

CSF β -amyloid predicts prognosis in patients with multiple sclerosis

Article (Accepted Version)

Pietroboni, Anna, Caprioli, Michela, Carandini, Tiziana, Scarioni, Marta, Ghezzi, Laura, Arighi, Andrea, Cioffi, Sara, Cinnante, Claudia, Fenoglio, Chiara, Oldoni, Emmanuela, Basilico, Paola, Fumagalli, Giorgio, Colombi, Annalisa, Giulietti, Giovanni, Serra, Laura et al. (2018) CSF β -amyloid predicts prognosis in patients with multiple sclerosis. Multiple Sclerosis. ISSN 1352-4585

This version is available from Sussex Research Online: <http://sro.sussex.ac.uk/id/eprint/77336/>

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:

Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

CSF β -amyloid predicts prognosis in patients with multiple sclerosis

ABSTRACT

Background: The importance of predicting disease progression in multiple sclerosis (MS) has increasingly been recognised, hence reliable biomarkers are needed.

Objectives: To investigate the prognostic role of cerebrospinal fluid (CSF) Amyloid beta₁₋₄₂ (A β) levels by the determination of a cut-off value to classify patients in slow and fast progressors. To evaluate possible association with white (WM) and grey matter (GM) damage at early disease stages.

Methods: Sixty patients were recruited and followed-up for three to five years. Patients underwent clinical assessment, CSF analysis to determine A β levels, and brain MRI (at baseline and after 1 year). T1-weighted volumes were calculated. T2-weighted scans were used to quantify WM lesion loads.

Results: Lower CSF A β levels were observed in patients with a worse follow-up EDSS ($r=-0.65$, $p<0.001$). The multiple regression analysis confirmed CSF A β concentration as a predictor of patients' EDSS increase ($r=-0.59$, $p<0.0001$). Generating a receiver operator characteristic curve, a cut-off value of 813 pg/ml was determined as the threshold able to identify patients with worse prognosis (95% CI 0.690-0.933, $p=0.0001$). No differences in CSF tau and NfL levels were observed ($p>0.05$).

Conclusions: Low CSF A β levels may represent a predictive biomarker of disease progression in MS.

INTRODUCTION

Multiple sclerosis (MS) is the most common chronic inflammatory disease of the central nervous system (CNS). Although traditionally regarded as a white matter (WM) demyelinating disease, axonal loss is critically involved in MS pathophysiology since early clinical stages^{1,2}. The mechanisms underlying axonal damage, however, are not entirely clear and no reliable prognostic biomarker of disease progression are currently available.

Magnetic resonance imaging (MRI) is an invaluable tool for the diagnostic work-up of MS patients^{3,4}. However, no strong correlation has been found between conventional MRI measures and clinical outcomes of progression^{5,4}.

When taking into account the hypothesis of neurodegeneration as a major contributor to MS disability, β -amyloid₁₋₄₂ ($A\beta$) has recently become an interesting candidate for its putative role in this process. Amyloid-Precursor Protein (APP) has been detected in MS plaques with a higher APP immunoreactivity in actively demyelinating than in chronic lesions, thus indicating a modification of APP metabolism across disease stages⁶. Moreover, APP was found upregulated in both acute and chronic MS lesions and has been regarded as a sensitive marker of axonal damage^{7,8}. Reduced CSF $A\beta$ levels have already been reported in MS patients^{9,10,11,12} although the interpretation of these findings remains controversial¹³. In addition, a previous study on the mouse model of MS suggested a possible protective role of increased serum $A\beta$ levels¹⁴. On the other hand, a recent study in a relatively small group of MS patients revealed that lower baseline levels of CSF $A\beta$ are predictive of a more severe disease progression over a 3-year follow-up¹².

In this scenario, aims of the current study were: 1) to confirm the prognostic role of CSF $A\beta$ levels in a larger cohort of MS patients with a longer clinical follow-up; 2) to compare the prognostic role of CSF $A\beta$ levels with a validated predictive index of disease progression, namely the Bayesian Risk Estimate for MS at Onset (BREMSO)¹⁵; 3) to determine a cut-off level of CSF $A\beta$ with the ability of correctly classifying MS patients in slow and fast progressors; 4) to assess whether patients with lower CSF $A\beta$ levels show any peculiar radiological features at baseline and at follow-up.

