UNIVERSITY OF MEDICINE AND PHARMACY

"CAROL DAVILA", BUCHAREST

DOCTORAL SCHOOL

FIELD OF MEDICINE

CORRELATIONS BETWEEN PREDICTOR MARKERS FOR MULTIPLE SCLEROSIS

SUMMARY OF THE THESIS

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Year 2023

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Tiu VE, Popescu BO, Enache II, Tiu C, Terecoasa E, Panea CA. Serum and CSF Biomarkers Predict Active Early Cognitive Decline Rather Than Established Cognitive Impairment at the Moment of RRMS Diagnosis. Diagnostics (Basel, Switzerland). 2022 Oct;12(11). **IF 3.992** – Chapter 5

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Summary of the doctoral thesis

Introduction

This PhD thesis aims to perform an in extenso analysis of the main risk factors associated with a negative prognosis for patients with relapsing remitting multiple sclerosis.

The general part focuses on a brief overview of the data currently available regarding the pathophysiology of the disease, reviewing the evidence regarding inflammatory and neurodegenerative immune processes. It is followed by a brief recap of the most important clinical factors, as well as humoral and imaging biomarkers in use today in multiple sclerosis centers around the world.

The special part then analyses the potential of these biomarkers to predict the evolution of patients on an ultra-short term (6 months-one year) in terms of disability progression and cognitive function.

The stakes are high: identifying patients with a significant risk of immediate clinical deterioration following RRMS diagnosis. Correctly selecting patients can influence the therapeutic decision at the time of immunomodulatory therapy initiation, as well as the management plan, with an important impact in terms of the long-term evolution for the patients.

Current state-of-knowledge in the pathophysiology of multiple sclerosis

Multiple sclerosis is a pathology of the central nervous system, affecting both the white and the gray matter, involving processes of neuroinflammation, demyelination and neurodegeneration. (1,2)

There is an ongoing dispute in the academic world about the initial pathophysiological process that triggers the disease. Some authors consider MS to be an autoimmune disease, while other authors consider MS to be a neurodegenerative disease that secondaryly generates an immune response of varying intensity. (3)

The two theories, probably the most important at the moment regarding the origin of multiple sclerosis, are called "inside-out" (neurodegenerative theory) and "outside-in"

(autoimmune theory). Their implications are remarkable, and confirming either one would change the way we relate to MS in terms of treatment and predicting factors. (4)

It should be noted that, at this point, the auto-immune nature of multiple sclerosis relies mostly on indirect and circumstantial evidence. (5–7)

The outside-in theory is based on the involvement of immune cells in the pathophysiology of the disease, as there is vast proof of important roles for T lymphocytes, B lymphocytes, Th lymphocytes type 17, Nk cells etc. (8–11) Studies that looked at possible genetic mutations that predispose to MS identified more than 200 autosomal variants of susceptibility, most of them involving the immune system, and only a small proportion mutations regarding cells originating in the central nervous system. (12)(13)

B lymphocytes play an important role in what has been described as the "compartmentalized immune response" in MS, an increased synthesis of oligoclonal populations of immunoglobulins exclusively at the level of the central nervous system. This process is revealed by the detection of oligoclonal bands or immunoglobulin index in the cerebrospinal fluid. Decades of clinical studies and research have not yet been able to detect a clear, direct link between a self-antigen in the central nervous system and an autoantibody identified in the cerebrospinal fluid or blood of patients with multiple sclerosis. (14) New data suggest that, as a general rule, this immune response is non-specific and directed towards antigens independent of the central nervous system, but showing a cross-reactivity with self-antigens at this level. (15,16)

This cross-reaction is most often attributed to a viral trigger, the strongest evidence for this being related to the Epstein Barr virus (EBV). The risk of MS increases by a RR of 2.17, 95% CI 1.97-2.3 after infectious mononucleosis, and patients with MS have an increased EBV seropositivity rate relative to the general population (almost 100% vs. 80-90%). (17,18,19) A recent study from 2022 also showed that EBV infection brings a Hazard Ratio (HR) of 32.4 to develop MS in those with a genetic predisposition. (20) The mechanism incriminated by some authors is the molecular mimicism between the nuclear antigen 1 EBV (EBNA1) and the glial cellular adhesion molecule (GlialCAM), with a study demonstrating that clonal B cells can produce antibodies that cross-react with EBNA1 and GlialCAM. (21,22). Another argument in favor of immune theory is the disease response to immunosuppressive therapies. (23)

