Supplementary material (Supplementary document S3)

Title: Neuronal and glial CSF biomarkers in multiple sclerosis: A systematic review and meta-analysis

Journal: Reviews in the Neurosciences

Authors: Sara Momtazmanesh, Parnian Shobeiri, Amene Saghazadeh, Charlotte Teunissen, Joachim Burman, Levente Szalardy , Peter Klivenyi, Ales Bartos, Adelaide Fernandes, Nima Rezaei*

Corresponding author: Nima Rezaei, MD, PhD, Research Center for Immunodeficiencies, Children's Medical Center, Dr. Gharib St, Keshavarz Blvd, Tehran, Iran

E-mail: rezaei_nima@tums.ac.ir

Table of Contents

Association of neuronal and glial damage biomarkers with imaging biomarkers.....	2
Effects of DMTs on neuronal and glial damage biomarkers	3
Blood levels of neuronal and glial damage biomarkers in MS.....	4

Association of neuronal and glial damage biomarkers with imaging biomarkers

Lesion load can be associated with CSF levels of neuronal and glial damage biomarkers. The CSF levels of NFL correlated with the formation of new MRI lesions in two years (Hakansson et al., 2017). Correlation of T1 lesion load, which indicates axonal and myelin loss, with CSF NFL (Burman et al., 2014), GFAP (Burman et al., 2014), t-tau (Kosehasanogullari et al., 2015) and correlation of T2 lesion load with NFL (Hakansson et al., 2017, Burman et al., 2014, Bergman et al., 2016) and t-tau (Brettschneider et al., 2006, Kosehasanogullari et al., 2015) have also been reported in MS.

Patients with a higher number of gadolinium-enhancing brain lesions, which are indicative of active and inflamed lesions, were found to have higher CSF levels of NFL (Novakova et al., 2017, Burman et al., 2014), GFAP (Burman et al., 2014), and t-tau (Brettschneider et al., 2005). Moreover, the number of gadolinium-enhanced lesions correlated with CSF NFL (Gil-Perotin et al., 2019, Burman et al., 2014) and CHI3L1 levels (Comabella et al., 2010). However, in some studies including PMS patients no correlation was found between the number of gadolinium-enhancing lesions and CSF t-tau (Mori et al., 2011) or CHI3L1 levels (Gil-Perotin et al., 2019).

CSF levels of neuronal and glial damage biomarkers may also differ between patients with different brain volume. Patients with low total gray matter and total brain volume had higher CSF NFL and GFAP levels compared to those without cerebral atrophy (Burman et al., 2014, Tortorella et al., 2018). Total and peripheral gray matter volume also correlated inversely with NFL levels (Tortorella et al., 2018). Moreover, baseline NFL and t-tau levels were predictors of brain atrophy after three years of follow up (Mellergard et al., 2017).

Effects of DMTs on neuronal and glial damage biomarkers

Reduction in CSF levels of NFL after DMTs (natalizumab, fingolimod, mitoxantrone, or rituximab) is reported (Gunnarsson et al., 2011, Axelsson et al., 2014, Sehr et al., 2019). However, two recent meta-analyses found no significant difference between CSF NFL levels in treated and untreated patients (Bridel et al., 2019, Martin et al., 2019). Natalizumab and mitoxantrone are also reported to decrease CSF CHI3L1 levels (Malmstrom et al., 2014). Albeit, Burman et al. reported no significant difference in CSF levels of CHI3L1 between treated and untreated patients (Burman et al., 2016). Notably, GFAP levels seem not to be affected by DMTs (natalizumab, mitoxantrone, or rituximab) (Axelsson et al., 2014, Gunnarsson et al., 2011).

For monitoring treatment response, serum biomarkers have the advantage of not requiring the invasive procedure of lumbar puncture, which makes them more suitable for conducting frequent measurements. Serum NFL levels are reported to reduce as a result of taking DMTs and, therefore, are suggested as a potential marker for monitoring treatment response (Disanto et al., 2017, Novakova et al., 2017, Piehl et al., 2018, Sehr et al., 2019). Moreover, Serum levels of S100B have also been suggested as a marker for the assessment of immunosuppressive therapies in MS (Bartosik-Psujek et al., 2011). However, serum levels of t-tau may not be a suitable marker for this application (Bartosik-Psujek et al., 2011).

