in different stages of the disease process (Paschali, Messinis, Kargiotis, Vassilakos, & Papathanasopoulos, 2010) idiopathic basal ganglia calcinosis (Paschali et al. 2009b) and in macrophagic myofascitis (Van der Gucht et al. 2015).

Regarding the utilization of brain perfusion SPECT in MS and the association between perfusion rates and cognitive dysfunction in this population, the literature is scarce and indefinite. One of the first studies to be published examined the regional distribution of 99mTc HMPAO and the relationship to neuropsychological measures in RRMS patients supplementary to magnetic resonance imaging (MRI) (Pozzilli, Passafiume, & Bernardi, 1991). This study revealed significant hypoperfusion in the frontal and left temporal lobes of MS patients.
Moreover, an association was found between tracer uptake in the left temporal lobe and impaired verbal memory and verbal fluency. The study concluded that according to the tracer uptake pattern, a predominantly frontal and greater left than right temporo-parietal perfusion deficit was evident in MS patients compared to healthy controls.

Another study published in 1993 that used \(^{99m}\)Tc-HMPAO as flow tracer, reported reduced rCBF in the frontal grey matter that correlated with neurological disability, and reduced frontal grey and white matter perfusion that was associated with cognitive dysfunction. Furthermore, MS patients with a progressive disease course had significantly reduced rCBF in the frontal gray matter compared to RRMS patients and healthy controls. Diagnostically, however, \(^{99m}\)Tc-HMPAO SPECT was found to have low sensitivity for MS (Lyce, Wikkelso, Bergh, Jacobson, & Andersen, 1993).

Horiuchi & Mitsuo, (1999), report abnormal brain perfusion SPECT findings in an interesting case study of a 38-year-old woman with MS. By utilizing Iodine-123 N-isopropyl-p-iodoamphetamine (\(^{123}\)I-IMP) they found an increased accumulation of this tracer in the left cerebellar hemisphere 14 days after neurological symptom onset. After steroid therapy, her neurological symptoms improved and SPECT conducted at two months did not reveal any abnormalities. Seven months later she showed a relapse of the neurological symptoms and T2 weighted MRI revealed high intensity areas in the midbrain and pons and she was diagnosed with definite MS. The authors suggest that the findings noted on SPECT may possibly be contributed to the inflammatory process of the cerebellum in this MS case and acute cerebellar ataxia.

In a recent study, Assadi et al. (2010), evaluated the efficacy of SPECT imaging with Tc-99m MIBI or Tc-99m ECD to detect abnormal perfusion in MS patients and compared these values with results obtained on MRI (T1, T2, and fluid-attenuated inversion recovery) with and without the use of gadolinium contrast. The authors report that MRI was able to detect an
average of 10.47, 3.7, 5.3, 1.7, and 0.9 lesions in the periventricular white matter, juxtacortical white matter, corpus callosum, cerebellar peduncles and brainstem. On the contrary, brain SPECT imaging with Tc-99m MIBI or Tc-99m ECD did not detect any abnormality.

In another recent study, Taghizadeh Asl, Nemati, Chabi, Salimipour, Nabipour & Assadi, (2016), evaluated cerebral perfusion by utilizing $^{99m}$Tc-ECD in secondary progressive MS (SPMS) patients with moderate to severe disability pre-and post-hyperbaric oxygen therapy (HBOT). Results were compared by statistical parametric mapping (SPM). The study found a significant relationship between the severity of perfusion impairment, disease duration and Expanded Disability Status Scale (EDSS) status. Ninety - two percent of patients had abnormal SPECT studies pre-HBOT, with a significant pre-to-post treatment improvement on perfusion scans that was, however, not demonstrated in the clinical assessment of the SPMS patients. The authors conclude that SPECT was able to highly detect decreased cerebral perfusion in their progressive disease course MS sample with moderate to severe disability, and because of its accessibility, relatively low cost, practical ease and provision of objective quantitative information, it may be used in order to complement other diagnostic modalities such as MRI and clinical assessment in disease surveillance and monitoring.

1.9.9 Cognitive impairment in MS and everyday functioning

Many disease variables associated with MS may negatively influence activities of daily living (ADL) and everyday functioning capacity. The extent of disability associated with MS is widely variable and some patients are minimally affected, while others may progress rapidly to total disability with regard to everyday functional activities. Approximately 70% of MS patients will have impairments in everyday functioning (Goverover, 2016), and deficits in motor, sensory, cognitive, social and psychological functioning all contribute in this respect. MS patients are typically affected in three domains of everyday functioning. These encompass personal or self-care skills, instrumental ADLs such as ability to drive, cook or manage finances, requiring
cognitive and motor skills, and advanced ADLs requiring a combination of intact motor, cognitive and psychosocial skills (e.g. retaining employment, social or community integration).

Although evidence indicates that cognitive dysfunction in MS patients is highly related to everyday functioning abilities (Kalmar, Gaudino, Moore, Halper & DeLuca, 2008. Goverover, Chiaravalloti, DeLuca, 2016), there is lack of consensus regarding the best approach to the assessment of everyday activities in this population. In general, reports of everyday life activities are significantly limited in MS, due mostly to a lack of reliable and sensitive measures of everyday life functioning capacity. For example, the use of self-report questionnaires may introduce bias due to affective symptomatology and perception. On the other hand, some commonly used performance-based measures, such as cooking, may not reflect the actual needs or tasks of most male MS patients.

Despite these limitations, several studies evaluating the performance between cognitive dysfunction and everyday functioning abilities have been conducted. In one such study that evaluated associations between cognitive function and objective performance on measures of everyday functioning (Executive Functions Performance Test - EFPT) in MS, Goverover et al. (2005), reported that MS patients had significantly more difficulties in simple and more complex cooking abilities, using the phone, taking medication, and paying the bills, compared to healthy participants. Moreover, significant correlations were found between the EFPT subtests of bill payment and cooking ability, and measures of new learning and mental processing speed. In another similar study, Kalmar et al. (2008), found significant differences in EFPT performance between cognitively intact and cognitively impaired MS patients, and healthy controls. The study concludes that persons with and without cognitive dysfunction differ regarding their functional capacity and that several aspects of cognition may predict functional status in this population.
In an interesting study by Goverover, O’Brien, Moore, & DeLuca, (2010), utilizing an actual reality (AR) approach through the use of everyday tasks requiring the internet (e.g., booking an airline ticket, purchasing cookies and ordering pizza), the authors report significant correlations between these tasks and performance on mental processing speed (SDMT), concluding that this measure contributes significantly to predicting everyday functioning capacity in MS.

Another study by Goverover, et al. (2016), showed that even brief cognitive screening batteries such as the BICAMS (composed of only 3 measures), can predict everyday functioning activities in persons with MS. Specifically, the authors found that better performance on the BICAMS was related with more independent actual reality task performance (i.e. accessing the internet to purchase an airplane ticket or cookies).

In a recent study, Goverover, Hass, & DeLuca, (2016), examined the ability of MS patients to manage their finances. The authors found that MS patients demonstrated and reported more difficulties in managing their finances compared to healthy controls. Moreover, MS patient’s difficulties in handling their finances were associated with the severity of cognitive dysfunction. As this important everyday task requires intact mental processing ability and executive-attentional abilities, domains usually impaired in MS individuals, these findings may serve as potential intervention indicators when planning cognitive rehabilitation interventions.

1.10 Interventions for cognitive dysfunction in MS

It is common knowledge amongst clinicians working with MS individuals that cognitive impairment will ultimately affect the majority of patients, independently of disease subtype and disability status, and that cognitive deficit can occur from early on in the disease course, even in the absence of major physical disability (Messinis, et al. 2010). Moreover, as mentioned previously, cognitive dysfunction exerts a significant negative influence on everyday functioning.
abilities in this population, limiting their capacity to complete simple and more complex everyday activities such as cooking a meal, driving a car, handling their finances, using the internet to order or purchase items and other related activities (Kalmar et al. 2008. Goverover et al. 2010. Goverover et al. 2016). In this respect, it becomes obvious that interventions to alleviate, stabilize, reduce or compensate for cognitive impairment are of an extremely high priority, in order to provide these individuals with the necessary mechanisms to better handle their everyday functioning disabilities.

As will be documented in the sections that follow, the evidence up till now is only modest regarding the efficacy of pharmacological agents on cognitive dysfunction (Roy, Benedict, Drake & Weinstock-Guttman, 2016) and non-pharmacological interventions such as cognitive rehabilitation also provide incomplete evidence on whether they might improve or stabilize cognitive impairment and especially over long follow up periods (Mitolo, Venneri, Wilkinson, & Sharrack, 2015). Despite this general consensus, there are studies that have reported the efficacy of pharmacological agents (see Mokhber et al. 2014) and cognitive rehabilitation (see Rosti-Otajarvi & Hamalainen, 2014) in reducing MS associated cognitive deficits. Moreover, studies utilizing the computerized cognitive training software RehaCom have reported that specific cognitive training in RRMS patients improved performance in previously impaired cognitive domains when compared with interventions that applied a generic training protocol, suggesting that targeted cognitive rehabilitation may improve specific cognitive functions (see for e.g. Mattioli et al. 2015). What will however become evident in the sections that follow is that the ability to make conclusions based on the overall body of evidence is limited by the heterogeneity of patient samples, interventions, and outcome measures.
1.10.1 Pharmacological treatments and cognition

As mentioned previously, cognitive deficits occur in all MS subtypes, are evident at the earliest stages of the disease, and tend to worsen over time (Messinis et al. 2010). Despite the high prevalence rates and the significant impact of cognitive impairment on quality of life and everyday functioning abilities in these individuals, there are currently no proven symptomatic pharmacological treatments (Messinis et al. 2010. Lyros, Messinis, Papageorgiou & Papathanasopoulos, 2010. Amato et al. 2013. Yamout et al. 2015. Roy et al. 2016).

Current pharmacological interventions for MS patients whether cognitively intact or cognitively impaired include disease modifying therapies/treatments (DMTs) or symptom management therapies. Disease modifying treatments for MS were initially presented in the early 1990s with the positive results of phase 3, placebo-controlled trials reporting that recombinant interferons (IFNs) and glatiramer acetate produce significant positive effects on relapse rate and disability as measured by EDSS progression (Roy et al. 2016). To date, seven DMTs are available and have received approval as first-line therapy in patients with relapsing remitting MS (RRMS), without particular restrictions. These include IFN-beta 1a IM, IFN-beta 1a SC, IFN-beta 1b SC, pegylated interferon beta 1a, Glatiramer Acetate- GA, teriflunomide, and dimethyl fumarate (DMF) (Yamout et al. 2015. Hydelburg & D Aversa, 2014. Roy et al. 2016).

On the other hand, three additional DMTs with specific restrictions have been approved as first line therapy. Fingolimod has been approved for initial treatment of RRMS patients in the USA, but in Europe is used only with patients that do not adequately respond to first line therapies or that show an aggressive disease from its onset. Natalizumab has approval as second line therapy or in patients that present with an aggressive disease from onset. Alemtuzumab has been approved in European patients as first line therapy with active disease as evidenced by clinical and neuroimaging features, whereas in the USA it is utilized as second
line therapy in patients not responding adequately to 2 or more approved DMTs (Yamout et al. 2015. Wiendl & Meuth, 2015. Roy et al. 2016).

In general, cognitive studies incorporating the impact of DMTs have shown modest beneficial effects. However, some studies have found DMTs to prevent or minimize the progression of cognitive decline (actual improvements may also occur, if inflammatory mediators are causing reversible dysfunction) (Lyros et al. 2010. Messinis et al. 2010. Wiendl & Meuth, 2015). Moreover, they improve clinical features (relapses, disability progression) and MRI (T1 and T2) measures of the disease (Yamout et al. 2015).

In the pivotal trial of interferon beta-1b, Pliskin et al. (1996), reported significant improvements on delayed visual memory between the second and fourth years of the trial only for those patients treated with high-dose IFNβ-1b, but not for low dose treated patients. In another study that evaluated IFNβ-1a, at doses of 30 μg IM on a weekly basis, Fischer et al. (2000), revealed significant differences on mental information processing speed and episodic memory 24 months after treatment was initiated. In a study that assessed the efficacy of glatiramer acetate at 12 and 24 months after treatment initiation, the authors report no significant differences between treatment arms on a comprehensive neuropsychological battery. This study was however confounded as it included cognitively reserved patients at baseline (Weinstein, Schwid, Schiffer, McDermott, Giang, & Goodman, 1999).

In another study that compared the efficacy of different types of interferon beta on cognitive dysfunction (Mokhber et al. 2014), the authors conclude that treatment with INFbeta-1a (Avonex and Rebif) were more efficient in resolving cognitive deficits in MS patients compared to INFbeta-1b (Betaferon). However, in general only minor positive effects on cognition were observed 12 months after initiation of treatment.

In another interesting study, Voshkuhl et al. (2016), utilizing voxel-based morphometry and volumetry, revealed that treatment with GA plus estriol reduced relapses by 32%, 24
months after treatment initiation. Moreover, a major reduction in gadolinium enhancing lesions compared with glatiramer acetate plus placebo treatment was recorded. Furthermore, the study showed that higher estriol levels were associated with improved scores on the PASAT. Other findings were demonstrated regarding gray matter sparing in the estriol plus glatiramer acetate group, and a strong association between PASAT improved scores and sparing of gray matter was found.

Recently, MS patients treated with natalizumab for 12 months were compared to patients on stable first-line treatment (interferon beta therapy) and healthy control subjects. Results showed that patients treated with natalizumab did not truly improve on cognitive function after this period of treatment (Sundgren, Piehl, Wahlin & Brismar, 2016).

However, one of the main challenges in conducting studies with these pharmacological agents for evaluating cognition in MS is that the change in cognition over time at the group level progresses very slowly. As an example, in an 18-year longitudinal study conducted by Strober et al. (2014), it was reported that only 18% of patients cognitively intact at baseline neuropsychological assessment showed cognitive deficits at follow up, thus highlighting the extremely slow change over time.

Another limitation is that the earlier major randomized controlled trials which attempted to assess the effects of DMTs on cognitive function were not specifically designed to address cognition. This methodological issue caused major biases, since there is evidence that patients with cognitive impairment in the beginning of a clinical trial are more likely to show cognitive deterioration over the course of the trial (Messinis et al. 2010). This issue has been overcome to a certain extent by more recent trials assessing the impact of DMTs on cognition (Roy et al. 2016). Other methodological issues, such as education and gender effects, practice effects from repeated testing or multinational studies may have further compounded the effects of confounding variables (Amato et al. 2013. Messinis et al. 2010)
One other major limitation of current DMTs is that they are only effective against relapsing disease course (i.e. RRMS or SPMS with relapses), as they only target components of the immune system actively involved in relapses. Although evidence from a small study suggested beneficial effects of mitoxantrone on physical disability and cognitive status in progressive MS patients (Ozakbas, Idiman, Kaya, Poyraz, & Poyraz, 2008), these patients or generally patients that are unresponsive to interferon therapies or glatiramer acetate, remain essentially untreated. Further research is desperately required to identify target molecules in order to develop new pharmacological therapies that will ameliorate progressive types of the disease.

At present, there is also insufficient evidence from clinical trials concerning the efficiency of symptomatic treatments for cognitive dysfunction in MS (Doraiswamy & Rao, 2004. Roy et al. 2016). Despite this, various medications used in the treatment of cognitive dysfunction associated with Alzheimer’s disease (AD) and Parkinson’s disease have shown some positive effects on cognition in MS. Specifically, three acetylcholinesterase inhibitors (AChEIs), donepezil, rivastigmine and galantamine (all licensed for treatment of cognition in AD and rivastigmine also licensed for PD) and a fourth agent, memantine (an NMDA receptor antagonist), have been investigated in MS as off-label treatments (Doraiswamy & Rao, 2004).

The AChEIs inhibit the action of the enzyme cholinesterase, thus reducing the breakdown of acetylcholine, and increasing both the level and duration of action of this neurotransmitter. In a randomized controlled trial involving 69 MS patients over a 24-week period, donepezil (10mg daily) improved learning and memory in memory-impaired MS patients compared with placebo (Krupp et al. 2004), and the clinical benefit was evident to patients and physicians, as reported in another study (Christodoulou, Melville, & Scherf, 2006). In a repeat of the donepezil study, however, Krupp et al. (2011) did not find significant between group differences (treatment vs. healthy controls) on a measure of episodic memory (Selective
Reminding Test), 24 weeks after treatment was initiated and concluded that donepezil is actually ineffective in treating episodic memory deficits in RRMS patients.

Rivastigmine, a cholinesterase inhibitor that inhibits both butyrylcholinesterase and acetylcholinesterase, used to treat mild-to-moderate dementia in AD and PD, appeared to have benefitted MS patients. A report from an fMRI study noted that rivastigmine had positive effects on brain function (increased brain activity predominantly in the left medial prefrontal cortex) when performing a cognitive task (a counting Stroop task) in MS patients (Parry, Scott, Palace, Smith & Mathews, 2003).

In a more recent study Peyro Saint Paul et al. (2016), did not find significant differences between memantine and placebo treated patients after 52 weeks of treatment, on either the PASAT or SDMT. Further studies with larger samples and longitudinal designs are required in order to determine the efficacy of these types of treatments on cognition in MS.

It is evident from the available data that current pharmacological approaches are not sufficiently efficacious in alleviating or treating cognitive decline in MS, therefore, reinforcing the importance of utilizing non-pharmacological interventions such as cognitive rehabilitation in order to improve cognitive functions and everyday functioning capacity in this population. In the section that follows the efficacy of cognitive rehabilitation approaches in MS will be discussed and both strengths and limitations to such approaches will be presented.

