"The PROnostic Value of Cholesterol Esters in the Progression of Multiple Sclerosis" (PROCOLEM)

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1- Resumen divulgativo para pacientes:

Los resultados de nuestro estudio mostraron que los pacientes con más afectación tras 10 años de enfermedad (los que tenían una enfermedad más agresiva, los que puntuaban >4 puntos en la escala EDSS, los que tenían lesiones medulares y en los que su enfermedad estaba activa bien fuera a nivel de brotes, nuevas lesiones en la Rm o progresión de la discapacidad) tenían unos niveles más elevados del éster de colesterol 18:2 (CE18:2) en el LCR del momento del diagnóstico. En base a estos hallazgos, pensamos que el CE18:2 podría ser un biomarcador pronóstico de la enfermedad. Por lo tanto, podría servir de ayuda a la hora de elegir un tratamiento de moderada, alta o muy alta eficacia en cada caso. Además, interpretamos que estos niveles elevados de CE18:2 en pacientes con una EM más avanzada reflejarían una mayor tasa de destrucción de la mielina o bien un defecto en la vía de remielinización. Son necesarios más estudios en esta línea para dilucidar los mecanismos subyacentes y las implicaciones clínicas de estos hallazgos y potencialmente allanar el camino para el desarrollo de estrategias terapéuticas personalizadas para pacientes con EM.

2-Abstract

Background: Multiple Sclerosis (MS) is an autoimmune disease in which autoantibodies recognize myelin, triggering focal inflammation in nervous tissue. MS is classified by the course of the disease: Relapsing-Remitting MS (RRMS; active lesions, which are limited in time and reversible); Secondary Progressive MS (SPMS; irreversible with increasing disability after an RRMS phase); and Primary Progressive MS (PPMS; irreversible with increasing disability without an RRMS phase). Disease Modifying Therapies (DMTs) are highly efficient in reducing the number of relapses in RRMS patients. However, there are no DMTs capable of modifying the progression of disability in SPMS. A lack of cholesterol

esterification (CE) is a potential disease mechanism, since it is implicated in the remyelination process and can be used as a surrogate biomarker to select potential candidates for DMT targeting this mechanism. We have previously observed by non-targeted lipidomics (LC-MS QTOF) differences in 18:2 and 20:2 cholesterol ester levels in CSF of patients with agressive versus bening course of the disease. The aim of this study is to analyze the potential prognostic biomarker of 18: 2 (CE18:2) and 20: 2 cholesterol esters (CE20:2) by a more sensitive and specific targeted method (triple quadrupole). With this information, we can improve patient follow-ups and design new clinical trials for the early treatment of SPMS.

Material and Methods: This is a retrospective case-control study consisting of 127 MS patients (21 PPMS and 106 RRMS), who were diagnosed with MS using McDonald's revised criteria (Thompson et al., 2018), and 58 controls (subjects with different characteristics: non-demyelinating and non-inflammatory neurological diseases). The CSF samples were obtained at the time of diagnosis of the disease. There was then a follow up period of a minimum of 10 years to obtain the degree of disability, which was assessed using the EDSS scale. In this study, patients with MS were classified into 4 groups based on the severity of their illness (benign/aggressive) and its progression (progressive /non-progressive).

Results: It was not possible to quantify the ester 20:2. However, we found higher levels of 18:2 cholesterol ester in patients with: BOC+, aggressive forms of MS, spinal cord lesions, EDSS >4.5 vs ≤4, and no NEDA after 10 years of evolution of the disease.

Conclusions: Our findings demonstrate the utility of using targeted lipidomic analysis to identify specific cholesterol esters, such as cholesteryl ester 18:2, which could serve as potential markers for disease progression in MS. This CE accumulation could indicate either a higher rate of myelin destruction or a failure in one of the steps following cholesterol esterification in the remyelination pathway. Furthermore, the toxic effect of free CE would tend to inhibit the remyelination process. These results build upon previous untargeted lipidomic studies and underscore the importance of cholesterol ester metabolism in MS pathogenesis. Further research is needed to elucidate the underlying mechanisms and clinical implications of these findings and to potentially

pave the way for the development of personalized therapeutic strategies for MS patients.

3-Background to the subject and the scientific interest of the project.