Blood levels of neuronal and glial damage biomarkers in MS

Blood levels of NFL correlated with CSF levels in MS patients (Ayrignac et al., 2020, Disanto et al., 2017, Jens et al., 2016, Novakova et al., 2017, Piehl et al., 2018). A recent meta-analysis showed that blood levels of NFL were significantly higher in patients with MS compared to controls (Cai and Huang, 2018). However, some individual studies did not find any significant difference (Hogel et al., 2020). Serum levels of NFL were associated with age, EDSS, disease duration, and relapse rate (Hogel et al., 2020, Disanto et al., 2017, Delcoigne et al., 2020). NFL serum levels were also higher in patients with relapse than remission (Novakova et al., 2017) and were associated with disease activity on MRI (Varhaug et al., 2018). Blood levels of NFL may also aid in differentiating different subtypes of MS. Higher serum levels were reported in SPMS compared to RRMS (Hogel et al., 2020). However, no difference between these groups in serum NFL levels was reported as well (Baldassari et al., 2019).

Like NFL, serum levels of GFAP correlated with its concentration in the CSF, and MS patients were found to have higher serum GFAP levels compared to HC. Serum GFAP levels were associated with age, EDSS, MRI lesion load, and disease duration (Abdelhak et al., 2018, Hogel et al., 2020, Ayrignac et al., 2020). The correlation with clinical severity remained significant after correction for age (Abdelhak et al., 2019). Notably, patients with gadolinium-enhancing lesions had higher serum GFAP levels compared to others (Disanto et al., 2017).

Serum levels of other neuronal and glial damage biomarkers are less explored in MS. A recent study found lower levels of t-tau in the serum of RRMS patients compared to controls with a negative correlation with EDSS (Mirzaii-Dizgah et al., 2020). However, no difference in serum levels of CHI3L1 between MS patients and controls was reported (De Fino et al., 2019, Uysal et al., 2014). CHI3L1 serum levels did not correlate with clinical severity progression and new MRI

lesions in RRMS (Varhaug et al., 2018). However, Huss et al. found a positive correlation between serum levels of CHI3L1 and EDSS scores only in PMS (Huss et al., 2020). Additionally, higher levels of S100B were also detected in the serum of MS patients (Bartosik-Psujek et al., 2011).

References:

