

Cerebrospinal fluid adenosine triphosphate metabolites in multiple sclerosis

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Abbreviations: ATP = adenosine triphosphate, BSP = brain-specific proteins, CSF = cerebrospinal fluid, CTRL = control. EDSS = expanded disability status scale, ELISA = enzyme linked immunoabsorbant assay, HPLC = High Performance Liquid Chromatography, MS = multiple sclerosis, Nf = neurofilament, NfH = neurofilament heavy chain, PP = primary progressive, RR = relapsing remitting, SP = secondary progressive.

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Abstract

Background: Increased axonal energy demand and mitochondrial failure have been suggested as possible causes for axonal degeneration and disability in multiple sclerosis (MS).

Objective: To test whether ATP depletion precedes clinical, imaging and biomarker evidence for axonal degeneration in MS.

Methods: A longitudinal study including 21 patients with MS. High Performance Liquid Chromatography (HPLC) was used to quantify biomarkers of the ATP metabolism (oxypurines and purines) from the cerebrospinal fluid (CSF) at baseline. The Expanded Disability Status Scale (EDSS), MRI brain imaging measures for brain atrophy (ventricular and parenchymal fractions) and CSF biomarkers for axonal damage (phosphorylated and hyperphosphorylated neurofilaments) were quantified at baseline and 3-year follow up.

Results: Central ATP depletion (sum of ATP metabolites $> 19.7 \mu\text{mol/L}$) was followed by more severe progression of disability if compared to normal ATP metabolites (median 1.5 vs 0, $p < 0.05$). Baseline ATP metabolite levels correlated with change of EDSS in the pooled cohort ($R=0.66$, $p=0.001$) and subgroups (RR patients: $R=0.79$, $p < 0.05$ and SP/PP patients: $R=0.69$, $p < 0.01$). There was no relationship between central ATP metabolites and either biomarker or MRI evidence for axonal degeneration.

Conclusion: The data suggests that an increased energy demand in MS causes a quantifiable degree of central ATP depletion. We speculate that the observed clinical disability may be caused by depolarisation associated conduction block.

1 Introduction

Progression of disability in multiple sclerosis (MS) is hypothesised to be ultimately linked to axonal loss.¹ One of the mechanisms driving axonal degeneration has suggested to result from virtual hypoxia (reviewed in reference²). [There is some evidence](#) that the increased expression of sodium channels along demyelinated axons requires an increased amount of adenosine triphosphate (ATP) even at a resting state (reviewed in reference³). This places an increased energy demand and may result in an additional energy penalty of neuroelectrical conduction in the damaged axons. It has been proposed that the exhaustion of ATP stores results in loss of protective ion-pump function leading to intracellular Ca^{2+} overload which then activates a “death cascade”.^{2,4} Consequently the disintegrating axons release their cellular content into the adjacent body fluid compartment, the extracellular fluid from where they diffuse into the cerebrospinal fluid (CSF) (reviewed in reference⁵). [The mentioned Editorial by Waxman³ refers to a landmark paper by Kapoor *et al.* on axonal vulnerability. Kapoor *et al.* demonstrated convincingly how electrically active axons degenerate in a biochemically hostile environment. Invariably](#) following axonal loss and neurodegeneration, atrophy of the brain ensues and disability progresses (reviewed in reference⁶).

Energy available to cells depends crucially on availability of ATP. In MS there is accumulating evidence for a mismatch between energy production and energy consumption [which has been linked genetically and histologically to mitochondrial dysfunction.](#)⁷⁻¹⁰ Immunohistochemical and histochemical studies suggested that particularly complex IV of the respiratory chain may be affected in demyelinated axons.⁸ These authors

also showed that inhibition of complex IV augmented glutamate mediated axonal injury,⁸ a mechanism well recognised from cell biology.¹¹ Mitochondrial failure is known to be enhanced by oxidative stress,¹² for which there is convincing post-mortem evidence in MS.^{7,9} The biochemical consequence of mitochondrial failure is a metabolic imbalance between ATP consumption and ATP production.^{13–15} There is a strict correlation between ATP depletion and concomitant increase of ATP breakdown products (purines and nucleosides).^{16–19}

