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Häggtoft, Walina Lydia

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**UNIVERSITY OF SPLIT
SCHOOL OF MEDICINE**

Walina Lydia Häggtoft

**SIPONIMOD TREATMENT OF PATIENTS WITH MULTIPLE SCLEROSIS
AT THE DEPARTMENT OF NEUROLOGY IN SPLIT**

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Assist. Prof. Sanda Pavelin, MD, PhD

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LIST OF ABBREVIATIONS

- ALC – absolute leukocyte count
- BBB – blood brain barrier
- CD – cluster of differentiation
- CIS – clinical isolated syndrome
- CNS – central nervous system
- DIS – dissemination in space
- DIT – dissemination in time
- EDSS – expanded disability status scale
- EBV – Epstein-Barr virus
- FDA – Food and Drug Administration
- EU – European Union
- HSV – herpes simplex virus
- MBP – myelin binding protein
- MCH – major histocompatibility complex
- MRI – magnetic resonance imaging
- MS – multiple sclerosis
- OLs – oligodendrocytes
- OPC – oligodendrocytes progenitor cell
- PLP – proteolipid protein
- PRMS – primary progressive multiple sclerosis
- PNS – peripheral nervous system
- RRMS – relapsing relapsing multiple sclerosis
- SPMS – secondary progressive multiple sclerosis
- TLR – toll-like receptor
- PMS – progressive multiple sclerosis
- VZV – varicella-zoster virus

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1. INTRODUCTION

1.1. Epidemiology and etiology of multiple sclerosis

Multiple sclerosis affects approximately 2.3 million people worldwide. Primarily affecting the brain and spinal cord, it causes neurological disability, diminishes the quality of life, imposes a financial burden on global healthcare, and ultimately reduces life expectancy (1–4). Despite the limited understanding of the cause of MS, significant progress has been made in recent years regarding its etiology and clinical course. This chronic neurological inflammatory disease is typically diagnosed during adulthood, between the ages of 20 and 30 years, predominantly in women living in geographical areas further from the equator (4). Consequently, it is unsurprising that MS is more prevalent in northern Europe and the United States (5). Epidemiological studies have suggested that a combination of genetic predisposition and environmental factors increases the susceptibility to the disease, suggesting a multifactorial component (6).

1.2. Risk factors associated with multiple sclerosis

Multiple risk factors have been identified for MS. These include various infections such as the Epstein-Barr virus, vitamin D deficiency, insufficient exposure to sunlight, cigarette smoking, obesity, and elevated estrogen levels. MS pathology is driven by an inflammatory response elicited by T-helper cells and autoreactive lymphocytes. In areas with sufficient sunlight, ultraviolet rays can enhance regulatory T-lymphocyte function by their suppression, consequently reducing the number of these specialized lymphocyte cells. Thus, the risk of MS is reduced by regulating immune processes. In addition, sunlight exposure is essential for the synthesis of vitamin D, a natural inhibitor of autoimmune mechanisms involved in MS pathogenesis (7–9).

The susceptibility to MS development depends mainly on genetic predisposition and environmental factors. Nevertheless, variations in specific genes, such as HLA-DRB1 on the MHC II HLA-DRB1-1501 allele, significantly influence MS mitigation (4,10). Results from previous studies have shown an increased MS risk in patients with this allele compared to individuals who are noncarriers. This has implications for understanding the development of MS. MHC plays an essential role in the immune system through antigen presentation for T lymphocytes and MS risk. Other genes implicated in increased MS risk also code for immune system components such as chemokines, cytokines, and immunomodulators (4,12).

1.3. Pathogenesis of multiple sclerosis

To understand the disease mechanism of MS, additional clarification is required regarding the composition, function, and involvement of myelin sheath in the immunological processes. Mainly composed of lipids, myelin acts as a protective layer around axons and plays a fundamental role in the CNS by facilitating the reception and transmission of electrical impulses. Myelin insulates neurons and protects their integrity (13,14).

1.4. Myelin sheath: composition and functions

Myelin sheaths are found in the PNS and the CNS. In the CNS, the glial cells surrounding axons are called oligodendrocytes, responsible for myelin production. Their counterparts are found in the PNP and are called Schwann cells. OLs are specialized myelinating cells that differentiate from oligodendrocyte progenitor cells and are found in various areas of the CNS. Myelin is composed of a multilamellar membrane that extends from the neuron's cytoplasm and wraps around the neuron in concentric circles. Like other cell types, OLs undergo various stages of migration, proliferation, and differentiation. Several cellular and molecular changes occur during the maturation of OL. One significant sign that marks the maturation of OLs is the expression of specific myelin proteins, such as myelin essential protein, myelin-associated glycoprotein, and proteolipid protein. Previous studies have shown the implication of myelin proteins in MS pathogenesis. It is worth mentioning that myelin is also composed of lipids that provide insulating properties to its structure and contribute to its function and stability, which are essential for the effective conduction of action potentials (10,15). In short, myelin is a membrane that acts as an electrical insulator to increase the conduction speed of impulses in the CNS. Therefore, the demyelination of neurons leads to the slowing of electrical impulses, a process evident in the pathology of MS (10,14,15,16).

1.5. Autoimmune processes in multiple sclerosis

The innate and adaptive immune systems represent two arms of the human immune system, each comprising specialized immune cells that work together to defend the body against pathogens and infections. Innate immunity is the first line of defense and offers a rapid but nonspecific response. However, it has limitations in entirely eradicating infections. Conversely, the adaptive immune system, also known as acquired immunity, orchestrates a slower response and is characterized by its accuracy and effectiveness. When the innate immune fails to eliminate invading pathogens, the adaptive immune system assumes control by employing precision to mount a targeted defense (8,17,18). MS, both arms of the immune system contribute to disease development. Inflammation is a critical component of complex immunopathology. Therefore, understanding the interplay between these two systems is essential for comprehension of the pathogenesis of MS. Although both innate and adaptive immune dysfunction contribute to disease development, dysfunction of the adaptive immune system plays a more prominent role (29).

1.6. Role of the adaptive immune system in multiple sclerosis

Cells of the acquired immune system comprise T and B lymphocytes, which make up most of the adaptive immunity cells. These cells undergo proliferation and differentiation into effector cells under the regulation of chemokines and cytokines (8,17). T-lymphocytes are produced in the bone marrow from the same progenitor as B-lymphocytes but mature in the thymus. The process of maturation and selection occurs once thymocytes reach the thymus. The expressions of cell surface receptors, such as the toll-like receptor, cluster of differentiation (CD4), and significant histocompatibility are indications of maturity (18). Following T-cell selection, naïve T lymphocytes migrate to the lymphatic system, activated through antigen presentation (18). The human immune system is characterized by its ability to differentiate between foreign and self-tissues, which allows it to mount potent defenses without harming the body. This ability is achieved via a mechanism known as tolerance. There are two types of tolerance: central tolerance, which occurs during T and B lymphocyte development in the thymus, and peripheral tolerance, which occurs in the lymphoid tissues (19).