As for the inside-out theory, electron microscopy studies have shown that the first abnormalities of the myelin sheath in the initial phases of the disease seem to involve the internal myelin sheath of seemingly unaffected and myelinated axons, away from foci of inflammation. Moreover, this uniform change in the structure of the internal myelin sheath often occurs while the external layer of myelin is still perfectly intact. It is not clear, however, whether these structural changes are the expression of a myelinopathy or secondary to a lesion of oligodendrocytes. (24,25) Some authors have proposed that the first event in multiple sclerosis is the disturbance of the blood-brain barrier (BBB) through a mechanism still uncertain, possibly a viral infection (with EBV), but with multiple other possible triggers such as trauma, stress, systemic inflammation, astrocytic dysfunction, etc., to which individuals with genetic susceptibility will subsequently trigger the disease through a pathological astrocytic response. (26)

Groups of authors consider, moreover, in the spirit of the inside-out theory, that primary progressive multiple sclerosis (PPMS) and RRMS are just different phenotypic forms of the same disease, the only difference between them being the genetic predisposition of RRMS patients towards an exaggerated and long-lasting immune reaction in response to the constant release of highly immunogenic compounds such as myelin detritus. The proposed mechanisms for the degenerative element of the disease is the compartmentalized leptomeningeal inflammation behind a relatively intact BBB, oxidative stress leading to mitochondrial damage, chronic microglial activation and oligodendrocytic dysfunction with axonal lesions. (3,27)

This concept of a primary neurodegenerative disease (inside-out) has evolved in recent years towards what a group of authors consider to be "smoldering MS". G. Giovannoni et al. argues that there is evidence now that the neurodegenerative processes are present from the earliest stages of the disease and continue to be present throughout it, regardless of inflammatory activity. It is known at this time that most of the disability occurs independently of relapses in RRMS, being present from the very beginning. (28)

We can conclude that there are strong arguments in favor of both theories, but we cannot permanently eliminate any of them. In either variant, multiple sclerosis appears to have an extremely complex mechanism, with an intricate interaction between environmental factors and genetic susceptibility.

For these reasons, multiple sclerosis remains a disease with an unpredictable evolution (29,30), a disease where a better understanding of the underlying pathophysiological mechanisms is needed in order to be able to identify and evaluate the potential of the various prognostic factors.

Prognosis factors in multiple sclerosis

Humoral biomarkers

In this study we analyzed serum biomarkers (neurofilaments), as well as CSF biomarkers (amyloid Aβ42, neurofilaments, oligoclonal bands, immunoglobulin index).

The beta amyloid 1-42 (Aβ42), derived from the cleavage of amyloid precursor proteins (APP) (31) is a biomarker rather known for its use in Alzheimer's disease, where a decrease in Aβ42 plays a diagnostic and a prognostic role. (32–34) The role of amyloid proteins in MS is still uncertain. (35) Some authors have demonstrated that it is a potential predictive biomarker of cognitive function in MS (36–38) and a recent study has shown that low values in the CSF predict a medium-term worse outcome (EDSS>3.0 after 5 years of follow-up)(39) and up to 3 years in another study, (40) with a suggested role as a predictive biomarker for the risk of disease progression at 3–5 years. (41)

Neurofilaments are a physiological proteic component of the axonal structure, with a role in maintaining mitochondrial stability, microtubule content, dendritic structure and function, and in regulating neurotransmitters in glutamatergic and dopaminergic synapses. Of the 5 identified subunits, the light chain is best studied so far. (42,43) The physiological circulating level of neurofilaments is normally extremely low, but in acute, active lesions, their concentration increases.

Although it is not a specific biomarker for MS, it has been shown to excellently correlate with numerous clinical parameters in RRMS, including cortical atrophy, fatigability and progression of clinical disability and cognitive dysfunction, both in the short, medium and long term (>10 years of follow-up). (42,44–49) It is recommended to adjust raw sNfL values according to age and body mass index, for example by using an online calculator as the one provided by Prof. dr. Jens Kuhle’s team. (50)

The compartmentalized synthesis of immunoglobulins in the central nervous system can be paraclinically objectified by means of oligoclonal bands and immunoglobulin index.