Therefore exhaustion of the ATP results in an increase of the end products of the metabolic pathway.^{16–19} The dominant end products of this pathway are oxypurine (uric acid, hypoxanthine, xanthine) and purine nucleosides (inosine, adenosine, guanosine).^{16–19} Higher CSF concentrations of total purine nucleosides and total oxypurines in patients with MS compared to control subjects provide indirect evidence for increased ATP consumption in MS.²⁰ This finding rises the question whether increased *in vivo* ATP consumption in patients with MS could precede axonal degeneration as suggested by the energy insufficiency/virtual hypoxia hypothesis.²

In this prospective, longitudinal study we tested if ATP depletion at baseline would predict the development of axonal degeneration assessed by three methods: a clinical scale for disability (EDSS), MRI brain atrophy measures and protein biomarkers for axonal damage.

2 Materials and methods

This study was approved by the IRB and written informed consent was obtained from all patients.

Two CSF samples (baseline and three year follow up) were available from 21 patients of a previously reported cohort²¹ with clinically definite

MS.²²

The Expanded Disability Status Scale score (EDSS)²³ was recorded at baseline and 3-year follow up. Progression of disability was calculated as $\Delta\text{EDSS} = \text{follow up EDSS} - \text{baseline-EDSS}$.

MRI examinations were performed at 1.0 T or 1.5 T at baseline and followup. In brief, as measures for brain atrophy we used (1) the parenchymal fraction (PF), calculated as the ratio of the whole brain parenchyma to the intracranial volume and (2) the ventricular fraction (VF) calculated as the ratio of the ventricular volume to the whole brain parenchyma.²⁴ The change between baseline and follow up was calculated as $\Delta\text{PF} = \text{follow up-PF} - \text{baseline-PF}$ and $\Delta\text{VF} = \text{follow up-VF} - \text{baseline-VF}$.

Samples of CSF were obtained by routine lumbar puncture. Aliquots of CSF were centrifuged to remove cellular debris and immediately stored at -70°C until assayed. CSF levels of ATP metabolites (uric acid, hypoxanthine, xanthine, inosine, adenosine, guanosine) were quantified by High Performance Liquid Chromatography (HPLC).²⁰ The sum of the ATP metabolite levels was considered high if above the range observed in the control population (mean 13.87, standard deviation 5.83; cut-off $> 19.7 \mu\text{mol/L}$ ²⁰). CSF biomarker levels for axonal damage, the phosphorylated neurofilament heavy chain (NfH^{SMI35}) and hyperphosphorylated neurofilament heavy chain (NfH^{SMI34}) were quantified by ELISA²⁵ at baseline and 3-year follow up. The change between baseline and follow up was calculated as $\Delta\text{NfH} = \text{follow up NfH} - \text{baseline-NfH}$.

Data analysis All statistical analyses and graphs were done using SAS software (version 9.1.3, SAS Institute, Inc., Cary, North Carolina, USA). Because of non-Gaussian distribution the median values and the 25–75

% interquartile range (IQR) were shown. Independent variables were compared using the non-parametric Kruskal–Wallis test. Analyses of covariance were performed with general linear models. The linear relationship between continuous variables was evaluated using the Spearman correlation coefficient.

3 Results

The baseline characteristics of the patients are summarised in Table 1. One-third (7/21) of the MS patients had normal CSF ATP metabolite levels ($< 19.7 \mu\text{mol/L}$). Two-thirds (14/21) of the MS patients had evidence for ATP depletion (Table 1). There was no significant difference between the two groups in terms of age, gender distribution, disease duration or treatment with disease modifying drugs. The median EDSS appeared to be slightly higher in patients with normal ATP stores, but data distribution was overlapping and no statistical significant difference was found (Table 1). This observation was however taken into account for a post-hoc covariate analysis (see below). Likewise there was no statistical significant difference between MRI atrophy measures (PF, VF) and axonal damage biomarkers (NfH^{SMI34}, NfH^{SMI35}).