Central tolerance involves the elimination of autoreactive lymphocytes in the thymus and comprises both positive and negative selection processes. Peripheral tolerance occurs beyond the primary lymphoid organs, such as the lymph nodes, and includes several mechanisms to prevent autoreactivity. These mechanisms include peripheral deletion, clonal anergy, and the suppression of autoreactive T lymphocytes through the action of T-regulatory lymphocytes (Tregs) (12–14). The presence of central and peripheral tolerance mechanisms in the immune system provides a safeguard against self-antigen attack. However, peripheral tolerance acts as a backup mechanism if central tolerance fails. In cases where both mechanisms fail, this can lead to the development of autoimmune diseases such as MS (19).

1.6.1. Blood-brain barrier and inflammation of multiple sclerosis Pathogenesis

The blood-brain barrier functions as a selectively permeable membrane that maintains the integrity of the brain parenchyma. Its unique structure consists of a network of blood vessels and specialized cells that regulate the passage of microorganisms, immune cells, immune mediators, and specific drugs into the CNS. The CNS is considered an immune privilege, implying a distinct and independent immune system separated from the periphery. The BBB contributes to the maintenance of CNS privilege (20).

Risk factors associated with MS serve as triggers for disease initiation and play a crucial role in its pathogenesis. It has been hypothesized that MS initiation and pathogenesis can be attributed to molecular mimicry. The theories surrounding the initiation of MS pathogenesis have been associated with molecular mimicry. There are two proposed hypotheses, both resulting in the demyelination of neuronal axons in the CNS. The main difference between these theories lies in the initiation site. The outside-in theory posits that MS pathogenesis begins from the periphery, triggered by molecular mimicry of antigens, which includes the myelin sheath protein, amongst other proteins. Viral and environmental factors have also been identified as triggers. Concisely, a breach in the tolerance mechanisms activates an immune response that causes the recruitment of effector lymphocytes in the CNS. Upon breaching the BBB, a cascade inflammatory response causes damage in myelin sheaths (21,22). The compromised BBB mediates for an interplay of immune cells, cytokines, and chemokines, ultimately leading to vasodilation and increased entry of immune mediators from the periphery. However, the inside-out hypothesis suggests that an immune response is triggered by a foreign antigen within the brain. This immune response leads to the release of chemokines and cytokines from the BBB, causing vasodilation and promoting the migration of immune cells from the periphery to the brain (9). This chain of events leads to the differentiation of activated T lymphocytes into

cytotoxic T cells, which damage OLs by releasing cytotoxic substances. In summary, the initiation and pathogenesis of MS are complex processes involving the interplay of various factors. Whether it is an outside-in or inside-out hypothesis, both mechanisms ultimately lead to demyelination and the characteristic inflammatory reaction observed in MS.

Following an injury to OLs, the axonal repair is initiated through the remyelination of the CNS by OPCs. However, as the disease progresses, the death of OLs halts remyelination, resulting in irreversible damage and inefficient signal transmission. Demyelination results in the loss of myelin sheets, leading to the formation of plaques that can either be initially active or eventually become chronically inactive. They are characterized by inflammation, axonal loss, and sclerosis in both cases. On imaging, these plaques appear as hyperintense lesions on T2 weighted images and hypointense lesions on T1 images. Clinically, the manifestations of these plaques include motor and sensory deficits, disequilibrium, and vision dysfunction (25).

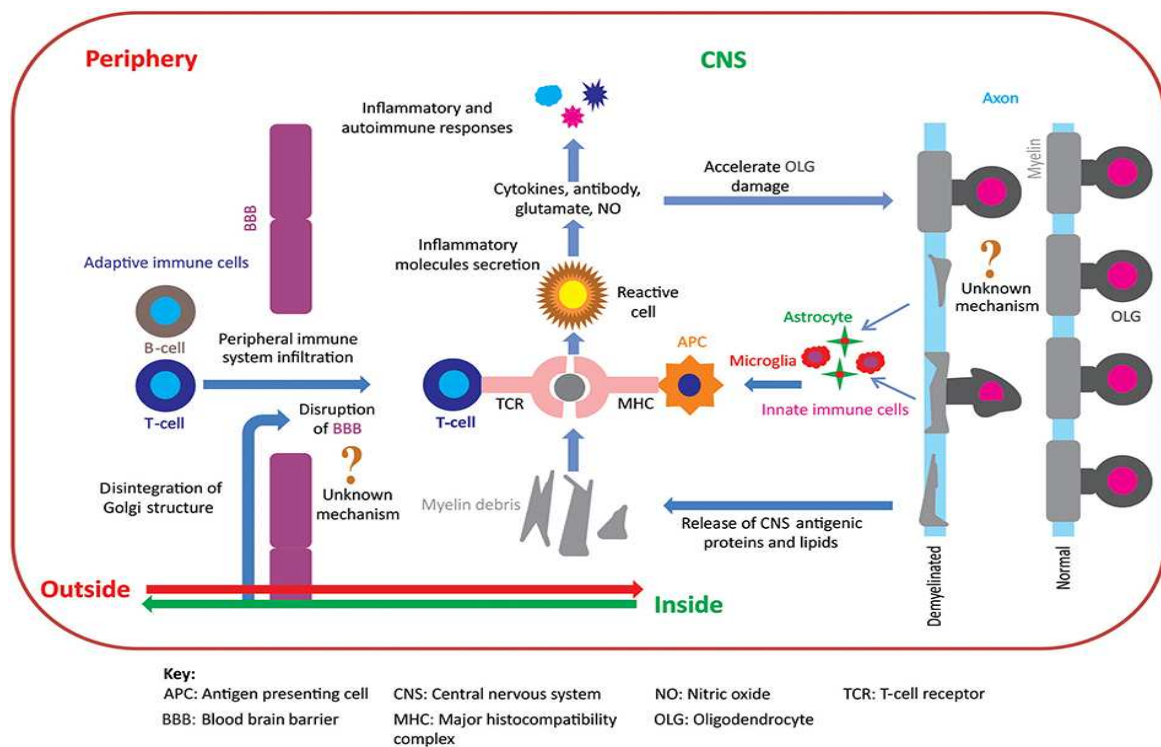


Figure 1: Pathogenesis of MS.

The figure shows both the inside-out and outside-in theories. The inside theory begins within the CNS, and the outside theory begins from the periphery. Source: Hurwitz BJ. The diagnosis of multiple sclerosis and the clinical subtypes. *Ann Indian Acad Neurol.* 2009;4:226-30.