Oligoclonal bands are an important diagnostic biomarker in multiple sclerosis, being positive for about 90% of patients. (51) They are considered a predictive biomarker for multiple aspects of the disease, including the risk of developing the disease following a clinically isolated syndrome (52), as well as an increased risk of inflammatory activity, a rate of accelerated atrophy and faster accumulation of disability. (51) The immunoglobulin index, rarely used in the international literature, is a predictive biomarker for increased disease activity in the early stages. (53)

Imaging Biomarkers

The imaging biomarkers used in our study were related to magnetic resonance imaging (MRI) and optical coherence computed tomography (OCT).

The role of MRI as a biomarker for diagnosis, evaluation of the therapeutic response, evaluation of adverse reactions to medication and prediction of disease evolution is indisputable in multiple sclerosis. (54,55) For objective reasons that are largely related to the technical limitations of the images acquired for the study, we decided to use only negative prognostic parameters derived from classical imaging techniques. Thus, according to a consensus recently published by an ECTRIMS working group, we selected ⩾20 lesions in hyper-signal T2/FLAIR at the time of diagnosis, ⩾2 lesions with Gadolinium contrast enhancement (GdE) at the time of diagnosis, ⩾3 new lesions in hypersignal T2/FLAIR follow-up, ⩾1 GdE lesion at one year follow-up. We decided not to use as imaging biomarkers the presence of visible T1 black holes or infratentorial or spinal lesions. (56)

Computed tomography in optical coherence is a non-contact imaging method used to obtain high-resolution transverse section images of the retina. (57) Of the standard measurements obtained by this examination, the most extensively correlated with the pathophysiological mechanisms of multiple sclerosis are the thickness of the retinal nerve fiber layer (RNFL) and the ganglion macular cell layer together with the internal plexiform layer (GCL-IPL). Probably reflecting the neurodegenerative processes associated with MS involving the entire CNS, the two parameters correlate (independently of optic neuritis history) with the medium and long-term disability, the RNFL also having a good individual predictive power. (58) The RNFL also showed predictive power for cognitive decline in patients with MS (those with a lower RNFL thickness have a higher risk of experiencing medium- and long-term cognitive decline). (59,60)

The GCL-IPL layer has been less explored as a predictive biomarker in MS. It is considered a good predictor of diffuse axonal degeneration in CNS neurodegenerative diseases, and a correlation between its average thickness and the course of Alzheimer's disease (60,61) has been proven.

Clinical prognosis factors

According to the ECTRIMS consensus on risk factors for aggressive forms of the disease, we decided to consider as elements of negative prognosis the following: male sex, age at onset>35 years, severe, frequent relapses during the first 5 years, short intervals between relapses, rapid accumulation of disability between relapses, EDSS of 3.0 or higher in the first year of evolution of the disease. (56)

We also decided to evaluate the prognostic role for the BREMSO (Bayesian Risk Estimate for MS at onset) score. This score is designed to predict the risk of a long-term unfavorable evolution (EDSS score≥6.0 to ten years and the transition to SPMS). The necessary data are age, sex, sphincter damage at onset, sensory motor or motor damage at onset, the number of functional systems affected at onset, and incomplete recovery after onset. (62)

Another score of interest is the RoAD (Risk of Ambulatory Disability) score. It calculates the risk of patients experiencing a significant level of disability (EDSS=6.0 or more) at ten years of follow-up under immunomodulatory treatment. The score is based on data available after 1 year of follow-up from the time of diagnosis of RRMS and initiation of DMT, respectively. The variables required are: the sex and age of the patient, the duration of the symptoms, the basal EDSS score, the EDSS score at 1 year of follow-up, the number relapses during the first year while under DMT and the appearance of new hyperintense T2/FLAIR lesions on the one-year follow-up MRI.63)

Hypothesis

This study explores the correlation between negative prognostic factors in relapsing remitting multiple sclerosis (RRMS) at the time of diagnosis.

The main assumption is that there is a correlation between these risk factors and the principal clinical measurements at the time of diagnosis and up to one year-follow-up.

The secondary hypotheses are that there is a correlation between at least some of the main prognostic biomarkers used in multiple sclerosis, as well as that predictive models can be develop based on identified biomarkers, with the aim to predict the ultra-short-term (6 months-one-year) evolution of RRMS patients following diagnosis.

Objectives

The objectives were established individually for each of the 2 sub-studies included in this doctoral thesis.

Thus, for the initial study, which followed the correlation of predictive biomarkers with the cognitive function of patients with RRMS, the objectives were as follows:

The main objective was to identify clinical prognostic factors and/or imaging and humoral biomarkers that correlate with the active cognitive decline of patients with RRMS.