Multiple sclerosis (MS) is a highly complex disease which affects the central nervous system (CNS) and is characterized by multifactorial pathogenesis. It has a very heterogeneous clinical presentation (Karussis, 2014) and is the leading cause of disability in young adults (Zéphir, 2018). One very active field of current research involves searching for new biomarkers that will help us to better understand the etiology of MS, aid in its diagnosis and prognosis, and facilitate the implementation of personalized medical treatments (Comabella & Montalban, 2014; Grecchi et al., 2012; Housley, Pitt, & Hafler, 2015; Reinke et al., 2014). One of the most important hypotheses concerning the origins of MS is that this disease is a consequence of an autoimmune response to autoantigens in genetically susceptible individuals (Ortiz et al., 2013; Sospedra & Martin, 2005). In MS, the immune system has been shown to attack myelin, causing demyelination and axonal damage to neurons (Sospedra & Martin, 2005). The myelin sheath is rich in lipids (70-85% by dry weight), containing about 700 different lipid species and - in particular - sphingolipids and glycerophospholipids (Cermenati et al., 2015; O'Brien & Sampson, 1965), which play putative roles in MS and in other demyelinating diseases (Schmitt, Cantuti Castelvetri & Simons, 2015). As a result, several authors have suggested that lipid mediators could be involved in autoimmune attacks on neurons (Gonzalo et al., 2012; Kanter et al., 2006). To date, several different patterns of lipid antibodies have been identified which are related to different stages of the disease (Kanter et al., 2006). In addition, lipid-specific oligoclonal IgM bands have been associated with several relevant conditions observed throughout the course of the

disease. These include: cerebral atrophy, increased injury burden from early stages of the disease, and disability (Villar et al., 2015; Villar et al., 2005).

Higher concentrations of phospholipids and lower levels of sphingolipids have also been reported in the brain tissue of MS patients (Wheeler, Bandaru, Calabresi, Nath, & Haughey, 2008), as have altered levels of certain ceramides (Moscatelli & Isaacson, 1969).

It is not yet clear, however, whether the components of cerebrospinal fluid (CSF) contribute to the destruction of myelin and to axonal loss, or if its presence is the consequence of these processes (Dutta et al., 2006). Whatever the case, the results of different studies suggest that fatty acids (GAs) are important regulators of axon/neuron well-being (Klosinski et al., 2015; Tafferner et al., 2016). Different lipid species play different roles in the CNS: cholesterol drives synaptogenesis in CNS neurons; GAs are precursors of structural and signalling lipids; corticosteroids and prostaglandins are mediators of inflammation; and glycerophospholipids and glycosphingolipids are involved in membrane biogenesis (Cermenati et al., 2015). Changes in lipid metabolism have been reported in various CNS diseases (Adibhatla & Hatcher, 2007) and lipidomics is an important resource for the study of multifactorial diseases (Castro-Perez et al., 2010). Under pathological conditions, cells can undergo metabolic changes that could alter their lipidomes. Such changes can be detected in the cerebrospinal fluid (CSF), as it reflects the brain environment thanks to its close contact with that organ and its nutritional functions.

Our group recently published an article in which we concluded that the description of the lipidomic profile of the CSF at the time of diagnosis could help us better understand the pathophysiology of MS in its early stages (Nogueras et al., 2019). This study has also helped us to define the role of lipid metabolism in disease progression and has led us to propose new biomarkers for monitoring the disease, and also new therapeutic targets. It should be noted that fingolimod, which is an effective treatment for MS, is an antagonist of sphingosine-1-phosphate (sphingolipid). This highlights the importance of this family of lipids, whose levels in the CSF seem to be altered after the onset of disease.

Subsequent analysis of our unpublished data revealed that two cholesterol esters (18:2 and 20:2) could serve as potential prognostic biomarkers, as they exhibited statistically significant differences in their levels in the cerebrospinal fluid (CSF) of patients with aggressive courses of MS compared to in those with benign courses. 18:2 cholesterol had a high specificity (0.8 in ROC curve analysis), while 20:2 cholesterol showed a high degree of sensitivity (0.8 in ROC). However, our research had certain limitations and these data were obtained from a cohort with a relatively small sample size (N = 36). Furthermore, a non-directed approach was followed, which made reproducibility rather limited. We also used equipment whose availability is limited (implying difficulties with validation by other laboratories) and identification was not carried out using a pure standard for the particular metabolite. Even so, the fact that we obtained two results from the same family with the same outcome reinforces the idea that the combined use of the two cholesterol esters could offer a useful prognostic biomarker for the disease. Our research also pointed to a pathological mechanism in a certain group of patients. This could also be used to define groups in clinical trials involving drugs aimed at modulating cholesterol esterification. Along these lines, the importance of cholesterol esterification for the correction of myelin defects is a subject that has recently been discussed as an essential step for remyelination in MS (Gouna et al., 2021). One strategy for counteracting neurodegeneration is to promote neuroprotection by improving myelin regeneration and thus restoring nerve conduction and metabolic support to the axon (Lubetzki et al., 2020). Failure to produce remyelination contributes to axonal loss and to the progression of disability in MS. Failures in the repair process could be due to ongoing toxic neuroinflammation and/or to an inhibitory lesion microenvironment that prevents the differentiation of oligodendrocyte progenitor cells into myelin-forming oligodendrocytes (Ghorbani et al., 2021).