1.7. Diagnosis criteria for multiple sclerosis

The diagnosis of MS is not based on a single test or set of clinical features but rather on a combination of certain factors. Comprehensive diagnostic procedures generally include a medical history, neurological examination, laboratory results, and magnetic resonance imaging (MRI) of the CNS. To establish a diagnosis, the McDonald's criteria must be utilized. These criteria were first published in 2001 and modified in 2017 and require evidence of both dissemination in time and dissemination in space of CNS inflammation. DIT indicates that neurological findings on MRI show that attacks occur more than once. In contrast, DIS indicates that neurological lesions affect different areas of the nervous system, including the brain and spinal cord. Diagnosis relies on a comprehensive evaluation incorporating clinical manifestations, radiological evidence from MRI, and positive laboratory findings from cerebrospinal fluid analysis (21–24).

1.8. Phenotypes of multiple sclerosis

1.8.1. Disability and Extended Disability Status Scale

In MS, “sclerosis” refers to the pathological process of scar tissue formation resulting from CNS inflammation. This scarring disrupts the transmission of neural signals, thereby contributing to the neurological symptoms of MS. Importantly, the effects of sclerosis extend beyond the CNS and can affect multiple organ systems, resulting in symptoms. Symptoms include paresis, paresthesia, and ataxia. There is evidence of disability when these symptoms begin to impair the quality of life and ambulatory function significantly. The Expanded Disability Status Scale is widely used for evaluating MS progression and monitoring treatment effectiveness. Developed by Kurtzke in 1955 and modified in 1985, the EDSS measures the degree of disability in patients with MS, ranging from 0 to 10, with higher scores indicating more significant disability and ambulatory impairment. A complete neurological examination is conducted, including an assessment of ambulatory ability, resulting in a numerical score. Although the EDSS does not consider cognitive function or upper-extremity disability, it is an effective tool in clinical trials for assessing eligibility for DMTs and predicting disease prognosis. In addition to the EDSS, the number of years since the onset of symptoms or diagnosis is used to calculate the Multiple Sclerosis Severity Score, which is an evaluation of disease severity based on disability accumulation over a period (25–27).

1.8.2. Remitting -relapsing disease

MS is a complex disease characterized by waves of relapse. These attacks are clearly defined as new or increasing neurological symptoms followed by periods of remission. Timely and accurate diagnosis of MS is essential for better treatment outcomes. The clinical subtypes of MS, including progression and relapse, are crucial concepts that need to be understood. Relapses or flare-ups occur when symptoms suddenly worsen due to fresh inflammatory activity in the central nervous system. These symptoms can last several days or weeks and may subside after treatment. Remitting-relapsing RRMS is the most common type of MS that presents with loss of neurological function over some time and returns to baseline via mechanisms such as remyelination. It has been observed that RRMS may begin with the CIS as patients untreated with CIS are prone to MS development associated with unfavorable prognosis. RRMS is characterized by relapses and remissions, where patients experience remission between relapses, with no new or active flare-ups and no subsequent deterioration in function between attacks. However, in instances where a baseline is not achieved, the presenting symptoms accumulate and gradually progress, leading to secondary progressive multiple sclerosis SPMS, which is a form of progressive MS (5,11,22).

1.8.3. Progressive multiple sclerosis

When MS progression is characterized by a gradual worsening of symptoms over months or even years, it is progressive. This presentation can be either active or inactive depending on whether there is new or ongoing damage or no new damage made apparent through clinical neurological examinations and MRI findings. This pattern of disease development observed clinically and in previous studies mirrors the primary progression of Multiple Sclerosis. PPMS is a subtype of MS, and the clinical diagnosis depends on its timing and course. This form of MS presents with a downhill course and gradual accumulation of neurological deficits without any apparent exacerbation or remission. The difference between these two types of progressive MS is that a remitting relapsing course characterizes the SPMS disease pattern. In contrast, PPMS lacks these remissions and is usually associated with poor outcomes. There is a thin line between the points of transition from RRMS to SPMS, which can lead to challenges in establishing the diagnosis of SPMS, as it is typically made in hindsight based on worsening symptoms in patients who have already been diagnosed with RRMS. In summary, diagnostic challenges may hinder early diagnosis and lead to inadequate treatment (23,24,29,30).

1.9. Treatment of secondary progressive multiple sclerosis

In addition, several approved drugs are disease-modifying therapies and are available in different formulations for treating RRMS. Some of these drugs have been used to treat SPMS but have been proven to lack effectiveness and the inability to halt disease progression. There are several FDA-approved drugs for SPMS therapy in the USA, including cladribine, mitoxantrone, and interferon medications. SPMS usually begins with RRMS; therefore, early therapeutic intervention and accurate diagnosis are necessary to prevent RRMS progression to SPMS and to ensure effective therapy to halt and prevent disease progression. In contrast to the rest of the world, siponimod is the only clinically approved medication for treating patients diagnosed with SPMS in Croatia. Siponimod is a sphingosine-1-phosphate receptor modulator that has proven efficacy in both the CNS and periphery. The EU and the FDA have approved the use of siponimod in treating SPMS in adults (29,30).

1.10. Siponimod for treatment of active secondary multiple sclerosis

1.10.1. Sphingosine -1 phosphate

To better understand the mechanism of action of siponimod, it is wise to explore sphingosine-1-phosphate (SIP1) and its function in cellular processes. Sphingosine -1 phosphate plays a role in cellular processes such as proliferation, migration, differentiation, and survival. It is synthesized from sphingosine through phosphorylation, with the cell membrane as its principal source. Upon its release from the cell membrane, SIP serves as a ligand for the family of G-protein coupled receptors termed SIP receptors. Five types of SIP1 receptors have been identified based on their expression on different cell types, including immune cells. The receptor-ligand binding of SIP on immune cells leads to a sequence of intracellular events important for immune response regulation. SIP1 receptors are expressed in multiple tissue types such as myocytes, brain, spleen, and eyes, which can explain adverse effects in these organ systems (20,29,30).

1.10.2. Pharmacodynamics and pharmacokinetics

Despite its numerous adverse effects, siponimod reduces the occurrence of post-therapy relapses and neurological lesions in patients with SPMS. Marketed under its brand name, Mayzent, the mode of action involves targeting autoreactive T and B lymphocytes, critical players in immunological processes that cause damage to the myelin sheath in the CNS. Ligand-receptor binding causes internalization and degradation of receptors, inhibiting lymphocyte migration from lymphoid organs into the CNS. Siponimod is characterized by its dual effect as it is effective both on the periphery and the CNS, with its primary site of impact on the periphery and its ability to cross the BBB and act on the CNS.