MATERIALS AND METHODS

Subjects

Seventy patients with a new diagnosis of relapsing-remitting MS (RRMS) according to the 2010 revised McDonald criteria³ have been recruited from January 2013 to January 2015. A fraction of the population (n=48) has already been included in a previous study¹². All patients underwent clinical assessment, brain MRI and lumbar puncture (LP). LP was always performed in an acute phase of disease, [i.e. clinical relapse or the presence of new and/or gadolinium enhancement lesions at MRI](#), and before starting any treatment, including corticosteroids. From this cohort, 60 patients have been clinically followed-up for 3 or 5 years (n=60 and n=35 respectively), while 10 patients were lost to follow-up. The main demographic and clinical characteristics of all subjects are summarized in Table 1 and 2.

Forty-four out of 70 patients agreed to undergo an extra MRI scan for research purposes at baseline and at 1-year follow-up (details of the MRI acquisition protocol are reported below).

For each recruited patient, the Expanded Disability Status Scale (EDSS) score was assessed at baseline and [at remission](#) during each follow-up visit at six month intervals. An EDSS cut-off score at 3-year follow-up equal to 3.0 was used to classify MS patients into two different groups of disease severity, as previously described¹².

For all patients, the BREMSO score was also calculated. The higher the BREMSO score, the higher the risk of future disability¹⁵.

The current study was approved by the Institutional Review Board of the Fondazione Cà Granda, IRCCS Ospedale Maggiore Policlinico (Milan, Italy). All MS patients and control subjects gave their written informed consent for this research before entering the study.

CSF collection

CSF samples were collected by LP in the L3/L4 or L4/L5 interspace for all patients at diagnosis. Following LP, CSF samples were centrifuged in 8000 rpm for 10 minutes. The supernatants were aliquoted in polypropylene tubes and stored at -80 °C until use. CSF cell counts, glucose, and proteins were determined. Albumin was measured by rate nephelometry. Oligoclonal bands (OCB) were

evaluated by isoelectrofocusing. CSF A β ₁₋₄₂ and total tau were measured by using two commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits (Fujirebio, Ghent, Belgium). Then, all statistical analyses were performed between baseline A β levels and clinical and radiological outcomes measured at diagnosis and at follow-up evaluations. CSF neurofilament light chain (NfL) levels were measured using the Uman Diagnostics NF-light ELISA kit (Umeå, Sweden) as described by the manufacturer. It uses two highly specific, non-competing monoclonal antibodies (mAB47:3 and mAB2:1) to quantify soluble NfL.

MRI acquisition

Forty-four patients underwent a MRI examination for research purposes (at baseline and at 1 year follow-up) using an Achieva 3T scanner (Philips, The Netherlands). The acquisition protocol included: 1) a T1-weighted scan (TR 9.90 ms; TE 4.61 ms; Flip angle 8°; slices thickness 1 mm; gap 0); 2) Fluid attenuated inversion recovery (FLAIR) images (TR 11000 ms; TE 125 ms; Flip angle 90°; slices thickness 1 mm; gap 0); 3) a T2-weighted scan (TR 2492 ms; TE 78 ms; Flip angle 90°; slices thickness 4 mm; gap 0).

WM damage

To quantify the macroscopic WM LL, the lesions of all patients (n=44) were first identified on FLAIR scans by consensus of three trained and independent observers (MC; AP; MS). Lesions were then outlined using a semi-automated local thresholding contouring software (Jim 7.0, Xinapse System, Leicester, UK, <http://www.xinapse.com/>). For each dataset, the WM LL was calculated and used for correlation analyses. The same procedure was applied to 1-year follow up scans.

Brain volumetrics

All 3D T1-weighted scans (n=44) were first visually inspected to exclude the presence of macroscopic artefacts. Data of 23 scans were processed using an optimized voxel-based morphometry (VBM) protocol in Statistical Parametric Mapping 12 (SPM12; Wellcome Department of Imaging Neuroscience; www.fil.ion.ucl.ac.uk/spm/). Segmentation and normalization produced a GM probability map¹⁶ in Montreal

Neurological Institute (MNI) coordinates. To compensate for compression or expansion during warping of images to match the template, GM maps were modulated by multiplying the intensity of each voxel by the local value derived from the deformation field¹⁷. All data were smoothed using a 8-mm full width half maximum (FWHM) Gaussian kernel.

