TRABAJO FIN DE GRADO

Grado en Medicina

"ASSESSMENT OF BRAIN ATROPHY WITH THE CORPUS CALLOSUM INDEX IN PATIENTS WITH MULTIPLE SCLEROSIS TREATED WITH TERIFLUNOMIDE"

Autores:

Sara Duque González

Rebeca de Luis Sosa

Alba Rodríguez González

Tutores:

Dra. Montserrat González Platas

Dr. Carlos Emilio González Reimers

Servicios de neurología y Medicina Interna . Sección Enfermedades Desmielinizantes. Complejo Universitario Hospitalario de Canarias

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SECCIÓN MEDICINA

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Sara Duque González

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Supervisors:

Montserrat González Platas, PhD

Carlos Emilio González Reimers, PhD

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ABSTRACT

Multiple sclerosis (MS) is an autoimmune, demyelinating, neurodegenerative, and the most common inflammatory disease of the Central Nervous System. It is considered the second cause of neurological disability in young adults. The treatment of MS consists on achieving the control of the relapsing forms and limiting unwanted immune responses associated with disease propagation.

Teriflunomide (Aubagio®) is an immunomodulator approved for the treatment of relapsing-remitting MS (RRMS). Several studies have demonstrated benefits on both clinical and MRI outcomes, such as the decrease of inflammatory activity, disabilities and brain atrophy. Brain atrophy is the result of the persistent axonal degeneration which can appear in the cortex or in other brain structures.

In this project, we aimed to study the improvement of the disease in patients treated with Teriflunomide. For this purpose, MRI was used to measure the Index of Corpus Callosum (ICC) correlating it with brain atrophy and the cognitive status of the MS patients.

SUMMARY

ABSTRACT: The multiple sclerosis modifying treatments (MDT) act fundamentally in the inflammatory processes of the disease, decreasing relapses and delaying disability. Some treatments also reduce the speed of the progression of cerebral atrophy, with an uncertain effect on the evolution of the disease.

Teriflunomide reversibly inhibits dihydroorotate dehydrogenase (DHDH), a mitochondrial enzyme necessary for the synthesis of de novo pyrimidine, decreasing the number of activated T and B cells. It showed an effect in the suppression of the inflammatory activity of the magnetic resonance and the deceleration of the progression of cerebral atrophy. The median annualized percentage change in brain volume using normalization of atrophy shows lower brain volume loss for teriflunomide 14 mg at month 12 and 24 (reductions of 36.9% - 30.6%) in the study TEMSO.

OBJECTIVES: To study the effectiveness of teriflunomide 14 mg / day in MS patients to slow the progression of cerebral atrophy using the CCI. As secondary objectives, clinical parameters: EDSS, disability, time until the first relapses after the start of treatment, side effects, tolerance.

METHODS: 53 relapsing MS patients agreed to participate in the study and signed their consent. Clinical and MRI examinations were collected from their clinical records. CCI was carried out according to the method described by *Figueiras F, et al. 2006.*

RESULTS: After an average time of follow-up 27,24 month, the CCI remain stable, keeping it stable in most patients. EDSS remained stable. Dropout by side effects drug occurred in the first months (elevation of liver enzymes, diarrhea and weight loss, abdominal pain) 8 patients. The rest are mild and diminish with time. The time to present the first outbreak was 13,25 months in 8 patients.

CONCLUSIONS: MS Patients treated with teriflunomide remain stable with little brain atrophy and disability.

KEY WORDS: TERIFLUNOMIDE, CORPUS CALLOSUM INDEX, BRAIN ATROPHY, MULTIPLE SCLEROSIS.

RESUMEN

RESUMEN: los tratamientos modificadores de la enfermedad (TME) en Esclerosis Múltiple actúan fundamentalmente en el proceso inflamatorio de la enfermedad, disminuyendo las recaídas y retrasando la discapacidad. Algunos tratamientos también reducen la velocidad de progresión de la atrofia cerebral, con efecto incierto sobre la evolución de la enfermedad.

La teriflunomida inhibe de forma reversible la dihidroorotato deshidrogenasa, una enzima mitocondrial necesaria para la síntesis de novo de pirimidinas, disminuyendo el número de células T y B activadas. Con ello, se produce un efecto en la supresión de la actividad inflamatoria en resonancia magnética y la reducción de la progresión de la atrofia cerebral. La mediana del porcentaje anual del cambio en el volumen cerebral utilizando la normalización de la atrofia, muestra una menor pérdida de volumen cerebral con 14 mg de teriflunomida al 12º y 24º mes (reducción del 36.9%-30.6%) en el estudio TEMSO.

OBJETIVOS: estudiar la efectividad de la ingesta diaria de 14mg de teriflunomida para frenar la progresión de la atrofia cerebral, utilizando el Índice del Cuerpo Calloso. Como objetivos secundarios se valorarán algunos parámetros como: EDSS, discapacidad, tiempo hasta la primera recaída tras el comienzo del tratamiento, efectos secundarios y tolerancia.

MÉTODOS: 53 pacientes con Esclerosis Múltiple Recurrente-Remitente accedieron a participar en el estudio y firmaron el consentimiento informado. Se recogieron datos clínicos y de RMN de sus historiales médicos. Se realizó el cálculo del ICC de acuerdo al método descrito por *Figueiras F, et al. 2006.*

RESULTADOS: después una media de seguimiento durante 27,4 meses, el ICC se mantiene estable en la mayoría de los pacientes. El EDSS también permaneció estable. 8 pacientes abandonaron el tratamiento durante los primeros meses por los efectos secundarios (elevación de las enzimas hepáticas, diarrea, pérdida de peso o dolor abdominal), mientras que el resto fueron leves y disminuyeron con el paso del tiempo. La media de tiempo hasta la presentación del primer brote fue de 13,25 meses en 8 pacientes.