With their central role in the inflammatory process that damages myelin sheath within the CNS, T, and B lymphocytes are targeted and modified via cellular processes involving a series of signaling pathways once Siponimod binds to its target receptors SIP1 and SIP5. The modified cellular processes include proliferation, cell survival, migration, and angiogenesis. Therefore, the series of events described above results in the inhibition of lymphocyte migration to the CNS, thereby preventing the initiation of an inflammatory response. The cumulative result of immune suppression prevents all inflammatory processes that target and damage myelin proteins and causes disability accumulation, which is the main feature of the SPMS disease pattern.

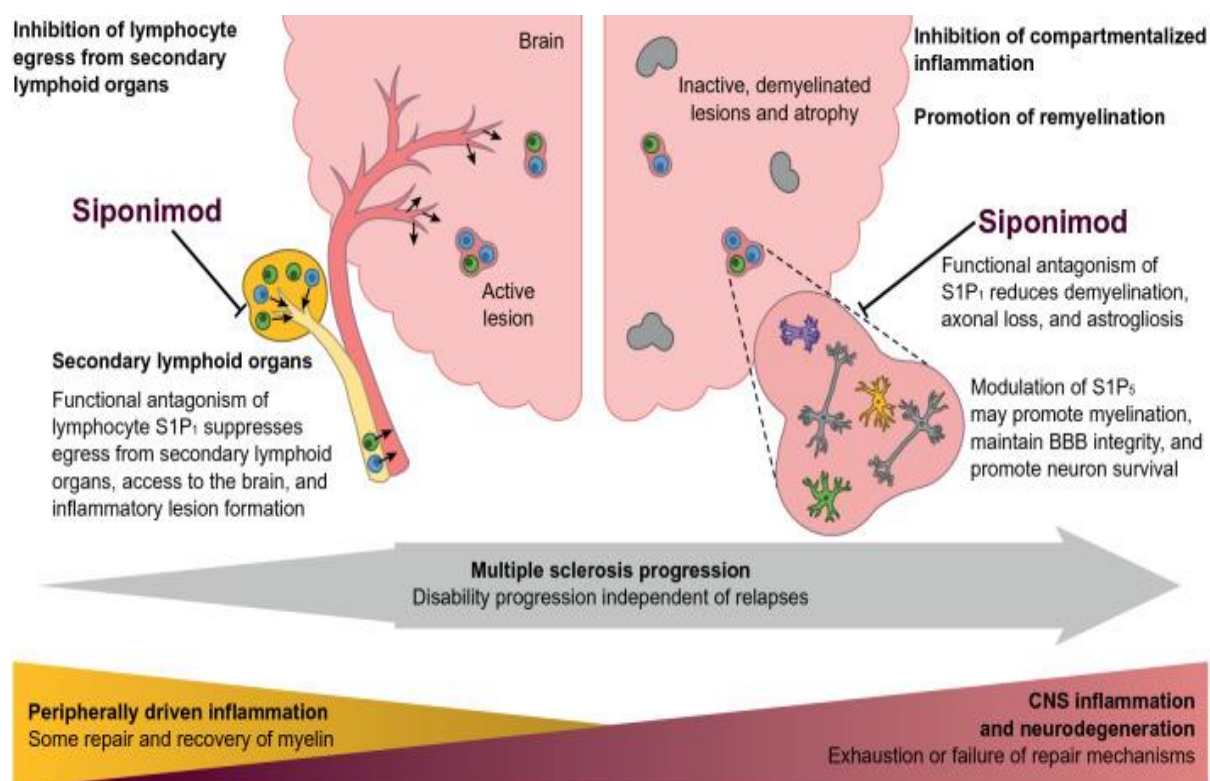


Figure 2: Siponimod mechanism of action.

Siponimod inhibits the regression of lymphocytes from secondary lymphoid organs and promotes remyelination in the CNS. Source: Cohan SL, Benedict RHB, Cree BAC, DeLuca J, Hua LH, Chun J. The two sides of siponimod: evidence for brain and immune mechanisms in multiple sclerosis. Vol. 36, CNS Drugs. Adis; 2022. p. 703–19.

Siponimod reaches its peak plasma concentration approximately four hours post-oral ingestion. Drug metabolism involves two enzymes from the cytochrome p450 family in the liver. Most metabolism is carried out by the CYP2C9 enzyme, with a minor contribution of CYP3A4. Drug elimination occurs via the biliary or gastrointestinal system. The maximum daily dose for siponimod is 2 mg; however, individual doses are based on genotypes that influence drug metabolism. Due to the mechanism of action previously described for siponimod, its administration reduces ALC. This increases susceptibility to infections, but ALC typically recovers by the 10th day of therapy. There are three genotypes: *1 CYP2C9, *2 CYP2C9, *3 CYP2C9, one of each allele inherited from each parent. Patients with the 3*3 genotype are classified as poor metabolizers and have an increased risk of drug toxicity. Conversely, individuals with the 1*3 and 2*3 genotypes should take a lower dose of Siponimod to achieve efficacy. Additionally, siponimod can interact with other medications that induce or inhibit the CYP2C9 and CYP3A4 enzymes. Rifampin, an antibiotic that is commonly used to treat tuberculosis, reduces siponimod plasma concentration, whereas fluconazole and grapefruit inhibitors increase drug levels. Gradual titration of siponimod dosage is essential, with

adjustments based on individual metabolism, but it also helps monitor for first-dose bradycardia, a potential adverse effect of therapy initiation. It is recommended that patients with favorable genetic profiles who are efficient metabolizers should receive a total daily dose of 2 mg and patients with an unfavorable genetic half dose of 1 mg (28–32).

1.10.3. Adverse effects of siponimod

Patients undergoing treatment with siponimod have reported a range of adverse effects, primarily affecting tissues expressing the drug's target S1PRs. Siponimod operates by suppressing the immune system and hindering the migration of lymphocytes to the periphery, which reduces activated lymphocytes (ALC) (30,32,33,35). Notably, treatment has been linked to an increased susceptibility to various pathogens, including HSV, Varicella Zoster Virus, COVID-19, PML, and *Cryptococcus neoformans*. Additionally, the cardiovascular system is negatively affected by S1PR modulation, as endothelial cells and cardiomyocytes express these receptors, leading to hypertension, atrioventricular block, and bradyarrhythmia (36). Siponimod administration also affects other organ systems, notably the respiratory system, and increases the risk of respiratory diseases. Although the precise mechanism remains unclear, the drug's immunomodulatory action may compromise the host's ability to combat respiratory infections or exacerbate pre-existing respiratory conditions. Consequently, close monitoring of siponimod users for respiratory symptoms such as dyspnea and decreased lung function is imperative, especially considering the increased risk of COVID-19 and associated respiratory complications (35). Macula edema is a common occurrence within the initial three to four months of siponimod treatment, is often observed in individuals with diabetes, and can be asymptomatic. It was reversible upon discontinuation of the medication (38,39). While severe permanent liver damage is rare, elevated liver enzymes have been documented that occur approximately six months after the initiation of therapy, returning to normal levels approximately three months after cessation of the drug (40). Additionally, research suggests reduced vaccine efficacy among patients receiving S1PR modulators such as siponimod (35). Owing to its potential teratogenicity and the risk of congenital disabilities, siponimod is contraindicated during pregnancy and lactation.