We derived for each scan the WM (WMF) and GM fractions (GMF), which were calculated, respectively, as the ratio of total WM and GM volume to the total intracranial volume (TIV). We first used unpaired t-tests to assess between group differences of WMF and GMF at baseline, and paired t-tests to assess within group differences between baseline and 1 year follow-up scans. In particular, we divided the patients into two groups, based on their GMF Δ (Δ =1 year follow-up GMF minus baseline GMF), which could be either stable or reduced. Additionally, due to the increasing interest in GM loss in MS^{18,19,20}, whenever any global difference in GMF at baseline was found, we run a voxel-wise analysis to clarify the regional patterns of anatomical distribution. For this purpose, flexible factorial models were created in SPM, in which age, gender and WM LL were always entered as covariates of no interest. We tested for between (cross sectional) and within (longitudinal) group differences of regional GM volumes by using unpaired and paired (one sample and two time points: baseline and after 1 year) T-contrasts respectively. We accepted as significant differences surviving the family wise error (FWE) correction at cluster level ($p < 0.5$).

Statistical analysis

All statistical analyses were performed using SPSS 21.0 for Windows (SPSS Inc., Chicago, IL, USA), Graph Pad PRISM 6.0, MedCalc and SPM12, [including correction for age and gender](#).

CSF A β concentrations obtained at baseline were compared in MS patients divided in two different subgroups: those with a follow-up EDSS score < 3 ($n=43$) and those with a follow-up EDSS score ≥ 3 ($n=17$). Due to the non-normal distribution of data, all between-group comparisons were tested by non-parametric inferential statistical analyses (Kruskal-Wallis test and Mann-Whitney U test). For all analyses, the statistical threshold was set up at $p < 0.05$.

Spearman correlation coefficient between WM LL and EDSS scores at follow-up and between GMF and CSF A β levels at baseline and at follow-up was assessed.

To calculate the best CSF A β value, able to predict disease progression, a receiver operator characteristic (ROC) curve was generated. The cut-off value of A β has been measured with MedCalc that provides the Youden's index to determine the point of the ROC curve for which (sensitivity + specificity) is maximal.

Hierarchical multiple regressions analysis between EDSS score at follow-up as dependent variable and CSF A β levels as explanatory variable was performed using SPSS. The regression model was adjusted in order to control for the potential effect of age, gender, EDSS score at baseline, disease duration, WM LL, and GMF. For linear and multiple regressions analyses and for correlation analyses the statistical threshold was set to $p < 0.05$.

RESULTS

Clinical variables and CSF biomarkers

When dividing the patient cohort according to individual disease severity over 3 to 5-year follow-up, those patients with a follow-up EDSS score ≥ 3 had lower CSF A β levels than those with a follow-up EDSS score < 3 (650.8 ± 204.8 pg/ml vs 941.9 ± 280.2 pg/ml; $p < 0.0001$; Figure 1). Interestingly, the retrospective comparison of their baseline EDSS scores did not reveal any significant difference ($p > 0.05$).

CSF A β levels correlated with the EDSS score at 3-year follow-up ($n=60$; $r=-0.59$, < 0.0001 ; Figure 2a), and such correlation became even stronger when considering the available EDSS scores at 5-year follow-up ($n=35$; $r=-0.65$, $p < 0.0001$; Figure 2b). [No correlation between CSF A \$\beta\$ levels and EDSS score at baseline was observed \(\$p > 0.05\$ \).](#)

The multiple regression analysis (adjusted for the potential effect of age, gender and disease duration) to predict patients' BREMSO score showed CSF A β concentration as the best predictor ($r=-0.60$, $p < 0.0001$; $\beta=-0.56$; Figure 2c).

The multiple regression analysis to predict patients' increase in the EDSS score at follow-up confirmed CSF A β concentration as best predictor ($r=-0.59$, $p < 0.0001$, $\beta=-0.52$).

We calculated the best CSF A β value able to predict disease progression with a ROC curve analysis and it turned out to be 813 pg/ml. The area under the curve (AUC) was 0.811 (95% CI 0,690-0,933, p=0.0001; Figure 3).

We split our cohort into two groups based on CSF A β levels (A β ^{low} under 813 pg/ml and A β ^{high} over 813 pg/ml).

With respect to CSF tau and NfL levels, no significant between-group differences were observed (p>0.05, data not shown).

WM damage

At baseline MRI, there were no differences in WM-LL between A β ^{low} and A β ^{high}. These data were confirmed also at 1-year follow-up MRI.

The EDSS score at follow-up correlated with WM LL (r=0.31; p=0.04).