There were also two secondary objectives: the first is related to the use of these prognostic factors to create a predictive model with better sensitivity and specificity than those observed for each biomarker individually.

Another secondary objective was to identify prognostic factors that would correlate with the cognitive status of the patients at the time of diagnosis or at one year follow-up.

For the second study included in the doctoral thesis, which followed the correlation of predictive biomarkers with the early progression of disability (as measured by the EDSS-plus score), the following objectives were established:

The main objective was to identify prognostic factors for the risk of experiencing progression of the EDSS-plus score at one year follow-up.

The secondary objective was to use these predictive biomarkers to create a multivariate model of the risk of EDSS-plus progressor status at a year follow-up, as well as exploring the correlation between negative prognostic factors.

Research methodology

The study had an observational, prospective, cohort-type design, which included all consecutive patients diagnosed with RRMS in (or redirected to) the neurology clinic of the Bucharest University Emergency Hospital between January 2020 and December 2021.

Patients could be included if they had been diagnosed with RRMS in the previous 6 months according to the McDonald 2017 criteria of dissemination in time and space. (64) The exclusion criteria concerned protocol-abiding medical contraindications, inability to sign the informed consent, or degree of disability considered too high at the time of the assessment.

64 patients were recruited, of which 2 did not follow protocol and were excluded from the study, and 10 patients did not complete the entire follow-up period at the time of writing the thesis and could not be included in the final analysis.

The study included 4 visits: at inclusion, at 3 months, at 6 months and respectively 12 months after the moment of inclusion. Patients were clinically evaluated by EDSS and EDSS-plus scale, cognitively by the Montreal Cognitive Assesment - MOCA(65), Brief Visual Memory Test- Revised - BVMT-R(66), SDMT (67)), as well as a screening test for depression by means of the Beck Depression Inventory 2 (BDI-II) (68) . The inclusion visit required the collection of CSF and serum samples, while the rest of the visits required only serum samples collection. The MRI and OCT imaging evaluation was performed at the inclusion and at the one-year visit.

Biomarkers analyzed from cerebrospinal fluid (CSF) were: light chain of neurofilaments (NfL), beta-amyloid – Beta-42 fraction, oligoclonal bands, immunoglobulin index. The main serum biomarker tracked was NfL.

The study was endorsed by the Ethics Committee of the hospital by decision number 6973 from 05.02.2019. All patients signed the informed consent to voluntarily participate in this study.

Statistical analysis of the data was done using SPSS 26.0 for Windows (SPSS Inc., Chicago, IL, USA) and Microsoft Excel 2019 (Microsoft Corp., Seattle, Washington, USA).

The biological samples were processed according to BioMD-eu international recommendations. (69)

Correlations between predictive biomarkers and cognitive decline at the time of RRMS diagnosis

The cohort of patients ultimately included 52 patients, with demographic data similar to those reported by much larger patient registries. (70.71) Thus, the median age at inclusion was 30.5 years, with 37 women (71.2%) and 15 men (28.8%) respectively, representing an F:M ratio of 2.46.

At the time of inclusion, 22 patients (44.2%) met the neurocognitive impairment criteria, decreasing to only 9 patients (17.3%) after cognitive testing at one year follow-up. Although the incidences fall within data reported in the literature (72), it is particular that there is a 58% decrease in the incidence of neurocognitive impairment after one year of follow-up. The explanation is probably multifactorial, involving the phenomenon of learning and accommodation to the tests applied, (73,74) and distancing from the time of diagnosis, often associated with significant transient impairment of cognitive capacity. (75)

Depressive disorder, present in a mild to moderate form in 7 patients (13.4%) did not correlate with any of the factors analyzed, and was not included as a potential factor of error. The level of education also did not have a significant impact on cognitive performance in the analyzed group.

However, there is a moderate negative correlation between almost all cognitive tests performed at inclusion and one year of follow-up and the age of the patients, which is why age was taken into account as covariate/confounder in subsequent multivariate analyses.

In terms of correlation with clinical status and disease progression, cognitive tests showed a significant correlation with the one-year EDSS score, but not with the initial EDSS

score or EDSS-plus progressor status. The data are in line with those reported in the literature. (76,77)

Given that the majority of patients (45 patients, 86.5%) were initiated with moderately effective therapies, no correlation was noted in our study between cognitive performance and the type of DMT used. Although the effect may not be seen due to the small group of patients on high efficacy therapies, a recent meta-analysis has shown that the moderately positive effect on cognition is not significantly different between highly effective and moderately effective immune therapies, nor between the different types of pharmacological agents used. (78–80)

The one-year SDMT test showed significant correlations with the average thickness of the RNFL and GCL-IPL layers, the immunoglobulin index and the smoking status.