CONCLUSIONES: los pacientes con Múltiple Esclerosis tratados con teriflunomida se mantienen estables con ligera atrofia cerebral y discapacidad.

PALABRAS CLAVE: TERIFLUNOMIDA, ÍNDICE DEL CUERPO CALLOSO, ATROFIA CEREBRAL, ESCLEROSIS MÚLTIPLE.

INTRODUCTION

Background

Multiple sclerosis (MS) is an autoimmune, demyelinating, neurodegenerative and the most common inflammatory disease of the Central Nervous System (CNS). *(Gold R, et al. 2011).* Around 85% of all MS cases follow a relapsing/remitting course, which implicates periods of remission after relapses.

Its incidence has raised over last years because of better case ascertainment and the improvement in the diagnostic tools and criteria used. Nowadays, over 2.5 million people around the globe suffer from MS and it affects young adults between 20 and 40 years of age who live in Europe and North America. The prevalence depends on the country and it is estimated in 90,2 cases per 100,000 in Spain (including Canary Islands). *(Izquierdo G, et al. 2015).*

It affects those with a genetic predisposition: major histocompatibility complex class II phenotype and human leukocyte antigen HLA-DRB1*15:01. In the last group of patients caucasian ethnic group is a factor that increase 1,6 the risk.

Also certain lifestyle and environmental factors increase the risk of MS, such as Epstein Barr Virus (OR~3,6), low vitamin D levels (OR~1,4), adolescent obesity (OR~2), night work (OR~1,7), low sun exposure (OR~2) or infectious mononucleosis (OR~2), among others. Smoking (active or passive) increase the risk between 1,6 and 5 depending on the genetic predisposition. Oral tobacco can also neutralize the effect of some biological treatments.

However, it is known that HLA-A*02 (class I variant) is a protective factor. In fact, the absence of HLA-A*02 and the presence of HLA-DRB1*15:01 has a combined OR of ~5. Other potential protective factors include high coffee consumption, alcohol consump-tion, and serological evidence of cytomegalovirus (CMV) infection. *(Olsson T, et al. 2016).*

All these factors produce an immunological response that activate the lymphocytes in and outside CNS. The disease appears when the breaking of the blood-brain-barrier occurs, which activate the mechanism of MS.

Symptoms and diagnosis

Symptoms in MS vary depending on the location of the demyelinating damage and the stage of the disease. The most common symptoms are those related to sensory disturbances, such as crawling sensation, paresthesias, dysesthesias, L'Hermitte sign or numbness *(Guía de Actuación en Pacientes con esclerosis múltiple, 2015) (Figure 1).* Moreover, there are other symptoms that affects the MS patients including motor and visual disturbances, cerebellum disorders, urinary and sexual dysfunction, fatigue and neuropsychiatric disorders; among others *(Saguil, 2014).* An extended explanation of possible symptoms in MS are numbered in *table 1.*

SIGNS AND SYMPTOMS SUGGESTIVE OF MS	
Sensory Disturbances	Crawling sensation, numbness dysesthesias, paresthesias, L'Hermitte sign, trigeminal neuralgia, allodynia.
Motor Disturbances	Loss of strength, weakness, spasticity, paralysis, hyperreflexia, muscular atrophy.
Visual Disorders	Blurry vision, optic neuritis, oscillopsia, optic atrophy, diplopia.
Cerebellum disorders	Incoordination, ataxia, trembling.
Brainstem dysfunction	Difficulty swallowing, nystagmus, vertigo, hearing loss, tinnitus.
Urinary and sexual dysfunction	Frequency, urgency and urinary incontinence, bladder retention, repeated infections, erectile dysfunction, dyspareunia, anorgasmy.
Neuropsychiatric disorders	Memory and attention failures, depression, irritability, decreased mental processing capacity, epilepsy.
Fatigue	

Table 1. Signs and symptoms suggestive of MS.

MS diagnosis is always of exclusion, based on the symptoms, and it is confirmed by MRI results.

In the presence of abrupt or transient symptoms, prominent cortical failures, peripheral neuropathy and other organ involvement, the diagnosis of MS should be questioned.

Diagnostic tools are changing constantly in order to achieve an early diagnosis with the same sensibility and specificity. There are different diagnosis criteria but in this project the 2010 McDonald Criteria has been used *table 2*.

Clinical presentation	Additional data needed for MS diagnosis
≥2 attacks; objective clinical evidence of ≥ 2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of prior attack.	None.
≥2 attacks; objective clinical evidence of 1 lesion.	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.
1 attack; objective clinical evidence of ≥ 2 lesions.	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.
1 attack; objective clinical evidence of 1 lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by: • For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of CNS (periventricular, juxtacortical, infratentorial, or spinal cord); or await a second clinical attack implicating a different CNS site. • 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.
Insidious neurological progression suggestive of MS (PPMS)	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 DIS 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.

Table 2. 2010 Mcdonald criteria for MS diagnosis (Polman CH, et al. 2011).

Treatments of MS

Treatment of MS is challenging and it is based on achieving the control of the relapsing forms and limiting unwanted immune responses associated with disease propagation, that is the reason why immunosuppressants are used during the remitting periods since mid-1990s. *(Bar-Or A, et al. 2014.)* However, the risk of infection and cancer is increased due to the immune-suppression.