Brain volumetrics

For all subjects, we measured WMF and GMF from both, baseline and 1 year follow-up T1-weighted volumes. Although a trend of volume reduction was observed, it did not reach the statistical significance (p>0.05, data not shown). In contrast, when comparing patients who did reduce their GMF against the patients who did not, the former had lower CSF A β levels (865.4 \pm 206.4 pg/ml vs. 1062.0 \pm 141.8 pg/ml; p=0.04; Figure 4). In other words, the patients with a reduction of their GMF after 1 year were the same who had lower CSF A β levels. The VBM analysis performed to assess regional GM atrophy between baseline and 1-year follow-up MRI scan showed a pattern of relative atrophy mainly involving the prefrontal, cingulate, parahippocampal and cerebellar cortex (P_{FWE corr} at cluster level <0.0025). In particular, the specific areas we identified were: bilateral cerebellar cortex, bilateral dorsolateral and medial prefrontal cortex, left orbitofrontal cortex, bilateral cingulate cortex, and right parahippocampal cortex (Figure 5).

DISCUSSION

Following our previous findings¹², we recruited here a larger cohort of MS patients with a longer follow-up, classifying them as $A\beta^{\text{low}}$ and $A\beta^{\text{high}}$ based on their CSF $A\beta$ levels. With respect to the clinical outcome, $A\beta^{\text{low}}$ showed a higher risk of disease progression. Interestingly, patients showing a higher EDSS score at 5-year follow-up were not the same who showed a higher EDSS score on their first attack (baseline). Also, when dividing the patient cohort into two groups according to their follow-up EDSS score (cut-off ≥ 3), the retrospective comparison of their baseline EDSS scores did not reveal a significant difference. In line with this evidence, patients' EDSS score at baseline was able to predict patients' disability at 3-year, but not at 5-year follow-up. This suggests that the severity of the first MS relapse does not reflect the future evolution of the disease, and that other pathophysiological mechanisms are likely implicated. Interestingly, CSF $A\beta$ levels came out as a predictor of disease disability¹², whose strength became increasingly stronger when prolonging the follow-up intervals from 3 to 5-year. Keeping all these data together, they indicate that a higher EDSS score at baseline in the absence of reduced CSF $A\beta$ levels does not necessarily indicate a worse prognosis. Although our findings need to be replicated on a larger cohort of patients, we argue that lower CSF $A\beta$ levels may be associated with or may even induce the activation of more aggressive pathogenic mechanisms that are already in place since the early stages of disease. With respect to the underlying processes that might combine reduced CSF $A\beta$ levels with a worse features, the spectrum of hypotheses appears extremely broad. On one hand, the inflammation increases β -site-APP-cleavage-enzyme-1 (BACE1) activity²¹, which was previously found increased in patients with reduced CSF $A\beta$ levels²². BACE1 is also involved²³ in the cleavage of neuregulin-1 (NRG1)²³, a protein that plays a crucial role in myelin repair. On the other, CSF $A\beta$ reduction could depend on APP deposition around injured axons, although there is no evidence of $A\beta$ deposition in MS plaques. Recent studies showed that APP-expressing axons are partially myelinated, suggesting that acute axonal damage may, at least partially, occur independently from demyelination²⁴. In other words, dysregulation of BACE1 and NRG1 might represent the myelin repair processes, while APP expression might be regarded as a biomarker of acute axonal injury. However, recent studies have shown that $A\beta_{1-42}/A\beta_{1-40}$ ratio is not altered in MS, in contrast to $A\beta_{1-42}$ alone²⁵. Therefore, we acknowledge that the $A\beta_{1-42}/A\beta_{1-40}$ ratio should be considered before attributing too much influence of $A\beta_{1-42}$ to the mechanism of disease progression.

In our cohort of patients, we also identified a cut-off value of CSF A β able to discriminate between individuals with a higher from those with a lower risk of clinical progression.

Interestingly, no correlations were found between WM-LL and A β levels at baseline. These data gain interest since we previously demonstrated in a paper about CSF biomarkers of neurodegeneration and WM damage in patients with Alzheimer's disease (AD)²⁶ that CSF A β levels were instead a predictor for WM-LL accumulation. The myelin model combines the occurrence of WM metabolic damage with A β deposition²⁷. It is fascinating how several factors might be involved in this model. To take an example, we mention Apolipoprotein E (ApoE), the second most important risk factor for late onset AD after age. In the brain, ApoE is the primary transporter of endogenously produced lipids that are essential for myelin function²⁸. A reduced capacity to mobilize these essential lipids and recycle them into myelin repair processes may link together WM damage and A β deposition²⁷. Regarding MS, there are currently insufficient data on this topic, which could be a matter of further studies.