1.10.2 Cognitive rehabilitation and cognition

The goals of non-pharmacological treatments for MS-related cognitive deficits are similar to those of the immune-modulating drugs. In other words, these interventions are used with the intent of preventing the progression of cognitive dysfunction and promoting a therapeutic ‘milieu’ in which optimal cognitive functioning can occur, and include specific approaches which are known to be effective in remediating cognitive disorders of any etiology (Messinis et al. 2010). Cognitive rehabilitation or ‘rehabilitation of individuals with cognitive impairment’
(Sohlberg & Mateer, 2001, p. 3) include specific approaches designed to assist the MS patient to better cope with existing cognitive impairments or to improve a specific cognitive skill. It focuses on two main approaches: the restorative or functional training approach (i.e. ameliorating patients’ deficits in processing and interpreting information – e.g. when cognitive training is used to enhance attention or memory performance). The restorative approach depends on the brain’s capability of cortical reorganization following injury (i.e. that the brain possesses some degree of plasticity). The second is the compensatory or strategy training approach (e.g. modifying the patient’s environment, using a calendar and set phone reminders). These approaches have different goals and limitations, and may be used in isolation or in combination. For example, in patients with extensive tissue loss, neural plasticity might be hampered and no or little effect will result from restorative or functional training. In that particular patient, compensatory or strategy training might help the patient to work around the problems that are present. As for most MS patients, especially those with a relapsing disease course, it is expected that restorative or functional training will lead to improved cognitive functioning on neuropsychological measures, improved functioning in everyday life activities, and ultimately will lead to an improvement in network efficiency (Hulst & Langdon, 2017).

Several studies have investigated the effectiveness of cognitive rehabilitation interventions in patients with MS, including computer-based training and neuropsychological counseling, but with inconsistent results. The majority of studies found improvements in specific cognitive domains, but the evidence provided in the literature remains inconclusive (Mitolo et al. 2015). A significant limitation in providing evidence on the efficacy of studies involving cognitive rehabilitation is the great variability in the methods or strategies utilized for treatment, the measures used to assess cognition and other secondary outcome variables and the lack of ecologically valid outcome measures in order to assess the efficiency of these interventions in everyday functioning ability.
Utilizing a six-week cognitive rehabilitation programme that combined restorative and compensatory approaches (Jonsson, Korfitzen, Heltberg, Ravnborg, & Byskon-Ottosen, 1993), found improved visual perception, but not attention in a group of cognitively impaired MS patients. In another study that involved 14 MS outpatients receiving extensive (17 weeks) cognitive rehabilitation treatment showed no improvement in cognition following treatment (Fowley et al., 1994). A study utilizing a computer-based program (AIXTENT), which allows for specific training of four attention domains (alertness, divided attention, selective attention and vigilance) showed significant improvements in MS patients with selective attentional deficits at baseline (Plohman et al. 1998). Another study utilizing a computer-based training program (BrainStim) that recruited 30 MS patients (receiving four 45-minute sessions per week) and 20 matched healthy controls demonstrated improvements in mental processing speed and working memory tasks for both groups (Vogt et al. 2009). Sastre-Garriga et al. (2011), after a relatively short duration of training (5 weeks) found that patients who received cognitive treatment had improved performance on the backward version of the digit span and a composite score of cognitive outcome.

Applying a technique known as the Story Memory Technique (SMT), Chiaravalotti, Moore, Nikelshpur & DeLuca, (2013) provided class 1 evidence that this technique applied for 5 weeks/ twice weekly (10 sessions) with an emphasis on teaching context and imagery to facilitate learning, improved episodic memory in MS patients relative to controls and moreover produced increased f-MRI activation during a memory task in frontal and parietal regions. Positive effects were additionally observed for objective measures of everyday memory function, general contentment, and executive functioning. These positive outcomes were sustained for a period of 6 months.

A computer software known as Rehacom (RehaCom Cognitive Therapy Software. https://www.rehacom.co.uk), has been utilized extensively in Europe over the last couple of
years for the purpose of providing computer assisted cognitive rehabilitation. This software which has over 20 modules is available in many languages, including Greek. Clinical trials utilizing this software in MS patients with cognitive impairments have shown positive results, especially as regards the domains of attention and executive functions. More specifically, in a study conducted by Mattioli, Stampatori, Zanotti, Parrinello, & Capra, (2010) in MS individuals with baseline cognitive deficits on the Wisconsin Card Sorting Test (WCST) and PASAT, after 12 weeks of training with the Rehacom (3 days a week for approximately 1 hour), patients that received treatment showed significant improvement in measures of attention, mental information processing and executive functions relative to untreated healthy controls. A nine month follow up assessment of these patients revealed stability regarding the initial treatment effects, indicating the efficacy of this intervention over time (Mattioli, Stampatori, Scarpazza, Parrinello, & Capra, 2012). Similar positive results were presented by Fillipi et al. (2012), who also found significant pre-to post treatment improvements in the RehaCom treated group, in mental information processing, executive functions and attention. This and other similar studies have reported positive outcomes in MS patients treated with this software, and moreover, associations between functional neuroimaging (f-MRI) findings with changes in neurocognitive measures have been reported (Fillipi et al. 2012. Bonavita et al. 2015. Cerasa et al. 2013).

In a multicenter Italian study, RehaCom was utilized to provide specific intensive cognitive training for 12 months. Results showed that MS patients treated with this modality had improved scores post treatment on the SDMT, PASAT, and episodic memory measures relative to MS patients who received aspecific psychological therapy for the same period of time (Matiolli et al. 2015). Even more importantly, in a two-year follow-up study, the authors revealed that MS patients who had been treated with the specific intensive training via the RehaCom retained these beneficial effects for 24 months after the treatment (Matiolli et al. 2016).
A positive note regarding the efficacy of cognitive rehabilitation interventions is that recent f-MRI studies documenting alterations in cortical activation associated with cognitive rehabilitation raise the intriguing possibility that compensatory cerebral reorganization may underlie the relatively lasting benefits of cognitive rehabilitation (see for e.g. Fillipi et al. 2012. Chiaravalotti et al. 2013). Cognitive impairment in individuals with MS is hypothesized to be the result of network collapse. As structural damage in the brain increases with disease progress in MS patients, and as whole network efficiency decreases with increasing underlying structural damage, ultimately a critical threshold is exceeded, beyond which the network is unable to function normally and cognitive impairment occurs.

In an intriguing study, Draganski, Gaser, Busch, Schuierer, Bogdahn, & May, (2004), were the first to reveal the potential for inducing structural changes in the brains of healthy individuals, where they described significant increases in the grey matter volume of brain areas responsible for complex visual movements induced by juggling training.

Utilizing an RRMS sample, Penner, Kappos, Rausch, Opwis & Radu, (2003) published a study of cognitive training and associated fMRI findings. Pre-to post cognitive treatment activation patterns revealed an increase in activation of the same areas noted prior to cognitive training and in additional areas activated after the training. This change was considered adaptive because the change was related to the specific task that the patients performed. Similar findings were reported by Sastre-Garriga et al. (2011) who reported that training with a cognitive rehabilitation program was associated with increased brain activity in the cerebellum of cognitively impaired MS patients relative to healthy controls. This result was also considered an adaptive change as the applied cognitive tasks were specifically related to the cerebellum.

In the study conducted by Chiaravalloti et al. (2013), described previously, the modified story memory technique was reported to significantly improve new learning and episodic memory in MS individuals. However, utilization of fMRI also demonstrated improvement in
neuropsychological task performance and increased activation in the frontal, parietal, precuneus, and parahippocampal regions, with the activated regions being specific to the cognitive tasks that were performed. More recently, Huiskamp et al. (2016) presented findings of an fMRI protocol utilizing a working memory task after training with the modified story memory technique in MS patients. Treatment with this technique showed increased activation in brain areas related to the specific task that was performed, including the inferior parietal lobe, the dorsolateral prefrontal cortex, and the supplementary motor area. However, similar positive results were not noted for the behavioral data, complicating the interpretation of the positive fMRI findings.

While the previously mentioned positive results regarding the efficacy of cognitive rehabilitation interventions in MS individuals cannot be overstated, it is important to note that a recently published Cochrane Review that included 15 studies and 989 MS participants regarding the efficacy of memory retraining techniques with or without the assistance of computer software, concluded that there is only limited evidence on the effectiveness of memory rehabilitation in this population. The authors further suggest that more RCTs of high methodological quality be conducted with the utilization of ecologically valid outcome assessments (das Nair, Martin & Lincoln, 2016). Another Cochrane Review that included 20 studies and 966 MS participants evaluating the effectiveness of neuropsychological rehabilitation in MS (Rosti-Otajarvi, & Hamalainen, 2014), reported low-level evidence for the positive effects of neuropsychological rehabilitation in this population. However, the authors reported that the comparability of the 20 studies reviewed was limited due to heterogeneity of interventions and outcome measures. It should be noted however, that the majority of studies included in this review did show some evidence of positive effects on cognitive outcome measures.
Despite the limitations noted by the previously mentioned Cochrane reviews, a growing body of literature supports the efficacy of cognitive rehabilitation for individuals with MS and more randomized controlled trials are needed to support existing and new rehabilitation techniques. Cognitive rehabilitation appears to be useful for all patients with MS regardless of disease course and level of cognitive impairment, although studies including exclusively MS patients with progressive disease course are limited. Future clinical trials utilizing cognitive rehabilitation interventions in progressive MS patients should become a priority.

Although it is never too late to introduce cognitive rehabilitation, even in patients with severe cognitive dysfunction, rehabilitation is recommended early in the disease course so that benefits can be achieved from the existing functional networks. As areas of increased activation associated with a given cognitive task may not always imply a beneficial response, our knowledge considering adaptive and maladaptive changes when interpreting functional imaging findings in MS patients will need to be enriched with future studies.

1.11 Aims and study hypotheses

As previously mentioned cognitive impairment is frequently encountered in MS affecting between 40-65% of individuals, irrespective of disease duration, severity of physical disability, and at both the earlier and later disease stages (Potagas et al. 2008. Messinis et al. 2010. Panou et al. 2012), with a tendency to worsen over time (Smestad et al. 2010). Moreover, cognitive dysfunction in this population may have a significant negative impact on quality of life (Putzki et al. 2009), activities of daily living and independence (Engel, Greim, & Zettl, 2007) and employment status (Papathanasiou et al. 2015). Furthermore, past and current pharmacological treatments have shown inconsistent findings in alleviating cognitive impairment in individuals with MS requiring further clarification (Roy et al. 2016). This inconsistency regarding the effects of pharmacological interventions on cognition, coupled with the reduced ability to effectively handle everyday tasks, loss of employment and social
interaction capacity, and overall poorer quality of life, prioritizes the need for utilizing potentially more effective non-pharmacological, neurobehavioural interventions to address cognitive dysfunction and everyday functioning abilities.

Neurobehavioral interventions utilizing cognitive rehabilitation have shown favorable effects on MS patients cognitive performance and other related skills, and in some cases, have managed to generalize these positive effects to MS individual’s everyday life functioning ability (see for e.g. Plohman et al. 1998, Chiaravalloti et al. 2013). While as described previously there is evidence to support cognitive rehabilitation interventions in the MS population, the results of past and present clinical trials have been marred by numerous methodological limitations. These include lack of appropriate control groups and objective neuropsychological status assessment at baseline, utilization of inappropriate randomization methods, single site studies, inconsistency regarding the specific target of the rehabilitation intervention and outcome measures (especially as regards the use of ecologically valid measures) (see Rosti-Otajarvi, & Hamalainen, 2014). Therefore, it becomes obvious that there is a need for rigorous new cognitive rehabilitation studies that may overcome some of these limitations and provide robust evidence regarding the efficiency of such interventions.

In the present study, the efficiency of a 10 week (2 days a week for approximately 1 hour) multicenter randomized controlled trial utilizing the RehaCom software (RehaCom Cognitive Therapy Software. https://www.rehacom.co.uk) on cognitive functioning in Greek relapsing remitting MS (RRMS) patients was investigated. As most of our patients that took part in the intervention (will be described in detail in the special section that follows), were impaired in more than one cognitive domain, but mostly on episodic memory, information processing / attention and executive functions, the intervention was balanced over the 10-week period in order to train all domains equally. We hypothesized that patients receiving the specific 10-week intervention will show improved pre-to post intervention performance on neuropsychological
measures in the related trained cognitive domains relative to control group participants who received only usual clinical care. Moreover, we hypothesized that these positive training effects on specific cognitive domains (episodic memory, information processing / attention and executive functions), would be retained over time (6 months in this case) providing evidence on the long-term benefits of such interventions. We also hypothesized that control participants will show either further cognitive decline or remain cognitively stable as the period of the intervention may be inadequate to produce significant cognitive changes in these patients.

We described earlier on that brain perfusion SPECT utilized mainly in nuclear medicine is able to detect reduced blood flow or changes in regional cerebral blood flow (rCBF) at injured or abnormal sites, thus providing a potential early marker of functional impairment. With this aspect in mind and coupled with the indefinite and scarce literature of brain perfusion SPECT in multiple sclerosis and the possible association between perfusion rates and cognitive dysfunction in this population, we evaluated these patients with this method. All patients that were eligible to take part in the study were additionally invited to take part in a perfusion brain SPECT study after written informed consent. A cohort (n=31) of the initially recruited (n=58) RRMS patients who were eligible for inclusion in the study accepted our invitation and also underwent SPECT imaging a maximum of two days after the neuropsychological assessment and before initiation of the 10-week intervention. We hypothesized that MS patients with cognitive deficits would show greater hypoperfusion rates on brain perfusion SPECT in several predefined Brodmann areas and lobes of the brain compared to healthy controls, according to an established normative database; NeuroGam (GE Medical System, Segami Corp., Columbia, MD, USA). Moreover, we hypothesized that associations between hypoperfusion in several predefined Brodmann areas and lobes of the brain and neuropsychological performance on specific cognitive domains/measures would be established. A final hypothesis was that MS patients with more severe cognitive decline (impaired on 2 or more cognitive domains
according to established norms) would show greater hypoperfusion rates and a different pattern of hypoperfusion compared to patients with less severe cognitive decline (impaired on 1 cognitive domain according to established norms) on SPECT imaging. In the special section that follows the study will be presented and findings will be discussed along with relative strengths and limitations.
2. Methods

2.1 Participants

Fifty – eight clinically stable outpatients diagnosed with relapsing remitting MS (RRMS) were included in the study after meeting specific inclusion criteria. These patients were randomly assigned to either receive treatment with the RehaCom software (IG; n= 32) or placed in the control group condition (CG; n=26) and received usual clinical care. Demographic and clinical characteristics of both groups at baseline are provided in Table 2.1

All patients met criteria for the diagnosis of MS according to the McDonald et al. (2001) criteria. Additional study inclusion criteria were: (i) patient age between 21 and 60; (ii) educational level of at least 6 years (primary school graduates in Greece) (iii) relapsing-remitting MS (RRMS); (iv) EDSS score of between 0-5; (v) cognitive deficit on at least one domain of the Central Nervous System Vital Signs neuropsychological screening battery, Papathanasiou et al. (2014); and (vi) native Greek speakers (vii) provision of written informed consent to take part in the study. Exclusion criteria were as follows: (i) ongoing major psychiatric disorders (e.g., psychotic symptoms or disorders, illegal drug or alcohol abuse); (ii) presence of another neurological disorder (e.g., dementia, stroke, epilepsy, traumatic brain injury resulting in a loss of consciousness for more than 30 minutes); (iii) Mini –Mental State Examination score (MMSE) ≥ 24; (iv) IQ score of ≤ 80 on the Greek validated Wechsler abbreviated Scale of Intelligence (WASI); Messinis & Papathanasopoulos, 2012; (v) one or more exacerbations in the 3 months’ prior to enrollment, immunological or immunosuppressant treatment initiated within 4 months prior to enrollment or treated with cognitive rehabilitation in the 12 months prior to enrollment; (vi) Initiation of psychotropic medications or medications for spasticity, tremor, bladder disturbances and fatigue. If already taking such medications, doses
and schedules had to be held constant during the study period; (vii) normal or corrected hearing and vision.

2.2 Procedure

Between March of 2014 and December of 2015, 98 patients who had been previously diagnosed with MS based on the McDonald criteria (McDonald et al. 2001), attending either the outpatient neurology department at the University Hospital of Patras in Greece or the “Society of friends of patients with multiple sclerosis” situated in Ioannina, who reported cognitive difficulties or were judged by clinical neurological evaluation to have cognitive deficits, were referred for neuropsychological assessment at the outpatient memory and neuropsychological unit of the same hospital or the laboratory of audiology, neurootology and neurosciences of the Higher Educational Institute of Epirus, Ioannina, Department of Speech and Language Therapy. Clinicians assessing patients at both sites were supervised by the clinical neuropsychologist (LM) and lead consulting neurologists (PP) in Patras and (GN) in Ioannina.