The treatment of MS should be given as soon as possible to maximize the benefits in the evolution of the illness. *(Dargahi N, et al. 2017).*

Relapses period

Relapses periods affect 85% of patients with MS. It is thought that infections and stress play a role. *(Sá MJ, et al. 2012).* These periods are treated with corticosteroids intravenously, plasma exchange or adenocorticotropic hormone injection. Nowadays, high doses intravenous methylprednisolone are used as the first line treatment *(González Feria, et al. 2016).* Corticosteroids treatments usually last between 3 and 10 days and if the recovery is not enough, a new sequence of corticosteroids or intravenous immunoglobulin can be used. Otherwise, there are no evidences of benefits (such as the decrease of the number of relapses or interruption of MS development) for long period treatment with corticosteroids. Moreover, the risk of side effects like peptic ulcer, acne, osteoporosis, and psychoses is higher with long treatment; hence they are not used during remitting periods.

Disease-modifying therapy

Modified MS agents has demonstrated moderate effectivity by preventing relapses periods. However, there are no tools available to prevent the degenerative/chronic state of the disease. The effect of the improvement of the disability is not as important as the previous.

It has been demonstrated that the use of immunosuppressants reduces the amount of relapses forms, decrease the number and size of the Resonance Magnetic Imaging (RMI) lesions, as well as stabilize or defer MS disability. MS treatment with immunosuppressants appeared in the 90's with two injectable agents: Interferons-ß (IFN-ß) (Avonex®) and Glatiramer Acetate (GA) (Copaxone ®) *(Miller A, et al. 2016).*

Treatment	Dosage	Mechanism of action	Effect	Side effects
Azathioprine (Off label) *(Elion GB, et al. 1993).* *(Filippini G, et al. 2013).*	2.5 mg/kg daily or depending on levels of thiopurine methyl-transferase (TPMT). Oral.	Purine analogue which blocks the de novo purine synthesis pathway.	It reduces outbreaks and disability progression.	Hypersensitivity reactions, increase in mean corpuscular volume (MCV) and leukopenia, infections, nausea, vomits, pancreatitis, liver toxicity, reversible pneumonitis, hair loss.
Interferon-ß (IFN-ß) (Avonex ®) 90's *(Miller A, et al. 2016), (Garg N, et al. 2015), (Rudick RA, et al. 1997), (Dargahi N, et al. 2017).*	30 µg once a week. Intramuscular.	Anti-viral and anti-tumoral agent, which modifies the expression of pro- and anti-inflammatory cytokines in the brain as well as reduces the amount of inflammatory cells that go through the blood brain barrier. Furthermore, it reduces IL-17 cytokine and Th17 population, which participate in the immunopathophysiology of MS.	It reduces outbreaks and brain injuries in MRI.	Flu-like symptoms and even liver dysfunction, thyroid abnormalities, leukopenia or anemia, injection site reactions.
Glatiramer Acetate (GA) (Copaxone ®) 90's *(Miller A, et al. 2016), (Garg N, et al. 2015), (Dargahi N, et al. 2017), (Rudick RA, et al 1997), (La Mantia L, et al 2010).*	20 mg daily or 40 mg three times weekly. Subcutaneous.	It transforms Th1 cells into Th2 cells that suppress inflammatory responses.	It reduces outbreaks and brain injuries in MRI. Limited effect on disability progression.	Systemic reaction, flu-like symptoms and even liver dysfunction.
Mitoxantrone *(Bar-Or A, et al. 2014), (Garg N, et al. 2015).*	12 mg/m2 every 3 months. Limit dose: 140 mg/m2. Intravenously	Intercalates with DNA and causes single-and-double-strand breaks, and inhibits DNA repair through inhibition of DNA topoisomerase II. Cytotoxic to stimulated T and B lymphocytes.	It reduces relapse frequency and disability.	Blue coloration of sclera and urine. Necrosis if extravasation occurs. Infections, tumors, secondary leukemias,cardiotoxicity, infertility.

Natalizumab (Tysabri ®) 20's *(Dargahi N, et al. 2017), (Miller DH, et al. 2003), (Polman CH, et al. 2006), (Garg N, et al. 2015).*	300 mg every 4 weeks. Intravenously	Antibody agent, approved in 2006, against the cellular adhesion molecule alfa4-integrin, which reduces T cell. As a result, neuroprotective effects and anti-inflammatory response occur.	It reduces relapse rate and disability progression.	Infections, progressive multifocal leukoencephalopathy, herpesviral encephalitis, hypersensitivity, headache, fatigue.
Fingolimod (Gilenya ®) 20's *(Garg N, et al. 2015), (Miller A, et al. 2016), (Kappos L, et al. 2010).*	0.5 mg once daily Oral	It prevents the exit of the lymphocytes from the lymph node, reducing its infiltration into de CNS.	It reduces relapse rates, disability progression and brain injuries in MRI.	Bradyarrhythmia, lymphopenia, macular edema, infections, hypertransaminasemia, high blood pressure, posterior reversible encephalopathy syndrome (PRES), breathing impairment. Even though, it is considered as a good alternative for patients with highly active Remiting-Relapses forms who prefer oral treatment.
Dimethyl fumarate (DMF) // (Tecfidera ®) 20's *(Dargahi N, et al 2017), (Garg N, et al. 2015).*	240 mg twice daily. Oral.	It decreases the amount of inflammatory cells that cross the blood brain barrier, as well as activate a nuclear factor (erythoroid 2-related factor) that reduces the oxidative and inflammatory cells damage.	It reduces outbreaks and brain injuries in MRI.	Diarrhoea, nausea, abdominal pain, lymphopenia.
Alentuzumab 20's *(Miller A, et al. 2016), (Dargahi N, et al. 2017).*	1st cycle: 12 mg once daily during 5 days (60 mg). First year. 2nd cycle: 12 mg once daily during 3 days (36 mg). Second year. Intravenously	Its targets are CD52 molecule located in the surface of T and B cells so the depletion from circulation occurs.	It reduces outbreaks and brain injuries in MRI and delays disease progression.	Rash, headache, fever, nausea and chills. Infections, late autoimmune reactions, thyroiditis,idiopathic thrombocytopenic purpura, glomerulonephritis, cytopenia, even certain cancers (blood or thyroid cancer).