With respect to GMF, taking into consideration the relative small number of MRI scan, our findings seem to corroborate the hypothesis that lower CSF A β levels associate not only with worse clinical features, but also with worse radiological prognostic conditions. In particular, those patients with a relative reduction of GMF at follow-up MRI had lower CSF A β levels than those who had not. Replication in a larger cohort of patients is needed to clarify this point.

In our study, regional VBM analysis were globally comparable in A β^{low} and A β^{high} , but interestingly, among patients with a reduction of GMF after one year, a pattern of relative atrophy mainly involving the prefrontal, cingulate, parahippocampal and cerebellar cortex was found. It is known that GMF decreases progressively during MS course²⁹ and appears to be associated with accumulation of physical and cognitive disabilities^{18,19}. This GMF loss occurs in varying degrees, and with a different regional distribution among disease phenotypes, but it is already detectable at the earliest disease stages²⁰. A widespread pattern of GM atrophy was found in several previous VBM studies. In particular, basal ganglia structures, prefrontal cortex, posterior cingulate gyrus and cingulate gyrus were often identified as regions of significant GM loss in MS³⁰. Recent studies also reported hippocampal and parahippocampal atrophy in MS patients³¹. Therefore, as concluded in a recent meta-analysis of VBM

works³⁰, GM atrophy is documented by neuroimaging studies in MS and appears to correlate with clinical disability more closely than WM changes. Whether regional GM atrophy is linked to specific pathophysiological processes or it is the result of differences in susceptibility to inflammation, is still matter of debate.

Certain limitations exist for this study. First, as it known, CSF A β levels could have a clear variability in CSF between repeated analyses also in the same laboratory³¹. Second, it would be necessary to prolong the follow-up time to minimize the risk of confounding factors such as the aggressiveness of the acute first clinical attack. Third, this is an exploratory study and a larger cohort of patients is needed to confirm the findings.

In conclusion, this study suggests that CSF A β levels may represent a significant feature in MS, as they may be a predictive progression biomarker. Moreover, we propose a possible cut-off value of CSF A β levels to identify patients with a high or low risk of disease progression. As part of a fascinating hypothesis, we have speculated that lower CSF A β levels may be associated with a decreased ability to remyelinate CNS axons, with an early WM and GM damage, and a with a higher probability of clinical disease progression. Nevertheless, the exact role played by A β remains to be determined. In particular, it remains to be clarified whether it plays a causal role or represents the epiphenomenon of a neuroaxonal reparative process, or it plays a proper protective role.

FUNDING ACKNOWLEDGEMENT

This research was supported by Fondazione Monzino and the Italian Ministry of Health (“Ricerca Corrente” to ES).

Conflict of interest statement

None declared.

BIBLIOGRAPHY

1. Frischer JM, Bramow S, Dal-Bianco A, et al. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 2009;132:1175-89.

2. Bjartmar C, Kidd G, Mork S, Rudick R, Trapp BD. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann Neurol* 2000;48:893-901.
3. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. *Ann Neurol* 2011;69:292-302.
4. Filippi M, Rocca MA, Ciccarelli O, et al. MRI criteria for the diagnosis of multiple sclerosis: MAGNIMS consensus guidelines. *Lancet Neurol* 2016;15:292-303.
5. Filippi M, Paty DW, Kappos L, et al. Correlations between changes in disability and T2-weighted brain MRI activity in multiple sclerosis: a follow-up study. *Neurology* 1995;45:255-60.
6. Gehrman J, Banati RB, Cuzner ML, Kreutzberg GW, Newcombe J. Amyloid precursor protein (APP) expression in multiple sclerosis lesions. *Glia* 1995;15:141-51.
7. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998;338:278-85.
8. Ferguson B, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997;120 (Pt 3):393-9.
9. Augutis K, Axelsson M, Portelius E, et al. Cerebrospinal fluid biomarkers of beta-amyloid metabolism in multiple sclerosis. *Mult Scler* 2013;19:543-52.
10. Mori F, Rossi S, Sancesario G, et al. Cognitive and cortical plasticity deficits correlate with altered amyloid-beta CSF levels in multiple sclerosis. *Neuropsychopharmacology* 2011;36:559-68.
11. Mattsson N, Axelsson M, Haghighi S, et al. Reduced cerebrospinal fluid BACE1 activity in multiple sclerosis. *Mult Scler* 2009;15:448-54.
12. Pietroboni AM, Schiano di Cola F, Scarioni M, et al. CSF beta-amyloid as a putative biomarker of disease progression in multiple sclerosis. *Mult Scler* 2017;23:1085-91.
13. Gentile A, Mori F, Bernardini S, Centonze D. Role of amyloid-beta CSF levels in cognitive deficit in MS. *Clin Chim Acta* 2015;449:23-30.