Table 2.1 Demographic and clinical characteristics of the sample at baseline

<table>
<thead>
<tr>
<th></th>
<th>MS RehaCom group (n=32)</th>
<th>SD</th>
<th>MS Control group (n=26)</th>
<th>SD</th>
<th>t/U</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.03 (43.16 - 48.90)</td>
<td>7.97</td>
<td>45.15 (41.26 - 49.05)</td>
<td>9.65</td>
<td>.379</td>
<td>56</td>
<td>.706</td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.12 (10.87 - 13.38)</td>
<td>3.47</td>
<td>12.73 (11.46 - 14.01)</td>
<td>3.15</td>
<td>-.945</td>
<td>.345</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>10 (31.25)</td>
<td></td>
<td>8 (30.76)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>22 (68.75)</td>
<td></td>
<td>18 (69.24)</td>
<td></td>
<td>.002</td>
<td>1</td>
<td>.969</td>
</tr>
<tr>
<td>EDSS</td>
<td>3.14 (2.66 - 3.61)</td>
<td>1.32</td>
<td>3.11 (2.71 - 3.51)</td>
<td>1.09</td>
<td>-.126</td>
<td>.899</td>
<td></td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>13.31 (11.46 - 15.17)</td>
<td></td>
<td>11.27 (9.39 - 13.14)</td>
<td></td>
<td>-1.515</td>
<td>.130</td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>27.97 (27.54 - 28.39)</td>
<td>1.17</td>
<td>28.42 (28.06 - 28.79)</td>
<td>.90</td>
<td>-1.578</td>
<td>.115</td>
<td></td>
</tr>
</tbody>
</table>
WASI (IQ) | 102.31 (99.49-105.14) | 7.83 | 103.96 (100.37-107.55) | 8.89 | -.959 | .338

Premorbid intelligence
WASI (Voc) T-score
Fatigue (FSS) | 4.38 (4.04-4.48) | 1.80 | 4.35 (3.98-4.55) | 1.75 | -.297 | .486

BDI-FS | 4.31 (3.31-5.32) | 2.78 | 4.46 (3.01-5.91) | 3.09 | .178 | 56.859

Medication at Enrolment
Interferon | 25 (78.12) | 17 (65.38)
Fingolimod | 2 (6.25) | 3 (11.53)
Natalizumab | 5 (15.63) | 6 (23.07)

Notes: All values are raw scores. 
Abbreviations: EDSS: Expanded Disability Status Scale; MMSE: Mini Mental State Examination; WASI: Wechsler Abbreviated Scale of Intelligence; WASI (VOC): Vocabulary subscale of the Wechsler Abbreviated Scale of Intelligence; FSS: Fatigue Severity Scale; BDI-SF: Beck Depression Inventory Fast Screen; SD: Standard Deviation; CI: Confidence Interval; df: degrees of freedom; t: Independent sample t-test; U: Mann-Whitney U; x²: chi-squared

After being initially evaluated on a brief screening neuropsychological battery (Central Nervous System Vital Signs – CNSVS; Gualtieri & Johnson, 2006. Papathanasiou et al. 2014) patients with a diagnosis of RRMS that were found to have cognitive deficits on at least one domain of the CNSVS (performance between the 2nd and 8th percentile based on CNSVS demographically corrected normative data, see figure 2.1) were informed of the opportunity to participate in a 10 week cognitive rehabilitation intervention by the lead consulting neurologists (PP) and (GN) or clinical neuropsychologist (LM) supervising the study, and were invited to take part after providing written informed consent.
In order to overcome the limitations of recruiting patients from only one site, Southwestern Greece in this particular case, and to provide a more representative sample of MS patients, RRMS patients included in the intervention protocol, as mentioned previously, were also recruited from a second site, the national Society of MS attendees in Northwestern Greece known as the “Society of friends of patients with multiple sclerosis” situated in Ioannina, by following the exact same protocol as the patients recruited from Southwestern Greece. Eligible patients were randomized by a computer – generated, site stratified, independent randomization schedule to either undergo cognitive rehabilitation (intervention group) with the RehaCom software, or were placed in the placebo arm (control group) and spent the same portion of time (10 weeks) receiving usual clinical care. Before initiating the intervention (pretreatment) patients in both groups were administered a flexible battery of...
neuropsychological tests and measures of mood. Both groups were then evaluated within one week after completing the intervention (post treatment) and the RehaCom treated group was also evaluated at a six month follow up (see figure 2.2). All patients were non-compensated volunteers.

In both settings qualified clinicians which had previously attended training sessions in order to ensure uniform test administration and application of the rehabilitation intervention, following a strict protocol, under the supervision of an experienced clinical neuropsychologist (LM), administered the screening CNSVS battery and the flexible comprehensive neuropsychological battery of tests with well validated psychometric properties in MS individuals, and all other measures (excluding the EDSS scale which measures disability and was administered by specialist neurologists) at pre, post and 6 month follow up evaluation. Moreover, they conducted the rehabilitation interventions for the entire 10-week duration. The participants and clinicians taking part in the assessments and intervention were not blind to the allocated treatments. However, scoring of neuropsychological measures at baseline, post treatment and 6 months follow up was performed by two blinded observers, in order to avoid inter-rater variability.

Thirty-two participants diagnosed with RRMS completed the intervention, whereas twenty-six were included in the control group and received usual clinical care for the entire 10 weeks as mentioned previously. Six months following the intervention and continuing with usual clinical care for this time period, only patients that had undergone cognitive rehabilitation were evaluated in a follow up session in order to establish the effects of the intervention over time. Patients assigned to the control condition were for ethical reasons provided the opportunity to participate in a cognitive rehabilitation intervention similar to the one utilized in this study once they completed the research protocol. The research protocol was approved by the ethics committee of the University of Patras Medical School and was conducted in accordance with
the principles of the Declaration of Helsinki (WMA, 2013). Patients recruited from both sites provided written informed consent to take part in the study.

Figure 2.2 Consort Flow diagram
2.3 Instruments – Outcome assessment

2.3.1 Clinical assessment

Clinical characteristics of MS patients were assessed by specialist neurologists with significant experience in the MS population. These neurologists provided the diagnosis of MS based on the McDonald et al. (2001) criteria, type of disease course, disability rating on the EDSS scale (Kurtzke, 1983), fatigue rating on the Greek validated Fatigue Severity Scale (FSS) (Bakalidou, Skordilis, Giannopoulos, Stamboulis, & Voumvourakis, 2013), types of medications patients were taking, duration of illness, differential diagnostic issues, and also screened patients to ensure eligibility of inclusion and exclusion criteria (with the exception of cognitive criteria and mood). If deemed necessary patients were also assessed psychiatrically to ensure correct differential diagnosis of behavioral and mood disorders and to exclude patients with ongoing major psychiatric disorders.

2.3.2 Initial screening assessment of cognitive functions and intelligence level

All MS patients that were referred for neuropsychological assessment were initially screened on a brief neuropsychological battery (Central Nervous System Vital Signs – CNSVS; Gualtieri & Johnson, 2006. Papathanasiou et al. 2014) in order to evaluate their cognitive status and to determine whether they had impaired cognitive performance (one of the study inclusion criteria) on any of the CNSVS tested domains, defined as performance between the 2nd and 8th percentile based on demographically corrected normative data, see figure 2.1).

The CNSVS battery provides core neuropsychological assessment utilizing seven neuropsychological tests. A brief description of each measure is presented below followed by a description of the primary domain scores. The first of these tests, the Verbal Memory Test, involves learning immediate and delayed recognition for 15 words. These words (drawn from a reservoir of 100 words) are presented, individually, on a computer screen every two seconds.
For the immediate recognition trial, the participant has to identify those words nested among 15 new words. Then, after been assessed with the remaining six tests (approximately 30 minutes’ duration), there is a delayed recognition trial. The same paradigm is followed for the Visual Memory Test, which measures recognition memory for figures (immediate and delayed recall), drawn from a reservoir of 45 designs.

For the Finger Tapping Test, participants are asked to press or tap the space bar with their index finger (separately for right and left hands) as many times as they can for a period of 10 seconds, over three trials, with a preceding practice trial. The scores produced are the average number of taps for the right and left hands. This test examines psychomotor speed and fine motor control.

The Symbol Digit Coding Test assesses information processing—psychomotor speed, complex attention, and visuo-perceptual speed. The participant is required to identify eight different symbols corresponding to the numbers 1 through 9, and to type in the correct number under the corresponding symbol in empty boxes presented on the screen (drawn from a reservoir of 32 symbols). Scoring is the number of correct and incorrect responses generated in 2 minutes.

The Stroop Test examines executive function, simple and complex reaction time, information processing speed, psychomotor speed, and inhibition–disinhibition. It contains three parts that involve responding to words and colours. In the first part, the words RED, YELLOW, BLUE, and GREEN (which are written in black colour on the computer screen) appear randomly on the screen and the participant presses the space bar as soon as the word is seen. In the second part, the words appear on the screen printed in colour. The participant is asked to press the space bar when the colour of the word matches what the word says (e.g., the word RED printed in red ink) but not responding when the colour of the word does not
match what the word says (e.g., RED printed in blue ink). In the third part, the participant is asked to press the space bar when the colour of the word does not match what the word says (e.g., RED printed in blue ink) but not responding when the colour of the word matches what the word says (e.g., RED printed in RED ink). Scores include simple reaction time (part 1), complex reaction time (parts 2 and 3), and a commission error score (part 3).

The Shifting Attention Test examines executive function, reaction time, and psychomotor and information processing speed. It is a measure of the ability to shift from one instruction set to another quickly and accurately. Participants are instructed to match geometric objects either by shape or by colour. The participant is asked to match one of two bottom figures to a figure at the top of the screen based on one of two rules that are presented (e.g., “match to shape” or “match to colour”). The test continues in this manner for 90 seconds. Shifting Attention Scores include correct matches, errors, and response time.

The Continuous Performance Test is a measure of vigilance and sustained attention. The participant is asked to respond to the target stimulus “B” but not to any other letter while stimuli are presented randomly for 5 minutes. Scoring is correct responses, commission errors, omission errors, and choice reaction time.

Correct responses from the verbal and visual memory tests provide Verbal Memory and Visual Memory domains scores, respectively, as well as the Composite Memory domain score. The total of right and left taps from the Finger Tapping Test and the total correct responses on the Symbol Digit Coding Test generates a composite score for Psychomotor Speed. Averaging the two complex reaction time scores from the Stroop Test generates a domain score for Reaction Time, which can be considered as measuring information— processing speed in a test of executive function. The number of correct responses on the Shifting Attention Test, minus the number of errors on the Shifting Attention Test and the
Stroop Test, is used to create a domain score for Cognitive Flexibility. The domain score for Complex Attention is generated by adding the number of errors committed in the Continuous Performance Test, the Shifting Attention Test, and the Stroop Test. The overall summary score, called the *Neurocognition index*, is the average of the domain scores.

Intelligence level of MS patients at this stage was estimated by administering the vocabulary and matrix reasoning subscales of the Greek adapted version of the Wechsler Abbreviated Scale of intelligence (WASI; Messinis & Papathanasopoulos, 2012; Wechsler, 1999). The vocabulary subscale is a good measure of crystallized intelligence, correlates well with general intellectual ability, and is relatively insensitive to cortical insults (i.e., is considered a good measure of premorbid intellectual ability). For this reason, the demographically corrected T-score of the vocabulary scale was used as an estimate of premorbid intelligence level in this study. The Matrix Reasoning subscale is a measure of nonverbal fluid reasoning and correlates well with general intellectual ability. These two subscales yield an estimated full-scale IQ. An IQ score on the WASI of ≤ 80 was one of the study exclusion criteria.

At this screening stage patients were also administered the Greek validated version of the Mini Mental State Examination (MMSE) (Fountoulakis, Tsolaki, Chantzi, & Kazis, 2000). The MMSE assesses a restricted set of cognitive functions simply and quickly and is utilized as a dementia screening measure in everyday clinical practice. Recently, Solias, Skapinakis, Degleris, Pantoleon, Katirtzoglou, & Politis, (2014), provided MMSE “cutoff scores” for discriminating demented patients in Greece based on age and education-corrected norms. Item analyses of this measure have identified five distinct though related domains: concentration or working memory (serial 7s and spelling of “world” backwards; for the Greek validated version, the word is “petra” (stone) backwards; (Fountoulakis et al., 2000), language and praxis (naming, following commands, and construction), orientation, memory (delayed recall of 3 items), and attention span (immediate recall of 3 items) (Lezak et al. 2012). The MMSE was
used to evaluate MS patients overall cognitive impairment. An MMSE score of ≥ 24 was one of the stipulated study inclusion criteria.

2.3.3 Neuropsychological assessment

Both groups of patients were administered a comprehensive flexible battery of neuropsychological tests at baseline and within one week of completing the RehaCom treatment phase. The RehaCom treated group was also assessed 6 months following the completion of the rehabilitation intervention after receiving only usual clinical care for this period. The main criterion for selecting the cognitive measures to be utilized in this study, were their use specifically for this population in routine clinical care and for research purposes (see for e.g. Rao et al. 1991; Benedict et al. 2002; Langdon et al. 2012; National Institute of Neurological Disorders and Stroke, 2017). Moreover, the selected cognitive measures assess domains that are normally impaired in MS individuals, independent of disease duration and disability status. This included tests of attention, mental processing speed, verbal fluency/language, verbal and visuospatial memory, and executive functions. All neuropsychological tests were administered using standard procedures in single sessions. To minimize retest effects, alternative forms of the tests were used when available. Table 2.2 provides a summary of the utilized neuropsychological test battery arranged by cognitive function/domain assessed.
Table 2.2 Comprehensive neuropsychological battery that was administered arranged by cognitive function/domain assessed

<table>
<thead>
<tr>
<th>Cognitive functions / domain assessed</th>
<th>Neuropsychological test used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal memory</td>
<td>Selective Reminding Test (SRT)</td>
</tr>
<tr>
<td>Visuospatial Memory</td>
<td>Brief Visuospatial Memory Test-Revised (BVMT-R)</td>
</tr>
<tr>
<td>Verbal fluency / Expressive language</td>
<td>Greek Verbal Fluency Test (phonemic and semantic fluency)</td>
</tr>
<tr>
<td>Attention / Processing speed</td>
<td>Symbol Digits Modalities Test (SDMT)</td>
</tr>
<tr>
<td></td>
<td>Trail Making Test Part A</td>
</tr>
<tr>
<td>Executive functions</td>
<td>Response inhibition</td>
</tr>
<tr>
<td></td>
<td>Stroop Neuropsychological Screening Test (SNST) – (colour word task)</td>
</tr>
<tr>
<td></td>
<td>Set shifting</td>
</tr>
<tr>
<td></td>
<td>Trail Making Test Part B (TMT-B)</td>
</tr>
</tbody>
</table>

Note: All measures utilized in the study have been adapted for native Greek speaking adults and demographically corrected normative data have been published (with the exception of the BVMT-R that has been adapted in Greece, but normative data are not yet available). The BVMT-R and SDMT have been validated in Greek MS patients.


2.3.3.1 Verbal learning / memory

A Greek adapted version of the Selective Reminding Test (SRT) (Zalonis, Kararizou, Christidi, Kapaki, & Triantafyllou, 2009) was administered to assess verbal learning and episodic verbal memory. What distinguishes this verbal learning test from other similar tests is that it provides a distinctive form of feedback (Zalonis et al. 2009a). The differentiation of retention, storage and
retrievable may also be accomplished with this measure. Moreover, as the SRT is considered more of a procedure rather than a specific test, it has been administered in many different ways (see Lezak et al. 2012). The SRT was included in the Rao Brief Repeatable Neuropsychological Battery (BRB) (Rao et al. 1991), the most widely used and validated battery in the MS population for many years. The test has been utilized clinically for many years in the MS population to evaluate verbal memory, and a plethora of empirical articles evaluating cognition in MS have included this test as an outcome measure (see for e.g. Potagas et al. 2008). In this study, the procedure used to administer the test is the one originally described by Rao et al., (1991) and more recently, Zalonis et al. (2009). More specifically, a list of 12 unrelated words is read aloud to the patient at a rate of approximately one word per second, who is then asked to repeat as many words as possible in any sequence. The examiner than provides any words not recalled, after which the patient attempts to recall the entire list. The procedure typically continues until the patient recalls all words on three successive trials or to the 6th trial (i.e. for the 6-trial version, as the original was the 12-trial version) followed by a 20-minute interval, after which the patient tries to recall and replicate the list again. For this study, the dependent variables used were, long term storage (SRTLTS), reflecting the number of words recalled on two or more consecutive trials (i.e. without intervening reminding). Every word recalled on two or more consecutive trials is calculated in the total SRTLTS score, and every new attempt, independently of whether it is still recalled. The second dependent variable is the delayed recall trial (SRTDR) which refers to the total number of words recalled after 20-minute delay period.

2.3.3.2 Visual/spatial episodic memory

In order to assess visual / spatial episodic memory we used the Brief Visuospatial Memory Test-Revised (BVMT-R), (Benedict, 1997). The BVMT-R has been included in the comprehensive neuropsychological battery known as the Minimal Assessment of Cognitive
Function in MS (MACFIMS), following a consensus meeting in 2001, (Benedict et al. 2002), and is widely used in clinical practice and neuropsychological research with MS patients. On this nonverbal test, patients are shown a page containing six visual designs. After observing the designs for 10 seconds, the patient is provided with a blank sheet of paper and is asked to draw each of the designs in the correct location. Each design is scored with zero, one or two points, depending on accuracy and location. The Total recall score (BVMT-RT) is the sum of the individual scores on three consecutive trials. After 20-minute waiting period, the patient is asked to recall and draw the designs again (delay recall trial). For this study, as the BVMT-R is a non-verbal culture friendly instrument and does not require adaptation, only the test instructions were translated by two independent bilingual Greek–English speaking clinicians and then back translated by an independent bilingual psychologist checking for possible errors. As the test comes with 6 equivalent alternate forms, we used form one for baseline assessment of both groups, form two for post treatment assessment of both groups, and form three for the 6 months follow up assessment of the RehaCom treated MS patients. Dependent variable for this study was the total recall score (BVMT-RT). The BVMT-R has been validated in Greece with MS individuals, by Polychroniadou et al. (2016) in the recent Greek BICAMS validation study.

2.3.3.3 Verbal fluency / expressive language

Assessment of verbal fluency / expressive language was accomplished with the Greek Verbal Fluency Test (Kosmidis, Vlahou, Panagiotaki, & Kiosseoglou, 2004). The Greek Verbal Fluency Test consisted of two parts: semantic and phonemic fluency. On the semantic part (categories), we asked participants to generate as many different animals, fruits, and objects as possible, each in a time period of 60 seconds. On the phonemic part (letters), we asked participants to generate as many words as possible beginning with the Greek letters “χ” (chi), “σ” (“sigma), and “α” (alpha), each in a time period of 60 seconds, excluding proper nouns and variations of the same word. Dependent variables in the present analyses were the total number of words
produced on the semantic part and the total number of words produced on the phonological task. The verbal fluency task was included in the Rao Brief Repeatable Neuropsychological Battery (BRB) (Rao et al. 1991), and the more recently developed Minimal Assessment of Cognitive Function in MS (MACFIMS) battery (Benedict et al., 2002), and is one of the most widely used and psychometrically stable cognitive measures in clinical practice and research related to the MS population (Benedict & Walton, 2012).

2.3.3.4 Attention / processing speed

The Greek Trail Making Test Part A (Trails -A) (Vlahou & Kosmidis, 2002. Zalonis et al. 2008), was administered as a measure of divided attention and visuomotor scanning speed. In this task, the participants had to connect encircled numbers in ascending order as quickly as possible. The dependent variable included the time (in seconds) to complete Trails A. This type of Trail making Test format is part of the MACFIMS battery (Benedict et al.2002), incorporated as one of the stand-alone tests in the Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplin & Kramer, al. 2001), number sequencing condition. It is one of the most widely used instruments in the neuropsychological literature and widely used since the development of the MACFIMS battery in research involving cognitive aspects in MS. Moreover, it correlates well with other timed visual tests, such as the visual version of the Symbols Digit Modalities Test (SDMT) ($r = .65$; Lezak et al. 2012) and part B of the Trail making Test (TMT B) ($r = .73$; Lezak et al. 2012), that will be described in the sections that follow.