Our hypothesis is that differences in the levels of cholesterol esters in the CSF of MS patients with more aggressive progressions indicate a deficit in remyelination which aggravates the injury, leading to a higher degree of disability. Absolute quantification by lipidomics, carried out at the time of diagnosis, could be used to predict disease progression in patients.

The main objective of this work was to quantify 18: 2 and 20: 2 cholesterol esters in MS patients with varying degrees of progression and to analyse their potential as prognostic biomarkers.

Studying lipids could be an important tool and help to better understand the biological processes that occur at the onset of MS. It could even aid in the diagnosis/prognosis of MS and pave the way for new therapeutic targets such as remyelination.

4-Materials and methods (including the approximate duration of the study).

The patients included in this study were diagnosed at the multiple sclerosis unit of the Arnau de Vilanova University Hospital in Lleida. Patient follow-up was conducted through biannual visits. Assessment included measurements using the Expanded Disability Status Scale (EDSS) score, a progression test [either a 25-step test (T25-FW) or the Nine-Hole Peg Test (9-HPT)] and a neuro-psychological study. We included (1) patients who met the diagnostic criteria for MS based on the 2010 McDonald criteria, and (2) patients for whom a cerebrospinal fluid (CSF) sample was available at the time of diagnosis. The EDSS scores of all the patients were validated by a Neurostatus-EDSS certified physician. Any relapses were recorded by the treating neurologist at the onset of new, or recurrent, MS symptoms lasting for ≥24 hours. Confirmed and sustained disability worsening (CDW) events were based on EDSS and defined by an increase in EDSS (≥ 1.5 points for patients with a baseline EDSS of zero; ≥ 1.0 points for patients with a baseline EDSS of 1-5; and by 0.5 points for patients with a baseline EDSS of ≥5.5) which was confirmed by another EDSS assessment made at least 3 or 6 months after the onset of the worsening. We categorized CDW events as either RAW or PIRA.

i. RAW was related to a 3- or 6- month CDW event with an onset within 90 days of the investigator-reported relapse (irrespective of the EDSS confirmation)
ii. PIRA was defined as a 3- or 6- month CDW event with either no prior relapse or an onset more than 90 days after the last investigator-reported relapse (irrespective of the EDSS confirmation). In addition, to qualify as a PIRA

event, there could be no relapse within 30 days (either before or after) of the EDSS confirmation.

NEDA3 (No Evidence of Disease Activity 3) refers to a state in which a patient shows no evidence of disease activity across three key parameters. These parameters commonly include:

-Clinical Stability: Absence of relapses or of any worsening of symptoms.

-No Disability Progression: No advance or deterioration in disability status.

-No Radiological/MRI Activity: No new lesions or enhancement in magnetic resonance imaging (MRI) scans.

This was a retrospective case-control study involving 127 MS patients (21 PPMS and 106 RRMS) who were diagnosed using McDonald's revised criteria (Thompson et al., 2018) and 58 controls (subjects with different characteristics: non-demyelinating and non-inflammatory neurological diseases). CSF samples were obtained at the time of the diagnosis of the disease and there was a follow up period of a minimum of 10 years to obtain the degree of disability, which was assessed using the EDSS scale. In the study, patients with MS were classified into 4 groups based on severity (benign/aggressive) and disease progression (progressive /non-progressive):

- **Benign**: EDSS ≤3 with 10 years of evolution without any progression of disability except for random outbreaks and without high-efficacy treatments.

- **Aggressive**: EDSS ≥6 after 10 years of evolution or with high-efficacy treatments over 10 years.

- **Non-progressive forms**. Patients with 10 or more years of evolution with no evidence of progression: increases of less than 1 point on the EDSS scale, without considering relapses (>0.5 if EDSS was 6 or more), progression <20% in the 25-step test (T25-FW) or

in the Nine-Hole Peg Test (9-HPT), and with the same number of, or more, symbols in the Symbol Digit Modalities Test (SDMT).

- **Progressive forms.** Patients with 10 or more years of evolution, with evidence of disease progression (increase >1 point on the EDSS scale in the previous year without any relapses (>0.5 if EDSS was 6 or more) or progression > 20% in the 25-step test (T25-FW) or in the Nine-Hole Peg Test (9-HPT), or 5 or fewer symbols in the SDMT.

The study was evaluated by the local ethics committee of the Arnau de Vilanova University Hospital (Lleida, Spain): CEIC-2719. This was carried out in accordance with the Code of Ethics of the World Medical Association (Helsinki Declaration). Informed consent was obtained from all participants at the time of their lumbar punctures and included a subsection in which we were given the samples for future research. All the patients signed to give their consent. 18: 2 and 20: 2 cholesterol ester standards were purchased with the C13 isotope (from Merck, Avanti Polar Lipids or similar). A Triple Quad 6420 LC / MS Agilent Technologies (TQD) mass detection system was used for sample analysis. The processing of the samples was carried out by the Lipidomics Service of IRBLleida.