Ocrelizumab (Ocrevus ®) *(Kappos L, et al. 2011), (Barun B, et al. 2012), (Sorensen PS, et al. 2015).*	600 mg once every 6 months. Intravenously	Evidences: humanized monoclonal antibody against CD20 which produces B-cell depletion by complement-dependent lysis and/or antibody-dependent cytotoxicity.	It reduces relapse rate, disability progression and MRI measures of disease activity.	Infusion-related reactions during first infusion, infections, progressive multifocal leukoencephalopathy (PML).
Rituximab *(Barr TA, et al. 2012), (Sorensen PS, et al. 2015).*	1000 mg with a 15-day interval each 6 months. Intravenously.	It causes B-cell depletion so pro-inflammatory T-cell responses are reduced.	It reduces gadolinium-enhanced lesions and relapse rates.	Infections, infusion-related reactions during first infusion.
Simvastatin (SPMS without MDT) (Off label) *(Chataway J, et al. 2014).*	80 mg per day Oral	Reduction of antigen presentation to MHC class II so it regulates the activation of T cells and the proliferation Th1 to Th2 downwards. Blockade of adhesion molecules and inhibition of the leukocytes migration through the blood-brain barrier. Reduction of nitric oxide producción in the CNS and the activation of astrocytes and microglia.	It reduces MRI activity and MRI brain atrophy by 43 %.	No differences between placebo group in a randomised, placebo-controlled, phase 2 trial.
Cladribine (Mavenclad ® TM) *(Siddiqui MK, et al. 2017), (Schreiner T. L, et al. 2012).*	Cladribine tablets: 0.875 mg/kg over 4-5 days of a 28-day period ar weeks 1, 5, 48 and 52 (total dose: 3,5 mg/kg during a period of 96 weeks). Oral	Inhibition of purine synthesis (T, B and macrophages).	It reduces outbreaks, lesions in RMI and delays disease progression.	Dose-dependent decreases in blood cells as thrombocytopenia or lymphocytopenia. Infections, neoplasm.

Biotin *(Sedel F, et al. 2015), (Tourbah A, et al. 2016).*	100-300 mg per day. Oral	Oligodendrocytes: activation of the Acetyl-CoA carboxylase (ACC) which increase the fatty acids synthesis in order to produce an increase in ATP synthesis needed for the transmission of nerve impulses.	It improves progressive symptoms: vision (80%), gait (90%) and cerebellar symptoms.	Transient diarrhea infections, hyperthyroidism, headache, nausea, back pain, dizziness.

Table 3. Treatment of MS.

Teriflunomide

Teriflunomide (Aubagio®) is one of those immunosuppressants that has demonstrated the decrease of inflammatory activity, brain atrophy and disabilities. It is an oral drug and an active metabolite of leflunomide. Its mechanism of action is based on the reversible inhibition of dihydroorotate dehydrogenase (DHODH), a mitochondrial enzyme implicated in the *de novo* synthesis of pyrimidine for DNA replication for T- and B- lymphocytes proliferation (*figure 1*). Consequently, these cells implicated in MS cannot go through the blood-brain barrier. Teriflunomide is considered to be a cytostatic drug because DHODH inhibition does not affect homeostatic hematopoietic cell lines (also known as "salvage pathway") because they have their own pyrimidine cellular pool. Teriflunomide produces not only the inhibition of DHODH, but also the cytokines, protein tyrosine-kinases; as well as it modifies the expression of cell surface molecules. Moreover, it is thought teriflunomide inhibits the Janus tyrosine kinase (JAK) enzymes JAK1 and JAK3 and reduces transforming growth factor α-induced nuclear factor κB activation.

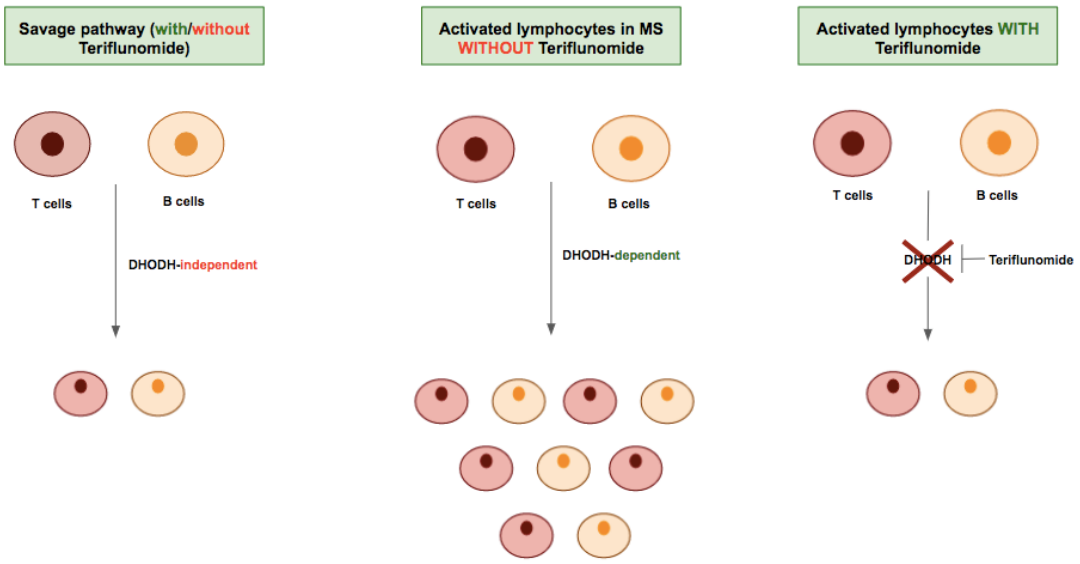

Figure 1. Mechanism of action of teriflunomide.