14. Grant JL, Ghosn EE, Axtell RC, et al. Reversal of paralysis and reduced inflammation from peripheral administration of beta-amyloid in TH1 and TH17 versions of experimental autoimmune encephalomyelitis. *Sci Transl Med* 2012;4:145ra05.
15. Bergamaschi R, Montomoli C, Mallucci G, et al. BREMSO: a simple score to predict early the natural course of multiple sclerosis. *Eur J Neurol* 2015;22:981-9.
16. Ashburner J, Friston KJ. Unified segmentation. *NeuroImage* 2005;26:839-51.
17. Ashburner J, Friston KJ. Why voxel-based morphometry should be used. *NeuroImage* 2001;14:1238-43.
18. Roosendaal SD, Bendfeldt K., Vrenken H, et al. Grey matter volume in a large cohort of MS patients: relation to MRI parameters and disability. *Multiple Sclerosis* 2011;17:1098–1106.
19. Ceccarelli A, Rocca MA, Pagani E, et al. A voxel-based morphometry study of grey matter loss in MS patients with different clinical phenotypes. *NeuroImage* 2008;42:315-22.
20. Lansley J, Mataix-Cols D, Grau M, et al. Localized grey matter atrophy in multiple sclerosis: a meta-analysis of voxel-based morphometry studies and associations with functional disability. *Neurosci Biobehav Rev.* 2013;37(5):819-30.
21. Hong HS, Hwang EM, Sim HJ, et al. Interferon gamma stimulates beta-secretase expression and sAPPbeta production in astrocytes. *Biochem Biophys Res Commun* 2003; 307: 922–927.
22. Mulder SD, van der Flier WM, Verheijen JH, et al. BACE1 activity in cerebrospinal fluid and its relation to markers of AD pathology. *J Alzheimers Dis* 2010; 20: 253–260.
23. Fleck D, Van Bebber F, Colombo A, et al. Dual cleavage of neuregulin 1 type III by BACE1 and ADAM17 liberates its EGF-like domain and allows paracrine signaling. *J Neurosci* 2013; 33:7856–7869.
24. Schultz V, van der Meer F, Wrzos C, et al. Acutely damaged axons are remyelinated in multiple sclerosis and experimental models of demyelination. *Glia.* 2017 Aug;65(8):1350-1360.
25. Spitzer P, Lang R, Oberstein TJ, et al. A Specific Reduction in A β (1-42) vs. a Universal Loss of A β Peptides in CSF Differentiates Alzheimer's Disease From Meningitis and Multiple Sclerosis. *Front Aging Neurosci.* 2018 May 24;10:152.

26. Pietroboni AM, Scarioni M, Carandini T, et al. CSF β -amyloid and white matter damage: a new perspective on Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2017 Oct 20 [Epub ahead of print].
27. Bartzokis G. Alzheimer's disease as homeostatic responses to age-related myelin breakdown. *Neurobiol Aging*. 2011 Aug;32(8):1341-71.
28. Dietschy JM, Turley SD. Thematic review series: brain Lipids. Cholesterol metabolism in the central nervous system during early development and in the mature animal. *J Lipid Res*. 2004 Aug;45(8):1375-97. Review.
29. Amato MP, Portaccio E, Goretti B, et al. Association of neocortical volume changes with cognitive deterioration in relapsing-remitting multiple sclerosis. *Archives of Neurology* 2007;64:1157–1161.
30. Rocca MA, Agosta F, Sormani MP, et al. A three-year, multi-parametric MRI study in patients at presentation with CIS. *J Neurol* 2008;255:683-691.
31. Mattsson N, Andreasson U, Persson S, et al. CSF biomarker variability in the Alzheimer's Association quality control program. *Alzheimers Dement* 2013;9:251-61.