The Symbols Digit Modalities Test (SDMT) (Smith, 2002. Argirokastritou et al. 2005, normative study in Greek adults) was used to assess mental processing speed and working memory. In this study patients viewed a key with nine numbers, each paired with a unique symbol. Below the key, the rest of the page presents the symbols in random order, each paired with an empty space. Patients are requested to respond with the number that matches each symbol as rapidly as possible. This non-verbal or written version measures visual/spatial processing speed and
working memory. This test may also be completed in an audio or verbal format following the same procedure, with the exception that responses are verbal and not written. The verbal format is preferred when patients have pronounced motor disability or motor slowing. It is one of the most widely used instruments in the MS neuropsychological literature and widely used either as a stand-alone test or in neuropsychological batteries (e.g. BRB; Rao et al., (1991). MACFIMS; Benedict et al. (2002). BICAMS; Polychroniadou et al. (2016). In this respect, it is considered the test with the highest sensitivity for detecting cognitive decline in MS (Niccolai et al. 2015). Moreover, as mentioned previously it correlates highly with other timed visual tests, such as the TMT parts A and B (Lezak et al. 2012). Recently, it was validated in Greece with MS individuals, by Polychroniadou et al. (2016) in the Greek BICAMS validation study. Dependent variable for use in the study was the total number of correct written substitutions in 90 seconds.

2.3.3.5 Executive Functions (response inhibition)

In order to evaluate the response inhibition component of executive functions we used the Stroop Neuropsychological Screening Test (SNST) – (colour word task) (Trennery et al. 1989. Zalonis et al. 2009b. Messinis, 2012). The SNST basically assesses the speed of reading names of colors. It is a measure of executive function, requiring the subject to inhibit an overlearned response in favor of an unusual one. The SNST consists of two tasks (color task and color-word task). Both tasks include 112 color names - red, green, blue, tan, arranged in 4 columns of 28 names. In the color task, participants are required to read all the words aloud as quickly as they can, starting at the top of the first column. The Color–Word task which was utilized in this study requires the patient to name aloud, as quickly as possible, the color of the ink (red, green, blue, tan), in which the word is printed, and is allowed up to 120 seconds. In case of successful completion of the Color–Word task before the time of 120 seconds has elapsed, the total number of items completed is recorded (Zalonis et al. 2009b. Messinis,
The dependent variable for this test was the total number of correct items/responses completed in 120 seconds. The STROOP format is also part of the MACFIMS battery (Benedict et al. 2002), and is incorporated as one of the stand-alone instruments in the Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplin & Kramer, al. 2001). It is considered a popular neuropsychological measure and has been used widely in neuropsychological research. Since the development of the MACFIMS battery various STROOP formats have been utilized widely in specialized MS clinical and research settings (Delis, Kaplin & Kramer, et al. 2001.Benedict et al. 2002).

2.3.3.6 Executive Functions (Set shifting)

The Greek Trail Making Test Part B (Trails -B) (Vlahou & Kosmidis, 2002. Zalonis et al. 2008), was administered as a measure of set shifting / mental flexibility. In this task, patients were asked to connect encircled numbers, alternating between numbers and letters, in ascending order as quickly as possible. The dependent variable in this study included the time (in seconds) to complete Trails B. The TMT B test format is also part of the MACFIMS battery (Benedict et al. 2002), incorporated as one of the stand-alone tests in the Delis-Kaplan Executive Function System (D-KEFS; Delis, Kaplin & Kramer, al. 2001), number letter switching condition. As the TMT part A, it is one of the most widely used instruments in the neuropsychological literature and widely used since the development of the MACFIMS battery in research involving cognition in MS. Moreover, it has high correlations with other timed visual tests, such as the written version of the Symbols Digit Modalities Test (SDMT) (Lezak et al. 2012).

2.3.4 Assessment of mood

The Beck Depression Inventory Fast Screen for Medical Patients (BDI-fast screen), (Beck, Steer, & Brown, 2000. Messinis & Tsakona, 2004), was administered in order to assess the severity of depression. The BDI fast screen is a 7-item self – report case-finding instrument that
screens for severity of depression that corresponds to the psychological or nonsomatic criteria for diagnosing major depression disorders as listed in the DSM-IV (American Psychiatric Association, 1994) in adults and adolescents. It consists of seven items extracted from the 21-item Beck Depression Inventory – II (Beck, 1996). The administration procedure used was the one suggested by Beck et al. (2000), using a Greek translated and adapted version (Messinis & Tsakona, 2004), with Cronbach’s internal reliability coefficient (a = 0.82). The dependent variable in this study included the sum of the highest ratings for each of the seven items (maximum score = 21). The BDI-fast screen has been validated in multiple sclerosis patients (Benedict, Fishman, McClellan, Bakshi & Weinstock-Guttman, 2003). More specifically, it discriminated individuals with MS that were receiving treatment for depression from untreated MS patients with neurological symptoms. Moreover, the authors with their findings support its concurrent and discriminative validity in the MS population (Benedict, et al. 2003).

2.3.5 Assessment of fatigue

Fatigue was assessed with the Fatigue Severity Scale (FSS), a 9 item self-assessment scale (Krupp, LaRocca, Muir Nash, & Steinberg, 1989). The scale was recently adapted and validated in Greek MS patients and found to be reliable and valid for this population (Bakalidou et al. 2013). Respondents indicate the fatigue level they experienced throughout the last two weeks. The questions are related to how fatigue interferes with certain activities and rates its severity. The items are scored on a 7-point scale with 1 = strongly disagree and 7 = strongly agree. The scoring is done by calculating the average response to the questions (adding up all the answers and dividing by nine). The minimum score = 1 and maximum score possible = 7. A higher score is indicative of greater fatigue severity. In a recent validation study by Learmonth, Dlugonski, Pilutti, Sandroff, Klaren, & Motl, (2013), a mean FSS score ≥4 was indicative of substantial fatigue in 77% of the MS patients.
2.3.6 Single-Photon Emission Computed Tomography (SPECT)

As described previously thirty-one RRMS patients from a larger cohort of MS individuals that were eligible to take part in the study, irrespective of whether they were eventually randomized to the Rehacom treatment or standard care control group, consented to additionally undergo perfusion brain SPECT scanning. This process was conducted in two diagnostic centers, the nuclear medicine departments at the University hospital of Patras, and at the University hospital of Ioannina. An identical SPECT imaging and image analysis protocol was utilized in both institutions. Seventeen patients were scanned in Patras and 14 in Ioannina. Demographic and clinical characteristics of the 31 RRMS enrolled patients are provided in Table 2.3.

Table 2.3 Demographic and clinical characteristics of the RRMS sample evaluated by brain single-photon emission computed tomography (SPECT) (n=31)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
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<tbody>
<tr>
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<td>Education (years)</td>
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<td></td>
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<tr>
<td>EDSS</td>
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</tr>
<tr>
<td>Disease duration (years)</td>
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</tr>
<tr>
<td>MMSE</td>
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<td>WASI (IQ)</td>
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</tr>
<tr>
<td>Fatigue (FSS)</td>
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<td>1.60</td>
</tr>
<tr>
<td>Depression (BDI-FS)</td>
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<td>2.66</td>
</tr>
<tr>
<td>CI patients</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>RCI patients</td>
<td>12</td>
<td></td>
</tr>
</tbody>
</table>

Notes: All values are raw scores. Cognitively impaired MS patients (CI): failed ≥ 2 cognitive tests on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean; Relatively cognitively impaired MS patients (RCI): failed 1 cognitive test on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean.

Abbreviations: EDSS: Expanded Disability Status Scale; MMSE: Mini Mental State Examination; WASI: Wechsler Abbreviated Scale of Intelligence; FSS: Fatigue Severity Scale; BDI-FS: Beck Depression Inventory Fast Screen; SD: Standard Deviation; CI: Cognitively impaired; RCI: Relatively cognitively impaired
The procedure that was followed for Perfusion brain SPECT in this study has been described previously by our scientific group in patients with Parkinson's disease (see Paschali et al. 2009). More specifically, brain perfusion SPECT scanning was performed 30 minutes following intravenous injection of 740 MBq \(^{99m}\text{Tc}\) HMPAO (Ceretec, GE Healthcare Ltd., Buckinghamshire, United Kingdom) in a dimly lit, quiet room with the patients lying supine with eyes closed. Brain SPECT was performed with double-head gamma cameras (Infinia Hawkeye 4, GE Healthcare, Milwaukee, USA) equipped with low-energy, high-resolution parallel-hole collimators. The head of each patient was held with fixation strips attached to a specially constructed carbon fiber head holder, which allowed the camera detector to rotate very close to the head. The data were collected into a 128×128 matrix, through 360° rotation at steps of 3° for 20 s per view. SPECT image reconstruction was achieved via filtered back projection using a Butterworth (order 10, cut-off 0.45 cycles.cm\(^{-1}\)) and ramp filter. Attenuation correction based on Chang’s method (Chang, 1978) was performed on each slice, with a uniform attenuation coefficient of 0.11.

Analysis of rCBF SPECT was performed by NeuroGam (GE Healthcare, Waukesha, WI, USA), a dedicated statistical parametric mapping software. This involves intensity-thresholding and spatial normalization of the images to a standardized stereotactic (Talairach) 3-dimensional (3D) space. After anatomical standardization and voxel normalization to cerebellum perfusion mean values, which is considered to be the least affected area in degenerative diseases, NeuroGam compares each patient’s rCBF SPECT against a database of gender- and age-matched healthy controls and creates a z-score map, thus allowing for the objective demonstration of the extent and magnitude of rCBF changes. When for technical reasons (e.g., patients with short necks) the cerebellum was not within the camera’s field-of-view, we used as anatomical standardization site the area with the highest perfusion rate in the brain. For the analysis of an individual study, the computer programme calculates a voxel by
voxel z-score using the following equation: 
\[ z\text{-score} = \frac{([\text{control mean}] - [\text{individual value}])}{\text{(control standard deviation SD)}} \].

The z-score maps are displayed either in the standard cuts or by overlay on a 3-D anatomical topographic representation by means of a specific colour scale. This is equivalent to presenting the individual data items in terms of SD from a normal age-matched database. Abnormal perfusion areas are defined as those with decreased uptake (below 2 SD of the normal mean uptake in area >50% pixels) (see figure 2.3). Dependent variables that were investigated for this study included the right and left frontal, parietal, temporal and occipital lobes, and the predefined left and right Brodmann areas (BA), mainly in the prefrontal cortex; BA 9 (posterior lateral prefrontal cortex); BA 10 (medial prefrontal cortex); BA 11 and BA 12 (orbitofrontal cortex).

### 2.3.7 Treatment intervention

As noted previously MS patients that were eligible to take part in the study were randomized to either receive specific computerized cognitive remediation training – cognitive rehabilitation (n=32), over a period of 10 weeks, with 2 weekly 60 minute sessions on an individual basis or usual clinical care – standard treatment (n=26) for the same time period. The study is a multicentric (2 centers), randomized controlled trial investigating the efficacy of cognitive functional training in RRMS patients. This approach aims to improve cognitive functioning by restoring or improving network efficiency in the brain.
Figure 2.3 Example of a z-score perfusion map of an elderly normal individual displayed on a 3D anatomical topographic representation by means of a specific colour scale (standard deviation from normal age-and-gender matched database)

2.3.7.1 Treatment Intervention: computer-assisted cognitive rehabilitation

(RehaCom® modules)

The treatment consisted of 20 individualized one hour sessions over a 10-week period, with a frequency of two sessions per week. The rehabilitation program was conducted by trained clinicians, either speech and language therapists or psychologists, supervised by a clinical neuropsychologist (LM), on a desk top computer with a large screen. The computer was connected with a special input panel using the commercially available RehaCom software package (RehaCom Cognitive Therapy Software. https://www.rehacom.co.uk), which has been utilized extensively in Europe over the last couple of years for the purpose of providing computer assisted cognitive rehabilitation. The panel – keyboard that is utilized limits the interference of motor and coordination impairments. Moreover, the software which has over 20
modules is available in many languages, including Greek. In Greece, the software is available commercially at Ostracon. For more details about this product see the Ostracon website at (http://ostraconmed.com/ostracon-proionta/gnostiki-apokatastasi/rehacom/gia-ton epaggelmata/).

It provides the opportunity to train patients on several levels of difficulty and length of sessions, and according to whether the patient succeeds or fails the task, the difficulty levels are automatically adjusted to meet the patient’s needs. Once the training is completed the therapist can review the session from the results screen. The data can be presented in a variety of ways including charts, graphs and comparisons. The most common format results are: level of progression, number of mistakes and time utilized for each cognitive task. By analyzing the data thoroughly, the therapist is able to identify particular weaknesses of the patient e.g. noticing auditory stimuli, and address this further in the training.

For this specific study, as most of our MS patients that took part in the intervention, were impaired in more than one cognitive domain, but mostly on episodic memory, information processing / attention and executive functions, the intervention was balanced over the 10-week period in order to train all domains equally. In order to train attention, we used two modules. The first module is called *attention and concentration*, training mainly selective attention, and in this procedure a separately presented picture is compared to a matrix of pictures. The patient has to recognize a picture (symbols, items, animals or abstract figures) and respond by selecting it from a matrix. This activity trains the ability to differentiate and concentrate simultaneously. The matrices are either 3 pictures (1 x 3 matrix), 6 pictures (2 x3 matrix) or 9 pictures (3 x 3 matrix) depending on the level of difficulty that we want to train at (see figures 2.4 and 2.5, for examples of a 1 by 3 screen on level 1 and a 3 by 3 matrix screen on level 18. The selected picture on figure 2.5 is framed and the sign signals a correct choice providing feedback to the patient.
Figure 2.4 Example of a screen with a 1 by 3 matrix on level 1 (Greek edition)

Figure 2.5 Example of a screen with a 3 by 3 matrix on level 18
The second module used to train attention is a more naturalistic or ecologically valid cognitive task called divided attention. In this task, the patient works through the cognitive training as the driver of a train shown on the lower part of the screen. He sits in the steeple cab (or driver's cab) of the train and can observe the railway like looking through the windscreen of the driver's cab, and has the following task: He must carefully observe the control panel of the train and the countryside, as it flashes past, and react to different events as they occur. At first, only the acceleration of the train is to be regulated. Later, and with increasing levels of difficulty more tasks are added; in which different levels of attention and particular reactions are expected from the trainee. The driver's panel contains a speedometer, a so-called “Deadman’s lamp” and the “emergency stop lamp”. On the speedometer, a “target speed” is set that the client must keep. As soon as a lamp lights up, the client has to press the corresponding button on the RehaCom Panel (e.g. the stop button). If a relevant object appears on the railway, the client also has to react to it (e.g. stopping at a red signal) (see figure 2.6)

In order to train memory, we also used two different modules. For training visuospatial memory, we used the topological memory module. In the so-called “memorizing phase”, a variable number of cards (depending on the level of difficulty) with concrete pictures or geometric figures are displayed on the screen. The client has to memorize the position of the pictures. After a preset time – or manually by pressing the OK button – the pictures of the matrix are hidden (turned face down). The client must find the picture matching the one indicated on the right side of the screen. Altogether, 464 pictures of concrete objects, geometric figures, and letters are available. The number of simultaneously displayed cards varies from 3 to a maximum of 16 (see figure 2.7)
Figure 2.6 Example of the divided attention task on level 14

Figure 2.7 Example training on level 9, of the topological memory task
In order to train verbal episodic memory, we used the *verbal memory* module. In this task, a short story is presented on the screen. The client has to memorize as many details of the story as possible (names, numbers, events, objects). The learning phase is completed by pressing the OK button. After that, the client must answer questions about the content of the story.

More than 80 short stories are available. Depending on the setting, either the computer or the therapist selects a story for the client. An extension of the pool of stories is possible by using an integrated editor (see figures 2.8 and 2.9).
Figure 2.8 Example of training on level 1, of the verbal memory module (Greek edition)

Figure 2.9 Example of training on level 3, of the verbal memory module
Executive functions were trained with two respective modules. The first module called *logical reasoning* trains abstract logical thinking ability and conclusive thinking. The task requires that from several symbols (pool of answers), the client has to find out the one that correctly continues a given sequence of symbols. A sequence of symbols (circles, triangles, squares, etc.) of different shape, color, and size are displayed on the screen being in a regular relation to each other. If the answer is wrong, special pieces of information about the type of error (shape, color, and/or size) are provided. The principle behind the training is that the problem-solving tasks are graphic and vivid. The patient learns to recognize the concepts underlying each problematic situation and uses these concepts to find a solution to the logic problem (see figure 2.10).

The second module used to treat executive function is a more ecologically valid highly realistic training exercise called *shopping*. The patient performs the same tasks on the computer that he would have to do while going shopping in a supermarket. Specifically, the trainee gets a shopping list of articles that he has to look for in a supermarket and put into a trolley. When all articles are in the trolley, the client can leave the supermarket by using the “cash” button. Beyond a certain level of difficulty, additional demands are made on the client’s mathematical abilities (a certain amount of money is specified, the products are marked with prices, etc.). This training module currently uses more than 100 articles illustrated photo-realistically (food, household objects, etc.). These articles appear on shelves from which the client must choose them. The training programme disposes of a voice output, which means all articles are named when selected (see figure 2.11).
2.3.7.2 Control group: Standard clinical care

MS patients that were randomized to receive standard or usual clinical care continued taking their prescribed medication and all other related treatments (e.g. physiotherapy, psychotherapy), and all other clinical or referral services were available to them as usual for the entire 10 weeks that the intervention group received cognitive training. As in the University Hospital of Patras or the laboratory of audiology, neurootology and neurosciences of the Higher Educational Institute of Epirus, specific interventions for cognitive difficulties in MS patients are
not offered on a standard basis, these patients did not receive any specific cognitive rehabilitation for their cognitive problems. This group of patients for ethical reasons was offered the opportunity to undertake cognitive rehabilitation after completion of the study period.

2.4 Statistical Analysis

We initially computed the basic descriptive statistics and the 95% confidence intervals of the demographic (age, education level, gender, and Wechsler Abbreviated Scale of intelligence: full 2 scale IQ and Vocabulary subscale T-score); clinical (Expanded Disability Status Scale, Mini Mental State Examination, Beck Depression Inventory-fast screen, duration of illness, medication regimen at enrolment, Fatigue Severity Scale) and neuropsychological variables (Trail Making Test parts A and B; Selective Reminding Test; Brief Visuospatial Memory Test-Revised; Symbol Digits Modalities Test; Greek Verbal Fluency Test (semantic and phonemic); Stroop Neuropsychological Screening Test: colour word task).