Its once-daily oral bioavailability is 100% at doses of 5-25 mg/day the plasma half-life is 10-18 days with 1-2 hours peak after the intake. It is distributed in plasma bound to albumin.

It's contraindicated in pregnant women, women of childbearing potential and during breastfeeding, based on observations of teratogenicity in rats and rabbits. If there are pregnancy desires, it is necessary to quit the treatment during 8 months before conception, because it undergoes enterohepatic circulation. If pregnancy or serious side effects appear, cholestyramine or activated charcoal should be taken.

Its side effects are less common than other drugs; that is the reason why its discontinuation is not as frequent. The great majority of the side effects appear during the first 6 weeks, with no further deterioration while patients remained on therapy. The most common are: alanine aminotransferase increase, nausea, diarrhea and alopecia (hair thinning). Nevertheless other side effects can appear such as increased of blood pressure, anxiety, anemia, menorrhagia or peripheral neuropathy. Even though it can decrease the white blood cells count (specially neutropenia), it is not related with an increase of the incidence of serious infections, including serious opportunistic infections. There are no increased risk of malignancy during the treatment. As it only inhibits those cells implicated in MS, it is not considered as an immunosuppressant. *(Antochi F, et al. 2013), (Miller A, et al. 2016), (Dargahi N, et al 2017), (Oh J, et al. 2013), (Chan A, et al. 2016).*

The efficacy, safety and tolerability profile of teriflunomide was demonstrated in three phase III, double-blind, placebo-control trials: TEMSO *(O`Connor P, et al. 2011),* TOWER *(Confavreux C, et al. 2014)* and TOPIC *(Miller AE, et al. 2014).* The TOPIC *(Miller AE, et al. 2014).* study showed that the time to a second clinical episode (relapse) in patients with a first clinical demyelinating event, was significantly lower. Otherwise, TEMSO *(O`Connor P, et al. 2011)* and TOWER *(Confavreux C, et al. 2014)* studies established that teriflunomide 14 mg once-daily significantly reduced the annualized relapse rate and the risk of confirmed disability progression for at least 12 weeks, with favorable safety profile in Relapsing-Remitting MS. Furthermore, they proved the decreased of total lesions volume, gadolinium-enhancing lesions, unique active lesions and brain atrophy, using MRI technique *(Chan A, et al. 2016), (Miller A, et al. 2012).*

Immunopathophysiology

Brain Atrophy

The failure of axonal conduction at the side of the lesion is the main reason of the relapses periods. This is caused by axonal chances that appears because of the acute inflammation and demyelination. The first step that takes place is the rise of the inflammatory markers, such as cytokines, antibodies against ion channels, macrophages and nitric oxide which blocks the axons, specially those affected by demyelination. On the other hand, the myelin damage is segmental which means that exits affected and unaffected parts of the axon. Consequently, the axonal current is blocked and the propagation of the action potential is interrupted *(Sá MJ, et al. 2012).*

These both mechanisms destroy the neurons and cause the lost of neuronal function and brain atrophy. The symptoms of the disease appear when those elements take place *(Dargahi N, et al. 2017).*

It is widely known the relation that exists between MS and corpus callosum (CC) atrophy. CC is the major commissural structure that communicates cortical and subcortical neurons of the two cerebral hemispheres. It can be divided in four different regions: splenium, body, genu and rostrum. (*Erdoğan N, et al. 2005)*. It plays a significant role in the organization of complex commands involving bilateral task that requires inter-hemispheric connection. In MS patients, the morphology of the CC in damaged due to the injury of the communicating neurons since early stages. This resulting disconnection cause the cognitive impairment; mainly verbal and visual memory, information processing speed and executive tasks, as well as physical disability in MS.

CC is easily assessed by magnetic resonance imaging (MRI). Sagittal planes are usually used to study the changes and different stages of the CC in MS. It is measured using the corpus callosum index (CCI) which is obtained on a best mid-sagittal T1 image. It is based on drawing a straight line at greatest anteroposterior diameter of CC and a perpendicular at its midline, owing to points a, b and c: genu (a-a'), splenium (b-b'), body (c-c'), anteroposterior distance (a-b) (*figure 2*). This measures are placed in the following formula of CCI *(Figueiras F, et al. 2006).*