- ABDELHAK, A., HOTTENROTT, T., MORENAS-RODRIGUEZ, E., SUAREZ-CALVET, M., ZETTL, U. K., HAASS, C., MEUTH, S. G., RAUER, S., OTTO, M., TUMANI, H. & HUSS, A. 2019. Glial Activation Markers in CSF and Serum From Patients With Primary Progressive Multiple Sclerosis: Potential of Serum GFAP as Disease Severity Marker? *Front Neurol*, 10, 280.
- ABDELHAK, A., HUSS, A., KASSUBEK, J., TUMANI, H. & OTTO, M. 2018. Serum GFAP as a biomarker for disease severity in multiple sclerosis. *Sci Rep*, 8, 14798.
- AXELSSON, M., MALMESTRÖM, C., GUNNARSSON, M., ZETTERBERG, H., SUNDSTRÖM, P., LYCKE, J. & SVENNINGSSON, A. 2014. Immunosuppressive therapy reduces axonal damage in progressive multiple sclerosis. *Multiple Sclerosis*, 20, 43-50.
- AYRIGNAC, X., LE BARS, E., DUFLOS, C., HIRTZ, C., MALESKA MACESKI, A., CARRA-DALLIERE, C., CHARIF, M., PINNA, F., PRIN, P., MENJOT DE CHAMPFLEUR, N., DEVERDUN, J., KOBER, T., MARECHAL, B., FARTARIA, M. J., CORREDOR JEREZ, R., LABAUGE, P. & LEHMANN, S. 2020. Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity. *Sci Rep*, 10, 10923.
- BALDASSARI, L. E., PLANCHON, S. M., BERMEL, R. A., NAKAMURA, K., FISHER, E., FENG, J., SAKAIE, K. E., ONTANEDA, D. & COHEN, J. A. 2019. Serum neurofilament light chain concentration in a phase 1/2 trial of autologous mesenchymal stem cell transplantation. *Mult Scler J Exp Transl Clin*, 5, 2055217319887198.
- BARTOSIK-PSUJEK, H., PSUJEK, M., JAWORSKI, J. & STELMASIAK, Z. 2011. Total tau and S100b proteins in different types of multiple sclerosis and during immunosuppressive treatment with mitoxantrone. *Acta Neurologica Scandinavica*, 123, 252-256.
- BERGMAN, P., PIKET, E., KHADEMI, M., JAMES, T., BRUNDIN, L., OLSSON, T., PIEHL, F. & JAGODIC, M. 2016. Circulating miR-150 in CSF is a novel candidate biomarker for multiple sclerosis. *Neurol Neuroimmunol Neuroinflamm*, 3, e219.
- BRETTSCHNEIDER, J., MAIER, M., ARDA, S., CLAUS, A., SUSSMUTH, S. D., KASSUBEK, J. & TUMANI, H. 2005. Tau protein level in cerebrospinal fluid is increased in patients with early multiple sclerosis. *Mult Scler*, 11, 261-5.
- BRETTSCHNEIDER, J., PETZOLD, A., JUNKER, A. & TUMANI, H. 2006. Axonal damage markers in the cerebrospinal fluid of patients with clinically isolated syndrome improve predicting conversion to definite multiple sclerosis. *Mult Scler*, 12, 143-8.
- BRIDEL, C., VAN WIERINGEN, W. N., ZETTERBERG, H., TIJMS, B. M., TEUNISSEN, C. E., AND THE, N. F. L. G., ALVAREZ-CERMENO, J. C., ANDREASSON, U., AXELSSON, M., BACKSTROM, D. C., BARTOS, A., BJERKE, M., BLENNOW, K., BOXER, A., BRUNDIN, L., BURMAN, J., CHRISTENSEN, T., FIALOVA, L., FORSGREN, L., FREDERIKSEN, J. L., GISSLEN, M., GRAY, E., GUNNARSSON, M., HALL, S., HANSSON, O., HERBERT, M. K., JAKOBSSON, J., JESSEN-KRUT, J., JANELIDZE, S., JOHANNSSON, G., JONSSON, M., KAPPOS, L., KHADEMI, M., KHALIL, M., KUHLE, J., LANDEN, M., LEINONEN, V., LOGROSCINO, G., LU, C. H., LYCKE, J., MAGDALINO, N. K., MALASPINA, A., MATTSSON, N., MEETER, L. H., MEHTA, S. R., MODVIG, S., OLSSON, T., PATERSON, R. W., PEREZ-SANTIAGO, J.,