We also computed the frequency of impaired MS patients on rCBF brain perfusion SPECT scanning, using as criterion for impairment 2 SD below an age-and-gender-adjusted normative database for rCBF SPECT performance; and the frequency of impaired perfusion according to a hypoperfusion grading. Next the normality assumption of the data was tested using the Shapiro-Wilk test since it is more powerful than the most commonly used in practice Kolmogorov-Smirnov test (Thode, 2002). When the hypothesis of normality was rejected, the non-parametric Mann-Whitney test was used to examine the differences between our two groups (intervention and control group); otherwise the standard independent samples t-test was used. For the comparison of dependent populations, the non-parametric Friedman test was used whenever the normality assumptions were rejected and the paired samples t-test in all other cases. The Pearson Correlation coefficient was used in order to measure correlations between neuropsychological and disease variables, depression and fatigue. We also examined
correlations between rCBF brain SPECT and performance on cognitive measures using the non-parametric Kendall's tau test. Furthermore, due to the use of multiple cognitive measures to assess cognitive functions, we decided to calculate composite scores and formulate composite variables (cognitive domains) for verbal episodic memory, attention, verbal fluency, and processing speed, by transforming raw neuropsychological test scores obtained from the neuropsychological assessment to form composite domain z-scores. In order to extract the new composite variables, the internal consistency of these variables was measured using Cronbach’s Alpha. As the internal consistency of all extracted composite domains was considered acceptable ($\alpha > 0.60$), the new variables were derived as a weighted sum of the z-scores of the initial neuropsychological variables. We also applied a mixed effect ANOVA in order to compare the mean cognitive domain performance difference between the intervention and control group (between subject’s factor) and the time points (baseline and post treatment) that patients were cognitively evaluated (within subject’s factor). Moreover, the interaction of these two factors was evaluated by a two–way mixed ANOVA. Statistical analyses were conducted using the statistical package SPSS 22.0 for Windows.

3. Results

3.1 Comparison of demographic and clinical characteristics at baseline (pretreatment)

The 58 patients included in the study were randomly assigned to either the cognitive rehabilitation intervention (n=32) or standard care control group (n=26). In general, there was a higher proportion of females compared to males that took part in the study. The percentage of females was higher in both groups (68.75% for the rehabilitation and 69.23% for the control group), something that was expected due to the higher female to male ratio in the MS population in general. However, the proportion/ratio of females between the two groups was not significantly different, [$\chi^2(1) = .002, p=.969$]. We then investigated the normality distribution
of our data with the *Shapiro-Wilk normality test*. For the variables age and BDI-FS (depression level) the null hypothesis could not be rejected, therefore, we the used the parametric *independent samples t-test* to test group differences on this variable. In contrast for the variables level of education, WASI (full IQ 2 scale; intelligence level), WASI vocabulary scale T-score (estimated premorbid intelligence level), FSS (fatigue severity), EDSS (disability level), MMSE, and duration of illness, we rejected the null hypothesis and used the non-parametric Mann–Whitney U-test to compare these variables. We did not find significant differences between the two groups on baseline (pretreatment) assessment for the variables age \( [t (56) = .379, p = .706] \), educational level \([z = -.945, p = .345]\), intelligence level (WASI 2 scale full IQ) \([z = -.959, p = .338]\), estimated premorbid intelligence (WASI vocabulary scale) \([z = -.959, p = .338]\), depression level (BDI-SF) \([t (56) = .179, p = .859]\), fatigue severity level (FSS) \([z = -.697, p = .486]\), \([z = -.959, p = .338]\), disability level (EDSS) \([z = -.126, p = .899]\), general cognitive status \([z = -.1578, p = .115]\), and duration of illness \([z = -.1515, p = .130]\) (see Table 2.1 for a detailed description of baseline demographic and clinical characteristics). From the above analysis, we conclude that our two groups were well matched on baseline demographic variables and premorbid intelligence level, that may significantly influence outcome measures post treatment. They also did not differ on important disease – related variables such as duration and course (all had a relapsing remitting course), neurological disability (EDDS scale), depression (BDI-FS) and fatigue (FSS) severity, that have also been reported to negatively impact cognitive performance in MS patients.

### 3.2 Comparison of neuropsychological test scores at baseline (pretreatment)

In order to test the normality assumption of our cognitive score measures at baseline we used the *Shapiro-Wilk test*. For the variables SRTLR (verbal memory; long term storage) and TMT B (executive function; set shifting), the null hypothesis could not be rejected, therefore, we the
used the parametric independent samples t-test to test group differences on this variable. In contrast for the variables SRT delay (verbal memory; delay recall), TMT A (attention; processing speed), Greek VFT (semantic fluency) and (phonemic fluency), SDMT (processing speed; working memory), BVMT-R (visuospatial memory; total recall) and SNST – colour word task (executive function; response inhibition) we rejected the null hypothesis and used the non-parametric Mann–Whitney U-test to compare the variables. We did not find significant differences between the two groups on baseline (pretreatment) assessment for the variables SRTLR \([t (56) = .201, p = .842]\), TMT B \([t (56) = .201, p = .604]\), VFT (semantic) \([z = -.478, p = .633]\), (phonemic) \([z = -.335, p = .520]\), SDMT \([z = -.916, p = .360]\), BVMT-R \([z = -.989, p = .578]\) and SNST \([z = -.285, p = .679]\) . On the contrary, patients randomized to the intervention group verbally recalled significantly less words \((M_{\text{intervention group}} = 6.09 \text{ words vs. } M_{\text{control group}} = 7.15 \text{ words})\) after a 20-minute delay period; SRT delay score \([z = -2.289, p = .022]\) and required significantly longer duration \((M_{\text{intervention group}} = 73.50 \text{ seconds vs. } M_{\text{control group}} = 69.27 \text{ second})\) to correctly complete the Trails A test; \([z = -2.294, p = .020]\), relative to the control group. These findings imply that the intervention group was marginally more cognitively impaired at baseline assessment (see Table 2.4 for raw cognitive test performance scores of both groups at baseline, post treatment and 6 months follow up).

### 3.3 Comparison of neuropsychological test performance for the Rehacom MS treated group between baseline, post treatment and 6-month follow up

In order to establish possible changes in outcome cognitive measures over time due to an intervention effect, we conducted a within group comparison of the RehaCom MS treated group. As the cognitive scores were not normally distributed we used Friedman’s non-parametric test for comparison of medians between baseline, post treatment and 6 months follow–up performance, and the Wilcoxin test with holm correction to conduct the pairwise
comparisons. We found significant time effects for most of our variables from baseline to post treatment. Post hoc pairwise comparisons showed that the patients who received functional cognitive training had improved cognitive performance between baseline and posttest on the SRTLR (verbal memory; long term storage) \( (p < .001; \text{with a large effect size}; r = .539) \), SDMT (processing speed; working memory) \( (p < .001; \text{with a large effect size}; r = .522) \), SRTLR (verbal memory; delay recall) \( (p < .001; \text{with a medium effect size}; r = .481) \), BVMT-R (visuospatial memory; total recall) \( (p < .001; \text{with a medium effect size}; r = .469) \), VFT (semantic) \( (p = .003; \text{with a medium effect size}; r = .417) \), TMT A (attention; processing speed), \( (p < .001; \text{with a large effect size}; r = .573) \), TMT B (executive function; set shifting) \( (p < .001; \text{with a large effect size}; r = .506) \), and SNST – colour word task (executive function; response inhibition) \( (p < .001; \text{with a medium effect size}; r = .460) \). In contrast to the positive time effects of the intervention shown for most of our variables, cognitive training did not significantly improve phonemic fluency (even though patients improved their mean phonemic production rate from \( M_{\text{baseline}} = 31.88 \text{ words vs. } M_{\text{post treatment}} = 33.13 \text{ words} \)).

In order to establish whether the treated patients differed in terms of their baseline versus 6-month follow up performance we compared their cognitive measure scores at these time points. The results revealed that treated patients differed significantly on the SRTLTS \( (p = .000; \text{with a medium effect size}; r = .469) \), SRTDR \( (p < .001; \text{with a medium effect size}; r = .454) \), BVMT-R \( (p < .001; \text{with a medium effect size}; r = .436) \), TMT A \( (p < .001; \text{with a large effect size}; r = .509) \), TMT B \( (p < .001; \text{with a medium effect size}; r = .475) \) and SNST \( (p < .001; \text{with a medium effect size}; r = .448) \). On three of our outcome variables non-significant differences were established between baseline and follow up performance. The VFT (semantic) \( (p = .424) \), phonemic \( (ns) \) and SDMT \( (p = .222) \). Although patients improved their mean semantic fluency production rate from \( M_{\text{baseline}} = 41.03 \text{ words vs. } M_{\text{follow up}} = 42.06 \text{ words} \) and their mean digit symbol substitution rate in 90 seconds from \( M_{\text{baseline}} = 36.91 \text{ correct substitutions vs. } M_{\text{follow up}} = 37.93 \text{ correct substitutions} \).
up = 37.50 correct substitutions), this was insufficient to produce statistically significant changes.

To examine the long-term effect of the intervention over time we compared cognitive outcome performance between post treatment and 6 months’ follow-up. Our findings showed that for most of our variables there were non-significant differences between the positive cognitive gains found on post treatment and follow up. In contrast, the mean semantic fluency production rate was reduced from (M post treatment = 43.56 words vs. M follow up = 42.06 words) and mean digit symbol substitution rate in 90 seconds from (M post treatment = 40.03 correct substitutions vs. M follow up = 37.50 correct substitutions), producing statistically significant changes over this time period (see Table 2.5).

3.4 Comparison of neuropsychological test performance for the MS standard care control group between baseline and post treatment

In order to investigate possible changes in outcome cognitive measures over time in the standard treatment control group, we conducted a within group comparison. As the cognitive scores were not normally distributed we used the Wilcoxon signed rank nonparametric test to compare performance between baseline and post treatment. The results revealed that in the majority of measures there were no significant within group effects of time. An exception was performance on the mean phonemic fluency production rate that increased from (M baseline = 29.81 words vs. M post treatment = 29.95 words) [z= -2.365, p = .018]; the mean semantic fluency production rate that decreased from (M baseline = 40.50 words vs. M post treatment = 39.58 words) [z= -2.874, p = .004]; and Trails A completion time that increased from (M baseline = 60.27 second vs. M post treatment = 60.88 seconds) [z= -2.117, p = .034]. These findings although marginally different in some cases produced statistically significant changes over time, albeit
mostly with a negative direction. These results imply that this group did not show improvements over time, on the contrary, there were trends of a possible further cognitive decline in the 10-week period between baseline and post treatment assessments (see Table 2.6).
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<th>MS RehaCom group (n=32)</th>
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<td></td>
<td>Mean (95% CI)</td>
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<tr>
<td>T0</td>
<td>6.09 (5.44 - 6.75)</td>
<td>1.82</td>
<td>7.15 (6.65 - 7.66)</td>
<td>1.25</td>
</tr>
<tr>
<td>T1</td>
<td>8.22 (7.59 - 8.85)</td>
<td>1.75</td>
<td>7.12 (6.73 - 7.50)</td>
<td>7.12</td>
</tr>
<tr>
<td>T2</td>
<td>7.75 (7.11 - 8.39)</td>
<td>1.77</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BVMT-RT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>21.40 (17.10 - 24.30)</td>
<td>5.85</td>
<td>22.50 (17.80 - 25.20)</td>
<td>7.80</td>
</tr>
<tr>
<td>T1</td>
<td>24.50 (19.50 - 26.30)</td>
<td>6.02</td>
<td>20.80 (17.50 - 24.60)</td>
<td>6.85</td>
</tr>
<tr>
<td>T2</td>
<td>23.10 (18.90 - 25.20)</td>
<td>6.40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VFT phon</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>31.88 (28.92 - 34.83)</td>
<td>8.20</td>
<td>29.81 (23.39 - 30.23)</td>
<td>8.46</td>
</tr>
<tr>
<td>T1</td>
<td>33.13 (30.60 - 35.65)</td>
<td>7.01</td>
<td>29.95 (24.16 - 30.53)</td>
<td>7.88</td>
</tr>
<tr>
<td>T2</td>
<td>31.47 (29.20 - 33.74)</td>
<td>6.29</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>VFT sem</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>41.03 (38.09 - 43.97)</td>
<td>8.16</td>
<td>40.50 (36.69 - 44.31)</td>
<td>9.44</td>
</tr>
<tr>
<td>T1</td>
<td>43.56 (40.55 - 46.57)</td>
<td>8.34</td>
<td>39.58 (35.60 - 43.55)</td>
<td>9.83</td>
</tr>
<tr>
<td>T2</td>
<td>42.06 (39.05 - 45.08)</td>
<td>8.35</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SDMT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>36.91 (33.89 - 39.92)</td>
<td>8.36</td>
<td>37.42 (33.03 - 41.82)</td>
<td>10.87</td>
</tr>
<tr>
<td>T1</td>
<td>40.03 (37.48 - 42.58)</td>
<td>7.08</td>
<td>37.43 (33.44 - 41.40)</td>
<td>9.85</td>
</tr>
<tr>
<td>T2</td>
<td>37.50 (35.25 - 39.75)</td>
<td>6.25</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>TMTA</td>
<td>T0</td>
<td>T1</td>
<td>T2</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>73.50</td>
<td>59.53</td>
<td>60.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(65.08 - 81.92)</td>
<td>(52.86 - 66.20)</td>
<td>(53.28 - 67.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.35</td>
<td>18.49</td>
<td>19.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(52.05 - 68.48)</td>
<td>(52.67 - 69.10)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69.27</td>
<td>68.88</td>
<td>68.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.30</td>
<td>20.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Notes: All values are raw scores; T0: baseline assessment; T1: post treatment assessment; T2: 6-month follow-up assessment; MS control group was not assessed at 6 months follow up.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
|Abbreviations: SRTLTS: Selective Reminding Test – Long Term Storage; SRTDR: Selective Reminding Test – Delayed Recall; BVMT-RT: Brief Visuospatial Memory Test-Revised Total Recall; VFT phon: Greek Verbal Fluency Test - Phonemic Fluency; VFT sem: Greek Verbal Fluency Test - Semantic Fluency; SDMT: Symbol Digits Modalities Test; TMTA: Greek Trail Making Test Part A; Greek Trail Making Test Part B; SNST Stroop Neuropsychological Screening Test.
Table 2. 5 Comparison of neuropsychological test scores for the RehaCom MS treated group at baseline, post treatment and 6 months follow up

<table>
<thead>
<tr>
<th>Test</th>
<th>Baseline Mean</th>
<th>Baseline Median</th>
<th>Post treatment Mean</th>
<th>Post treatment Median</th>
<th>6 months follow up Mean</th>
<th>6 months follow up Median</th>
<th>Baseline vs. post treatment p values</th>
<th>Baseline vs. follow up p values</th>
<th>Post treatment vs. follow up p values</th>
<th>Effect size (r)</th>
<th>Effect size (r)</th>
<th>Effect size (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRTLTS</td>
<td>36.72</td>
<td>36.50</td>
<td>43.47</td>
<td>41.00</td>
<td>43.00</td>
<td>41.00</td>
<td>.000***</td>
<td>.539</td>
<td>.469</td>
<td>.94</td>
<td>.452</td>
<td>.094</td>
</tr>
<tr>
<td>SRTDR</td>
<td>6.09</td>
<td>6.50</td>
<td>8.22</td>
<td>8.00</td>
<td>7.75</td>
<td>7.00</td>
<td>.000***</td>
<td>.481</td>
<td>.454</td>
<td>.076</td>
<td>.222</td>
<td></td>
</tr>
<tr>
<td>BVMT-RT</td>
<td>21.40</td>
<td>21.00</td>
<td>24.50</td>
<td>23.90</td>
<td>23.10</td>
<td>23.00</td>
<td>.000***</td>
<td>.469</td>
<td>.436</td>
<td>.025</td>
<td>.320</td>
<td></td>
</tr>
<tr>
<td>VFT phon</td>
<td>31.88</td>
<td>32.00</td>
<td>33.13</td>
<td>33.50</td>
<td>31.47</td>
<td>31.50</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>VFT sem</td>
<td>41.03</td>
<td>40.00</td>
<td>43.56</td>
<td>42.00</td>
<td>42.06</td>
<td>40.50</td>
<td>.003**</td>
<td>.417</td>
<td>.424</td>
<td>.100</td>
<td>.018*</td>
<td>.326</td>
</tr>
<tr>
<td>SDMT</td>
<td>36.91</td>
<td>36.00</td>
<td>40.03</td>
<td>39.00</td>
<td>37.50</td>
<td>37.00</td>
<td>.000**</td>
<td>.522</td>
<td>.222</td>
<td>.153</td>
<td>.021*</td>
<td>.319</td>
</tr>
<tr>
<td>TMTA</td>
<td>73.50</td>
<td>70.00</td>
<td>59.53</td>
<td>62.50</td>
<td>60.31</td>
<td>66.00</td>
<td>.000***</td>
<td>.573</td>
<td>.509</td>
<td>.829</td>
<td>.027</td>
<td></td>
</tr>
<tr>
<td>TMTB</td>
<td>145.81</td>
<td>32.50</td>
<td>113.28</td>
<td>106.50</td>
<td>115.78</td>
<td>107.50</td>
<td>.000***</td>
<td>.506</td>
<td>.475</td>
<td>.284</td>
<td>.134</td>
<td></td>
</tr>
<tr>
<td>SNST</td>
<td>59.80</td>
<td>58.50</td>
<td>63.50</td>
<td>62.90</td>
<td>62.10</td>
<td>60.40</td>
<td>.000***</td>
<td>.460</td>
<td>.448</td>
<td>.385</td>
<td>.205</td>
<td></td>
</tr>
</tbody>
</table>

Notes: All values are raw scores; p < .001***; p < .01**; p < .05*; Friedman’s non-parametric test used for comparison of medians between baseline, post treatment and follow up; Wilcoxin test with holm correction used for pairwise comparisons. Effect size (r) for Wilcoxin test calculated as follows: \( r = \frac{z}{\sqrt{N}} \) (N= total number of samples); abs (r) 0.1 small size; 0.3 medium size; 0.5 large size; ns: Friedman’s test indicated no significant group effect for VFT phon.