1.10.4. Checklist for healthcare professionals

There are established protocols that are used to make sure guidelines are followed before Siponimod therapy is initiated. They were created to minimize the risk of contraindications, and it is of great importance that physicians adhere to this. A series of laboratory analyses, including liver enzyme assessments, complete blood counts, and CYP2C9 genotype testing, are necessary to determine the appropriate dosage. Ophthalmic evaluations are essential for individuals at a high risk of ocular edema, such as those with diabetes. Some patients may require close monitoring of their initial dosing to prevent excessive reduction in heart rate. Testing for Shingle immunity is crucial, and non-immune patients should receive appropriate vaccination before beginning treatment. Individuals with specific respiratory conditions may require spirometry or lung function testing, whereas ECG assessments should be performed in all patients. First-dose surveillance is recommended for all patients, specifically those with a resting heart rate of < 55 beats per minute, and cardiac abnormalities as significant ECG abnormalities and a history of myocardial infarction or heart failure (33,34,41-44).

2. OBJECTIVES

The primary objective of this study was to explore the impact of siponimod therapy on patients residing in the Split Dalmatia region of Croatia. This hypothesis guided our investigation:

- To assess whether siponimod treatment's benefits outweigh this patient group's disadvantages.

3. SUBJECTS AND METHODS

3.1. Ethical considerations and study design

This study focused on patients diagnosed with SPMS who live in the Split-Dalmatian region. Inclusion criteria were based on an established SPMS diagnosis and a therapeutic course of siponimod for at least six months. This was a retrospective study, and patient consent was not required. It was a single-center study with 14 participants, predominantly women aged between 47 and 66. The University of the School of Medicine Ethics Committee approved this study's ethical principles (USSM, ur. Reg No. 2081-147-01-06-LJ.Z.-24-02).

3.2. Data collection

Data were obtained using routinely gathered documentation from the Neurology Department at the University Hospital, Split, Croatia. The dataset recorded is intended for therapeutic evaluation to ensure security. It includes various parameters, such as EDSS scores recorded before siponimod therapy initiation, and annually to assess disease progression and effectiveness of treatment. EDSS scores measured disability across the eight functional systems and ambulation. This was achieved through a thorough clinical examination performed by a neurologist and scored on a scale of 1-10. Initially, the study included 15 patients; however, one participant was excluded from the analysis because of an allergic reaction to siponimod. Adverse effects reported by SPMS patients on siponimod therapy were actively screened by neurologists and documented during appointments, following the guidelines for biannual assessment, unless the patient's condition remained stable. Blood samples were obtained pre- and post-Siponimod therapy to evaluate the lymphocyte counts. This was achieved through venipuncture, and the results were measured in cells per microliter. MRI lesion screening was conducted annually according to guidelines. However, additional MRI screening was performed in cases with worsening EDSS scores. Additionally, demographic information, such as age, sex, therapy length, and comorbidities, was collected to provide a framework and potential influencing factors in evaluating treatment outcomes and patient safety.

3.3. Variable dosage of siponimod

The participants were administered varying doses of siponimod, which were CYP-dependent. Genetic polymorphisms are critical in determining siponimod dosing, especially CYP2C9, which affects siponimod metabolism and its potential efficacy. Three genotypes (*1 CYP2C9, *2 CYP2C9, and *3 CYP2C9) were identified, and individuals with the *3/*3 genotype were classified as poor metabolizers. Most participants were fast metabolizers and received a full dose of 2 mg, whereas slow metabolizers received a daily dose of 1 mg.

3.4. Statistical analysis

Specific parameters from the collected dataset relevant to this study were statistically analyzed. They included EDSSA scores at different points in time, lymphocyte counts, and adverse effects. The following statistical methods were used for accurate analysis: For normality of distribution, the Wilcoxon test and Spearman's correlations were used to examine the relationship variables. Statistical analysis was performed using DATAtab statistic software. Statistical significance was set at $P < 0.05$.

4. RESULTS

4.1. Treatment history, gender distribution, and age

This study had 14 participants, and the results were based on recorded patient data that are important for this study. Table 1 shows demographics and EDSS scores with their corresponding functional scores at different points: EDSS score before initiating siponimod treatment, EDSS within one year of treatment, and the current EDSS score when this study was conducted. Other parameters include the year of RRMS diagnosis, disease duration in months, lymphocyte counts pre- and post-siponimod treatment, and new MRI lesions. The sex distribution was imbalanced: 85.7% females (n=12) and 14.3% males (n=2), as presented in (Table 2). The percentages are based on the total valid responses, with no missing gender data. The bar chart in Figure 1 shows the trend of female predominance in our sample group.

Patients ages ranged from 47 to 66, with a median age of 60. The median EDSS score ranged from 3.5 to 8, with a median of 6.25. Results for descriptive analysis and demographics are presented in Table 3. The scatter plot in Figure 4 shows a weak positive trend between EDSS score and age but gives a visual understanding. Spearman's correlation was used to quantify the trend seen in the scatter plot. The results showed a weak correlation $r=0.252$ with no significance $P=0.406$ between patients' ages and EDSS scores, as seen in Table 4.

Table 1. Patient characteristics and treatment history

Sex	D-Y ^a	EDSS PTS ^b	FS PTSc	EDSS 1YST ^d	EDSS CSTe	FS CST ^f	Lym PRT ^g	Lym CST ^h	ST Length ⁱ	New MRI ^j
M	2000	6	4	6.5	6.5	4	1.64	0.45	43	0
F	2008	5	3	6	6	4	1.9	0.29	43	1
F	1994	6.5	4	6.5	6.5	4	1.89	0.4	43	0
F	2008	3.5	2	3.5	3.5	2	2.4	0.9	6	0
F	1991	6.5	2	6.5	6.5	2	2.76	2.5	6	0
F	1991	7	5	7	7	6	1.45	0.47	40	0
F	1998	6	5	6	8	8	1.28	0.3	38	0
F	1998	6.5	4.5	6	7.5	7	2.37	0.7	38	0
F	2011	5.5	4	5.5	6	6	2.09	0.85	30	0
F	1991	6	4	6	6.5	5	1.44	0.77	36	0
F	2009	4	2	4	4	2	2.5	0.49	23	0
M	2001	5	3	5	5	3	1.6	0.33	23	0
F	2003	6	4	6	6	4	2.4	NA	1	0
M	1014	6	4.5	6	6	4.5	1.75	0.9	10	0