Three years later those patients with evidence for central ATP depletion progressed significantly more on the EDSS (median +1.5 points) compared to those with normal central ATP stores (median 0 points, $p=0.035$, Table 2). No difference was found for either MRI atrophy measures (ΔPV , ΔVF) or axonal damage biomarkers ($\Delta\text{NfH}^{\text{SMI34}}$, $\Delta\text{NfH}^{\text{SMI35}}$).

Importantly, CSF ATP metabolites correlated significantly with the degree of disease progression on the EDSS in the entire cohort ($R=0.67$, $p=0.001$, Figure 1). This correlation was not influenced by the disease sub-

type. In fact the subgroup analysis revealed that significance was retained in RR disease ($R=0.79$, $p<0.05$) and SP/PP disease ($R=0.69$, $p<0.01$).

In a post-hoc analysis the baseline EDSS was identified as a relevant covariate for the statistical analyses on disease progression. Controlling for this covariate reduced the difference for CSF ATP metabolites below noise ($F=2.54$, $p=0.12$).

No correlations were found between the CSF ATP metabolite levels and the patients age, disease duration, MRI atrophy measures or axonal damage biomarkers (data not shown).

4 Discussion

The main finding of this prospective, longitudinal study was that patients who suffer from MS and have biomarker evidence for depletion of ATP energy stores also suffered from significantly more disability progression over the following 3 years compared to patients who had normal ATP energy stores. This finding is consistent with the concept that a central energy penalty may contribute to disability progression in MS.²

An unexpected finding of this study was that central ATP depletion did not lead to any detectable degree of axonal damage either by MRI atrophy measures or axonal damage biomarkers. This finding extends on a previous observation by Narayana *et al.* who found that a drop of NAA could be transient and was not necessarily followed by axonal loss.²⁶ This is interesting because both NAA and ATP are highly concentrated in mitochondria.²⁷ In fact, NAA synthesis depends on energy from the ATP metabolism. Decreased NAA peaks were correlated to disability in a number of studies (reviewed in²⁸). It is therefore conceivable that decreased ATP metabolites indicate dysfunction of the mitochondrial energy

metabolism reduced NAA synthesis and disability. This hypothesis would need to be tested prospectively combining CSF and serial proton magnetic resonance spectroscopic imaging in another cohort of MS patients. It needs to be borne in mind that the present like other clinical *in vivo* studies on this hypothesis²⁹ were based on indirect evidence and all findings are correlative at best. A statistical significant correlation is no proof of causality. Therefore the hypothesis should also need to be tested in an experimentally.

How can an ATP energy penalty lead to disability progression without quantifiable evidence (MRI and protein biomarkers) for neuro-axonal loss? Physiologically disability may result from neuroelectrical conduction block.³⁰ Biochemically it has been shown that synaptic mitochondria are considerably more vulnerable to an ATP deficit than non-synaptic mitochondria.^{31,32} It may therefore be possible that the here observed increase of ATP metabolites which we interpret as indirect evidence for central ATP depletion may suffice for blocking transmission at the synaptic level. In addition, conduction may be blocked due to the enhanced energy demand following increased expression of sodium channels.³ Finally, conduction may be blocked on a ganglionic level. Rush *et al.* showed for voltage gated sodium channels (NA_v1.7) that a single mutation (L858H) can cause hypopolarisation (increased threshold and attenuated repetitive firing) in one cell population and hyperpolarisation in another cell population.³³ Crucially, the polarisation pattern depended on the presence of NA_v1.8 channels.³³ Experimentally it has been shown that NA_v1.8 channels were upregulated in Purkinje cells of an animal model of MS.³⁴ In addition, there is human post-mortem evidence for altered expression of NA_v1.2 and NA_v1.6 channels in MS plaques.³⁵ It has therefore been pro-