Abbreviations: SRTLTS: Selective Reminding Test – Long Term Storage; SRTDR: Selective Reminding Test –Delayed Recall; BVMT-RT: Brief Visuospatial Memory Test-Revised Total Recall; VFT phon: Greek Verbal Fluency Test - Phonemic Fluency; VFT sem: Greek Verbal Fluency Test -Semantic Fluency; SDMT: Symbol Digits Modalities Test; TMTA: Greek Trail Making Test Part A; Greek Trail Making Test Part B; SNST: Stroop Neuropsychological Screening Test
Table 2.6 Comparison of neuropsychological test scores for the standard care MS control group at baseline and post treatment

<table>
<thead>
<tr>
<th>Test</th>
<th>Baseline Mean</th>
<th>Baseline Median</th>
<th>Post treatment Mean</th>
<th>Post treatment Median</th>
<th>Baseline vs. Post treatment z</th>
<th>p values</th>
<th>Effect size</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRTLTS</td>
<td>36.42</td>
<td>36.50</td>
<td>36.38</td>
<td>37.00</td>
<td>.187</td>
<td>.852</td>
<td>.026</td>
</tr>
<tr>
<td>SRTDR</td>
<td>7.15</td>
<td>7.00</td>
<td>7.12</td>
<td>7.00</td>
<td>.302</td>
<td>.763</td>
<td>.042</td>
</tr>
<tr>
<td>BVMT-RT</td>
<td>22.50</td>
<td>22.00</td>
<td>20.80</td>
<td>21.10</td>
<td>.304</td>
<td>.675</td>
<td>.034</td>
</tr>
<tr>
<td>VFT phon</td>
<td>29.81</td>
<td>28.00</td>
<td>29.95</td>
<td>28.50</td>
<td>-2.365</td>
<td>.018</td>
<td>.328</td>
</tr>
<tr>
<td>VFT sem</td>
<td>40.50</td>
<td>39.50</td>
<td>39.58</td>
<td>38.50</td>
<td>-2.874</td>
<td>.004</td>
<td>.399</td>
</tr>
<tr>
<td>SDMT</td>
<td>37.42</td>
<td>38.50</td>
<td>37.43</td>
<td>39.00</td>
<td>-.069</td>
<td>.945</td>
<td>.010</td>
</tr>
<tr>
<td>TMTA</td>
<td>60.27</td>
<td>57.00</td>
<td>60.88</td>
<td>58.50</td>
<td>-2.117</td>
<td>.034</td>
<td>.294</td>
</tr>
<tr>
<td>TMT B</td>
<td>111.54</td>
<td>110.00</td>
<td>110.96</td>
<td>107.50</td>
<td>1.042</td>
<td>.298</td>
<td>.144</td>
</tr>
<tr>
<td>SNST</td>
<td>58.70</td>
<td>57.40</td>
<td>59.10</td>
<td>57.60</td>
<td>.348</td>
<td>.780</td>
<td>.035</td>
</tr>
</tbody>
</table>

Notes: All values are raw scores; $p < .01^{**}; p < .05^*; Wilcoxon signed ranked non-parametric test used for comparison of medians between baseline and post treatment.

Abbreviations: SRTLTS: Selective Reminding Test – Long Term Storage; SRTDR: Selective Reminding Test –Delayed Recall; BVMT-RT: Brief Visuospatial Memory Test-Revised Total Recall; VFT phon: Greek Verbal Fluency Test -Phonemic Fluency; VFT sem: Greek Verbal Fluency Test -Semantic Fluency; SDMT: Symbol Digits Modalities Test; TMTA: Greek Trail Making Test Part A; Greek Trail Making Test Part B; SNST: Stroop Neuropsychological Screening Test
3.5 Comparison of composite cognitive domain performance in the RehaCom group at baseline, post treatment and follow up

Composite cognitive variables (domains) were calculated for verbal episodic memory, attention, verbal fluency, and processing speed, by converting raw cognitive scores into z-values. In order to extract the new composite variables, the internal consistency of these variables was measured using Cronbach’s Alpha. Since the internal consistency of all extracted composite domains was considered acceptable ($\alpha > 0.60$), the new variables were derived as a weighted sum of the z-scores of the initial neuropsychological variables. Figure 2.12 depicts domain performance differences over time for the RehaCom treated group.

**Figure 2.12** Composite cognitive domain performance (z-scores) in the RehaCom group at baseline, post treatment and follow up
As is evident from figure 2.12 we noted a significant reduction \((p = .046)\) in processing speed from baseline to post treatment assessment and a slight non-significant increase \((p = .067)\) from post treatment to follow up, but without reaching the baseline levels of processing speed capacity. Verbal fluency output on the other hand improved significantly from baseline to post treatment \((p = .034)\), but showed significant deterioration from post treatment to follow up \((p = .020)\). Attention which was a composite of timed scored measures showed a significant reduction in completion time from baseline to post treatment \((p = .018)\), and remained relatively stable over time from post treatment to follow up \((p = .290)\). Verbal episodic memory which reflects total word learning capacity and delayed recall of words, showed a significant increase \((p = .002)\), from baseline to post treatment and a non-significant decrease \((p = .702)\), from post treatment to follow up, but without dropping to baseline levels.

3.6 Comparison of composite cognitive domain performance between the RehaCom intervention and control group at baseline and post treatment

A mixed effect ANOVA was applied in order to compare the mean cognitive domain performance difference between the RehaCom intervention group and standard treatment control group (between subject’s factor) and the time points (baseline and post treatment) that patients were cognitively evaluated (within subject’s factor). Furthermore, the interaction of these two factors was evaluated by a two –way mixed ANOVA. We found a significant time x group interaction on all four domains that we evaluated (see Table 2.7).

Moreover, (figure 2.13) clearly depicts the significant composite domain performance differences favoring the intervention group, as an interaction of patient group by time. Specifically, on the verbal episodic memory domain the rehabilitation group demonstrated a significant increase in the estimated marginal mean over time, indicative of improved encoding, consolidation, acquisition and delayed recall of verbally learned material, relative to the control
group that demonstrated a significant reduction in this domain over the ten-week period. On the attention domain, a composite of timed scored measures, a significant reduction in the estimated marginal mean completion time from baseline to post treatment was noted, relative to the control groups performance which showed an increase in the estimated marginal mean completion time from pre-to post assessment. A significant reduction was also noted in the estimated marginal mean mental processing speed, from pre- to post treatment assessment for the intervention group, relative to the control group that demonstrated an increased mental processing speed capacity over this time period. The verbal fluency domain, a composite of phonemic and semantic fluency output, improved significantly for the group that received cognitive treatment, over the 10-week period, from baseline to post treatment, relative to the control group whose combined fluency output decreased over this time period.

**Table. 2.7** Two – way mixed effect ANOVA for cognitive domain performance: time (within subjects’ factor) and patient group: (between subjects’ factor)

<table>
<thead>
<tr>
<th></th>
<th>Verbal Episodic Memory</th>
<th>Attention</th>
<th>Verbal Fluency</th>
<th>Processing Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td>0.628</td>
<td>0.727</td>
<td>0.767</td>
<td>0.662</td>
</tr>
<tr>
<td><strong>Group</strong></td>
<td>0.171</td>
<td>0.099</td>
<td>0.047</td>
<td>0.522</td>
</tr>
<tr>
<td><strong>Time x Group</strong></td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>0.006</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*p < .001***
Figure 2.13 Composite cognitive domain performance (z-scores) in the RehaCom intervention and control group at baseline and post treatment.
3.7 Relationships between disease parameters, depression and fatigue level and neuropsychological performance in MS patients at baseline

We found significant negative weak correlations between neurological disability status (EDSS) and performance on the SRTLTS ($r = -0.387$, $p = 0.004$), BVMT-R ($r = 0.305$, $P = 0.010$) and SNST ($r = -0.312$, $p = 0.009$), and between disease duration and SRTLTS ($r = -0.286$, $p = 0.012$). We further established a negative relatively large correlation between disability status (EDSS) and performance on the SDMT ($r = -0.622$, $P = 0.011$). Depression and fatigue did not correlate significantly with any of the variables. No other significant correlations were noted between any of the variables.

3.8 rCBF SPECT image analysis using NeuroGam

The distribution of rCBF in our 31 RRMS patients showed significant differences when compared to an age- and gender-adjusted normative database (NeuroGam software). This software was applied on the reconstructed data for the semiquantitative evaluation of brain perfusion in lobar and predefined Brodmann areas of both hemispheres, as described previously. The criterion for impairment is set at 2 SD below the normative mean perfusion rate.

More specifically, regarding left and right hemisphere lobar perfusion, we found the highest impaired blood flow rates in the right insula of Reil 18/31 patients (58.10%), left frontal lobe 17/31 (54.80%), left insula of Reil 14/31 patients (45.20%) and left parietal lobe 12/31 patients (38.70%). A smaller percentage of patients also showed impaired blood flow in other lobar regions (see Table 2.8). Furthermore, an even larger number of patients had impaired perfusion rates in the predefined left and right Brodmann areas (BA), mainly in the prefrontal cortex. Results revealed that the highest percentage of impaired perfusion 24/31 patients (77.40%) was in the left (BA) 9 (posterior lateral prefrontal cortex) and 18/31 patients (58.10%) in the right Brodmann area 9. A significant percentage of patients 16/31 patients (51.60%) also
showed impaired perfusion in the right (BA) 10 (medial prefrontal cortex) and 14 (45.20%) in the right (BA) 12 (orbitofrontal cortex). Smaller percentages of impaired perfusion were also noted in other predefined Brodmann areas (see Table 2.8)

<table>
<thead>
<tr>
<th>Brain Regions (Lobar or Brodmann Area)</th>
<th>Frequency (Percentage) impaired</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal lobe left</td>
<td>17 (54.80)</td>
</tr>
<tr>
<td>Frontal lobe right</td>
<td>10 (32.30)</td>
</tr>
<tr>
<td>Insula of Reil left</td>
<td>14 (45.20)</td>
</tr>
<tr>
<td>Insula of Reil right</td>
<td>18 (58.10)</td>
</tr>
<tr>
<td>Parietal lobe left</td>
<td>12 (38.70)</td>
</tr>
<tr>
<td>Parietal lobe right</td>
<td>9 (29.10)</td>
</tr>
<tr>
<td>Temporal lobe left</td>
<td>10 (32.30)</td>
</tr>
<tr>
<td>Temporal lobe right</td>
<td>6 (19.40)</td>
</tr>
<tr>
<td>Occipital lobe left</td>
<td>5 (16.10)</td>
</tr>
<tr>
<td>Occipital lobe right</td>
<td>3 (9.70)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 9 left</td>
<td>24 (77.40)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 9 right</td>
<td>18 (58.10)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 10 left</td>
<td>19 (61.30)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 10 right</td>
<td>16 (51.60)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 11 left</td>
<td>8 (25.80)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 11 right</td>
<td>9 (29.00)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 12 left</td>
<td>12 (38.70)</td>
</tr>
<tr>
<td>Brodmann Area (BA) 12 right</td>
<td>14 (45.20)</td>
</tr>
</tbody>
</table>

**Notes:** Criterion for impairment was 2 standard deviations below age and gender adjusted normative data for rCBF SPECT performance (NeuroGam software; GE Medical System, Segami Corp., Columbia, MD, USA); Brodmann Area (BA) 9: Posterior lateral prefrontal cortex; Brodmann Area (BA) 10: Medial prefrontal cortex; Brodmann Area (BA) 11 and 12: Orbitofrontal cortex

**Abbreviations:** rCBFbrain SPECT: regional cerebral blood flow brain single photon emission computed tomography
In order to determine the perfusion severity level of our MS patients in both hemispheres of the cerebral lobes and predefined Brodmann areas, we introduced a perfusion grading system. The criterion for impaired perfusion was 2 SD below age and gender adjusted normative data for rCBF SPECT performance. This system graded hypoperfusion severity as follows: (0 to -1.6 SD, normal perfusion), (-1.7 to -2.0 SD, borderline hypoperfusion), (-2.1 to -2.5 SD, low hypoperfusion), (-2.6 to -3.0 SD, medium hypoperfusion), (-3.1 to -3.5 SD, high hypoperfusion). Based on this grading classification (Tables 2.9 and 2.10) provide the frequency and percentage (%) of patients according to perfusion severity rate on the lobar and BA areas respectively.

3.9 Lobar and Brodmann area perfusion severity rates of cognitively impaired and relatively cognitively impaired MS patients on rCBF brain SPECT

In order to establish the frequency and percentage (%) of MS patients based on perfusion rate levels (see grading system described previously) in the lobar and BA areas respectively, that presented with different severity of cognitive impairment, we divided the 31 patients that underwent SPECT imaging into two separate groups. The first group (n=19), cognitively impaired MS patients (CI): failed ≥ 2 cognitive tests on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean; the second group (n =12), relatively cognitively impaired MS patients (RCI): failed 1 cognitive test on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean. Based on this grading classification (Table 2.11) provides the frequency of patients according to cognitive impairment category and perfusion rate level on the lobar and BA areas respectively.
<table>
<thead>
<tr>
<th>Hypoperfusion severity</th>
<th>Left frontal lobe</th>
<th>Right frontal lobe</th>
<th>Left occipital lobe</th>
<th>Right occipital lobe</th>
<th>Left parietal lobe</th>
<th>Right parietal lobe</th>
<th>Left temporal lobe</th>
<th>Right temporal lobe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal perfusion</td>
<td>10 (32.3)</td>
<td>12 (38.7)</td>
<td>15 (48.4)</td>
<td>25 (80.6)</td>
<td>15 (48.4)</td>
<td>19 (61.3)</td>
<td>15 (48.4)</td>
<td>21 (67.7)</td>
</tr>
<tr>
<td>Borderline hypoperfusion</td>
<td>6 (19.4)</td>
<td>9 (29.0)</td>
<td>7 (22.6)</td>
<td>4 (12.9)</td>
<td>4 (12.9)</td>
<td>4 (12.9)</td>
<td>7 (22.6)</td>
<td>4 (12.9)</td>
</tr>
<tr>
<td>Low hypoperfusion</td>
<td>7 (22.6)</td>
<td>3 (9.7)</td>
<td>4 (12.9)</td>
<td>-</td>
<td>7 (22.6)</td>
<td>2 (6.5)</td>
<td>5 (16.1)</td>
<td>4 (12.9)</td>
</tr>
<tr>
<td>Medium hypoperfusion</td>
<td>5 (16.1)</td>
<td>4 (12.9)</td>
<td>3 (9.7)</td>
<td>1 (3.2)</td>
<td>1 (3.2)</td>
<td>2 (6.5)</td>
<td>2 (6.5)</td>
<td>1 (3.2)</td>
</tr>
<tr>
<td>High hypoperfusion</td>
<td>3 (9.7)</td>
<td>3 (9.7)</td>
<td>2 (6.5)</td>
<td>1 (3.2)</td>
<td>4 (12.9)</td>
<td>4 (12.9)</td>
<td>2 (6.5)</td>
<td>1 (3.2)</td>
</tr>
</tbody>
</table>

**Notes:** Criterion for impaired perfusion was 2 standard deviations below age and gender adjusted normative data for rCBF SPECT performance (NeuroGam software; GE Medical System, Segami Corp., Columbia, MD, USA); *Hypoperfusion grading:* (0 to -1.6 SD, normal perfusion), (-1.7 to -2.0 SD, borderline hypoperfusion), (-2.1 to -2.5 SD, low hypoperfusion), (-2.6 to -3.0 SD, medium hypoperfusion), (-3.1 to -3.5 SD, high hypoperfusion).

**Abbreviations:** rCBF brain SPECT: regional cerebral blood flow brain single photon emission computed tomography
<table>
<thead>
<tr>
<th>Hypoperfusion severity</th>
<th>Brodmann Area (BA) 9 left</th>
<th>Brodmann Area (BA) 9 right</th>
<th>Brodmann Area (BA) 10 left</th>
<th>Brodmann Area (BA) 10 right</th>
<th>Brodmann Area (BA) 12 left</th>
<th>Brodmann Area (BA) 12 right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal perfusion</td>
<td>6 (19.4)</td>
<td>9 (29.0)</td>
<td>10 (32.3)</td>
<td>12 (36.7)</td>
<td>17 (54.8)</td>
<td>13 (41.9)</td>
</tr>
<tr>
<td>Borderline hypoperfusion</td>
<td>2 (6.5)</td>
<td>4 (12.9)</td>
<td>3 (9.7)</td>
<td>6 (19.4)</td>
<td>5 (16.1)</td>
<td>7 (22.6)</td>
</tr>
<tr>
<td>Low hypoperfusion</td>
<td>8 (25.8)</td>
<td>5 (16.1)</td>
<td>9 (29.0)</td>
<td>5 (16.1)</td>
<td>3 (9.7)</td>
<td>4 (12.9)</td>
</tr>
<tr>
<td>Medium hypoperfusion</td>
<td>7 (22.6)</td>
<td>7 (22.6)</td>
<td>5 (16.1)</td>
<td>4 (12.9)</td>
<td>1 (3.2)</td>
<td>2 (6.5)</td>
</tr>
<tr>
<td>High hypoperfusion</td>
<td>8 (25.8)</td>
<td>6 (19.4)</td>
<td>4 (12.9)</td>
<td>4 (12.9)</td>
<td>5 (16.1)</td>
<td>5 (16.1)</td>
</tr>
</tbody>
</table>

**Notes:** Criterion for impaired perfusion was 2 standard deviations below age and gender adjusted normative data for rCBF SPECT performance (NeuroGam software; GE Medical System, Segami Corp., Columbia, MD, USA); *Hypoperfusion grading:* (0 to -1.6 SD, normal perfusion), (-1.7 to -2.0 SD, borderline hypoperfusion), (-2.1 to -2.5 SD, low hypoperfusion), (-2.6 to -3.0 SD, medium hypoperfusion), (-3.1 to -3.5 SD, high hypoperfusion).