5- Results

Although it was not possible to quantify ester 20:2, ester 18:2 was quantified, along with four other esters that are still pending identification.

First, we compared 18:2 cholesterol ester levels between the controls and the MS patients. We found higher levels in MS patients (321371 ± 19738 vs. 35889 ± 18652; $p > 0.05$), although the difference was not statistically significant (Figure 1).

MS patients with BOC+ had higher levels of the 18:2 cholesterol ester in their cerebrospinal fluid than those with BOC- (38602 ± 19947 vs 31538 ± 15153; $p < 0.05$) (Figure 2).

When we compared 18:2 cholesterol ester levels in the total group of MS patients (RR and PP) between benign (EDSS =/<3 with 10 years of evolution without progression of disability other than sporadic outbreaks) and aggressive (EDSS =/>6 at 10 years) forms, we found significant differences, with higher levels in the latter group (34307 ± 18473 vs. 41321 ± 19573; $p < 0.05$) (Figure 3).

Moreover, when we compared 18:2 cholesterol ester levels in patients with RRMS between benign (EDSS =/<3 with 10 years of evolution without progression of disability other than sporadic outbreaks) and aggressive (EDSS =/>6 at 10 years of evolution) forms, we found significant differences, with higher levels in the latter group (34477 ± 18711 vs. 44156 ± 22593; $p < 0.05$) (Figure 4).

Next, we attempted to determine the EDSS score at which the 18:2 cholesterol ester levels reached statistical significance, in both the total MS and RRMS groups. We found that patients with an EDSS score of ≤ 4 had lower levels of 18:2 cholesterol ester than those with EDSS scores of >4 (32079 ± 17519 vs. 39612 ± 19303; $p < 0.05$) in both the total MS group (Figure 5) and the RRMS group (32227 ± 17744 vs. 42044 ± 22471; $p < 0.05$) (Figure 6).

In the case of progression, we observed that patients without PIRA had lower levels of 18:2 cholesterol ester than those with PIRA in both the total MS group (33147 ±17771 vs 38834± 19879; $p > 0.05$) (Figure 7) and the RRMS group (33111 ± 17893 vs. 40638 ± 23341; $p > 0.05$) (Figure 8). However, these differences did not reach statistical significance. When we analysed the progression of cognitive impairment, we also failed to find any statistically significant differences between the groups. We found no differences when comparing patients with cognitive impairment and those with conserved cognition (31963 ± 17167 and 33218 ± 16237: $p<0.05$)

Regarding the presence of RAW, we did not find any differences between RRMS patients without RAW and those with RAW after the relapse that prompted the study (34674 ± 18905 vs. 36003 ± 19731; $p > 0.05$).

When we studied 18:2 cholesterol ester levels with respect to the radiological locations of demyelinating lesions, we found that MS patients who did not experience spinal cord injuries had lower levels of 18:2 cholesterol ester than those with spinal cord lesions throughout the 10-year development of the disease (23837 ± 12087 vs. 34402 ± 17075; $p < 0.05$) in both the total MS group and in the RRMS group (20769 ± 6036 vs. 34109 ± 18559; $p < 0.05$) (Figure 9 and 10). We did not find any statistically significant differences with regard to the other territories studied (supratentorial, brainstem, and cerebellum). We similarly found no differences regarding whether the relapse that prompted the study was spinal or had another location (32777 ± 25098 vs.34650 ± 14913; $p<0.05$).

Finally, we found that RRMS patients with NEDA-3 after 10 or more years of disease had lower levels of 18:2 cholesterol ester than those in the RRMS group who did not have NEDA-3 (25815 ± 13116 vs. 38527 ± 20321) (Figure 11).

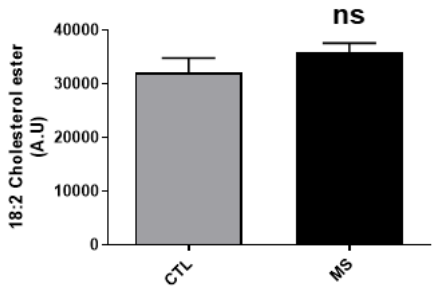

Figure 1. Levels of 18:2 cholesterol ester between controls and MS patients.

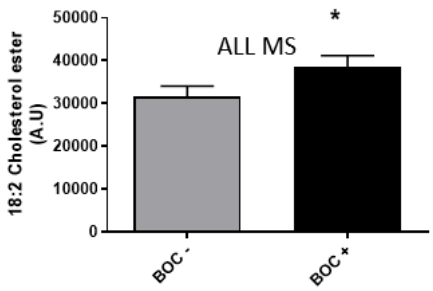

Figure 2. Comparison of 18:2 cholesterol ester levels between MS patients with BOC- and BOC+ in their cerebrospinal fluid.