$$CCI = \frac{\mathrm{AA'} + \mathrm{BB'} + \mathrm{CC'}}{\mathrm{AB}}$$

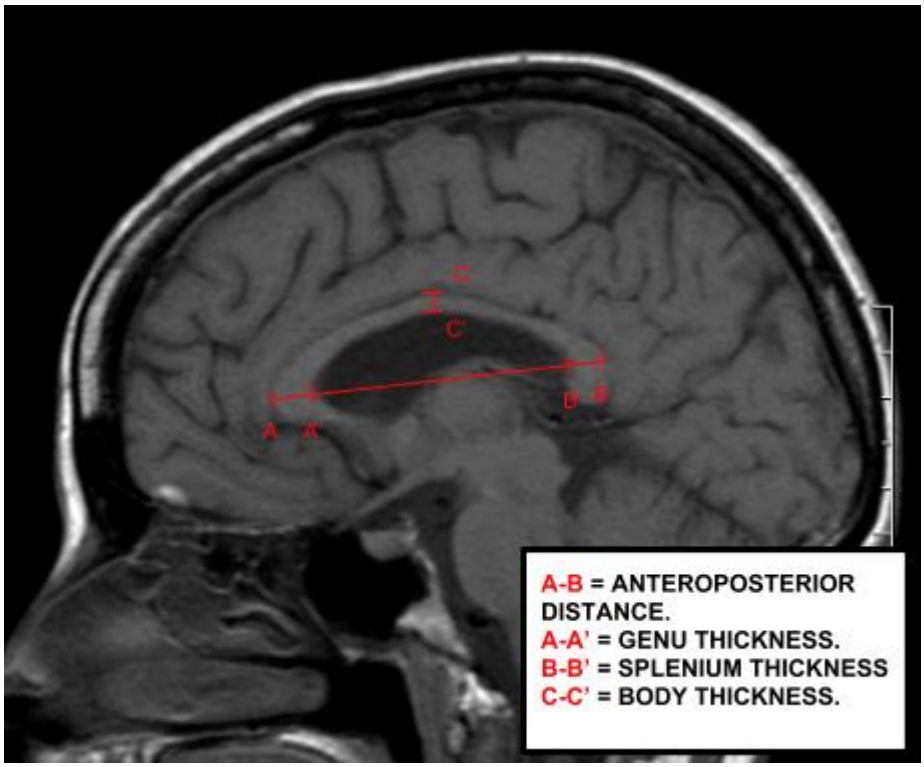

Figure 2. Measurement method of the CC described by Figueiras F, 2006. Interobserver correlation of 0.92. Interobserver correlation of 0.92.

OBJECTIVES

1. To analyze the cohort of patients with MS treated with teriflunomide and its neuroprotective effect with the measurement of the CCI.

2. To study reasons for early withdrawal of the treatment.

3. In case of withdrawal due to inefficacy, the time until the first outbreak will be also analyzed.

METHODS

Study design and ethical statement

In order to calculate the discriminant validity of the CCI, it was necessary to access the clinical-care data of the patients to stratify them by age and sex and to perform the CCI measurement by using images obtained by Magnetic Resonance Imaging collected in the patient's medical history; most of the individuals had already been informed about a previous study, so their profile was included in a pre-existing database that had already obtained the acceptance of the CHUC ethical committee. The study was carried out according to the provisions of the Helsinki Declaration.

The current study was classified as an observational, prospective and retrospective study by the *Agencia Española del Medicamento y productos sanitarios (AEMPS)* and it enrolled 53 patients who met McDonald criteria (2010) for MS treated with teriflunomide.

The study protocol was approved by the Ethics Committee of Hospital Universitario Canarias (San Cristóbal de La Laguna, Spain). The study was carried out according to the International Conference on Harmonization Guidelines for Good Clinical Practice and the Declaration of Helsinki. Every patient provided written informed consent before entering the study.

Montserrat Gonzalez Platas received payment for lecturing or travel expenses or research Grants or consultancy from Merck-Serono, Biogen, Sanofi-Genzyme, Roche, Bayer, Novartis and Allmiral. The remaining authors declare no conflicts of interest.

Patients

Patients treated with teriflunomide who signed the informed consent form will be included in the study. Patients proposed for the study are already treated with teriflunomide. In any case, the use of the drug will be promoted for inclusion.

Inclusion criteria

- Patients who have signed the informed consent form.
- Patients with RR MS who have taken teriflunomide 14 mg once daily during at least one week (2 dosis).
- Age >18 years.
- MS according to the McDonald criteria 2010.
- Expanded Disability Status Scale (EDSS) scores ≤ 5,5.
- Disease duration <30 years.
- This drug is in a special surveillance program, so it requires quarterly clinical visits the first year and analytical monitoring of liver function at the beginning of the treatment. The declaration of undesirable adverse effects will be at https://www.notificaRAM.es.
- None of the exclusion criteria.

Exclusion criteria

- Do not authorise the participation in the study.
- MS patients with hepatic impairment at the beginning of teriflunomide.
- Nursing mothers or pregnant women.
- Women of childbearing potential not using reliable contraception.
- Clinically significant infectious or neurological illness.
- Other pathology related to brain MRI abnormalities.
- Normal kidney function (creatinine clearance >59 mL/min).

Planning criteria for withdrawal from the study

- Discontinuation of treatment.
- Withdrawal of informed consent.

Variables

- Main variables
    - Basal CCI and evolution. This measure is not carried out by the radiology service (reports only refers to the subjective aspect of atrophy). However, in the standard protocol of resonance appears sequences sagittal T1 where we do the measures. The three main segments of the Corpus Callosum are the splenium, the trunk and the knee.
- Secondary variables
    - Age.
    - Gender.
    - Expanded Disability Status Scale (EDSS).
    - Previous treatments.
    - Reason for the change of previous treatments.

Data collection

Training in CCI evaluation techniques

We carried out a 1-hour theoretical-practical training course before the beginning of the essay. We were instructed to correctly evaluate a medial sagittal T1 sequence on conventional brain MRI. For that purpose, we should chose the most appropriate image that offers a better visibility of the anatomy of the corpus callosum to subsequently make the relevant measurements (knee, body, splenium and the anteroposterior distance of the CC), according to the method described by *Figueiras F, 2006.* After the description and display of the procedure by a qualified person, each author made 10 measurements of the corpus callosum under the supervision of the

instructor. The images evaluated were included in the study. At the end of the course, the skills acquired were evaluated before they performed the study independently.

CCI measurement

CCI measurement of the 53 patients included in the study were analyzed using MRI, starting with the one before the beginning of the treatment with teriflunomide until the dropout.

Statistical analysis

Statistical analyses were conducted using SPSS for Windows version 23.0 (IBM Corp., Armonk, NY) The demographic and clinical characteristics at baseline were compared using chi-square and Student's t-test. MRI characteristics were compared using non-parametric statistical methods due to sample size limitations, followed by post-hoc covariate-adjusted models.