- PIEHL, F., PIJNENBURG, Y. A. L., PYYKKO, O. T., RAGNARSSON, O., ROJAS, J. C., ROMME CHRISTENSEN, J., SANDBERG, L., SCHERLING, C. S., SCHOTT, J. M., SELLEBJERG, F. T., SIMONE, I. L., SKILLBACK, T., STILUND, M., SUNDSTROM, P., SVENNINGSSON, A., TORTELLI, R., TORTORELLA, C., TRENTINI, A., TROIANO, M., TURNER, M. R., VAN SWIETEN, J. C., VAGBERG, M., VERBEEK, M. M., VILLAR, L. M., VISSER, P. J., WALLIN, A., WEISS, A., WIKKELSO, C. & WILD, E. J. 2019. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. *JAMA Neurol.*
- BURMAN, J., RAININKO, R., BLENNOW, K., ZETTERBERG, H., AXELSSON, M. & MALMESTROM, C. 2016. YKL-40 is a CSF biomarker of intrathecal inflammation in secondary progressive multiple sclerosis. *J Neuroimmunol*, 292, 52-7.
- BURMAN, J., ZETTERBERG, H., FRANSSON, M., LOSKOG, A. S., RAININKO, R. & FAGIUS, J. 2014. Assessing tissue damage in multiple sclerosis: A biomarker approach. *Acta Neurologica Scandinavica*, 130, 81-89.
- CAI, L. & HUANG, J. 2018. Neurofilament light chain as a biological marker for multiple sclerosis: a meta-analysis study. *Neuropsychiatr Dis Treat*, 14, 2241-2254.
- COMABELLA, M., FERNANDEZ, M., MARTIN, R., RIVERA-VALLVE, S., BORRAS, E., CHIVA, C., JULIA, E., ROVIRA, A., CANTO, E., ALVAREZ-CERMENO, J. C., VILLAR, L. M., TINTORE, M. & MONTALBAN, X. 2010. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. *Brain*, 133, 1082-93.
- DE FINO, C., LUCCHINI, M., LUCCHETTI, D., NOCITI, V., LOSAVIO, F. A., BIANCO, A., COLELLA, F., RICCIARDI-TENORE, C., SGAMBATO, A. & MIRABELLA, M. 2019. The predictive value of CSF multiple assay in multiple sclerosis: A single center experience. *Mult Scler Relat Disord*, 35, 176-181.
- DELCOIGNE, B., MANOUCHEHRINIA, A., BARRO, C., BENKERT, P., MICHALAK, Z., KAPPOS, L., LEPPERT, D., TSAI, J. A., PLAVINA, T., KIESEIER, B. C., LYCKE, J., ALFREDSSON, L., KOCKUM, I., KUHLE, J., OLSSON, T. & PIEHL, F. 2020. Blood neurofilament light levels segregate treatment effects in multiple sclerosis. *Neurology*, 94, e1201-e1212.
- DISANTO, G., BARRO, C., BENKERT, P., NAEGELIN, Y., SCHÄDELIN, S., GIARDIELLO, A., ZECCA, C., BLENNOW, K., ZETTERBERG, H., LEPPERT, D., KAPPOS, L., GOBBI, C. & KUHLE, J. 2017. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Ann Neurol*, 81, 857-870.
- GIL-PEROTIN, S., CASTILLO-VILLALBA, J., CUBAS-NUÑEZ, L., GASQUE, R., HERVAS, D., GOMEZ-MATEU, J., ALCALA, C., PEREZ-MIRALLES, F., GASCON, F., DOMINGUEZ, J. A. & CASANOVA, B. 2019. Combined Cerebrospinal Fluid Neurofilament Light Chain Protein and Chitinase-3 Like-1 Levels in Defining Disease Course and Prognosis in Multiple Sclerosis. *Frontiers in Neurology*, 10.
- GUNNARSSON, M., MALMESTROM, C., AXELSSON, M., SUNDSTROM, P., DAHLE, C., VRETHEM, M., OLSSON, T., PIEHL, F., NORGREN, N., ROSENGREN, L., SVENNINGSSON, A. & LYCKE, J. 2011. Axonal damage in relapsing multiple sclerosis is markedly reduced by natalizumab. *Ann Neurol*, 69, 83-9.
- HAKANSSON, I., TISELL, A., CASSEL, P., BLENNOW, K., ZETTERBERG, H., LUNDBERG, P., DAHLE, C., VRETHEM, M. & ERNERUDH, J. 2017. Neurofilament light chain in cerebrospinal fluid and prediction of disease activity in clinically isolated syndrome and relapsing-remitting multiple sclerosis. *Eur J Neurol*, 24, 703-712.
- HOGEL, H., RISSANEN, E., BARRO, C., MATILAINEN, M., NYLUND, M., KUHLE, J. & AIRAS, L. 2020. Serum glial fibrillary acidic protein correlates with multiple sclerosis disease severity. *Mult Scler*, 26, 210-219.

