

Supplement Table 1. Post-hoc sample size power analysis.

Anti-EBNA-1 titer and T1 lesion MTR in MS			Anti-EBNA-1 titer and T1 lesion MTR in RRMS			Anti-EBNA-1 titer and NAGM MTR in RRMS		
r1=-0.287 vs. r2=0.00			r1=-0.524 vs. r2=0.00			r1=-0.308 vs r2=0.00		
power	Double sided testing	Single sided testing	power	Double sided testing	Single sided testing	power	Double sided testing	Single sided testing
60%	59	44	60%	17	13	60%	51	38
70%	73	56	70%	21	17	70%	63	49
80%	93	74	80%	26	21	80%	80	64
90%	123	101	90%	34	28	90%	106	87
95%	152	127	95%	41	35	95%	131	109

Legend: MS – multiple sclerosis, RRMS – relapsing remitting multiple sclerosis, EBNA-1 – Epstein Barr nuclear antigen-1, NAGM – normal appearing gray matter,

The power analysis was performed with the Simple Interactive Statistical Analysis (SISA) software.

Supplement Table 2. Associations between the level of humoral Epstein-Barr virus response (anti-EBNA-1 titer) and magnetization transfer ratio outcome measures in subsample of 3T-only MRI-scanned multiple sclerosis patients.

	MS (n=62)		RRMS (n=40)		SPMS (n=22)	
	r_s -value	p-value	r_s -value	p-value	r_s -value	p-value
T2 lesion MTR	-0.188	0.149	-0.189	0.249	-0.137	0.553
T1 lesion MTR	-0.353	0.022	-0.532	0.007	-0.053	0.834
NABT MTR	-0.15	0.315	-0.172	0.288	-0.118	0.601
NAWM MTR	-0.13	0.106	-0.117	0.473	-0.140	0.534
NAGM MTR	-0.207	0.244	-0.231	0.151	-0.124	0.584

Legend: MS – multiple sclerosis, RRMS – relapsing-remitting multiple sclerosis, SPMS – secondary progressive multiple sclerosis, EBNA-1 – Epstein Barr nuclear antigen-1, NABT – normal appearing brain tissue, NAWM – normal appearing white matter, NAGM – normal appearing gray matter.

Spearman's ranked correlation were performed. P values lower than 0.05 were considered significant and shown in bold.

Figure 1. Scatter-plot representation of the associations between anti-EBNA-1 titer levels and magnetization transfer ratio values of T1-hypointense lesions in all multiple sclerosis patients (left) and relapsing-remitting multiple sclerosis patients only (right).

Legend: MTR – magnetization transfer ratio, EBNA-1 – Epstein-Barr nuclear antigen-1. Due to the non-normal distribution of data, the anti-EBNA-1 titer was transformed using the natural logarithm and Pearson’s correlation is fitted.

Figure 2. Dichotomous comparison of anti-EBNA-1 titer between lower and higher NAGM MTR median values in MS subpopulations and in healthy controls.

Legend: EBNA-1 – Epstein-Barr nuclear antigen-1, NAGM – normal appearing gray matter, MTR – magnetization transfer ratio, RRMS – relapsing remitting multiple sclerosis, SPMS – secondary progressive multiple sclerosis, HCs – healthy controls.

P-values were derived with Mann-Whitney U test. Error bars demonstrate 95% confidence interval.

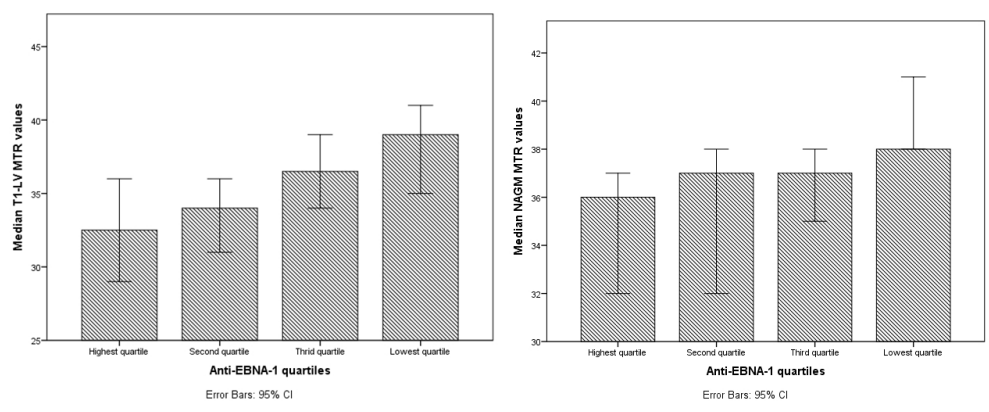
A significant difference (*) in anti-EBNA1 titer between RRMS with lower vs. higher half of MTR values.

Figure 3. Example of relapsing-remitting multiple sclerosis patients with very high anti-EBNA-1 titer and presence of T1-hypointensities

Legend: The patient had relatively low disease duration of 10 years, EDSS score of 3.5 and was on natalizumab treatment. At the time of serum sample examination, the patient presented with abnormally high titer of anti-EBNA-1 antibodies. The corresponding lesioned MRI features include 11 T1-hypointense lesions with total volume of 12.2ml and 12 T2-hyperintense lesions with total volume of 22.4ml. EDSS – Expanded Disability Status Scale, DMT – disease modifying therapy, EBNA-1 – Epstein Barr nuclear antigen-1.

Supplement Figure 1. Differences in T1 lesion MTR and NAGM MTR median values between anti-EBNA-1 quartiles in the total MS population.

Legend: MS – multiple sclerosis, NAGM – normal appearing gray matter, MTR – magnetization transfer ratio, EBNA-1 – Epstein Barr nuclear antigen-1.



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Legend: MS – multiple sclerosis, NAGM – normal appearing gray matter, MTR – magnetization transfer ratio, EBNA-1 – Epstein Barr nuclear antigen-1.

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