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

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Abstract

Autoimmune neurologic syndromes can be paraneoplastic (associated with malignancies and/or onconeural antibodies), or non-paraneoplastic. Their clinical presentation is often similar. As prognosis is related to malignancy treatment, better biomarkers are needed to identify patients with malignancy. We investigated cerebrospinal fluid (CSF) markers of neuronal (neurofilament light chain, NFL and total tau protein, T-tau) and glial (glial fibrillary acidic protein) damage. CSF-NFL and T-tau were increased in both paraneoplastic and non-paraneoplastic autoimmune syndromes. Patients with manifest malignancies were older, had less epilepsy, more focal central and peripheral neurological signs and symptoms, and worse long-term outcome, than those without malignancy. CSF-NFL-levels predicted long-term outcome but were not diagnostic for malignancy, after age adjustment.

Key words:

Autoimmune encephalitis, limbic encephalitis, neuronal damage markers, neurofilament light chain, NFL, total tau protein, glial fibrillary acidic protein, GFAP, NMDA-receptor antibodies, neuronal surface antigens, neuronal cell membrane antigens.

Introduction

Autoimmune encephalitis is a group of autoimmune brain disorders often but not always associated with more or less well-characterized antibodies directed against neuronal cell membrane or intracellular antigens, and sometimes related to underlying malignancies. The field has expanded tremendously during the last years and future research will probably reveal even more antibodies related to these disorders. The clinical presentation is variable and can be dramatic with a combination of symptoms such as epileptic seizures which, when sustained, may cause status epilepticus, psychiatric, cognitive, behavioral and movement abnormalities (for excellent reviews, see (1-5). Many patients initially need intensive care treatment, some develop persistent functional disabilities, a significant proportion recovers remarkably well, but a few may even die. The treatment for these disorders is symptomatic, directed at the prevailing symptoms, such as psychosis or epilepsy, and causal, directed at the underlying neuroimmunological abnormality and tumor removal, when present (6). In those patients who do not fully recover, it may be exceedingly difficult to determine the cause of treatment failure: still active disease due to insufficiently treated immunological processes, or remission with neurological sequels? The distinction has profound implications for the continuing treatment, with focus on either more aggressive immunosuppression with its serious potential side effects, or just intensified symptomatic treatment. There are no biomarkers for making this distinction straightforward. Antibody titers, when specific antibodies have been identified, CSF immunopathy findings, when present, and MRI abnormalities, when visible, offer some guidance, but are not entirely reliable for determining disease activity. For example, in anti-N-methyl-D-aspartate (NMDA) receptor encephalitis, although the antibody titers may decrease over time, they can decrease regardless of outcome, and might still be positive after recovery (7). MRI findings, if present, can be difficult to interpret, as they may be permanent sequels after remission and not a sign of still active disease (8, 9). There is still a need for better, more reliable biomarkers in autoimmune encephalitides.

Cerebrospinal fluid (CSF) neuronal damage and glial cell markers, such as neurofilament light chain (NFL), total tau protein (T-tau) and glial fibrillary acidic protein (GFAP), are constituent parts of neurons and glial cells. They are increased non-specifically in patients with both acute and chronic disorders causing neuronal damage aor death, and disintegration of astroglial cells (for reviews, see (19, 20, 16). Considering the pathophysiology and the presenting symptoms, it may be suspected that the disease processes in autoimmune encephalitis may damage the brain, thus making the study of CSF markers of neuronal damage and glial cells relevant even in this disorder. To the best of our knowledge, this has not been done yet.

The objective of this study is to investigate levels of CSF neuronal damage and glial cell markers in patients with autoimmune encephalitis and relate them to the presence of CSF immunopathy, antibodies to neuronal cell membrane or intracellular antigens, MRI findings, and outcomes.

Methods

Analysis of biographical, clinical and CSF data extracted retrospectively from patients' files.

Ethics:

Studies were approved by the regional ethics committee at the University of Gothenburg.