- HUSS, A., OTTO, M., SENEL, M., LUDOLPH, A. C., ABDELHAK, A. & TUMANI, H. 2020. A Score Based on NfL and Glial Markers May Differentiate Between Relapsing-Remitting and Progressive MS Course. *Front Neurol*, 11, 608.
- JENS, K., CHRISTIAN, B., ULF, A., TOBIAS, D., RAIJA, L., ÅSA, S., VICTOR, L., NIKLAS, N., KAJ, B. & HENRIK, Z. 2016. Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 54, 1655-1661.
- KOSEHASANOĞULLARI, G., OZAKBAS, S. & IDIMAN, E. 2015. Tau protein levels in the cerebrospinal fluid of the patients with multiple sclerosis in an attack period: Low levels of tau protein may have significance, too. *Clinical Neurology and Neurosurgery*, 136, 107-109.
- MALMESTROM, C., AXELSSON, M., LYCKE, J., ZETTERBERG, H., BLENNOW, K. & OLSSON, B. 2014. CSF levels of YKL-40 are increased in MS and replaces with immunosuppressive treatment. *J Neuroimmunol*, 269, 87-9.
- MARTIN, S. J., MCGLASSON, S., HUNT, D. & OVERELL, J. 2019. Cerebrospinal fluid neurofilament light chain in multiple sclerosis and its subtypes: a meta-analysis of case-control studies. *J Neurol Neurosurg Psychiatry*, 90, 1059-1067.
- MELLERGARD, J., TISELL, A., BLYSTAD, I., GRONQVIST, A., BLENNOW, K., OLSSON, B., DAHLE, C., VRETHEM, M., LUNDBERG, P. & ERNERUDH, J. 2017. Cerebrospinal fluid levels of neurofilament and tau correlate with brain atrophy in natalizumab-treated multiple sclerosis. *Eur J Neurol*, 24, 112-121.
- MIRZAI-DIZGAH, M.-H., MIRZAI-DIZGAH, M.-R. & MIRZAI-DIZGAH, I. 2020. Serum and saliva total tau protein as a marker for relapsing-remitting multiple sclerosis. *Medical Hypotheses*, 135, 109476.
- MORI, F., ROSSI, S., SANCESARIO, G., CODECA, C., MATALUNI, G., MONTELEONE, F., BUTTARI, F., KUSAYANAGI, H., CASTELLI, M., MOTTA, C., STUDER, V., BERNARDI, G., KOCH, G., BERNARDINI, S. & CENTONZE, D. 2011. Cognitive and cortical plasticity deficits correlate with altered amyloid-beta CSF levels in multiple sclerosis. *Neuropsychopharmacology*, 36, 559-68.
- NOVAKOVA, L., ZETTERBERG, H., SUNDSTROM, P., AXELSSON, M., KHADEMI, M., GUNNARSSON, M., MALMESTROM, C., SVENNINGSSON, A., OLSSON, T., PIEHL, F., BLENNOW, K. & LYCKE, J. 2017. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. *Neurology*, 89, 2230-2237.
- PIEHL, F., KOCKUM, I., KHADEMI, M., BLENNOW, K., LYCKE, J., ZETTERBERG, H. & OLSSON, T. 2018. Plasma neurofilament light chain levels in patients with MS switching from injectable therapies to fingolimod. *Multiple Sclerosis Journal*, 24, 1046-1054.
- SEHR, T., AKGUN, K., PROSCHMANN, U., BUCKI, R., ZENDZIAN-PIOTROWSKA, M. & ZIEMSEN, T. 2019. Early central vs. peripheral immunological and neurobiological effects of fingolimod-a longitudinal study. *J Mol Med (Berl)*, 97, 1263-1271.
- TORTORELLA, C., DIRENZO, V., RUGGIERI, M., ZOCCOLELLA, S., MASTRAPASQUA, M., D'ONGHIA, M., PAOLICELLI, D., CUONZO, F. D., GASPERINI, C. & TROJANO, M. 2018. Cerebrospinal fluid neurofilament light levels mark grey matter volume in clinically isolated syndrome suggestive of multiple sclerosis. *Mult Scler*, 24, 1039-1045.
- UYSAI, S., MERIC YILMAZ, F., BOĞDAYCIOĞLU, N., MUNGAN ÖZTÜRK, S. & AK, F. 2014. Increased serum levels of some inflammatory markers in patients with multiple sclerosis. *Minerva Med*, 105, 229-35.
- VARHAUG, K. N., BARRO, C., BJØRNEVIK, K., MYHR, K. M., TORKILDSEN, Ø., WERGELAND, S., BINDOFF, L. A., KUHLE, J. & VEDELER, C. 2018. Neurofilament light chain predicts disease activity in relapsing-remitting MS. *Neurol Neuroimmunol Neuroinflamm*, 5, e422.