A strong positive linear correlation is also observed between all the cognitive tests applied. Although the SDMT and BVMT-R have a long tradition in screening cognitive deterioration in patients with MS, the use of the MOCA test was not widely adopted despite the advantages that this scale presents (one of the easiest to administer validated cognitive screening scales in MS). Data from the few studies in the literature that have looked at the specificity and sensitivity of this scale in the detection of cognitive impairment in populations of MS patients show that MOCA is a valid alternative. (81,82) The data obtained in our study therefore support the use of the MOCA test for cognitive testing of MS patients, showing a strong correlation with SDMT and BVMT-R test results.

The neurocognitive impairment at inclusion did not correlate with any of the prognostic factors analyzed, but the neurocognitive impairment at one year follow-up showed a statistically significant moderate positive correlation (0.306, p=0.03) with the presence of oligoclonal bands in the CSF and a moderate negative correlation (-0.357, p=0.01) with the thickness of the GCL-IPL layer.

Active cognitive decline is a particular process in multiple sclerosis. The decrease in cognitive performance in MS seems to have multiple etiologies, as it is probably attributable to the degenerative phenomena that lead to the collapse of the neural network (83), but also to the transient global cognitive impairment that seems to accompany most of the relapses. (84) Rarely, there are purely cognitive relapses described that can have a significant effect on cognitive performance. (85)

In our group, 11 patients (21.2%) experienced active cognitive decline during the follow-up period. Among the clinical factors correlated with this cognitive outcome, the duration from the onset of symptoms played a role, patients with cognitive decline having a significantly shorter duration from the moment of onset of symptoms to diagnosis (0.63 years vs. 2.34; p=0.004). Rural environment was another incriminated factor, with 45% of the patients who experienced an active cognitive decline (5 patients) coming from rural areas. This has been reported by other studies previously. (86–88)

The adjusted Z-score of sNfL at inclusion (2.66 vs. 1.63, p=0.008), NfL in the CSF (2097 pg/ml vs. 1172 pg/ml, p=0.01) and adjusted Z-score of sNfL at 3 months (2.12 vs. 1.26, p=0.02) were significantly higher for the group of patients who experienced an active cognitive decline during the study period versus those who did not have a significant decrease in cognitive function. The Pearson correlation test also reflects a statistically significant moderate positive correlation for each of the 3 neurofilament samples.

A predictive model for the risk of neurocognitive impairment at one year follow-up was developed based on 7 variables: the patient's age, smoking status, BREMSO score, the average thickness of the RNFL layer, GCL-IPL, as well as the status of oligoclonal bands and the Aβ42 level in the CSF. The developed model has values $\chi 2(4) = 28,354$, $p < .0001$, with a sensitivity of 87.5% and a specificity of 95.1%.

A predictive model for the risk of cognitive decline at one year was also developed, based on 6 variables – living environment, BREMSO score, average RNFL layer thickness, GCL-IPL, as well as SNfL adjusted Z scores at inclusion and at 3 months. With a $\chi 2(4) = 21,960$, $p < .001$, a sensitivity of 92.5% and a specificity of 77.8%, the predictive model had a performance well above the results obtained at the individual analysis of prognostic factors.

The study was positive, fulfilling both the main and the secondary objectives. Our results prove that almost half of the patients with RRMS can meet the neurocognitive impairment criteria at the time of diagnosis and that, although a significant part of the patients will show a marked improvement in cognitive performance during the first year of follow-up, a subgroup of over 20% will experience acute cognitive decline during this period. Commonly used prognostic factors can be employed to develop predictive models regarding the risk of neurocognitive impairment and cognitive decline during the first year.

Predictive biomarkers for EDSS-plus score progression following RRMS diagnosis

This study tracks the evolution of 52 consecutive patients diagnosed with RRMS for a period of one year. Average age at the time of diagnosis was 30.5 years, ratio of women:men was 2.6:1, 71.2% of patients had an active lifestyle, a percentage of 80.8% lived in urban areas, and 61.5% had a higher level of education.