RESULTS

The demographic characteristics of our cohort are similar to other studies, with predominance of women (81%), 44 years old, without disability (EDSS 1.5), with RRMS and switched from another DMT (92%) *(table 4).*

The follow-up time of our cohort was 27,24 months. The dropout due to side effects (13%), described in technical sheet (elevation of liver enzymes, diarrhea and weight loss, abdominal pain), occurred during the first trimester, most of them during the first month. The rest are mild and diminish with time. Other side effects appeared in other patients that did not need special treatment.

15% of the patients showed relapses and brain activity in MRI (new T2 lesions and/or enhanced with gadolinium). The average time between the beginning of the treatment and the first relapse was 13,25 months *(table 4).*

	Participants (n=53)
Sex (women)—n (%) Sex (men)—n (%)	43(81.1) 10(18.9)
Age—M (sd) min-max	43.4385 (10.42) 23-82
EDSS at the beginning—min-max(med)	1.0-6.5(1.5)
Time evolution— min-max(med) month Dropout by side effects (7 patients) min-max(med) month Dropout by relapses (8 patients) min-max(med) month	(8-68) 27.24 (0.5-11) 4.07 (4-29)13.25
MS type —n (%) RR Overlap (Sjögren/RRMS)	 51 (96.2) 1 (1.9)
EDSS current moment—min-max(med)	0-6.5(1.5)
DMT—n (%) Without DMT "De Novo" With DMT	 4(7.54) 49(92.45)

Table 4: clinical and demographic characteristics.

Table 5 shows no differences between the first, second and third (3 years after the beginning of the treatment with Teriflunomide) MRI CCI measurement, before and after treatment. There are no evidences of progression of brain atrophy due to a possible neuroprotective effect of Teriflunomide. *(Zivadinov R, et al. 2018)* analyzed brain atrophy with a semiauthomatic method SIENA-SIENAX, it concluded that the brain volumen loss in patients treated with Teriflunomide decrease less than expected (MS patients). EDSS has remained stable during all the treatment period.

PRE-TREATMENT COMPARED WITH FIRST MRI POST-TREATMENT								
	Related differences					t	gl	Sig. (bilateral)
	Average	Standard deviation	Standard error	95 % confidence interval				
				Inferior	Superior			
Par 1 KNEE_PRE-KNEE_1	-,14885	1,40645	,19504	-,54040	,24271	-,763	51	,449
Par2 TRUCK_PRE-TRUCK_1	-,05294	,82363	,11422	-,28224	,17636	-,464	51	,645
Par3 SPLENIUM_PRE-SPLENIUM_1	-,38096	1,53734	,21319	-,80896	,04704	-1,787	51	,080
Par4 CCI_PRE-CCI_1	-,00662	,02766	,00384	-,01432	,00108	-1,726	51	,090
PRE-TREATMENT COMPARED WITH SECOND MRI POST-TREATMENT								
	Related differences					t	gl	Sig. (bilateral)
	Average	Standard deviation	Standard error	95 % confidence interval				
				Inferior	Superior			
Par 1 KNEE_PRE-KNEE_2	-,11618	1,40276	,24057	-,60562	,37327	-,483	33	,632
Par2 TRUCK_PRE-TRUCK_2	,00256	,55547	,09526	-,19125	,19637	,027	33	,979
Par3 SPLENIUM_PRE-SPLENIUM_2	-,19824	1,45736	,24993	-,70673	,31026	-,793	33	,433
Par4 CCI_PRE-CCI_2	-,00219	,02300	,00394	-,01022	,00583	-,556	33	,582
PRE-TREATMENT COMPARED WITH THIRD MRI POST-TREATMENT								
	Related differences					t	gl	Sig. (bilateral)
	Average	Standard deviation	Standard error	95 % confidence interval				
				Inferior	Superior			
Par 1 KNEE_PRE-KNEE_3	-,69455	1,27887	,38559	-1,55370	,16461	-1,801	10	,102
Par2 TRUCK_PRE-TRUCK_3	,10364	,88861	,26793	-,49334	,70061	,387	10	,707
Par3 SPLENIUM_PRE-SPLENIUM_3	-,05182	1,25087	,37715	-,89216	,78853	-,137	10	,893
Par4 CCI_PRE-CCI_3	-,00601	,01710	,00516	-,01749	,00548	-1,165	10	,271

Table 5: Brain atrophy study with CCI in the follow-up treatment with teriflunomide.

DISCUSSION

Patients treated with teriflunomide have similar demographic characteristics compared with others cohortes with a higher age average (10 years), due to most patients (92,45%) received other first line treatments before. The decisions to change the previous DMT to teriflunomide were inefficacy and desire of changing due to local side effects, both in similar proportion.

Our cohort of patients treated with teriflunomide are mainly women (81%). Due to an adequate contraceptive method during the treatment period, problems with pregnancy or breastfeeding were not cause of rejection of treatment.

Early dropouts occurs in 13 % of patients and were due to the side effects described in TEMSO *(O`Connor P, et al. 2011)*, TOWER *(Confavreux C, et al. 2014)*, among others. There was only one case of severe hepatopathy (class 3, 10 times the normal value) that were solved by withdrawal of the treatment during a sustained period of 3 months. This patient refused to the accelerated elimination procedure with the described methods (cholestyramine 8 g/8 hours or activated charcoal 50 g/12 hours).

There was no disability progression and the EDSS remained stable even though cognitive assessment data were not available. Only 15 % of patients had relapses during the study whereas 85% remained stable: NEDA 4 (no relapses, no disability, no new T2 lesions or T1 enhanced gadolinium lesion in MRI, no brain atrophy measured with CCI).

CONCLUSIONS

1. Neuroprotective effect of teriflunomide 14 mg daily in patients with MS has been confirmed. 85 % of our patients remain with a NEDA4.

