Cerebrospinal fluid markers of neuronal and glial cell damage in patients with autoimmune neurologic syndromes with and without peripheral malignancies

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Abstract

Autoimmune neurological syndromes (ANS) can be paraneoplastic or non-paraneoplastic. Their clinical presentation is often similar. Better biomarkers are needed to rapidly identify patients with tumors. We investigated cerebrospinal fluid (CSF) levels of commercially available markers of neuronal (neurofilament light chain = NFL, total tau protein = T-tau) and glial (glial fibrillary acidic protein) damage in patients with ANS. CSF-NFL and T-tau were increased in both paraneoplastic and non-paraneoplastic cases. Patients with malignancies had increased CSF-NFL, CSF-blood albumin ratio, and worse long-term outcome, compared with those without. CSF-NFL levels predicted long-term outcome but were not diagnostic for malignancy after age adjustment.

Key words:

Autoimmune neurologic syndrome, paraneoplastic neurological syndrome, non-paraneoplastic, autoimmune encephalitis, neurofilament light chain, tau protein

Introduction

Autoimmune neurological syndromes (ANS) may manifest with peripheral, as well as central nervous system dysfunction. ANS may be associated with peripheral tumors (i.e. be paraneoplastic – PaNS) or not (i.e. be non-paraneoplastic – non-PaNS).
Clinical presentation of ANS is variable and often does not allow direct identification of patients with malignancies. Detection of peripheral tumors can be difficult and involve many investigations that often need to be repeated. Identification and treatment of tumors is nonetheless imperative for improving long-term prognosis (Darnell and Posner, 2003, Graus and Dalmau, 2007, Gromadzka et al., 2013, Titulaer et al., 2011). Cerebrospinal fluid (CSF) analysis, brain magnetic resonance imaging (MRI) and antibody testing often cannot help in differentiating between patients with malignancies and those without (Psimaras et al., 2010, Graus et al., 2016, Dalmau et al., 2011). There is a clear need for better and clinically accessible biomarkers to rapidly identify patients with an underlying malignancy.

In patients with autoimmune encephalitis, increased CSF levels of neurofilament light chain (NFL) and total tau protein (T-tau) (neuronal damage markers), but not of glial fibrillary acidic protein (GFAP) (marker of glial damage) were recently found (Constantinescu et al., 2016). These markers were not yet studied separately in patients with underlying malignancies and those without. The aim of this study is to investigate patients with paraneoplastic and non-paraneoplastic ANS and compare those with malignancies with those without, in an attempt to identify characteristic clinical and laboratory patterns.

Patients and methods

Ethics

The retrospective design of this study was approved by the Regional Ethical Board at the University of Gothenburg. All medical procedures were performed only for clinical reasons.

Patients

Patients with autoimmune neurological syndromes were identified by searching patient files using combinations of two search terms: (1) "autoimmune, limbic, paramalignant, malignant or paraneoplastic" AND (2) "encephalitis, syndrome, phenomenon". Data of identified patients were then extracted and verified manually.

For study inclusion, following criteria were needed: (I) Definite or possible PaNS according to Graus et al., 2004 (Graus et al., 2004) OR possible autoimmune encephalitis according to Graus et al., 2016 (Graus et al., 2016); AND (II) Results from at least one CSF analysis; AND (3) Exclusion of other diagnoses with a similar clinical presentation (Asztely and Kumlien, 2012, Zuliani et al., 2012, Graus et al., 2008).

Diagnostic workup

Investigations included brain MRI, EEG, CSF and blood analyses. Oncoscreening included computer tomography (CT) of abdomen and chest, ultrasound of testes, gynecological evaluation and mammography in women. When no malignancy was found, a whole body positron emission tomography with whole-body CT (^{18}FDG-PET/CT) was performed. Oncoscreening was repeated at regular intervals, as recommended (Titulaer et al., 2011).

Cerebrospinal fluid analysis

Routine CSF investigation included: cell count, cytology, CSF levels of albumin, IgG and IgM with calculation of the CSF/serum albumin ratio, IgG index and IgM index, isoelectric focusing of paired

CSF and serum samples to identify CSF-specific oligoclonal IgG bands, and in cases with high IgM index agarose electrophoresis and western blotting to identify CSF specific oligoclonal IgM bands.