^a year at which RRMS diagnosis was established

^b EDSS scores pre-siponimod treatment

^c functional score (motoric) pre-treatment

^d EDSS scores within one year of siponimod treatment

^e current EDSS scores

^f functional score of current EDSS

^g lymphocyte count pre-siponimod treatment

^h current lymphocyte count ST length

ⁱ length on Siponimod treatment in months

^j new lesions found on magnetic resonance

Table 2. Sex distribution

Participants	Frequency	Percent	Valid Percent	Cumulative percent
Females	12	85.7	85.7	85.7
Males	2	14.3	14.3	100
Total	14	100	100	

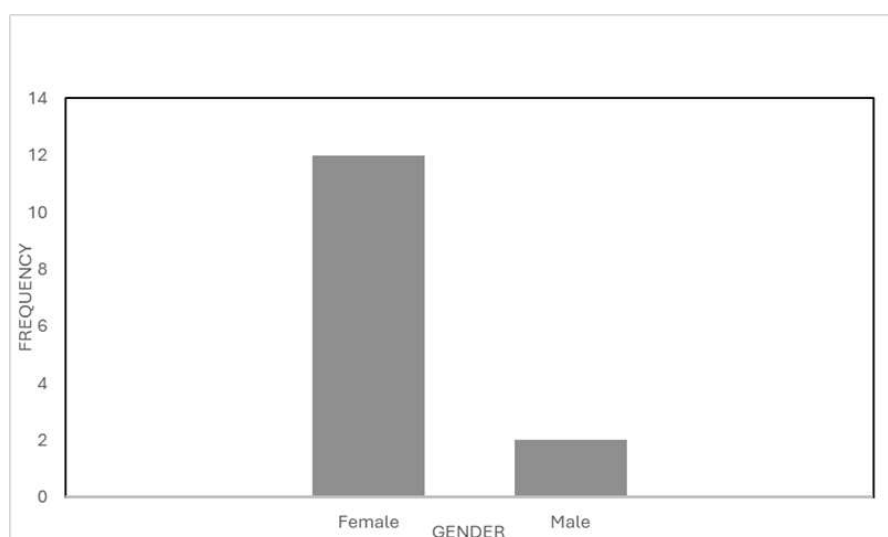


Figure 3. Sex distribution bar chart

Table 3. Descriptive analysis for EDSS and ages

	Median	Mean	Std. D	Min	Max
Age	60	58.07	6.486	47	66
EDSS	6.250	6.071	1.222	3.5	8

Table 4. Correlation between EDSS score and ages

Variable		EDSS	Age
EDSS	Spearman's rho	-	
	p-value	-	
Age	Spearman's rho	0.252	-
	p-value	0.406	-

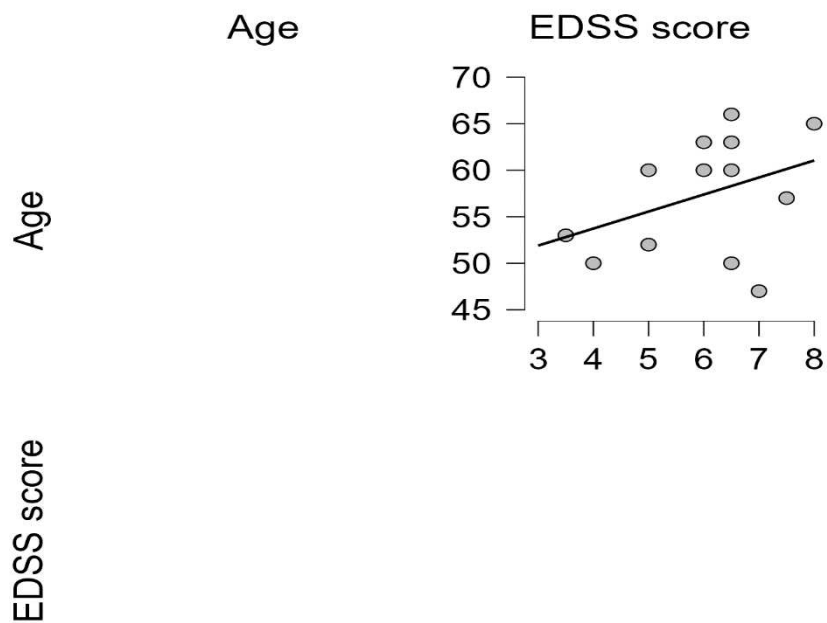


Figure 4. Relationship between EDSS scores and ages

4.2. Individual EDSS scores and analysis for clinical significance

There were no significant differences in the median EDSS scores before Siponimod treatment 6.0 and after one year of treatment 6.0. However, there was a slight increase in the median for the current EDSS scores, which was 6.25. Detailed statistics for the EDSS scores before Siponimod treatment, after one year of siponimod therapy, and the current EDSS scores can be found in Table 5. Individual patient data was examined to assess the response to treatment, using a threshold of ≥ 0.5 to determine clinically significant changes in disability status. The results indicated stability in the individual EDSS scores of eight patients, while six patients showed changes of ≥ 0.5 in EDSS scores from before treatment to the current EDSS scores. A more detailed data presentation can be found in Table 6.

Table 5. Descriptive statistics of EDSS scores before and after treatment

	EDSS^a pre-therapy	EDSS after one year	Current EDSS
Median	6	6	6.25
Minimum	3.5	3.5	3.5
Maximum	7	7	8
Interquartile Range	1.25	0.75	0.5

*Wilcoxon test with *P*-value set at 0.05

^a Expanded disability status scale.

Table 6. Individual EDSS of significance increase

Patient number	PST^a	1YST^b	CST^c	PST to CST \geq 0.5
1	6.0	6.5	6.5	Yes
2	6.5	6.5	6.5	No
3	5.0	6.0	6.0	Yes
4	3.5	3.5	3.5	No
5	6.5	6.5	6.5	No
6	7.0	7.0	7.0	No
7	6.0	6.0	8.0	Yes
8	6.5	6.5	7.5	Yes
9	5.5	6.0	6.0	Yes
10	6.0	6.0	6.5	Yes
11	4.0	4.0	4.0	No
12	5.0	5.0	5.0	No
13	6.0	6.0	6.0	No
14	6.0	6.0	6.0	No

^a EDSS score before siponimod treatment

^b EDSS score within one year on siponimod treatment

^c current EDSS score

4.3. Comparison of EDSS, correlations, and adverse effects

The Wilcoxon signed ranked test was used to evaluate the EDSS scores statistically. The results showed $W = 1.5$ and $P = .414$; there was no significant difference between EDSS scores before and within one year of Siponimod treatment. This means there was no significant change in disability within one year of Siponimod treatment.

When comparing EDSS scores from pre-Siponimod treatment to the current EDSS score, the results showed that none of the 14 patients' current EDSS score was lower than the pre-therapy EDSS score. However, the difference observed was statistically significant, $W = 0$ and $P = 0.026$ with six patients showing a worsening disability progression. Overall, no patient had a decrease in the current EDSS score compared to the EDSS score observed pre-siponimod treatment.