posed that MS has features of an acquired channelopathy.³⁶

To summarise, ATP depletion can cause depolarisation and thus conduction block on all three levels (synaptic, axonal and ganglionic). This would explain the relationship between loss of function and indirect CSF evidence for a central depletion of ATP stores without evidence for substantial axonal loss on a brain imaging or CSF protein biomarkers level. One limitation of this argumentation is that conduction block is understood to be reversible. On the background of our current understanding of conduction block it would therefore be difficult to explain how central ATP depletion related depolarisation leads to sustained disability. In fact in a post-hoc analysis the degree of baseline disability was identified as an important covariate for the statistical results. Therefore any future study aiming to test the validity of the model would need to compare groups of MS patients which are matched for their baseline EDSS prospectively. Another limitation of this study is that the PF/VF measurements were performed before semi-automated protocols such as SIENA had been validated.³⁷ Future studies could include more detailed and localised atrophy rates.

Taken together, our data suggests that central ATP depletion is associated with disability progression in MS, possibly due to depolarisation causing neuroelectrical conduction block. Considering the possibility of conduction block may be found useful in refining the virtual hypoxia hypothesis.²

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References

- [1] BD Trapp, JP Peterson, et al. Axonal transection in the lesions of multiple sclerosis. *N Eng J Med*, 338:278–285, 1998.
- [2] Bruce D Trapp and Peter K Stys. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. *Lancet Neurol*, 8(3):280–291, Mar 2009.
- [3] S.G. Waxman. Membranes, myelin, and the pathophysiology of multiple sclerosis. *N Engl J Med*, 306:1529–1533, 1982.
- [4] SG Waxman. Nitric oxide and the axonal death cascade. *Ann Neurol*, 53:150–103, 2003.
- [5] A Petzold. CSF biomarkers for improved prognostic accuracy in acute CNS disease. *Neurological Research*, 29:691–708, 2007.
- [6] Frederik Barkhof, Peter A Calabresi, David H Miller, and Stephen C Reingold. Imaging outcomes for neuroprotection and repair in multiple sclerosis trials. *Nat Rev Neurol*, 5(5):256–266, May 2009.
- [7] Maarten E Witte, Lars BÃ, Richard J Rodenburg, Jeroen A Belien, Rene Musters, Thierry Hazes, Liesbeth T Wintjes, Jan A Smeitink, Jeroen J G Geurts, Helga E De Vries, Paul van der Valk, and Jack van Horssen. Enhanced number and activity of mitochondria in multiple sclerosis lesions. *J Pathol*, 219(2):193–204, Oct 2009.
- [8] Don J Mahad, Iryna Ziabreva, Graham Campbell, Nichola Lax, Katherine White, Peter S Hanson, Hans Lassmann, and Douglass M Turnbull. Mitochondrial changes within axons in multiple sclerosis. *Brain*, 132(Pt 5):1161–1174, May 2009.