CSF immunopathy was defined as the presence of at least one of the following: (a) pleocytosis ($>3\text{x}10^6$ cells/L), (b) CSF-specific oligoclonal immunoglobulin G (IgG) bands (>1 CSF-selective band), (c) increased albumin ratio (>6.2), (d) increased IgG index (>0.63), or increased IgM index (>0,060). Increased CSF-albumin (>320 mg/L) and/or increased CSF to serum albumin ratio (>6.8) were considered as a sign of blood-brain barrier damage.

CSF-NFL, T-tau and GFAP were measured as previously reported (Constantinescu et al., 2016) – see also Supplementary section.

Neuronal surface antibodies and antibodies against intracellular antigens

Antibodies were measured in both serum and CSF. The following antibodies were examined: (1) Well-characterized onconeural antibodies against antigens -Hu, -Yo, -Ri, -Ma2/Ta,-CV2/CRMP5 and -amphiphysin; (2) Neuronal surface antibodies: antibody against N-methyl-D-aspartate receptor (NMDAR); γ-aminobutyric acid receptor B (GABABR); α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors 1 and 2 (AMPA1,2); leucine-rich glioma inactivated protein 1 (LGI1); contactin-associated protein 2 (CASPR2); (3) Other: anti-GAD and antimicrosomal antibodies (anti-TPO). Commercial kits (Euroimmun AG) were used for antibody measurement, as described in our previous study (Constantinescu et al., 2016). See also the Supplementary section.

Outcome

Outcome was assessed retrospectively from patient files, as the level of disability at the last documented follow-up, using the modified Rankin Scale (mRS) (Rankin, 1957, Farrell et al., 1991) and the Karnofsky Performance Status scale (Crooks et al., 1991).

Statistical analysis

SPSS statistics package was used for analysis. The Independent-Samples Mann-Whitney U-test, Kruskal-Wallis test, and cross-tabulation were used as appropriate. Associations were calculated with Fisher's exact test, Pearson Chi-Square test, partial correlations with age adjustment, and with logistic regression as appropriate. All tests were two tailed with $p \leq 0.05$ as the level of significance.

Results:

Demographics (Table 1)

Thirty-seven patients (14 males/ 23 females) fulfilled the inclusion criteria. Twelve had a peripheral malignancy and 25 did not. No gender differences were found, but patients with tumors were significantly older (median 72 vs. 42 years, p<0.001).

Clinical presentation

The following diagnoses were recorded: (1) Definite PaNS, N=14; (2) Possible PaNS, N=2; (3) Possible autoimmune encephalitis, N= 21. Patients with tumors were more likely to seek medical attention later, and to have more focal, specific, central and peripheral neurological signs and symptoms. Cognitive and psychiatric disturbances were equally common, but epilepsy and status epilepticus were more prevalent in patients without malignancies.

Malignancies

Twelve patients had malignancies. It could take several months until malignancy findings were positive and in one case malignancy was only found post mortem, 15 months after the onset of neurological symptoms.

Antibodies (Supplementary Table 2)

Single or multiple antibodies were found in serum or CSF in 7/12 (58 %) patients with malignancies and in 13/25 (52 %) patients without.

CSF immunopathy markers (Supplementary Table 3)

CSF-serum albumin ratio was significantly higher in patients with malignancies compared with patients without (p=0.035), as a sign of blood-brain barrier damage.

Brain imaging

There were no significant differences among patients with and without malignancies in respect to MRI findings (normal or pathological).

Brain damage markers (Table 2 and Figure 1 (a,b, c))

CSF-NFL and CSF-T-tau levels were high in both PaNS and non-PaNS patients, compared with reference values. CSF-GFAP was not significantly increased. CSF-NFL levels were significantly higher in patients with malignancies compared with those without (p=0,004). In the logistical regression model with age as a covariate, malignancies were not significantly correlated with any brain damage marker.