The group of patients seems to have a low level of activity of the disease prior to diagnosis, with a short duration from the onset of symptoms until the moment of diagnosis (median 1 year) and a median EDSS score at inclusion of 2 points.

When analyzing CSF biomarkers, we observe that 71.2% of patients have positive oligoclonal bands, while only 36.5% of patients have a positive immunoglobulin index. The usual data reported in the literature places the frequency of oligoclonal bands in MS around 80-90%, and for the immunoglobulin index around 60-70%. The discrepancy between reported data and our lot could be explained by both less active forms of the disease at the moment of diagnosis, but also to the sample processing technique and the average duration of 6-10 days until the samples are processed as opposed to other centers were this is performed immediately or after 1-2 days. (89.90)

A downward slope of the average values for the adjusted Z-score of sNfL is observed, from an initial value of 2.14 to 0.81 in the samples collected at one year. This phenomenon characterizes patients with stable EDSS score, without evidence of disease activity, without new relapses, under immunomodulatory therapy, who after 6-9 months of stable evolution show a normalization of the level of sNfL. (91,92)

The average thickness of the retinal nerve fiber layer (RNFL) was 95 μm, and of the Ganglion Cell Layer - Inner Plexiform Layer (GCL-IPL) of 79 μm, values slightly low from normal.

Regarding MRI parameters, 71.2% of patients presented at inclusion 20 or more hyperintense lesions in T2/FLAIR, and a percentage of 50% presented 3 new hyperintense T2/FLAIR lesions or more on the repeated MRI scan at one year follow-up. Only 23.1% of patients had 2 or more GdE lesions upon inclusion, with a decrease to 7.7% at the one-year follow-up.

We therefore observe a cohort of patients with a significant lesion load at the time of inclusion, and the presence of at least 3 new T2 / FLAIR lesions for 50% of the study lot at the one-year visit is an important alarm signal that shows an ineffective therapeutic control. This is most likely explainable by the inclusion in moderately effective therapies for 86.5% of patients. (93,94)

The average BREMSO score of 0.44, combined with the average RoAD score of 3 points, places most patients in a low-to-moderate risk group regarding a severe ten-year prognosis.

Beta-amyloid Aβ42 demonstrated significant correlations in our study with the adjusted Z-score of sNfL at 3 months. This has never been pointed out before in the literature, perhaps emphasizing a correlation between prolonged axonal degradation processes beyond the initial moment of onset and lower values of beta-amyloid Aβ42, perhaps suggesting a degradation process with a longer dynamic and associated with a worse prognosis.

The BREMSO score was notably correlated with serum and CSF neurofilaments values, as well as with the RoAD score. The RoAD score instead correlates with the age of the patients, as well as parameters of OCT and the presence of 20 lesions in hypersignal T2/FLAIR on the MRI from inclusion. This suggests a correlation of the BREMSO score with the biomarkers associated with acute axonal losses, and the RoAD score with the biomarkers associated with the neurodegeneration and progression of long-term atrophy in MS, in line with the demonstrated role of the score.

Neurofilament samples showed a strong or moderate correlation between inclusion visits and up to one year follow-up, with a linear relationship between samples, suggesting that axonal destructive processes persist for at least 12 months after the time of inclusion, even if the longitudinal trend is decreasing. Notably, male patients showed statistically significantly higher values for the adjusted Z-score of sNfL (2.1 vs. 1.1 at 3 months, 1.6 versus 0.75 at 6 months, 1.34 versus 0.56 at 12 months).

The absence of oligoclonal bands and immunoglobulin index proved to be a favorable prognostic factor, with patients with negative bands showing lower EDSS scores at inclusion than those with positive bands (2.3 vs. 1.3, p=0.001. Patients with negative index had an EDSS score lower at one year compared to those with a positive index (1.5 vs. 2.8, p=0.003).

The presence of a compartmentalized immune reaction in the CNS, objectified by oligoclonal bands or immunoglobulin index, appears to have correlation with the EDSS score. Thus, positive oligoclonal bands demonstrated a correlation with the EDSS score at inclusion, with patients with negative oligoclonal bands having an increased EDSS score risk almost 5 times lower than those with positive OCBs. The difference in the EDSS score between the two groups was 2.3 points for the positive OCB group, while the negative OCB group had a score of 1.4 points (p=0.001). Similarly, the one-year EDSS score was higher for those with a positive IgG index (2.8 average) compared to those with a <0.7 index who had a lower score (1.5 average, p=0.003). This aspect probably reflects a more active immune pathophysiological activity of the disease where the bands or index are present, and associated with a higher level of clinical disability.