Patients:

Patients were identified searching in the Sahlgrenska University Hospital Electronic Database (Melior) using the following identifiers: all in-patients treated at the Department of Neurology or at the Neurointensive Care Unit, Sahlgrenska University Hospital, between January 1, 2000 and May 20, 2014. All patients treated at this department are adults (age ≥ 18 years). Patients treated by the

Neurointensive Care Unit had to be there temporarily due to the seriousness of their medical condition. The search words used to scan patient files were: autoimmune encephalitis and variants of autoimmune encephalitis, such as limbic encephalitis, NMDA encephalitis. These words could be coded as diagnoses or just appear in the final discharge records as words, confirming, denying or just discussing these diagnoses. Manual verification of all search results was performed for identifying true patients with these diagnoses. Thereafter, data for these patients were extracted retrospectively from patients' files.
The diagnostic criteria for autoimmune/limbic encephalitis were the following: 1) Clinical symptoms from at least one of five core clinical domains (epileptic seizures, psychiatry and behaviour, cognition, disorders of consciousness, movement disorders); 2) At least one of following: a) Pathological findings on MRI brain performed according to a specified protocol for autoimmune encephalitis (for visualizing unilateral or bilateral T2/FLAIR hyperintensities in the hippocampus/medial temporal areas in addition to standard methods); b) CSF immunopathy (pleocytosis, presence of oligoclonal bands, increased immunoglobulin G or increased IgG index); c) Presence of antibodies typical for autoimmune encephalitis (neuronal surface antibodies, onconeuronal antibodies, glutamic acid decarboxylase (GAD) and thyroid peroxidase (TPO) antibodies). 3) Presence of tumor within 2 years from diagnosis; 4) Exclusion of other diagnoses with a similar clinical presentation.

Diagnostic work up:
The diagnosis was based on a combination of relevant symptoms and investigational findings and followed the recommendation of Zuliani et al., 2012 (3). Symptoms encompassed significant abnormalities from the above mentioned clinical domains. Status epilepticus was defined as ongoing motor or psycho-motor seizures, loss of consciousness, confusion or bizarre behavior and electro-encephalography (EEG) showing status epilepticus, repeated seizures without recovery of consciousness in between, or EEG showing status epilepticus on a patient sedated for treating epilepsy. In addition to routine investigations with respect to infections, metabolic disruptions, systemic rheumatological disorders, and other conditions known to mimic autoimmune brain disorders, patients were systematically investigated specifically for autoimmune encephalitis. These investigations included brain MRI with relevant sequences, CSF analysis in regard to immunopathy, blood and CSF analysis in regard to relevant antibodies (for details, see diagnostic criteria). At least one investigational finding had to be abnormal and congruent with a diagnosis of autoimmune encephalitis for this diagnosis to be made. If an alternative diagnosis could be considered more likely, a diagnosis of autoimmune encephalitis was not made. A malignancy work up was done in all patients, including computer tomography (CT) of abdomen and chest, ultrasound of testes in men, gynecological evaluation and mammography in women, and whole body FDG-PET-CT. All patients performed EEG at least once.

Cerebrospinal fluid analysis:
CSF was collected from all patients through lumbar puncture (LP) performed in the supine position. The CSF was always collected as part of the medical management, as deemed necessary on clinical grounds. For a majority of patients LPs were performed at the time for hospitalization which coincided most often with the time for disease onset (LP1), at 3±1 months (LP2) and at 12±3 months (LP3). According to the clinical situation and the wishes of the patient, the number of LPs could vary. Generally, the more serious the disease course was, the more LPs were performed. In some cases, with the patient's written informed consent, extra CSF was sent to a biobank and frozen to – 80° C for later use in both the clinical management of the patient, if needed, and for research purposes.

All analyses were performed as part of clinical routine testing by board-certified laboratory

technicians. The procedures used were accredited by the Swedish Board for Accreditation and Conformity Assessment. All samples were coded and the analyst was unaware of any patient data. The following routine CSF analyses were performed on all patients: cell counts, albumin and immunoglobulin G and M quantification, electrophoresis of CSF and serum, cytology. Neuronal damage and glial cell markers (NFL, GFAP, T-tau protein) were analyzed in all patients.