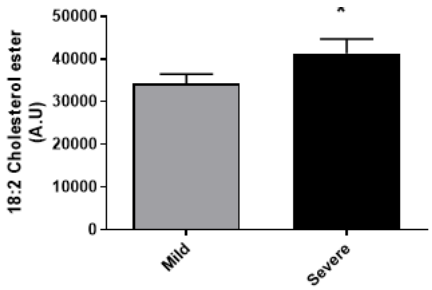

Figure 3. Comparison of 18:2 cholesterol ester levels between benign and aggressive cases in the total group (RR and PP) of MS patients.

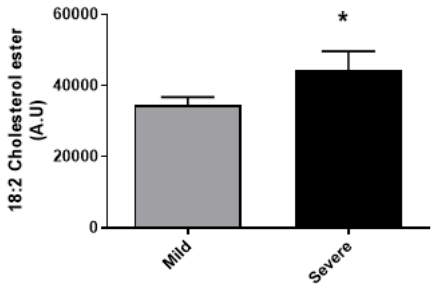

Figure 4. Comparison of 18:2 cholesterol ester levels between benign and aggressive cases in RRMS patients.

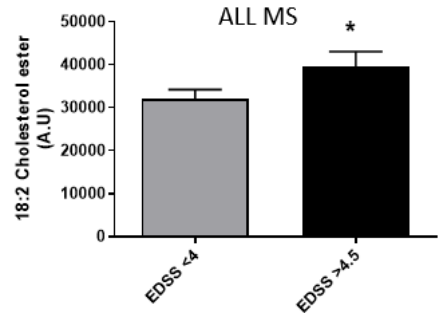

Figure 5. Comparison of 18:2 cholesterol ester levels between MS patients with EDSS ≤ 4 and those with EDSS > 4.

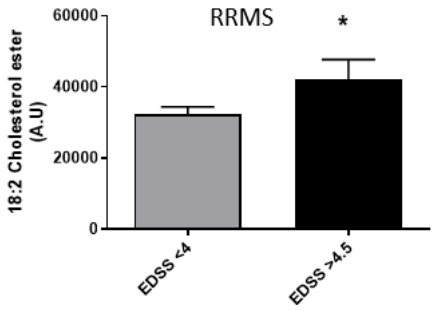

Figure 6. Comparison of 18:2 cholesterol ester levels between RRMS patients with EDSS ≤ 4 and EDSS > 4.

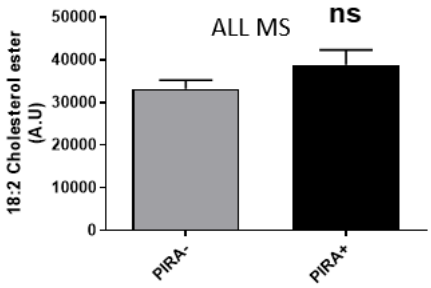

Figure 7. Comparison of 18:2 cholesterol ester levels between patients with and without PIRA in the total group (RRMS and PPMS).

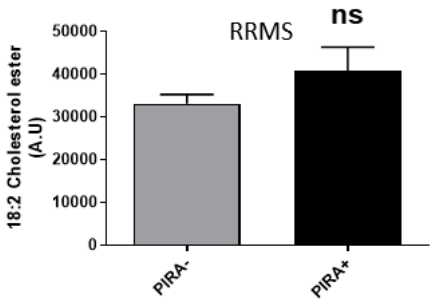

Figure 8. Comparison of 18:2 cholesterol ester levels between patients with and without PIRA in the RRMS group.

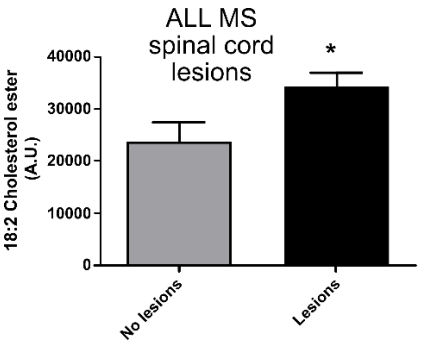

Figure 9. Comparison of 18:2 cholesterol ester levels between patients with and without spinal cord lesions in the total group (PPMS and RRMS).

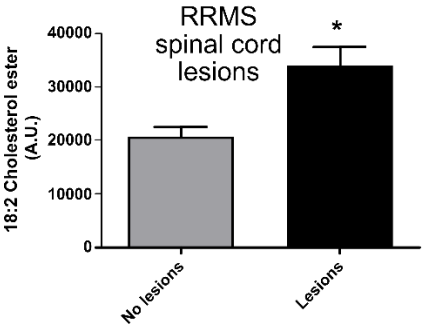

Figure 10. Comparison of 18:2 cholesterol ester levels between patients with and without spinal cord lesions in the RRMS group.