Also, the average thickness of the GCL-IPL layer correlated moderately negatively with the EDSS score at both inclusion and one year, while the RNFL layer showed a moderately negative association with only the EDSS score at one year.

A total of 20 patients (38.5%) were classified as progressors on the EDSS-plus scale after one year of follow-up. Progression purely due to the EDSS score was present in 50% of cases (10 patients), but 30% showed progression exclusively on 25FW and 5% only through 9HPT, with the rest showing various mixed forms of progression. This shows that a large percentage of patients show evidence of progression of disability from the onset, but also that 35% of patients would have experienced a significant clinical progression of disability that would have remained unnoticed if not performing and analyzing the 25FW and 9HPT. There were no statistically significant differences in the number of first-year relapses between the EDSS-plus and non-progressive groups of progressives.

Statistically significant correlations for the EDSS-plus progressor state involve the adjusted Z-score of sNfL at 6 and 12 months, as well as for the average thickness of the RNFL and GCL-IPL layer. The observation is especially important, because it reveals a double driver of the progression of disability, both through significant axonal loss and through diffuse neurodegenerative mechanism.

We built 2 predictive models. The first, based on 6 variables (average GCL-IPL layer thickness, RNFL, adjusted Z-scores of serum neurofilaments at inclusion, at 3 months and

at 6 months and the immunoglobulin index), had a $\chi 2(4) = 39,732$, $p < .0001$, with a sensitivity of 86.7% and a specificity of 90.6%.

The second model kept only 4 variables: the average thickness of the GCL-IPL score, the adjusted Z-score of neurofilaments at 3 and 6 months, and the immunoglobulin index. The performance of the second model was better, with a $\chi 2(4) = 44,638$, $p < .0001$, a sensitivity of 92.9% and a specificity of 96.9%.

This study is positive, fulfilling the main and the secondary objectives. Demonstrate that serum neurofilaments at the time of inclusion, at 3 and at 6 months have an important prognostic value, while also representing a biomarker of response to treatment.

The importance of using the EDSS-plus score from the moment of diagnosis of RRMS is also underlined. The progression of patients' disability during the first year after RRMS onset can be predicted with an excellent discriminating power based on biomarkers widely used in everyday practice, possibly guiding the choice of DMT.

Notably, 50% of patients with progression of disability did not experience relapses in the follow-up interval.

Conclusions and personal contributions

This doctoral study performs a careful evaluation of the main prognostic factors currently known for patients with RRMS at the time of diagnosis.

The two studies derived from the initial hypothesis analyze in extenso two important clinical aspects: the risk of cognitive deterioration in the first year after diagnosis, respectively the risk of progression of disability from the moment of diagnosis. Both studies are positive, meeting both the main and secondary objectives.

Thus, we demonstrate that common biomarkers can accurately predict these extremely important clinical endpoints, with major implications for the protocol we should apply to all newly diagnosed patients with RRMS. An important part of our work also involved the analysis of the interaction between humoral biomarkers, imaging and clinical risk factors.

This study has several important limitations:

-the number of enrolled patients is small for the proposed research target, leading to statistical artefacts where the number of outcomes was insufficient

-the follow-up period is a short (one year).

-two patients did not follow the study protocol and were excluded from the analysis, while ten patients did not complete the follow-up period

-a large number of patients were included in immunomodulatory therapies with pharmacological agents of moderate efficiency.

Personal contributions

-explored the correlation between predictor biomarkers and cognitive assessment tests used in MS, as well as demonstrating a good correlation between SDMT and BVMT-R tests with the MOCA test, thus supporting its use as a screening method for MS patients (Chapter 5.3.2)

-demonstrating active cognitive decline in newly diagnosed RRMS patients, and identifying predictive biomarkers for this phenomenon 9 months in advance (chapter 5.3.3)

-exploring through a prospective study for the first time in literature the correlations between imaging, humoral biomarkers and RoAD and BREMSO prognostic scores. (chapter 6.3.2)

-demonstrating the potential role of implementing the EDSS-plus score from the moment of RRMS diagnosis (chapter 6.3.4)

-creating predictive models with excellent discriminatory power regarding the risk of progression of disability one year after the diagnosis of RRMS, based on biomarkers widely used in everyday practice (chapter 6.3.5)

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