Serum Neurofilament Light Chain Levels are Increased at the Onset of PML in Natalizumab Treated MS Patients

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Introduction and purpose

- Several new immunoactive drugs for MS have reached the market or will be launched in the near future; this provides new opportunities for individualized and more effective treatment regimens; The more powerfull the treatments, the more challenging the large scale implementations for safety issues (cancer, infective diseases, etc);
- PML is the infectious complication that have threatened the most the use of some MS drugs, particularly Natalizumab; it's a devastating disease for which no biomarker for prediction of occurrence or early diagnosis has been introduced in clinical practice apart from JCV antibodies status and MRI;
- Neurofilament light chains (Nfl) are structural elements released in the extracellular space following axonal damage and are a candidate biomarkers of a disease with such an exstensive damage such as PML;
- The aim of the current study was to assess whether serum neurofilaments light chains are reliable biomarkers for the early recognition of PML during Natalizumab treatment.

Methods

- Patients and controls: 25 PML patients have been recruited from San Raffaele Hospital and St Josef-Hospital, Bochum, Germany. 112 untreated MS patients and 161 Natalizumab treated MS patients have been recruited as controls;
- Serum Neurofilament Light Chain Levels have been measured using a previously validated electrochemioluminescence (ECL). Mean intra-assay and inter assay coefficients of variation were 5.4% and 9.9%, respectively.
- Statistics: An unpaired Student t test was performed for comparison between 2 groups of patients and Wilcoxon signed-rank test for repeated measures. Univariate and multivariate regression analysis was used to determine predictors of Nfl levels. ROC curve analyses were used to determine the best cutoff values and relative sensitivities and specificities.

Results

Characteristic	MS Untreated (n = 112)	NTZ (n = 161)	NTZ-PrePML (n = 4)
Age at blood collection, mean yr ± SD	30.4 ± 8.5	35.4 ± 9.5	34.8 ± 10.1
Gender			
Females, No. (%)	80 (71)	119 (74)	3 (75)
Males, No. (%)	32 (29)	42 (26)	1 (25)
Time from last relapse, mean months ± SD	4.8 ± 6.2	23.7 ± 17.4	21.2 ± 19.4
Expanded Disability Status Scale score, median (IQR)	1.5 (1.0-2.0)	2.5 (1.0-3.5)	2.5 (1.0-3.5)
T2 lesions at brain MRI, No. (%)			
0-1	3 (3)	0 (0)	0 (0)
2-9	47 (42)	21 (42)	1 (25)
> 9	62 (55)	140 (87)	3 (75)
Presence of Gd-enhancing lesions at last MRI, No. (%)	33 (29)	7 (4)	0 (0)
Presence of CSF oligoclonal bands, No. (%)	100 (89)	145 (89)	4 (100)
Serum Neurofilaments in pg/ml, median (IQR)	24.2 (16.4-29.7)	19.7 (12.4-26.9)	20.3 (9.7-27.3)

Characteristic	n = 25
Age at PML onset, mean yr ± SD	38.9 ± 9.7
Gender	
Females, No. (%)	16 (64)
Males, No. (%)	9 (36)
JCV Ab positivity, No. (%)	25 (100)
N° Infusions, median (IQR)	30.0 (27.5 - 35.5)
EDSS at PML oset, median (IQR)	3.5 (3.0-5.5)
Number of CSF JCV copies, median (IQR)	286 (64-7400)
Time from onset of symptoms to CSF, mean months ± SD	1.2 ± 1.1
Time from first MRI to Serum, mean months ± SD	0.5 ± 1.2
Presence of Gd-enhancing lesions at first MRI, No. (%)	13 (52)
Serum Neurofilaments in pg/ml, median (IQR)	305.7 (98.9-432.1)

Table 1 (upper table). Baseline characteristics of the three cohorts of patients (MS patients untreated, n =112, MS patients treated with Natalizumab, n = 161, and MS patients treated with Natalizumab before developing PML)

Table 2 (table on the left). Characteristics of the 25 Natalizumab treated MS patients who developed PML at the clinical onset of the infectious complication.

Results

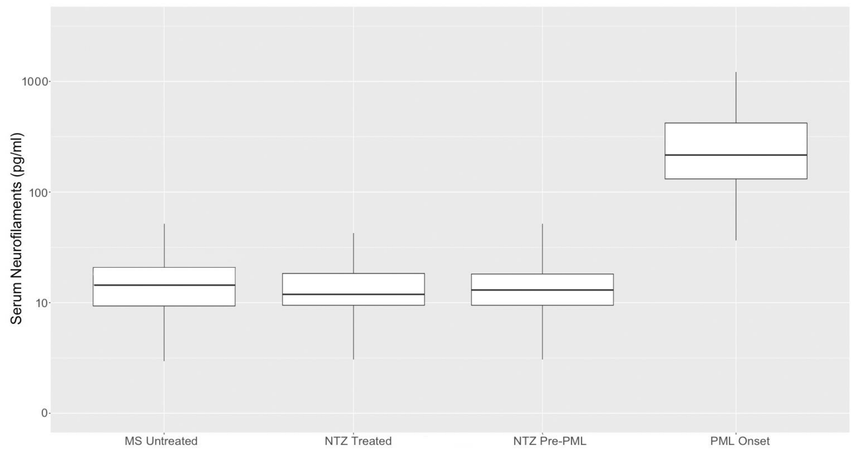

Figure 1. Boxplot of mean of Serum Neurofilament Light Chain Levels (pg/ml) in MS patients untreated, in MS patients treated with Natalizumab without or before the onset of PML, and in Natalizumab-treated MS patients at the onset of PML ($p<0.001$ for all comparisons).