- [9] Kimmy G Su, Gary Banker, Dennis Bourdette, and Michael Forte. Axonal degeneration in multiple sclerosis: the mitochondrial hypothesis. *Curr Neurol Neurosci Rep*, 9(5):411–417, Sep 2009.
- [10] Andrei Blokhin, Tamara Vyshkina, Samuel Komoly, and Bernadette Kalman. Variations in mitochondrial dna copy numbers in ms brains. *J Mol Neurosci*, 35(3):283–287, Jul 2008.
- [11] A. Almeida, S. J. Heales, J. P. Bola nos, and J. M. Medina. Glutamate neurotoxicity is associated with nitric oxide-mediated mitochondrial dysfunction and glutathione depletion. *Brain Res*, 790(1-2):209–216, Apr 1998.
- [12] Jake Jacobson, Michael R Duchen, John Hothersall, John B Clark, and Simon J R Heales. Induction of mitochondrial oxidative stress in astrocytes by nitric oxide precedes disruption of energy metabolism. *J Neurochem*, 95(2):388–395, Oct 2005.
- [13] Nele Vanlangenakker, Tom Vanden Berghe, Dmitri V Krysko, Nele Festjens, and Peter Vandenabeele. Molecular mechanisms and pathophysiology of necrotic cell death. *Curr Mol Med*, 8(3):207–220, May 2008.
- [14] Olivier Baud, Jianrong Li, Yumin Zhang, Rachael L Neve, Joseph J Volpe, and Paul A Rosenberg. Nitric oxide-induced cell death in developing oligodendrocytes is associated with mitochondrial dysfunction and apoptosis-inducing factor translocation. *Eur J Neurosci*, 20(7):1713–1726, Oct 2004.
- [15] Isabelle C J Lang-Rollin, Hardy J Rideout, Manish Noticewala, and Leonidas Stefanis. Mechanisms of caspase-independent neuronal

- death: energy depletion and free radical generation. *J Neurosci*, 23(35):11015–11025, Dec 2003.
- [16] S. M. Humphrey, D. G. Holliss, and R. N. Seelye. Myocardial adenine pool depletion and recovery of mechanical function following ischemia. *Am J Physiol*, 248(5 Pt 2):H644–H651, May 1985.
- [17] Leszek Domański, Krzysztof Safranow, Marek Ostrowski, Andrzej Pawlik, Maria Olszewska, Grazyna Dutkiewicz, and Kazimierz Ciechanowski. Oxypurine and purine nucleoside concentrations in renal vein of allograft are potential markers of energy status of renal tissue. *Arch Med Res*, 38(2):240–246, Feb 2007.
- [18] J. W. de Jong, E. Keijzer, T. Huizer, and B. Schoutsen. Ischemic nucleotide breakdown increases during cardiac development due to drop in adenosine anabolism/catabolism ratio. *J Mol Cell Cardiol*, 22(10):1065–1070, Oct 1990.
- [19] S. M. Humphrey, D. G. Holliss, and L. A. Cartner. The influence of inhibitors of the atp degradative pathway on recovery of function and high energy phosphate after transient ischemia in the rat heart. *J Mol Cell Cardiol*, 18 Suppl 4:55–59, Oct 1986.
- [20] Angela M Amorini, Axel Petzold, Barbara Tavazzi, Judith Eikelenboom, Geoffrey Keir, Antonio Belli, Gavin Giovannoni, Valentina Di Pietro, Chris Polman, Serafina D’Urso, Roberto Vagnozzi, Bernard Uitdehaag, and Giuseppe Lazzarino. Increase of uric acid and purine compounds in biological fluids of multiple sclerosis patients. *Clin Biochem*, pages 1001–1006, 2009.

- [21] A Petzold, MJ Eikelenboom, G Keir, et al. Axonal damage accumulates in the progressive phase of multiple sclerosis: A 3-year follow-up study. *J Neurol Neurosurg Psychiatry*, 76:206–211, 2005.
- [22] WI McDonald, A Compston, G Edan, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the International Panel on the diagnosis of multiple sclerosis. *Ann Neurol*, 50:121–127, 2001.
- [23] JF Kurtzke. Rating neurological impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, 33:1444–1452, 1983.
- [24] NF Kalkers, E Bergers, JA Castelijns, et al. Optimizing the association between disability and biological markers in MS. *Neurology*, 57:1253–1258, 2001.
- [25] A Petzold, G Keir, AJE Green, G Giovannoni, and EJ Thompson. A specific ELISA for measuring neurofilament heavy chain phosphoforms. *J Immunol Methods*, 278:179–190, 2003.
- [26] P. A. Narayana, T. J. Doyle, D. Lai, and J. S. Wolinsky. Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. *Ann Neurol*, 43(1):56–71, Jan 1998.
- [27] J. B. Clark. N-acetyl aspartate: a marker for neuronal loss or mitochondrial dysfunction. *Dev Neurosci*, 20(4-5):271–276, 1998.
- [28] Nicola De Stefano and Massimo Filippi. Mr spectroscopy in multiple sclerosis. *J Neuroimaging*, 17 Suppl 1:31S–35S, Apr 2007.