Tables (7 and 8) provide a detailed breakdown of the statistical analysis of EDSS scores at different points in time regarding correlations. The correlation between the following variables was tested. Disease duration, current lymphocyte count, and current EDSS score. According to Spearman's correlation, r , and P values were used to interpret relationship results. Results showed a weak positive correlation between disease duration and the current lymphocyte count, $r = 0.11$ and P -value $= 0.72$. This implies no meaningful relationship between disease duration and the current lymphocyte count in patients. In contrast, Disease duration and current EDSS scores showed a moderate positive correlation $r = 0.54$, with a P -value of 0.04 , suggesting that longer disease duration is associated with higher disability. No correlation was found between the current lymphocyte count and current EDSS scores $r = -0.29$ and P -value $= 0.312$; this means that there is no relationship between the patient current lymphocyte counts and disease disability. Results for relationships between disease duration, current lymphocyte count, and current EDSS scores were as follows: disease duration versus current lymphocyte count: correlation $= 0.11$, P -value $= 0.72$. For disease duration versus current EDSS: correlation $= 0.54$, P -value $= 0.048$, and between current lymphocyte count and current EDSS correlation $= -0.29$, P -value $= 0.312$. Only one statistically significant correlation was found: between disease duration and current EDSS scores, presented in Table 9. Records of adverse effects and lymphocyte counts are presented in Table 10. Most patients had elevated liver enzymes and reduced levels of lymphocytes after Siponimod treatment.

Table 7. Correlation results of EDSS pre-treatment versus EDSS within one year of treatment

		N	Mean Ranks	Sum
PTS-1YST^a	Negative Ranks	1	1.5	1.5
	Positive Ranks	2	2.25	4.45
	Ties	11		
	Total	14		
Wilcoxon-T				
	W	z	P*	r
PTS-1YST^a	1.5	-0.82	0.414	0.22

*Wilcoxon test with *P*-value set at 0.05

^a PTS-1YSTscore pre-Siponimod treatment and EDSS score within one year of Siponimod treatment.

Table 8. EDSS scores pre-treatment versus current EDSS scores

		N	Mean ranks	Sum of ranks
PTS-CST^a	Negative ranks	0	0	0
	Positive ranks	6	3.5	21
	Ties	8		
	Total	14		
Wilcoxon-T				
	W	z	P*	r
PTS-CST	0	-2.23	0.026	0.22

*Wilcoxon test with *P*-value set at 0.05

^a PTS-CST EDSS score pre-siponimod treatment and Current EDSS score.

Table 9. Correlation between disease duration, EDSS score, and lymphocyte count

		Disease duration	Current lymphocyte count	Current EDSS
Disease duration	Correlation	1	0.11	0.54
	<i>P</i>		0.72	0.048
Current lymphocyte count	Correlation	0.11	1	-0.29
	<i>P</i>	0.72		0.312
Current EDSS	Correlation	0.54	-0.29	1
	<i>P</i>	0.048	0.312	

^a Expanded Disability Status Scale

Table 10. Lymphocyte counts before and after siponimod treatment and adverse effects

Patient number	Side effects	Lymphocyte count before siponimod (/L)	Current lymphocyte count (/L)
1	Elevated liver enzymes	0.54	0.54
2	Decreased erythrocytes	1.89	0.29
3	Elevated GGT	1.9	0.9
4	Pancreatitis	2.4	2.5
5	Gastritis	2.76	0.47
6	Urogenital infections	1.45	0.7
7	Cholecystitis	2.37	0.3
8	Shoulder Pain	1.28	0.85
9	Elevated liver enzymes	2.09	0.77
10	Decreased cell count levels	1.44	0.78
11	0	2.5	0.49
12	Weight loss	1.6	0.33
13	0	NA	NA
14	All siponimod	1.75	0.9

5. DISCUSSION

Our study aimed to explore the impact of siponimod therapy on patients diagnosed with SPMS in Split Dalmatian municipality. To achieve our goal, we evaluated several vital parameters: EDSS scores at different time points, changes in lymphocyte count before and during therapy, new MRI lesions, and adverse effects reported by the participants. The study included 12 women and two men with SPMS.

The baseline EDSS scores varied between 3.5 and 8.0, with a median age of 60. Our results showed no increase in the median EDSS score from pre-siponimod treatment to the EDSS score recorded within a year of treatment, but a slight increase in the median of the current EDSS score was observed. To evaluate the efficacy of Siponimod more rigorously, the Wilcoxon signed ranked test was used to compare EDSS pre-treatment to EDSS within one year of treatment. The results showed $W = 1.5$ and $P = .414$, indicating no significant difference between EDSS scores before and within one year of Siponimod treatment. This implies that Siponimod treatment may stabilize EDSS scores within the first year and halt disability. A comparison was also made between the EDSS pre-treatment and current EDSS scores; $W = 0$ and $P = 0.026$ suggest Siponimod treatment's inability to halt disability progression in the long term. Six patients who also showed increased EDSS scores had an increase of six ≥ 0.5 , indicating clinical significance in disease progression. We also examined the relationship between disease duration, current EDSS scores, and lymphocyte counts. Disease duration and current EDSS scores showed a moderate positive correlation $r = 0.54$; P -value is 0.04. This suggests that a longer disease duration is associated with higher disease disability, which was already established in other studies(44). Results also showed a weak positive correlation between ages and EDSS scores. As this correlation was not statistically significant, a confident conclusion regarding this relationship cannot be reached.

Moreover, at therapeutic doses, siponimod can cause several side effects ranging from common issues such as headaches, hypertension, and abnormal liver values to severe conditions such as decreased lymphocyte counts, bradycardia induced after the first dose, macular edema, and progressive multifocal leukoencephalopathy (PML). Three patients reported abnormal liver test results, and the majority had lymphocyte counts below the normal range following siponimod treatment. However, no severe adverse effects such as PML were reported. The most prevalent adverse effects observed in our patient group were seen in the hepatic and gastrointestinal systems. Overall susceptibility is due to the immunosuppressive mechanism of siponimod, which leads to a decrease in the lymphocyte count. Results also showed that the hepatic and gastrointestinal adverse effects are likely at low lymphocyte counts ($< 1.0 \times 10^9/L$). This finding implies that a lower lymphocyte count may increase the risk of

experiencing adverse effects during siponimod treatment. Further investigation using larger sample sizes should be considered to substantiate these observations and better understand their clinical significance.