	Univariate analysis		
	Coefficient	95% CIs	p
Age at serum	0.9	-1.5 - 2.2	0.76
Gender, males vs females	5.4	-10.2 - 25.3	0.87
N° Infusions of Natalizumab	0.3	-2.8 - 4.1	0.83
EDSS at PML oset	20.6	-43.6 - 84.7	0.54
Number of CSF JCV copies	2.8e-5	-7.8e-3 - 8.4e-4	0.93
Time from onset of symptoms to CSF	-4.9	-142.3 - 132.3	0.93
Time from first MRI to Serum	-34.3	-172.3 - 103.7	0.59
Presence of gadolinium enhancing lesions	195.3	-66.8 - 457.5	0.13

Table 3 Predictors of Serum Neurofilament Light Chain Levels (pg/ml) at the clinical onset of PML in Natalizumab-treated MS patients. Univariate analysis was carried out with the use of linear regression models. Nfl levels were log transformed and the regression coefficients were back-transformed to the original scale

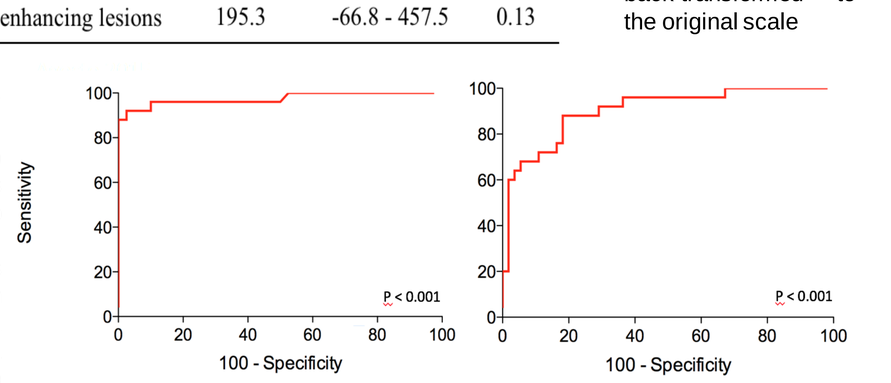

Figure 2. Receiver operating characteristic (ROC) curve analyses was performed to determine the best cut-off values to distinguish between Natalizumab treated MS Patients from PML Patients (left panel) and between relapsing MS patients and PML patients (right panel) on the basis of serum Neurofilament light chain levels.

Natalizumab vs PML Patients		
Cutoff (pg/ml)	41.3	
AUC	0.97	0.93 -1.00
Sensitivity	92	73.9 - 99.0
Specificity	97.5	88.8 - 99.9

Relapse vs PML		
Cutoff (pg/ml)	51.4	
AUC	0.90	0.83 - 0.98
Sensitivity	88.0	68.8 - 97.5
Specificity	81.8	89.1.8 - 90.9

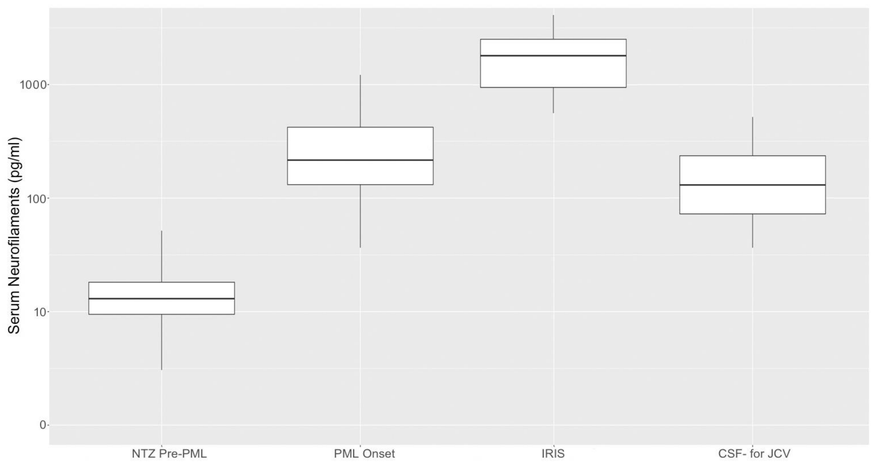

Figure 3. Longitudinal Analysis of Serum Neurofilament Light Chain Levels (pg/ml) before PML, at the clinical onset of PML, at the onset of immune reconstitution inflammatory syndrom e (IRIS) and the resolution of PML (negative CSF PCR for JC virus)

Conclusions

- PML is a severe complication of many immunoactive drugs that limits the use of established and emerging potentially beneficial therapies;
- Several candidate biomarkers have been proposed to monitor disease course and allow an early diagnosis of PML, but their clinical relevance remains uncertain;
- Serum neurofilaments, candidate biomarkers of neurodegeneration in MS, also appear to reflect ongoing disease activity;
- A 10 fold increase in serum neurofilaments levels in natalizumab treated MS patients is observed at the onset of PML, regardless of the presence of enhancing lesions or number of copies of JC virus;
- Serum neurofilament light chain levels beyond 41.3 pg/ml should suggest to further investigate the possible presence of ongoing MS activity or PML;

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