Outcome (Supplementary Table 1)

Level of disability at the last follow up (measured by Karnofsky Performance State scale and mRS) was significantly higher in patients with malignancies (p=0.003 and 0.002). In the group as a whole, disability was correlated with CSF-NFL (after age adjustment) (p=0.001), but not with CSF-T-tau or CSF-GFAP. Malignancies were associated with higher mortality (p=?).

Discussion

We found that levels of CSF-NFL and CSF-T-tau (neuronal damage markers) but not of CSF-GFAP (glial damage marker) were increased in both paraneoplastic and non-paraneoplastic ANS. Patients with underlying malignancies had significantly higher CSF-NFL levels, higher CSF-blood albumin ratios, more often focal neurological signs and symptoms, less often seizures and status epilepticus, worse long-term outcome, and were older compared with patients without detectable malignancies. After correcting for age, CSF-NFL was not significantly associated with malignancy, but it was related to the long-term outcome.

High levels of CSF-NFL and T-tau in ANS was an expected finding, because CSF-NFL and T-tau are increased non-specifically in patients with various brain disorders and indicate axonal and neuronal injury (Norgren et al., 2003, Blennow et al., 2010). Neuronal death due to inflammatory processes in ANS has already been demonstrated (Psimaras et al., 2010, Giometto et al., 1997). The novelty of our study is 1) to show for the first time the trend for increased neuronal injury (reflected in CSF-NFL levels) in PaNS, and 2) to suggest that long-term outcome in ANS appears to be correlated with CSF-NFL levels measured in proximity to disease onset. This expands our previous observations in autoimmune encephalitis (Constantinescu et al., 2016) and warrants the evaluation of prognostic significance of CSF-NFL in these disorders. Our findings indicate that severely elevated CSF-NFL, especially in older patients with ANS without status epilepticus could suggest the presence of an underlying malignancy. However, this finding needs to be confirmed in larger, prospective studies.

The absence of significant differences in regards to MRI findings, autoantibodies and markers of immunopathy between patients with malignancies and those without and the long delay to a malignancy diagnosis observed in one case illustrate again the need for better diagnostic biomarkers in this context, and reinforces further study of CSF-NFL's potential in this respect.

Major limitations of this study are the retrospective design and relatively low number of patients included. The choice of search terms may have focused too narrowly on autoimmune encephalitis and PaNS while omitting other types of autoimmune neurological syndromes. Due to these limitations, the study needs to be replicated prospectively on a larger patient cohort.

Tables

Table 1. Demographics. Numbers represent median (range). Groups were compared using the Fisher's exact test and Independent samples Mann-Whitney U-test.

	N (males/ females)	Age at LP (years)	Symptom duration at LP (months)	Follow-up time (years)
Malignancy	12 (5/7)	72 (53-81)	2.1 (0.2-27.6)	1.6 (0.3-8.8)
No malignancy	25 (9/16)	42 (17-75)	0,7 (0.0-24.4)	4.2 (0.6-10-3)
p	0.507	<0.001	0.21	0.008

Abbreviations (in alphabetical order): LP=lumbar puncture

Table 2. CSF-levels of brain damage markers in patients with paraneoplastic neurological syndromes, non-paraneoplastic neurological syndromes, in patients with malignancies, and in patients without malignances. Values are expressed in ng/L. The reference values are given for orientation and represent the upper limits of the reference range for the highest age groups (> 70 years for NFL, and > 60 years for GFAP and T-tau). These limits are lower for younger age groups. For details see Supplementary Table 4. Groups were compared using the Independent samples Mann-Whitney U-test. *Abbreviations (in alphabetical order):* CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; NFL = neurofilament light chain protein; Non-PaNS = non-paraneoplastic neurological syndrome; PaNS = paraneoplastic neurological syndrome (including definite and possible (Graus et al., 2004); T-tau = total tau protein.