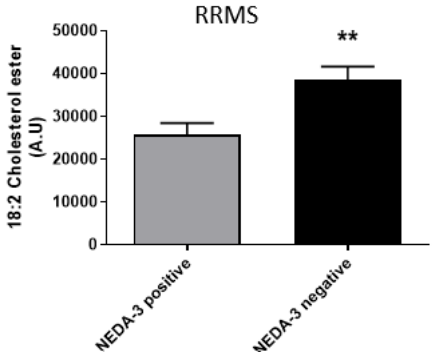

Figure 11. Comparison of 18:2 cholesterol ester levels between RRMS patients with NEDA-3 and without NEDA-3 after 10 years of disease duration.

6- Discussion:

We found significantly higher levels of CE18:2 in patients with a worse prognosis after 10 years of follow-up. This applied to both patients with aggressive courses of disease compared with those with benign forms, and also to those with EDSS >4 and ≤4. Moreover, we found significantly higher levels of CE18:2 in patients who had spinal cord lesions throughout the disease, those who had BOC+ in CSF, and finally, those who were not in NEDA 3 after 10 years of disease evolution.

It is known that brain cholesterol concentration remains relatively constant under normal conditions, with a small fraction replaced via de novo synthesis. Brain cholesterol turnover can be very slow in adult brains with a half-life of 0.5 to 5 years owing to the restrictive nature of the blood-brain barrier and CSF barriers. However, cholesterol levels may be altered in situations of disease and aging (Qian L, et al., 2022). When there is too much cholesterol in the body, it can be converted into what are known as cholesteryl esters and stored in special compartments within cells, called lipid droplets. In the brain, these cholesteryl esters account for only about 1% of the total cholesterol. This conversion process is facilitated by a protein called ACAT1, which is located in a cellular structure called the endoplasmic reticulum. ACAT1 is primarily active in neurons: a specific type of brain cell, and less so in glial cells: another type of brain cell. However, under certain conditions, such as when cholesterol levels are very high, or in the absence of a protein called ApoE, astrocytes may also produce cholesteryl esters using ACAT1 (Qian L, et al., 2022).

These lipid droplets play an important role. They not only protect cells from harmful levels of excess lipids but also act as storage units for cholesteryl esters and triglycerides. Through the mechanism of lipolysis, these stored molecules can then be converted back

into free cholesterol and fatty acids, when the cell needs them for tasks such as cell communication, building cell membranes, and generating energy (Ralhan et al., 2022

Based on our results, we believe that there is no failure in the cholesterol esterification process in MS patients, as even those with an aggressive course exhibited high levels of this CE. This CE accumulation could, instead, indicate a higher rate of myelin destruction or a failure during one of the steps that follows cholesterol esterification along the remyelination pathway. Between these two possibilities, we would lean towards the idea that this accumulation of CE could not be explained by the initial rate of myelin destruction. The reason of which is we did not find any correlation between CE18:2 levels and the presence of relapse-associated worsening (RAW) in the episode that prompted the CFS study. It was also not associated with the episode being spinal, or with the presence of asymptomatic lesions in the first MRI.

We therefore believe that this could indicate a subsequent defect in the remyelination pathway which causes these CE to accumulate in the cerebrospinal fluid (CSF) instead of being reintegrated into lipid membranes during remyelination. This defect could well be at the level of lipid droplet formation or lipolysis, or due to a loss of these CE through disruptions in the blood-brain barrier.

With the techniques used in our study, it was not possible to differentiate between the proportion of CE found in a free form and that present in lipid droplets. We think that this differentiation could be interesting since free CE are toxic and may inhibit the remyelination process. Expanding studies in this line could help to improve our understanding of this pathway.

7- Conclusions

Our findings demonstrate the utility of using targeted lipidomic analysis to identify specific cholesterol esters, such as cholesteryl ester 18:2, which could serve as potential markers for disease progression in MS. This CE accumulation could point to a higher rate of myelin destruction and/or a failure at one of the steps following cholesterol esterification in the remyelination pathway. Furthermore, it seems that the toxic effect of free CE should inhibit the remyelination process. These results build upon previous untargeted lipidomic studies and underscore the importance of cholesterol ester metabolism in MS pathogenesis. Further research is needed to elucidate the underlying mechanisms and the clinical implications of these findings and to potentially pave the way for the development of personalized therapeutic strategies for MS patients.