- [29] William T Regenold, Pornima Phatak, Michael J Makley, Roger D Stone, and Mitchel A Kling. Cerebrospinal fluid evidence of increased extra-mitochondrial glucose metabolism implicates mitochondrial dysfunction in multiple sclerosis disease progression. *J Neurol Sci*, 275(1-2):106–112, Dec 2008.
- [30] K. J. Smith. Conduction properties of central demyelinated and remyelinated axons, and their relation to symptom production in demyelinating disorders. *Eye*, 8:224–237, 1994.
- [31] G. P. Davey, L. Canevari, and J. B. Clark. Threshold effects in synaptosomal and nonsynaptic mitochondria from hippocampal ca1 and paramedian neocortex brain regions. *J Neurochem*, 69(6):2564–2570, Dec 1997.
- [32] G. P. Davey, S. Peuchen, and J. B. Clark. Energy thresholds in brain mitochondria. potential involvement in neurodegeneration. *J Biol Chem*, 273(21):12753–12757, May 1998.
- [33] Anthony M Rush, Sulayman D Dib-Hajj, Shujun Liu, Theodore R Cummins, Joel A Black, and Stephen G Waxman. A single sodium channel mutation produces hyper- or hypoexcitability in different types of neurons. *Proc Natl Acad Sci U S A*, 103(21):8245–8250, May 2006.
- [34] Matthew J Craner, Yuko Kataoka, Albert C Lo, Joel A Black, David Baker, and Stephen G Waxman. Temporal course of upregulation of na(v)1.8 in purkinje neurons parallels the progression of clinical deficit in experimental allergic encephalomyelitis. *J Neuropathol Exp Neurol*, 62(9):968–975, Sep 2003.

- [35] Joel A Black, Jia Newcombe, Bruce D Trapp, and Stephen G Waxman. Sodium channel expression within chronic multiple sclerosis plaques. *J Neuropathol Exp Neurol*, 66(9):828–837, Sep 2007.
- [36] S. G. Waxman. Acquired channelopathies in nerve injury and ms. *Neurology*, 56(12):1621–1627, Jun 2001.
- [37] D. R. Altmann, B. Jasperse, F. Barkhof, K. Beckmann, M. Filippi, L. D. Kappos, P. Molyneux, C. H. Polman, C. Pozzilli, A. J. Thompson, K. Wagner, T. A. Yousry, and D. H. Miller. Sample sizes for brain atrophy outcomes in trials for secondary progressive multiple sclerosis. *Neurology*, 72:595–601, 2009.

Table 1: *Baseline characteristics of MS patients. Patients were classified at baseline according to their central ATP metabolism. The median (IRQ) is shown, NS = not significant.*

	MS (all)	MS (normal ATP)	MS (depleted ATP)	
Age (years)	45 (38-51)	49 (40-55)	43 (35-48)	NS
Gender (F:M)	12:9	4:3	8:6	NS
Disease duration (years)	13.9 (9.5-19.1)	19.1 (12.6-22.0)	13.45 (7.4-17.0)	NS
IFN	8(38%)	3 (43%)	5 (34%)	NS
EDSS	4.0 (2.0-6.0)	6.0 (2.5-6.5)	2.25 (1.5-5.5)	NS
MRI PF	0.81 (0.79-0.83)	0.80 (0.77-0.83)	0.82 (0.79-0.83)	NS
MRI VF	0.03 (0.02-0.04)	0.03 (0.03-0.04)	0.03 (0.02-0.04)	NS
CSF NfH ^{SMI34}	9 (7-13)	11 (7-19)	9 (7-12)	NS
CSF NfH ^{SMI35}	78 (17-155)	139 (31-190)	575 (110-123)	NS
Number	21	7	14	

Table 2: Change from baseline to 3-year follow up. The median (IRQ) is shown.