|	NFL			GFAP			T-tau		
	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range
PaNS	29007	9277	781-127478	750	605	120-1650	1317	635	130-6910
Non-PaNS	6172	1440	180-54063	646	320	50-2450	1224	541	100-5690
p	<0.001			0.096			0.907		
Malignancy	33491	13297	2820-127478	878	700	120-1650	894	570	130-2520
No malignancy	7440	1800	180-54063	624	380	50-2450	1407	560	100-6910
p	0.001			0.630			0.733		
Reference values	<1850			<1250			<400		

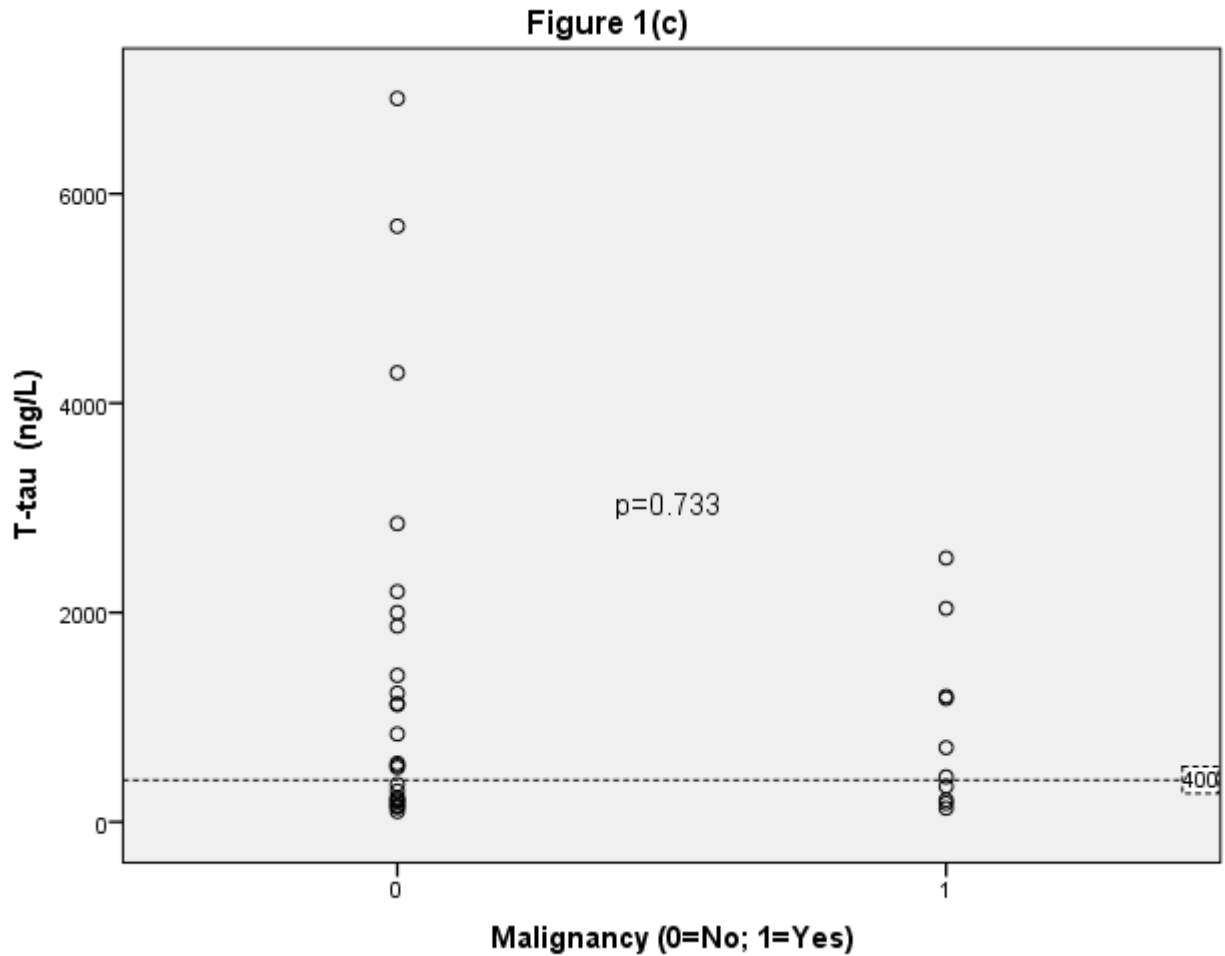# Figures

Figure 1(a)