8- Actividades divulgativas:

- Comunicación tipo póster en congreso ECTRIMS octubre 2023

- Comunicación oral en congreso SEN noviembre 2023

- Comunicación en Reunión RETREAT grupo neuroinmunología noviembre 2023

- Entrevista radio nacional de España 18/12/23

- Publicación artículo a lo largo del 2023-2024

Bibliography

Adibhatla, R. M., & Hatcher, J. F. (2007). Role of Lipids in Brain Injury and Diseases. *Future Lipidology*, *2*(4), 403–422. https://doi.org/10.2217/17460875.2.4.403

Beesley, R., Anderson, V., Harding, K. E., Joseph, F., Tomassini, V., Pickersgill, T. P., … Tallantyre, E. C. (2018). Impact of the 2017 revisions to McDonald criteria on the diagnosis of multiple sclerosis. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, *24*(13), 1786–1787. https://doi.org/10.1177/1352458518778007

Castro-Perez, J. M., Kamphorst, J., Degroot, J., Lafeber, F., Goshawk, J., Yu, K., … Hankemeier, T. (2010). Comprehensive LC-MS E lipidomic analysis using a shotgun approach and its application to biomarker detection and identification in osteoarthritis patients. *Journal of Proteome Research*, *9*(5), 2377–2389. https://doi.org/10.1021/PR901094J

Cermenati, G., Mitro, N., Audano, M., Melcangi, R. C., Crestani, M., De Fabiani, E., & Caruso, D. (2015). Lipids in the nervous system: from biochemistry and molecular biology to patho-physiology. *Biochimica et Biophysica Acta*, *1851*(1), 51–60. https://doi.org/10.1016/J.BBALIP.2014.08.011

Comabella, M., & Montalban, X. (2014). Body fluid biomarkers in multiple sclerosis. *The Lancet. Neurology*, *13*(1), 113–126. https://doi.org/10.1016/S1474-4422(13)70233-3

Dutta, R., McDonough, J., Yin, X., Peterson, J., Chang, A., Torres, T., … Trapp, B. D. (2006). Mitochondrial dysfunction as a cause of axonal degeneration in multiple sclerosis patients. *Annals of Neurology*, *59*(3), 478–489. https://doi.org/10.1002/ana.20736

Ghorbani S, Yong VW. The extracellular matrix as modifier of neuroinflammation and remyelination in multiple sclerosis. Brain. 2021 Aug 17;144(7):1958-1973. doi: 10.1093/brain/awab059.

Gonzalo, H., Brieva, L., Tatzber, F., Jové, M., Cacabelos, D., Cassanyé, A., … Portero-Otin, M. (2012). Lipidome analysis in multiple sclerosis reveals protein lipoxidative damage as a potential pathogenic mechanism. *Journal of Neurochemistry*, *123*(4), 622–634. https://doi.org/10.1111/J.1471-4159.2012.07934.X

Gouna, G., Klose, C., Bosch-Queralt, M., Liu, L., Gokce, O., Schifferer, M., … Simons, M. (2021). TREM2-dependent lipid droplet biogenesis in phagocytes is required for remyelination. *The Journal of Experimental Medicine*, *218*(10).

https://doi.org/10.1084/JEM.20210227

Grecchi, S., Mazzini, G., Lisa, A., Armentero, M. T., Bergamaschi, R., Romani, A., … Scovassi, A. I. (2012). Search for cellular stress biomarkers in lymphocytes from patients with multiple sclerosis: a pilot study. *PloS One*, *7*(9). https://doi.org/10.1371/JOURNAL.PONE.0044935

Housley, W. J., Pitt, D., & Hafler, D. A. (2015). Biomarkers in multiple sclerosis. *Clinical Immunology (Orlando, Fla.)*, *161*(1), 51–58. https://doi.org/10.1016/J.CLIM.2015.06.015

Kanter, J. L., Narayana, S., Ho, P. P., Catz, I., Warren, K. G., Sobel, R. A., … Robinson, W. H. (2006). Lipid microarrays identify key mediators of autoimmune brain inflammation. *Nature Medicine*, *12*(1), 138–143. https://doi.org/10.1038/NM1344

Karussis, D. (2014). The diagnosis of multiple sclerosis and the various related demyelinating syndromes: a critical review. *Journal of Autoimmunity*, *48–49*, 134–142. https://doi.org/10.1016/J.JAUT.2014.01.022

Klosinski, L. P., Yao, J., Yin, F., Fonteh, A. N., Harrington, M. G., Christensen, T. A., … Brinton, R. D. (2015). White Matter Lipids as a Ketogenic Fuel Supply in Aging Female Brain: Implications for Alzheimer's Disease. *EBioMedicine*, *2*(12), 1888–1904. https://doi.org/10.1016/J.EBIOM.2015.11.002

Lubetzki C, Zalc B, Williams A, Stadelmann C, Stankoff B. Remyelination in multiple sclerosis: from basic science to clinical translation. Lancet Neurol. 2020 Aug;19(8):678-688. doi: 10.1016/S1474-4422(20)30140-X.