Furthermore, the management of MS is achieved by reducing the occurrence of relapses and slowing disability progression. Regular monitoring of EDSS allows physicians to compare EDSS scores from the beginning of therapy and after therapy, which is a powerful predictive tool. This personal approach enables disease tracking and monitoring of the effectiveness of treatment. Our study results differ from previous findings, such as the EXPAND study by Kappos et al. (2018), which reported decreased disability progression in SPMS patients treated with Siponimod (51). Similarly, a review by Synnott et al. (2020) also reported the positive effects of Siponimod on patient outcomes (52).

While this study has provided insight into Siponimod's impact on SPMS patients from the Split-Dalmatian region, several factors, such as gender disparity, sample size, and age distribution in our population, may have caused the discrepancies observed in this study. Given this study's predominance of female participants, several factors should be considered. Gender imbalance may also limit the application of our findings to a broader population with SPMSs. Therefore, it is crucial to identify and consider the implications of gender disparities when assessing their impact on the applicability of our results. According to Greer and McCombe (2011), sex is significant in disease progression and response to treatment (47). Not only are females more susceptible to MS, but they mount a different immune response than males, which affects disease progression and treatment response; females often exhibit a pro-inflammatory immune response distinct from that of males, whereas males typically exhibit an adaptive immune response (45). Given these differences, could siponimod efficacy vary between sexes? This question merits further exploration, as its mechanism of action targets the lymphocyte's key players in the adaptive branch of immunity. The implication that sex-based immune response might influence siponimod therapy should be added to reasons that support personalized therapy strategies. As already proposed by Pathak (2016), factors such as disease severity, genetic predisposition, and overall health status greatly influence treatment and disease progression (49,50).

Most patients in this study belong to the elderly population. Aging has been associated with increasing axonal loss and ineffective therapeutic efficacy (44). Older patients with SPMS are not only prone to adverse effects but also present worsening disability despite treatment. This may have contributed to the discrepancies in our study, including other factors such as pharmacokinetics and commodities (46).

The study type might have influenced the results since a retrospective study is based on past data and factors that influence the results can be easily overlooked. Also, the limited sample size and the lack of a control group may limit the broad application of our findings. Our sample size was constrained by the limited number of individuals treated with siponimods in the Split Dalmatian region. Additionally, our patients may have been diagnosed and started therapy at later disease stages than those previous studies, which could have affected the findings since studies have suggested that favorable therapeutic outcomes can be achieved following early disease intervention. The progression in disability in some patients despite treatment raises the importance of early treatment intervention regarding siponimod therapy. With the above factors considered, future studies should aim for a better study design and account for demographic differences to understand Siponimod therapy's effectiveness better.

6. CONCLUSIONS

We studied the influence of siponimod on the disease's progression while monitoring its possible adverse effects. The ability of siponimod to completely halt and stabilize disease progression is limited. In our group, there is progression of the disease despite the use of medication, and a small number have an unchanged neurological status. The mean value of neurological disability already at the beginning of therapy was high, which, in addition to our results, indicates the need to include this drug as early as possible in patients with SPMS. Further follow-up of patients and examination of the effect of siponimod in a more significant number of patients is undoubtedly necessary to draw additional conclusions. All possible parameters that can affect the treatment with this drug, such as age, sex, duration of the disease, activity of the disease, and the start of therapy about the transition of the disease to the secondary progressive phase, must be considered.

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8. SUMMARY

Objective: This study aimed to evaluate the adverse effects of the only approved drug for SPMS treatment in the Split Dalmatian municipality. This was achieved by assessing changes in EDSS scores, lymphocyte counts before and after therapy, MRI findings, and reported adverse effects.

Materials and Methods: This retrospective study included 14 patients with SPMS. All participants had been on a siponimod for at least six months. Data collected included demographics and parameters such as EDSS scores, lymphocyte counts, and new CNS lesions. EDSS scores and lymphocyte counts before and after treatment were analyzed at different times (pre-treatment, within one year of treatment, and current EDSS scores).

Results: The study included 12 females (85.7%) and two males (14.3%), with a Median age baseline of 60. The baseline EDSS score ranged from 3.5 to 8. The median EDSS scores slightly increased from baseline after the first year of Siponimod treatment from 6 to 6.25. Results showed that the Wilcoxon test was statistically significant, with a $W = 0$ and $P = 0.026$, for the EDSS pre-treatment score compared with the current EDSS score. This indicates progressive disability despite ongoing siponimod therapy. Six participants showed a significant increase in the EDSS scores (≥ 0.5), which has clinical significance. Adverse effects were predominately seen in hepatic and gastrointestinal systems.

Conclusion: There was an increased disability in patients despite the use of siponimod, with some patients showing unchanged neurological status. Early therapy is recommended in SPMS, and further research encourages the exploration of parameters that affect treatment outcomes.

9. CROATIAN SUMMARY

Naslov: Liječenje siponimodom bolesnika s multiplom sklerozom na Klinici neurologije u Splitu

Ciljevi: Ovo istraživanje imalo je za cilj procijeniti nuspojave jedinog odobrenog lijeka za liječenje SPMS-a u Splitsko-dalmatinskoj županiji. To je postignuto procjenom promjena u EDSS rezultatima, broju limfocita prije i poslije terapije, MRI nalazima i prijavljenim nuspojavama.

Pacijenti i metode: Ova retrospektivna studija uključila je 14 pacijenata sa SPMS-om. Svi su sudionici bili na siponimodu najmanje šest mjeseci. Prikupljeni podaci uključivali su demografske podatke i parametre kao što su EDSS rezultati, broj limfocita i nove lezije CNS-a. EDSS rezultati i brojevi limfocita prije i poslije tretmana analizirani su u različitim vremenima (prije tretmana, unutar jedne godine od tretmana i trenutni EDSS rezultati).

Rezultati: Studija je uključivala 12 žena (85.7%) i 2 muškarca (14.3%), s početnom srednjom dobi od 66 godina. Osnovni EDSS rezultat bio je u rasponu od 3,5 do 8. Medijan EDSS rezultata neznatno je porastao u odnosu na početni rezultat nakon prve godine liječenja Siponimodom sa 6 na 6,25. Rezultati su pokazali da je Wilcoxonov rang s predznakom bio statistički značajan, s $W = 0$ i $P = 0,026$, za EDSS rezultat prije tretmana u usporedbi s trenutnim EDSS rezultatom. To ukazuje na progresivnu onesposobljenost unatoč kontinuiranoj terapiji siponimodom. Šest sudionika pokazalo je značajno povećanje EDSS rezultata ($\geq 0,5$), što ima kliničku važnost. Nuspojave su uglavnom uočene u jetrenom i gastrointestinalnom sustavu.

Zaključak: Došlo je do povećanja invaliditeta u bolesnika unatoč primjeni siponimoda s tim da su neki bolesnici pokazali nepromijenjen neurološki status. U SPMS-u se preporučuje rana terapija, a daljnja istraživanja potiču na istraživanje parametara koji utječu na ishod liječenja.