	MS (all)	MS (normal ATP)	MS (depleted ATP)	
Δ EDSS	0.05 (0-1.5)	0 (-0.5-1.0)	1.5 (0-2.0)	0.035
Δ MRI PF	0.015 (-0.005-0.015)	0.02 (-0.03-0.02)	0.01 (0-0.04)	NS
Δ MRI VF	-0.005 (-0.01-0)	-0.01 (-0.01-0)	0 (-0.01- 0)	NS
Δ CSF NfH ^{SMI34}	19 (-3- 51)	28 (10-95)	2 (-7- 51)	NS
Δ CSF NfH ^{SMI35}	0 (-78- 87)	-11 (-91- 68)	41 (-78- 104)	NS

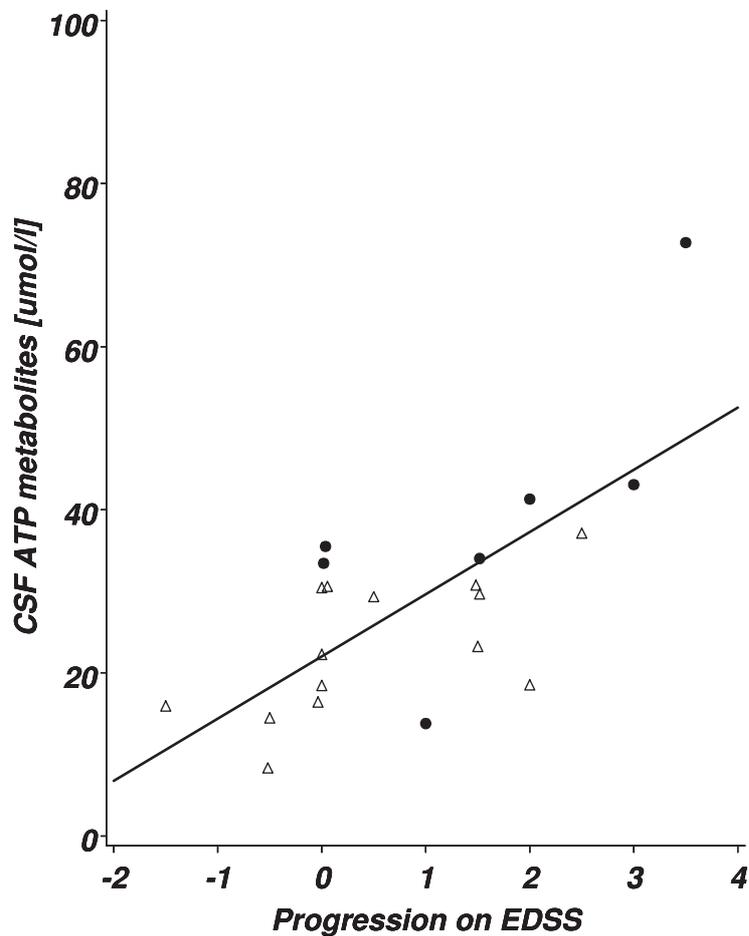


Figure 1: Baseline ATP metabolite levels correlated with the subsequent progression on the EDSS over the 3-year observation period in the pooled cohort (Spearman's $R=0.67$, $p=0.001$). Patients with RR disease are indicated by closed circles and patients with SP/PP disease by open triangles. Significance of the correlation was retained in the subgroup analyses (SP/PP disease: $R=0.69$, $p<0.01$; RR disease: $R=0.79$, $p<0.05$).