2. Two reasons for abandoning treatment were identified: an early one (13%) due to side effects occurrence and a lately one (15 %) because of inefficacy proved by relapse development and MRI activity. Time to the first relapse were 13,25 months.

ACQUIRED SKILLS

1. How to prepare a project in English.
2. How to prepare clinical records, specific neurological examination, EDSS assessment, including specific MS items, as well as to select the relevant information for the database.
3. How to measure CC with MRI and to calculate the CCI.
4. How to safeguard patients anonymity.
5. Even though the study was already designed, our incorporation allows to analyze images and record analytical controls and side effects occurrence.
6. A professional statistician was required for the study but we took an important part in the data analysis, the results and the discussion of the present study.

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APPENDIX

Servicio Canario de la Salud
HOSPITAL UNIVERSITARIO DE CANARIAS

Gobierno de Canarias

Titulo del Estudio: Valoración de la atrofia cerebral con el Index del cuerpo calloso, Siena, Sienax en pacientes con esclerosis múltiple tratadas con Teriflunomida.

Nombre del Investigador: Montserrat González Platas.
Centro del investigador: Complejo Hospitalario Universitario de Canarias.

Estimado paciente:

Su médico le invita a participar en un estudio de pacientes con Esclerosis Múltiple (EM) tratados con Teriflunomida (Aubagio®) cuyo objetivo es valorar la eficacia del tratamiento con Teriflunomida en paciente con EM en la disminución dela atrofia cerebral, medida con el Index del CC y comparada con medidas volumétricas (SIENA; SIENAX) y relacionarlo con el deterioro cognitivo y con la discapacidad mantenida.

Antes de que decida su participación, es importante que comprenda los motivos por los cuales se realiza este estudio y en qué consistirá.

Tómese el tiempo que necesite y lea atentamente la información de este documento que le presenta su médico y asegúrese de haberla comprendido bien. Coméntela con sus personas de confianza si lo desea.

Si algo no le queda claro o si desea recibir más información no dude en preguntar.

Su participación en este estudio es completamente voluntaria, pudiendo rechazar su participación o abandonar el mismo en cualquier momento y sin tener que dar por ello ninguna explicación. En este caso nunca se verá afectado el tratamiento que usted está recibiendo para su enfermedad ni existirá merma alguna en la calidad de la asistencia por parte de su médico.

Por favor, firme el consentimiento informado si está de acuerdo en participar en el estudio.

Introducción.

Le invitamos a autorizar los datos existentes en su historia clínica para valorar la eficacia de la Teriflunomida (Aubagio®), para ello recogeremos datos demográficos, status clínico, analítico, psicológicos y radiológicos; tratamientos previos y motivo del cambio.

Este estudio no es un ensayo clínico: Usted recibirá la atención sanitaria habitual de su médico. El protocolo recogerá datos retrospectivos sobre su salud y las afecciones que pueda tener antes y durante el periodo de recogida de datos.

Procedimiento y riesgos del estudio

Participar en este estudio no va a suponer para usted la toma de ninguna medicación adicional.

Tampoco supondrá ningún coste económico para usted ni será recompensado económicamente.

Para el estudio, su médico recogerá datos sobre sus antecedentes médicos, el uso de medicaciones, su estado de salud, efectos secundarios graves, imágenes de RM, etc.

Se introducirán sus datos médicos en una base de datos electrónica central. Esta información se almacenará y transferirá de forma estrictamente anónima, lo que quiere decir que nadie podrá relacionar estos datos con Usted personalmente. Su identidad también permanecerá confidencial si se publican los resultados del programa. "Ley de protección de datos de carácter personal 15/1999 de 13 de diciembre" (texto consolidado 5 de Marzo de 2011).

Otros detalles o dudas sobre el estudio que Usted quiera conocer le serán explicados por su médico o enfermera. Asimismo, cualquier dato relevante para Usted que pudiera surgir durante el estudio le será comunicado también por su médico.

Para cualquier información adicional puede ponerse en contacto con su médico:

Yo, el abajo firmante, he explicado íntegramente al paciente nombrado más arriba los detalles de este programa tal como viene descrito en el Protocolo.

Nombre del médico: Montserrat González Platas
Firma del médico:

La Laguna, a de del 2016

CONSENTIMIENTO INFORMADO POR ESCRITO
Yo,
.....
(Nombre y apellidos del participante en el estudio escritos por el paciente)

He leído la hoja de información sobre el estudio que me han entregado.
Lo he comentado con el médico Montserrat González Platas contestando a mis preguntas de forma satisfactoria.
Comprendo que mi participación es voluntaria
Comprendo que puedo retirarme del estudio:
−Cuando quiera.
−Sin tener que dar explicaciones.

−Sin que esto repercuta en mis cuidados médicos.
En las condiciones anteriores, presto libremente mi conformidad para participar en el estudio.

FECHA		NOMBRE Y APELLIDOS		FIRMA DEL PARTICIPANTE

FECHA		NOMBRE Y APELLIDOS DEL INVESTIGADOR		FIRMA DEL INVESTIGADOR

Universidad de La Laguna

ULL | Universidad de La Laguna | Facultad de Medicina

Trabajo Fin de Grado
Conformidad para la presentación de la memoria y defensa

Dr/Drs Montserrat González Platas y Carlos Emilio González Reimers, tutor/tutores del trabajo realizado por el alumno(s)/a(s) Sara Duque González, Rebeca de Luis Sosa y Alba Rodríguez González con el título ***"Assessment of brain atrophy with the corpus callosum index in patients with multiple sclerosis treated with teriflunomide"*** damos nuestra aprobación para la presentación de la memoria y a su defensa como Trabajo Fin de Grado.

La Laguna, 3 de mayo de 2018

Fdo: Montserrat González Platas

Fdo: Carlos Emilio González Reimers