Moscatelli, E. A., & Isaacson, E. (1969). Gas liquid chromatographic analysis of sphingosine bases in sphingolipids of human normal and multiple sclerosis cerebral white matter. *Lipids*, *4*(6), 550–555. https://doi.org/10.1007/BF02531040

Naudí, A., Cabré, R., Jové, M., Ayala, V., Gonzalo, H., Portero-Otín, M., … Pamplona, R. (2015). Lipidomics of Human Brain Aging and Alzheimer's Disease Pathology. In *International Review of Neurobiology* (Vol. 122, pp. 133–189). Academic Press Inc. https://doi.org/10.1016/bs.irn.2015.05.008

Nogueras, L., Gonzalo, H., Jové, M., Sol, J., Gil-Sanchez, A., Hervás, J. V., … Brieva, L. (2019). Lipid profile of cerebrospinal fluid in multiple sclerosis patients: a potential tool for diagnosis. *Scientific Reports*, *9*(1), 11313. https://doi.org/10.1038/S41598-019-47906-X

O'Brien, J. S., & Sampson, E. L. (1965). Lipid composition of the normal human brain: gray matter, white matter, and myelin. *Journal of Lipid Research*, *6*(4), 537–544.

Ortiz, G. G., Pacheco-Moisés, F. P., Bitzer-Quintero, O. K., Ramírez-Anguiano, A. C., Flores-Alvarado, L. J., Ramírez-Ramírez, V., … Torres-Sánchez, E. D. (2013). Immunology and oxidative stress in multiple sclerosis: clinical and basic approach. *Clinical & Developmental Immunology*, *2013*. https://doi.org/10.1155/2013/708659

Qian L, Chai AB, Gelissen IC, Brown AJ. Balancing cholesterol in the brain: from synthesis to disposal. Explor Neuroprot Ther. 2022;2:1-27. https://doi.org/10.37349/ent.2022.00015

Ralhan I, Chang CL, Lippincott-Schwartz J, Ioannou MS. Lipid droplets in the nervous system. J Cell Biol. 2021 Jul 5;220(7):e202102136. doi: 10.1083/jcb.202102136. Epub 2021 Jun 21. PMID: 34152362; PMCID: PMC8222944.

Reinke, S. N., Broadhurst, D. I., Sykes, B. D., Baker, G. B., Catz, I., Warren, K. G., & Power, C. (2014). Metabolomic profiling in multiple sclerosis: insights into biomarkers and pathogenesis. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, *20*(10), 1396–1400. https://doi.org/10.1177/1352458513516528

Schmitt, S., Cantuti Castelvetri, L., & Simons, M. (2015). Metabolism and functions of lipids in myelin. *Biochimica et Biophysica Acta*, *1851*(8), 999–1005. https://doi.org/10.1016/J.BBALIP.2014.12.016

Sospedra, M., & Martin, R. (2005). Immunology of multiple sclerosis. *Annual Review of Immunology*. Annu Rev Immunol. https://doi.org/10.1146/annurev.immunol.23.021704.115707

Tafferner, N., Barthelmes, J., Eberle, M., Ulshöfer, T., Henke, M., deBruin, N., …

Schiffmann, S. (2016). Alpha-methylacyl-CoA racemase deletion has mutually counteracting effects on T-cell responses, associated with unchanged course of EAE. *European Journal of Immunology*, *46*(3), 570–581. https://doi.org/10.1002/EJI.201545782

Thompson, A. J., Banwell, B. L., Barkhof, F., Carroll, W. M., Coetzee, T., Comi, G., … Cohen, J. A. (2018). Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *The Lancet. Neurology*, *17*(2), 162–173. https://doi.org/10.1016/S1474-4422(17)30470-2

Villar, Luisa M., Costa-Frossard, L., Masterman, T., Fernandez, O., Montalban, X., Casanova, B., … Álvarez-Cermeño, J. C. (2015). Lipid-specific immunoglobulin M bands in cerebrospinal fluid are associated with a reduced risk of developing progressive multifocal leukoencephalopathy during treatment with natalizumab. *Annals of Neurology*, *77*(3), 447–457. https://doi.org/10.1002/ANA.24345

Villar, Luisa María, Sádaba, M. C., Roldán, E., Masjuan, J., González-Porqué, P., Villarrubia, N., … Álvarez-Cermeño, J. C. (2005). Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS. *The Journal of Clinical Investigation*, *115*(1), 187–194. https://doi.org/10.1172/JCI22833

Wheeler, D., Bandaru, V. V. R., Calabresi, P. A., Nath, A., & Haughey, N. J. (2008). A defect of sphingolipid metabolism modifies the properties of normal appearing white matter in multiple sclerosis. *Brain: A Journal of Neurology*, *131*(Pt 11), 3092–3102. https://doi.org/10.1093/BRAIN/AWN190

Zéphir, H. (2018). Progress in understanding the pathophysiology of multiple sclerosis. *Revue Neurologique*, *174*(6), 358–363. https://doi.org/10.1016/J.NEUROL.2018.03.006

Vance JE. Dysregulation of cholesterol balance in the brain: contribution to neurodegenerative diseases. Dis Model Mech. 2012 Nov;5(6):746-55. doi: 10.1242/dmm.010124. Epub 2012 Oct 12. PMID: 23065638; PMCID: PMC3484857.