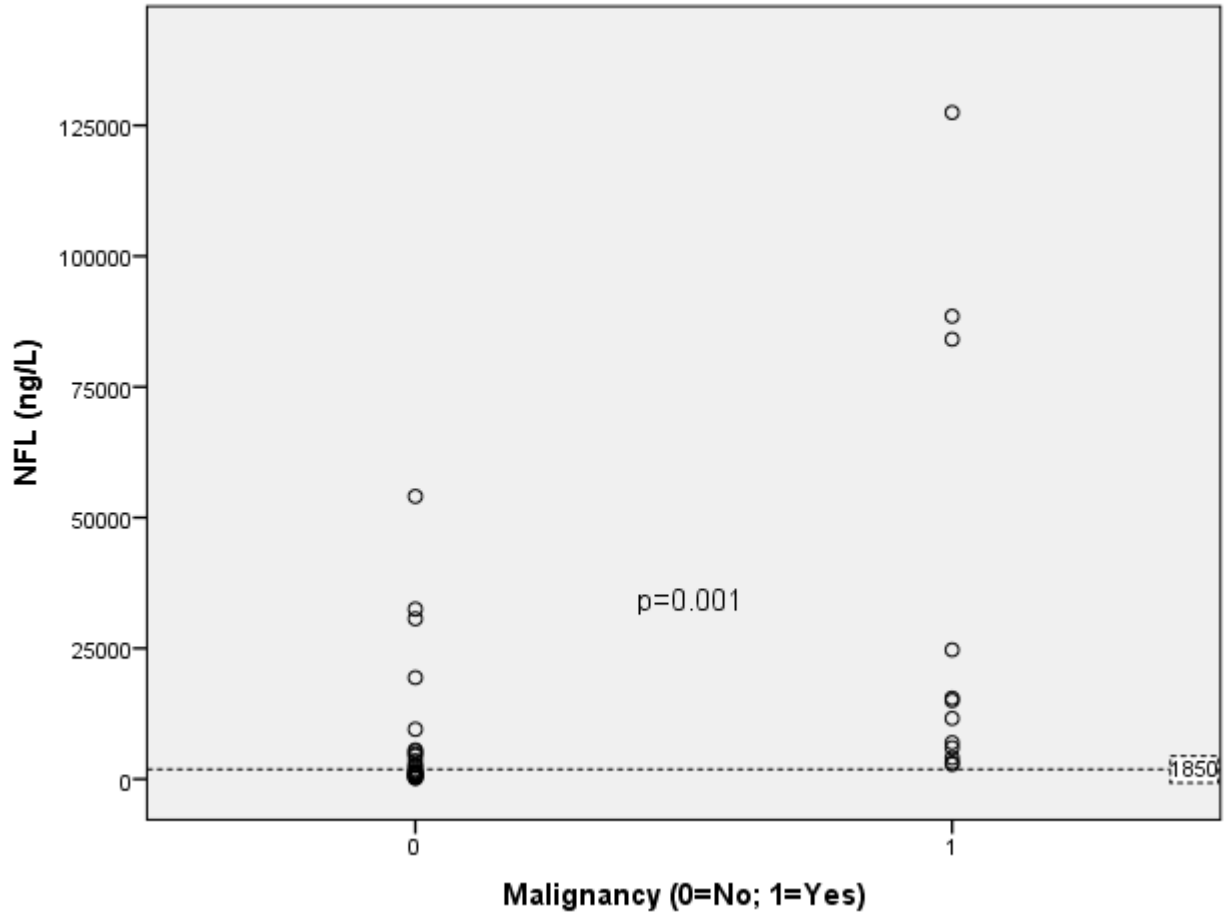
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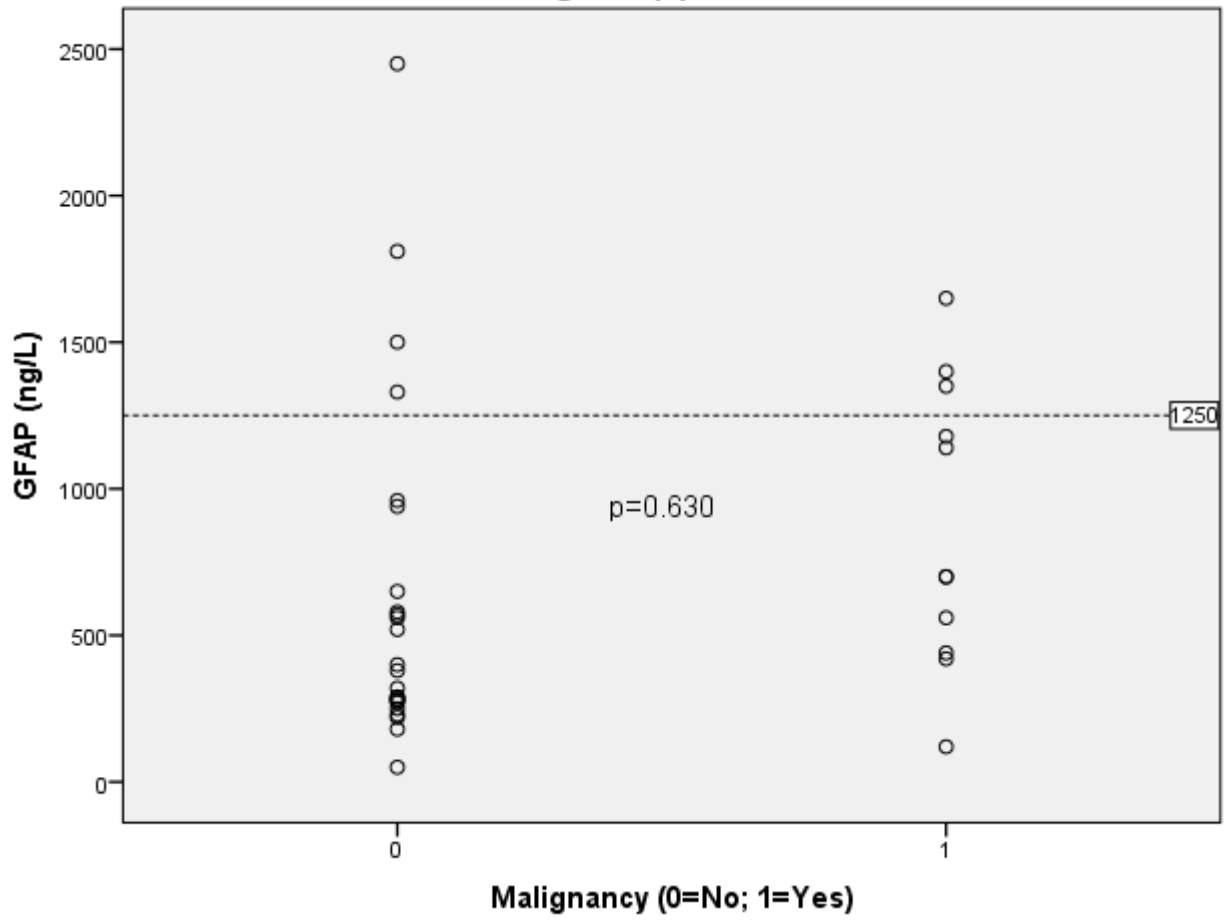
Figure 1 (a,b,c): Levels of brain damage markers in the cerebrospinal fluid (CSF) from patients with different types of autoimmune neurological syndromes, with and without peripheral malignancies. Dashed line represents the highest upper limits of the reference range. (a) Levels of neurofilament light chain (NFL) were significantly increased in patients with malignancies compared with those without. However, in a logistical regression model including age as a covariate, none of the brain damage markers (including CSF-NFL) were associated with malignancy; (b) Glial fibrillary acidic protein (GFAP) protein; and (c) total tau (T-tau) protein. **Abbreviations (in alphabetical order):** CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; NFL = neurofilament light chain protein; T-tau = total tau protein.

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