**Abbreviations:** rCBF brain SPECT: regional cerebral blood flow brain single photon emission computed tomography
Table 2.11 Lobar and Brodmann area hypoperfusion severity level rates of cognitively impaired and relatively cognitive impaired MS patients on rCBF brain SPECT

<table>
<thead>
<tr>
<th>Hypoperfusion severity</th>
<th>Cognitively impaired MS patients (CI) (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frontal Lobe L / R</td>
</tr>
<tr>
<td>Normal perfusion</td>
<td>15.8 / 26.3</td>
</tr>
<tr>
<td>Borderline hypoperfusion</td>
<td>31.6 / 26.3</td>
</tr>
<tr>
<td>Low hypoperfusion</td>
<td>15.8 / 21.1</td>
</tr>
<tr>
<td>Medium hypoperfusion</td>
<td>21.1 / 15.8</td>
</tr>
</tbody>
</table>
Relatively cognitively impaired MS patients (RCI) (n=12)

<table>
<thead>
<tr>
<th>Lobe</th>
<th>Normal perfusion</th>
<th>Borderline hypoperfusion</th>
<th>Low hypoperfusion</th>
<th>Medium hypoperfusion</th>
<th>High hypoperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal Lobe</td>
<td>75.0 / 83.3</td>
<td>25.0 / 16.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Occipital Lobe</td>
<td>91.7 / 100.0</td>
<td>8.3 / -</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Parietal Lobe</td>
<td>91.7 / 83.3</td>
<td>8.3 / 16.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Temporal Lobe</td>
<td>91.7 / 41.7</td>
<td>8.3 / 16.7</td>
<td>- / 25.0</td>
<td>16.7 / 25.0</td>
<td>16.7 / 8.3</td>
</tr>
<tr>
<td>BA 9 L / R</td>
<td>58.3 / 50.0</td>
<td>8.3 / -</td>
<td>16.7 / 25.0</td>
<td>8.3 / -</td>
<td>8.3 / 8.3</td>
</tr>
<tr>
<td>BA 10 L / R</td>
<td>66.7 / 56.3</td>
<td>16.7 / 33.3</td>
<td>8.3 / -</td>
<td>8.3 / -</td>
<td>- / 8.3</td>
</tr>
<tr>
<td>BA 12 L / R</td>
<td>66.7 / 41.9</td>
<td>25.0 / 22.6</td>
<td>25.0 / 22.6</td>
<td>- / 6.5</td>
<td>- / 16.1</td>
</tr>
</tbody>
</table>

Notes: Cognitively impaired MS patients (CI): failed ≥ 2 cognitive tests on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean; Relatively cognitively impaired MS patients (RCI): failed 1 cognitive test on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean. Perfusion rates are expressed in percentages (%). Criterion for impaired perfusion was 2 standard deviations below age and gender adjusted normative data for rCBF SPECT performance (NeuroGam software; GE Medical System, Segami Corp., Columbia, MD, USA); Hypoperfusion grading: (0 to -1.6 SD, normal perfusion), (-1.7 to -2.0 SD, borderline hypoperfusion), (-2.1 to -2.5 SD, low hypoperfusion), (-2.6 to -3.0 SD, medium hypoperfusion), (-3.1 to -3.5 SD, high hypoperfusion).

Abbreviations: rCBF brain SPECT: regional cerebral blood flow brain single photon emission computed tomography; BA: Brodmann Area; L: Left; R: right hemispheres
3.10 Comparison of cortical area perfusion between cognitively impaired and relatively cognitively impaired MS patients

In order to determine cortical area perfusion differences between cognitively impaired (n=19) and relatively cognitively impaired (n=12), MS patients in both hemispheres, we utilized the Mann Whitney U non-parametric test for non-normally distributed data. We found significant rCBF brain perfusion rate differences between the two groups on the left frontal lobe \( z = -3.653, p < 0.001 \), right frontal lobe \( z = -3.499, p < 0.001 \), left occipital lobe \( z = -3.212, p < 0.001 \), left parietal lobe \( z = -4.170, p < 0.001 \), right parietal lobe \( z = -2.804, p = .005 \), left temporal lobe \( z = -3.215, p < 0.001 \), right temporal lobe \( z = -2.327, p = .020 \), left (BA) 9 \( z = -3.416, p < 0.001 \), right (BA) 9 \( z = -2.890, p = .004 \), left (BA) 10 \( z = -2.348, p = .019 \), right (BA) 10 \( z = -2.632, p = .008 \), and right (BA) 12 \( z = -2.658, p = .008 \). In all significant cases, the cognitively impaired group demonstrated higher mean hypoperfusion rates compared to the relatively cognitively impaired group. Moreover, in most cases (with the exception of the occipital lobes) the mean perfusion rate of the cognitively impaired patients exceeded the criterion set for impaired perfusion (i.e. 2 standard deviations below age and gender adjusted normative data for rCBF SPECT performance) (see Table 2.12)
Table 2.12 Comparison of cortical area perfusion between cognitively impaired and relatively cognitively impaired MS patients: Mean values (± standard deviation)

<table>
<thead>
<tr>
<th></th>
<th>Frontal Lobe</th>
<th>Occipital Lobe</th>
<th>Parietal Lobe</th>
<th>Temporal Lobe</th>
<th>Brodmann (BA) Area 9</th>
<th>Brodmann (BA) Area 10</th>
<th>Brodmann (BA) Area 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitively impaired MS</td>
<td>-2.41 ± 0.67</td>
<td>-2.17 ± 1.03</td>
<td>-1.15 ± 0.79</td>
<td>-2.14 ± 0.78</td>
<td>-2.06 ± 0.95</td>
<td>-2.96 ± 0.77</td>
<td>-2.43 ± 1.2</td>
</tr>
<tr>
<td>patients (CI) (n=19)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatively cognitively</td>
<td>-1.40 ± 0.54</td>
<td>-1.14 ± 0.57</td>
<td>-0.26 ± 0.34</td>
<td>-0.95 ± 0.65</td>
<td>-0.84 ± 0.53</td>
<td>-1.09 ± 0.56</td>
<td>-1.67 ± 0.61</td>
</tr>
<tr>
<td>impaired MS patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(RCI) (n=12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p value</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .005</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Notes: p < .001***; p < .01**; p < .05*; Cognitively impaired MS patients (CI): failed ≥ 2 cognitive tests on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean; Relatively cognitively impaired MS patients (RCI): failed 1 cognitive test on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean. Criterion for impaired perfusion was 2 standard deviations below age and gender adjusted normative data for rCBF SPECT performance (NeuroGam software; GE Medical System, Segami Corp., Columbia, MD, USA); Mann Whitney U non-parametric test used for comparisons between cognitively impaired and relatively cognitively impaired MS patients.

Abbreviations; L: Left; R: right hemispheres
3.11 Relationships between neuropsychological and rCBF brain SPECT variables at baseline

Relationships (intercorrelations) between neuropsychological and rCBF brain SPECT variables at baseline assessment were computed with the non-parametric Kendall’s Tau-b test. We found significant moderate correlations between performance on a measure of phonemic verbal fluency (VFT-phonemic) and hypoperfusion rate in the left (BA) 9 region (posterior lateral prefrontal cortex) ($r = 0.515, p = 0.012$); and left temporal hypoperfusion rate and performance on a measure of verbal memory SRTLTS (total number of words recalled) ($r = 0.630, p < .001$).

Moreover, we noted statistically significant strong correlations between a measure of executive function (response inhibition) SNST and hypoperfusion in the left frontal lobe ($r = 0.720, p < .001$), and another measure of executive functioning (set shifting) and hypoperfusion in the left frontal lobe ($r = 0.712, p = 0.004$). No other significant correlations were noted between any of the variables.

3.12 Examples of three cases depicting z-score perfusion maps and associated neuropsychological performance

3.12.1 Case A

Diagnosed with RRMS female patient, 46 years old, with 12 years of education, EDSS disability score = 4.5, with 15-year duration of illness, presently on Gilenya, and not employed. Presents with diffuse hypoperfusion and failed $\geq 2$ cognitive tests on the administered neuropsychological battery at baseline with performance $\leq 1.5$ SD below normative data mean.

Based on demographically corrected normative data the patient was impaired on the SDMT (processing speed/working memory); SRTLTS (episodic memory: total recall); SNST (executive function: response inhibition). The z-score perfusion map depicted diffuse hypoperfusion in both hemispheres with predominantly frontal and parietal lobe impairment (figure 2.14)
Figure 2.14 Case A: z-score perfusion map
3.12.2 Case B

Diagnosed with RRMS female patient, 28 years old, has 14 years of education, EDSS disability score = 3.5, 12-year duration of illness, presently on Gilenya, is employed part time. Presents with reduced regions of hypoperfusion and failed 1 cognitive test on the administered neuropsychological battery at baseline with performance ≤ 1.5 SD below normative data mean. Based on demographically corrected normative data the patient was impaired only on the SDMT (processing speed/working memory). The z-score perfusion map depicted reduced regions of hypoperfusion with predominantly left temporal and parietal regions (figure 2.15)

![Figure 2.15 Case B: z-score perfusion map](image)

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3.12.3 Case C

Diagnosed with RRMS female patient, 41 years old, 12 years of education, EDSS disability score = 2.0, with 10-year duration of illness, presently on interferon b-1a (Avonex), is employed. Presents with very few regions of hypoperfusion. This patient did not have impaired performance on any cognitive test on the administered neuropsychological battery at baseline i.e. performance ≤ 1.5 SD below normative data mean. The z-score perfusion map depicted reduced regions of mainly borderline and low hypoperfusion (figure 2.16).

![Figure 2.16 Case C: z-score perfusion map](image)

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4. Discussion

One of the most disabling characteristics of MS is the cognitive deterioration that patients manifest, even at the early stages of the disease, with prevalence rates ranging from 45-70% (Chiaravalloti & DeLuca, 2008). Despite the rarity of overt cognitive dysfunction, several cognitive domains are impaired varying considerably from mild to moderate severity (Lovera & Konver, 2012). The impact of cognitive dysfunction in this population is significant with negative consequences on activities of daily living, independence, loss of employment (Papathanasiou et al. 2014), and ultimately quality of life (Putzki et al. 2009). Moreover, past and current pharmacological treatments have shown inconsistent findings in alleviating cognitive impairment in MS patients requiring further clarification (Roy et al. 2016). This inconsistency regarding the effects of pharmacological interventions on cognition, coupled with the reduced ability to effectively handle everyday tasks, loss of employment and social interaction capacity, prioritizes the need for utilizing potentially more effective non-pharmacological interventions to adequately address cognitive dysfunction and everyday functioning abilities.

As the effects of immunomodulatory treatments on cognition in MS are not completely clarified, more recent neurobehavioural interventions that have managed to overcome some of the initial methodological biases and limitations have produced favorable outcomes (see Chiaravalloti et al. 2013. Mitolo et al. 2015). While there is evidence to support neurobehavioural cognitive rehabilitation interventions in the MS population, a recent Cochrane review (Rosti-Otajarvi, & Hamalainen, 2014), reported low-level evidence for the efficacy of neuropsychological rehabilitation in this population, but noted that methodological heterogeneity contributed significantly to their findings. Despite this limitation noted by the Cochrane review, cognitive rehabilitation appears to be useful for most patients with MS regardless of disease course and level of cognitive impairment, and new rigorous studies are needed to clarify its status in treating cognition in this population.
With this aspect in mind and based on previous research, we hypothesized that a computer assisted (RehaCom Cognitive Therapy Software. https://www.rehacom.co.uk), individual based, functional restorative intervention of 10-weeks duration (twice weekly for 60 minutes), aiming to restore the most frequent cognitive domains affected in MS (episodic memory, processing speed /attention, and executive functions), would yield significant benefits in the cognitive functioning abilities of these patients. We therefore selected therapeutic modules from the RehaCom software that focus on these specific cognitive domains and have been validated in numerous populations with cognitive dysfunction (see for e.g. Fernandez, Bringas, Salazar, Rodriguez & Garcia, 2012, for the software’s efficacy on acquired brain injury), patients with schizophrenia (Mak et al. 2013) and MS patients (Mattioli et al. 2010. Mattioli et al. 2012. Mattioli et al. 2015. Bonavita et al. 2015). In order to test our hypotheses, we conducted a multicenter randomized controlled trial that has been described in detail previously.

Our results revealed that the two groups (IG; intervention and CG; control) were well matched at baseline assessment on demographic and clinical characteristics that could negatively impact the outcome measures and bias the study results. Specifically, patients age, level of education, present and premorbid intellectual capacity, general cognitive status, disease course (all were RRMS patients), depression and fatigue levels, and disability level were well balanced before patients entered the rehabilitation process (IG) or continued with their standard treatment (CG). Regarding, the gender of the participants, there was a higher proportion of females compared to males in both groups, something that was expected as a consequence of the general higher female to male ratio in the MS population. However, the ratio of females between the two groups was well matched. Moreover, patients’ medical therapeutic scheme was well balanced between the two groups. As regards the pretreatment cognitive performance of the two groups, we found that patients randomized to the intervention
procedure had lower SRTDR scores, i.e. they marginally retrieved less words after a 20-minute delay period (a mean 6.09 words versus 7.15 of the control group), and took longer to complete the TMT part A. With the exception of these two measures patients were well matched on all other cognitive domains. The multicenter design was also an asset providing a more representative sample of patients from North and South-western Greece, and permitting the replication of the identical rehabilitative procedure in two different centers by well trained in the procedure clinicians.

After receiving 10-weeks of rehabilitation, and within one week after completing the intervention, both groups were assessed. We found that MS patients who engaged in the individualized functional cognitive training showed significant improvement in cognitive performance on verbal episodic memory (total recall of words) with a large effect, retrieval of acquired words, visuospatial memory, semantic fluency, processing speed / working memory, and response inhibition ability, with a medium effect. Visual attention/visuomotor scanning speed and set shifting ability also improved with a large effect. On the contrary, phonemic fluency showed a trend towards improvement, as phonemic output increased, without however, reaching statistically significant levels. After completing the cognitive intervention, these patients continued their usual treatment regimen and were tested again 6-months later. On this follow-up assessment, results revealed a significant reduction on semantic verbal fluency (VFT) and processing speed (SDMT) performance. All other measures remained relatively stable over the 6-month period following treatment and no significant differences were observed. Interesting performance differences were also noted between pretreatment and follow-up evaluations. Specifically, treated patients scored better on follow-up in episodic verbal and visual memory measures, divided attention / visuomotor scanning speed with medium effect sizes, and set -shifting /mental flexibility with a medium effect.
The positive pre-to-post treatment effects obtained by the RehaCom group, were not observed in the standard treatment control group, as most measures remained relatively stable over the 10-week period. An exception to this was the semantic fluency output performance that decreased to a statistically significant level and the completion time of TMT part A that significantly increased.

When we compared derived composite cognitive domain scores in the intervention group, our results revealed a significant decrease in processing speed ability from pre-to post treatment, which was not retained at follow up, but also did not drop to the low pretreatment levels of processing speed capacity. Verbal fluency generation improved significantly from before to after treatment, but this gain was not retained to follow up. The treatment procedure also produced positive changes in the attention domain, with improved performance been evident at post treatment, and remaining relatively stable over time for six months. Verbal episodic memory domain, which reflects total word learning ability and retrieval of these words after 20 minutes, increased after treatment, but decreased marginally in the following 6 months without dropping to pretreatment levels. When cognitive domain performance between the RehaCom intervention group and standard treatment control group was compared over time, a significant time x group interaction effect on all four domains was noted, with the intervention group outperforming the control group on all derived domains (processing speed, attention, verbal episodic memory and verbal fluency).

Taken together, these results support our efficacy hypothesis regarding the benefits derived from the specific 10-week intervention on neuropsychological measures in the related trained cognitive domains relative to control group participants who received only standard clinical care. Furthermore, our data revealed that the positive training effects were retained for the attention domain over six months, providing evidence on the long-term benefits of such interventions for attentional capacity. Although for the remaining three derived domains, the
positive effects were not retained at six months, the cognitive performance of treated patients did not drop to the levels recorded before the intervention.

Regarding our hypothesis that control participants who receive standard treatment over the 10-week intervention duration, will show either further cognitive decline or remain cognitively stable, we found that performance remained relatively stable over this short duration in most measures, possibly implying that the period of the intervention may be inadequate to produce significant cognitive changes in these patients. An exception to this, was the score on the semantic fluency task that deteriorated significantly and the time required to complete Trails A, that increased. These two results indicate a possible trend towards further cognitive decline, suggesting that patients on standard available immunomodulatory treatments, are possibly not sufficiently protected against ongoing cognitive decline, although other confounding factors such as depression severity or fatigue changes during this period may have also contributed to these findings. However, as our groups were well matched on possible demographic and clinical confounding variables, these findings are less likely to have been biased by such factors.

The positive findings regarding the amelioration of attention, processing speed and executive function reported in the present study, are in concordance with several other studies that have utilized the RehaCom software in cognitively impaired patients with multiple sclerosis. More specifically, Mattioli et al. (2010), reported the effectiveness of a 3-month intensive neuropsychological rehabilitation intervention with the assistance of the RehaCom software on attention, information processing and executive functions. As in our study, their patients had a relapsing-remitting course and relatively low mean disability levels (EDSS scores ≤ 4), and their two groups were well matched at baseline on possible confounding demographic and clinical variables. The similar findings between the two studies, especially as regards the cognitive domains that improved and the duration and frequency of the rehabilitation sessions,
combined with the use of many similar modules from the computer software, replicates these positive findings, strengthening the notion that domain specific functional training may benefit patients with RRMS of a relatively low disability status.

The same group, Mattioli et al. (2012), reported that the cognitive benefits experienced by their MS patients after 3-months of intensive neuropsychological rehabilitation with the assistance of the RehaCom software, persisted for 9-months after the rehabilitation onset and also generalized to an amelioration of depression and quality of life. In our study, similar long-term benefits of a shorter, however, duration (6-months), were noted in attentional capacity. Although for the other cognitive domains in our study, the positively changed performances failed to remain stable for the six-month period, performance of treated patients did not drop to the levels noted prior to the cognitive intervention, implying that the intervention actually contributed positively to their cognitive status, and possibly protected them from further cognitive decline, as expected for this disorder.

In addition to their cognitive and behavioral outcome variables, the authors of the previously mentioned studies provide functional MRI data, suggesting that possible neural correlates of the functional cognitive intervention are training induced activations of the prefrontal and cingulate cortices, brain structures known to be involved in attention and executive functions. The persistence of cognitive gains over the 9-month period they believe may be related to persistent brain plasticity mechanisms (Matioli et al. 2010b).

A structural and functional imaging study by Fillipii et al. (2012), that evaluated brain changes and neuropsychological functions in RRMS patients after undergoing computer assisted cognitive rehabilitation of 12 weeks duration, utilizing the RehaCom software, with an emphasis on attention, information processing, and executive functions, reported similar positive pre-to post treatment outcomes on the neuropsychological variables to our study. The two studies were closely balanced on disease duration and disability status of the RRMS
patients studied, and furthermore, both studies utilized a similar therapeutic module from the RehaCom software to train attention (i.e. the divided attention module). In addition to providing evidence regarding the efficacy of cognitive training with this software on neuropsychological measures, Fillipi et al. (2012), recorded modifications in the activity of the posterior cingulate cortex (PCC) and dorso-lateral prefrontal cortex (DLPC) during the Stroop task, as well as modifications of the activity of the anterior cingulum (AC) and PCC at rest, in the RehaCom treated group. This study showed that functional cognitive training has the potential to modify the activity of trained neuronal system areas in patients with RRMS, and due to its plasticity mechanisms, may recruit additional regions to compensate for cognitively demanding tasks.

More recently, Parisi et al. (2014), utilizing a similar intervention protocol as the one in the present study, provided evidence that domain specific training of attention, information processing and executive functions for 12 weeks (three days per week), with the assistance of the RehaCom software, produces increased functional connectivity in the anterior cingulate cortex of treated patients, while the control patients showed decreased activation.

In a similar intervention design to the present study, but with a shorter treatment duration (6 weeks/ twice weekly), of computer assisted cognitive rehabilitation with the RehaCom software (Cerasa et al. 2013), that placed an emphasis on training of attention, the treated group showed an improvement in attention capacity, and this positive change in performance was associated with increased activity in the posterior cerebellar and superior parietal lobule.

Other studies utilizing the RehaCom software have placed emphasis on verbal or visual learning and memory with relatively improved pre -to- post treatment performance differences, similar to the findings noted in our study. In one such study, Solari et al. (2004), utilizing computer – aided (RehaCom modules) of memory and attention, in a randomized, double blind controlled trial, noted an improvement in 45% of the studied patients receiving
treatment, on a word list generation task. Ernst et al. (2012), utilizing the RehaCom software, provided computer-assisted cognitive training for 6 weeks (once weekly), and reported significant improvements of autobiographical memory, that were associated with increased cerebral activity in posterior cerebral regions. More recently, Bonavita et al. (2015), utilizing the RehaCom package, and more specifically, similar training modules to the ones utilized in the present study (i.e. attention and concentration, divided attention, logical thinking and verbal memory) found improved cognitive performance after the training on visual and verbal memory and processing speed. These improvements in cognition were associated with increased functional connectivity in the posterior cingulate cortex (PCC) and inferior parietal cortex (IPC) of the default mode network (DMN), implying training induced adaptive cortical reorganization in the DMN. This network is considered highly relevant for human cognition under physiological conditions, and is the most consistent and commonly reported resting-state network in functional MRI studies of functional connectivity (FC) (Esposito et al. 2006).

Most of the studies mentioned previously had no or relatively short follow up periods ranging from 3 to 9 months after completion of the training intervention. The long-term persistence of a 15-week domain specific cognitive training intervention with the RehaCom was reported in a recent two year follow up study (Mattioli et al. 2016). The authors report that patients treated with specific cognitive modules aimed at ameliorating the related cognitive domains, showed significantly less impaired tests both at one and two year follow up assessments relative to the aspecific group (that received generic psychological intervention). These results further strengthen the available evidence regarding the long-term benefits of relatively short duration (15 weeks in this case) domain specific functional restorative training with the previously mentioned software program.
Other functional neuroimaging studies have revealed changes in brain activation on task-based functional magnetic resonance imaging (fMRI), change in functional connectivity and, for one study, microstructural changes by diffusion tensor imaging after cognitive rehabilitation (Chiaravalloti, Genova, and DeLuca, 2015. De Giglio, Tona, & De Luca, et al. 2016. De Giglio, Upadhyay, & De Luca, et al. 2016). These results suggest that restorative or functional training could modify brain functioning and improve network efficiency. The characteristics of change in brain activation and connectivity observed after cognitive rehabilitation interventions (homologous region adaptation, local activation expansion and extra-region recruitment) and the observed association with neuropsychological improvement suggest that adaptive neuroplasticity may occur after restorative training (Brochet, 2017).

To summarize, it is apparent from neuropsychological and functional neuroimaging studies, that individuals with multiple sclerosis presenting with mild to moderate cognitive deterioration, are able to profit from even fairly brief, but domain specific cognitive training, and the RehaCom software provides assistance in this respect. In our study, verbal episodic memory, visual attention/visuomotor scanning and processing speed were the functions that showed the largest pre-to-post treatment effect on test performance. The specific cognitive training in which the intervention group participated consisted of direct functional training of cognitive areas considered the most frequently and severely affected in MS. Moreover, the software modules included in the intervention are considered to have high ecological validity, implying that they simulate many real everyday life conditions, such as shopping in a supermarket, or simulating the driver of a train. Several of the modules utilized time restricted training tasks that also involved a working memory component, which assisted in improving processing capacity and processing speed, considered one of the most prevalent and difficult to treat cognitive functions in MS.
Although no formal post treatment or follow up questionnaire was used as an outcome measure to determine the personal benefit of each patient gained from the intervention, we informally asked treated patients to provide feedback regarding the intervention on four verbal questions at post treatment assessment; The questions were: (i) how much have you personally benefited from this type of treatment, (ii) have your cognitive difficulties improved after the program (iii) has this program helped you to improve your everyday life activities (for e.g. can you now remember more items of a shopping list without writing down the list, or do you now need less time to complete mental tasks or plan a trip) (iv) would you recommend this intervention to other MS patients. Patients had to rate their response on a Likert type scale from 1-5, where 1 was indicative of no benefit at all, and 5 large benefit. The intervention was rated very positively from the majority of treated patients, who stated that they had derived above average benefit and were objectively feeling more confident about their cognitive abilities. Most patients made special reference to their improved concentration and memory capacity and reduced forgetting rate. They generally felt more confident in performing everyday functional tasks and noted appreciable speed ameliorations in performing tasks that require more rapid actions. As the program was very well received from most patients (this is also evident from the fact that there were no drop outs in the treatment program), they said that they would gladly recommend it to other MS patients (which is what many patients actually did by recommending it to patient members of their local MS society).

Another significant component of the present study was the evaluation with brain perfusion SPECT at the pretreatment stage, in thirty-one of our RRMS patients, irrespective of whether they eventually received cognitive training or usual clinical care. We investigated the association between perfusion rates and cognitive dysfunction in this population, as the literature in this regard is scarce and indefinite. Moreover, SPECT is widely available, time efficient (completed in about an hour), and cost effective.
Our main hypotheses, regarding this evaluation procedure was that cognitively deteriorated MS patients would show greater hypoperfusion rates in the SPECT study in several predefined Brodmann areas and lobes of the brain relative to demographically matched healthy controls, according to an established normative database. Furthermore, we examined the regional distribution of $^{99m}$TC-HMPAO in several predefined Brodmann areas and brain lobes of these patients, and the relationships to neuropsychological performance. We also conducted a perfusion severity rate comparative analysis between RRMS patients with more severe cognitive decline (impaired on 2 or more cognitive domains according to established norms), and those with less severe cognitive decline (impaired on 1 cognitive domain according to established norms).

Our results showed that the distribution of rCBF in our RRMS patients was significantly more impaired in both hemispheres and widely spread over various regions, when compared to the NeuroGam demographically matched normative database. The highest hypoperfusion rates in the RRMS sample were recorded in the right insula of Reil, 18 patients (58.10%), left frontal lobe 17 patients (54.80%), and left insula of Reil 14 patients (45.20%). A smaller, but substantial percentage of patients also showed impaired blood flow in other lobar regions. Furthermore, even more patients had impaired perfusion rates in the predefined left and right Brodmann areas (BA), mainly in the prefrontal cortex. The highest hypoperfusion rates were noted in the left (BA) 9 (posterior lateral prefrontal cortex) 24 patients (77.40%), and in the right (BA) 9, 18 patients (58.10%). A substantial number of patients also recorded decreased rCBf in the right (BA) 10 (medial prefrontal cortex), 16 patients (51.60%), and 14 patients (45.20%) in the right (BA) 12 (orbitofrontal cortex). Other predefined Brodmann areas demonstrated smaller percentages of impaired perfusion rates.

Our findings corroborate previous reports by Pozzilli et al. (1991), and Lyce et al. (1993), who found predominantly frontal hypoperfusion. Moreover, they are in keeping with the
findings of both previously mentioned studies, and the Horiuchi & Mitsuo, (1999), SPECT study, that reported greater left hemisphere hypoperfusion in their MS patient. Our study however, extends previous findings in the MS population, by providing data regarding the severity of hypoperfusion in the lobar and BA regions. In terms of perfusion rate severity in the four lobes bilaterally, based on the grading system that we introduced, we found that between 3.2 – 12.9 % of our RRMS patients had high hypoperfusion severity (-3.1 to -3.5 SD below age- and gender -adjusted controls). The most frequently recorded high hypoperfusion rate was in the left and right parietal lobes (12.9%). Medium hypoperfusion severity (-2.6 to -3.0 SD), was recorded in between 3.2 – 16.1 % of patients, with the most frequent medium severity rate noted in the left frontal lobe (16.1%). Low hypoperfusion severity (-2.1 to -2.5 SD), was recorded in between 6.5 – 22.6% of RRMS individuals, with the most frequent low rates found in the left frontal lobe (22.6%). Borderline hypoperfusion (-1.7 to -2.1 SD), was revealed in between 12.9 – 29.0 % of the RRMS sample, with most frequent borderline cases found in the right frontal lobe.

Similarly, perfusion rate severity in the predefined (BA) areas in both hemispheres showed that between 12.9 – 25.8 % of our RRMS patients had high hypoperfusion severity. The most frequently recorded high hypoperfusion severity rate was in the right (BA) 9 area (posterior lateral prefrontal cortex) (25.6 % of our patients). Medium hypoperfusion severity was recorded in between 3.2 – 22.6 % of our patients, with the most frequent medium rates noted in both the left and right (BA) 9 area (posterior lateral prefrontal cortex) (22.6%). Low hypoperfusion severity rates were recorded in between 9.7 – 29.0% of the RRMS individuals, with the most frequent low rates found in the left (BA) 10 area (medial prefrontal cortex) (29.0%). Borderline hypoperfusion was noted in between 6.5 – 22.6 % of the RRMS sample, with most frequent borderline cases found in the right (BA) 12 region (orbitofrontal cortex) (22.6%).
Another novelty in the present study, is the establishment of a different pattern of cortical area hypoperfusion severity, between cognitively impaired and relatively cognitively impaired MS patients bilaterally. More specifically, our cognitively impaired patients recorded the highest borderline hypoperfusion severity bilaterally in the frontal lobes, highest medium severity in the left posterior lateral prefrontal cortex BA (9), most frequent low severity in the right occipital lobe, and largest high hypoperfusion severity rate in the right temporal lobe (42% of cases). On the contrary, less severely cognitively impaired patients, recorded the highest borderline hypoperfusion rates in the right medial prefrontal cortex BA (10) area, most frequent low severity in the right temporal lobe, highest medium severity in the right temporal lobe, and most frequent high hypoperfusion rates in the orbitofrontal cortex (only 16%). These results are indicative of qualitative (different cortical brain pattern of hypoperfusion) and quantitative (more frequent medium and high hypoperfusion rates in the severely cognitively impaired MS individuals) hypoperfusion differences between the two groups.

Equally interesting are the widespread brain perfusion differences noted between the two cognitively deteriorated groups in the left and right hemispheres. The more severely deteriorated individuals had significantly higher blood flow impairments on both the right and left hemispheres in most of the studied regions, with the exception of the right occipital lobe and the left (BA) 12 orbitofrontal region. Furthermore, in most cases (except occipital lobes), the mean perfusion rate of patients with more severe cognitive decline exceeded the criterion set for impaired rCBF.

Another significant contribution of the study is the important associations that we established between results of perfusion decrements and neuropsychological performance. In this respect, a moderate association was noted between performance on a language expression – verbal fluency task and level of impaired regional cerebral blood flow in the left posterior lateral prefrontal cortex. Moreover, we found a moderate association between
performance on a measure of episodic verbal learning—memory and severity of hypoperfusion in the left temporal lobe. This finding was not completely unexpected as the involvement of the temporal lobe and especially the left medial temporal lobe in episodic memory—that is, the ability to acquire explicit information—is well known. We therefore assume, that the deficits noted on this episodic memory test may have elucidated the relative hypoperfusion recorded in the SPECT study. These findings are in accordance with the Pozzilli et al. (1991) report, which noted an association between $^{99m}$Tc HMPAO uptake in the left temporal lobe and impaired verbal memory and verbal fluency. Reduced blood flow in the left temporal and frontal lobes may be a reflexion of cortical deactivation, secondary to disconnection from subcortical structures. Such evidence of disconnection from documented subcortical MS lesions has been already reported (Hersovitch, Trotter, Leman et al. 1984). Other studies have speculated that common periventricular MS lesions disrupt white matter fiber tracts, which interconnect prefrontal limbic system structures, which may result in memory and executive or “frontal” impairments in this population (Hofman et al. 2007).

Strong associations were also recorded between two measures of executive functions, the SNST, which mainly assesses response inhibition, and the Trails B which loads mainly on set-shifting ability, and severity of hypoperfusion in the left frontal lobes respectively. The strong associations recorded between performance on the executive function measures and impaired blood flow rates in the dominant for language left hemisphere and particularly the left frontal lobe, are not surprising, as both Trails B (contains alphabetic and number sequences) and SNST (verbally reading names of colours) have a heavy language load, that was most likely deteriorated in our MS patients. Although findings such as the ones noted previously may be intriguing, we do need to keep in mind that associations established with correlational methods, do not necessarily imply causal relationships.
In total, the findings of our SPECT study indicate the presence of widespread blood flow reduction, mainly in the frontal lobes and other related prefrontal areas, involving both hemispheres, but with a slight asymmetric left hemisphere predominance. Furthermore, we found associations between performance on domain specific cognitive measures of verbal episodic memory, executive function measures with a strong language loading, and left frontal and temporal lobe rCBF brain SPECT deficits. Moreover, MS patients with more severe cognitive decrements recorded different hypoperfusion patterns and perfusion severity rates relative to patients with less severe cognitive decline. We therefore conclude, that brain perfusion SPECT was able to highly detect decreased cerebral perfusion in our RRMS patients with mild to moderate disability status, and because of its accessibility, relatively low cost, practical ease and provision of objective quantitative information, it may be utilized to complement other diagnostic modalities such as neuropsychological assessment in surveillance and monitoring of cognitive decline in this population.

Although our study has several strengths, including its multicenter randomized controlled design, the well matched baseline clinical, demographic and cognitive characteristics of the two groups, the strict inclusion criteria, absence of comorbid conditions that may have biased the study outcome measures, the ecologically valid treatment intervention modules that were utilized from the RehaCom software, the non-invasive nature of the intervention, and the additional utilization of rCBF brain perfusion SPECT evaluations that included novel hypoperfusion severity analyses, it also bears several limitations. Firstly, the present study was not blinded. Secondly, the control group received only standard clinical care, whereas a placebo intervention applied to this group might have restricted the differentiation of the positive cognitive effects, due to the cognitive rehabilitation intervention. Thirdly, patients receiving the intervention were offered increased attention, clinical care and individualized contact on a frequent basis (twice weekly, for 10 weeks), which may have contributed to the treated patients’
general well-being, and possibly influenced the positive cognitive outcomes in this group. Fourthly, not all patients that took part in the study consented to being evaluated with brain perfusion SPECT, mostly fearing the potential negative effects of the radioactive tracer, therefore, significantly reducing the SPECT study sample to 31 participants. It is worth mentioning, however, that in the very few SPECT studies conducted in MS patients so far, the participants included in those studies were much less than the participants included in the present study. Moreover, when the 31 RRMS patients were allocated to two groups, based on the level of cognitive impairment, and compared in terms of perfusion severity rate, the results were derived from two smaller sub-cohorts of patients, that need to be replicated in larger samples. Furthermore, brain SPECT images may be blurred and the method has limited spatial resolution, potentially limiting its clinical value in some cases. These results are limited to RRMS patients and should not be generalized to MS patients with other disease subtypes.

Finally, we did not utilize formal healthy related quality of life or activity of daily living questionnaires as primary outcome measures. However, in order to evaluate the personal benefit of each patient gained from the intervention, we informally asked treated patients to provide feedback regarding the intervention on four verbal questions at the post intervention assessment. On these four questions, patients had to rate their response on a Likert type scale from 1-5, where 1 was indicative of no benefit at all, and 5 was indicative of a large benefit.

5. Conclusions and future directions

In this multicenter, randomized controlled trial we implemented a computer assisted functional training cognitive rehabilitation intervention of 10-weeks duration (twice weekly), on cognitively impaired RRMS patients, with relatively low disability status. Our data showed, that this short period of domain specific cognitive training (attention, processing speed, executive functions and episodic memory), can be helpful in ameliorating the trained functions, and that
effectiveness persisted at 6-months follow up for the attention domain. For the other trained domains, performance did not deteriorate to pretreatment levels after 6-months, implying a possible protective long-term effect of the intervention in terms of cognitive deterioration rate. The RehaCom software appears to have sufficient flexibility, dynamics, objectivity and ecological validity, to make a useful contribution to the clinical practice of cognitive rehabilitation in the MS population. Recent explorative functional neuroimaging studies, have reported findings suggesting that cognitive rehabilitation interventions, including those that incorporated the RehaCom software, may induce an increase in the brain activation of treated patients. The contribution of these studies, however, in assessing the impact of cognitive rehabilitation in MS warrant further investigation. Well-designed studies, with clearly defined MS patient populations (for e.g., the investigation of cognitive rehabilitation efficacy in progressive MS), and utilization of different cognitive rehabilitation techniques, duration and frequency of treatment interventions, with longer follow up periods, are required in order to elucidate the functional correlates of cognitive amelioration in MS individuals, and to make further progress in this rapidly advancing field of cognitive rehabilitation in MS. Finally, the present study determined the potential utility of brain perfusion SPECT in monitoring cognitively deteriorated RRMS patients, and suggest that it may be implemented as a complementary modality to neuropsychological assessment for this purpose.
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