During the study period three different methods were used for measurement of NFL concentration in CSF. The first was an in house ELISA with a limit of detection of 250 ng/L (ref: Rosengren LE, Karlsson JE, Karlsson JO, Persson LI, Wikkelso C. Patients with amyotrophic lateral sclerosis and other neurodegenerative diseases have increased levels of neurofilament protein in CSF. Journal of neurochemistry 1996;67:2013-2018), which was later improved to 125 ng/L (Zetterberg H, Hietala MA, Jonsson M, et al. Neurochemical aftermath of amateur boxing. Archives of neurology 2006;63:1277-1280). The third method was a novel ELISA, developed in collaboration with Uman Diagnostics (NF-light ELISA kit, UmanDiagnostics AB, Umeå, Sweden), with a limit of detection of 50 ng/L. The third method produces highly correlated results, although with higher concentrations. To correct for this bias the ratio of means between this method and its two predecessors was used for normalization (NFL old/NFL new = 0.32; for further details, see Skillbäck T, Farahmand B, Bartlett JW, Rosén C, Mattsson N, Nägga K, Kilander L, Religa D, Wimo A, Winblad B, Rosengren L, Schott JM, Blennow K, Eriksdotter M, Zetterberg H. Neurology. 2014 Nov 18;83(21):1945-53). The ELISA GFAP in the CSF were performed as previously described (11). The detection limit of the assay for GFAP was 32 ng/L. The upper limits of the reference range for neuronal damage markers changed June 1 2010. Until that date, they were for CSF NFL 250, 312 and 795 ng/L for the age groups <60, 60–69, and >70 years, respectively, for CSF GFAP 1250 ng/L and for CSF T-tau 400 ng/L. Beginning with June 1 2010 they were for CSF NFL 380, 560, 890, and 1850 ng/L for the age groups <30, 30-40, 40-60 and >60 years, respectively; for GFAP they were <175, <750 and <1250 ng/L for ages <20, 20-60 and ≥ 60 years; and for CSF T-tau they were <300 ng/L for age 18-45 years and < 400 ng/L for ages > 45 years. CSF total tau (T-tau) concentration was determined using a sandwich ELISA (Innotest hTAU-Ag, Fujirebio, Ghent, Belgium) specifically constructed to measure all tau isoforms irrespectively of phosphorylation status, as previously described (12). The detection limit of the T-tau ELISA was 75 ng/L. Over the study period, the NFL and GFAP assays have had a mean inter-assay coefficient of variation (CV) of 15.5% and the T-tau inter-assay CV has been below 10%.

Neuronal surface and paramalignant antibodies analysis:
Blood and CSF analyses were performed in regard to the following neuronal surface, paramalignant (onconeuronal) and other antibodies: NMDA receptor, G-aminobutyric acid B ($GABA_B$) receptor, a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) 1 and 2 receptors, Leucine-rich glioma inactivated protein (LGI) 1, contactin-associated protein-like (CASPR) 2, Hu, Yo, Ri, Ma2/Ta, CV2/CRMP5, amphiphysin, GAD, TPO. Only a part of these investigations were available before 2008.

Treatment:
Patients were optimally treated for their symptoms (such as epileptic seizures, psychiatric and behavioral abnormalities, disturbances in homeostasis). All identified malignancies were fully treated. Immunosuppressive treatment was given to all patients as deemed necessary according to the clinical situation. All standard immunosuppressants were available, as needed, including corticosteroids, intravenous immunoglobulins, plasmapheresis, cyclophosphamide, rituximab, azatioprin, mycophenolat-mofetil. As this paper does not focus on treatment aspects, no detailed information regarding treatments is presented.

Outcome:
Outcomes at onset, 3±1 and at 12±3 months (LP3) were evaluated retrospectively using the modified Rankin Scale (mRS) (13, 14).

Statistical analysis:
Nonparametric tests were used as CSF data was not normally distributed. To compare the levels of the different brain damage markers at the time for LP1, 2 and 3, Friedman's two way analysis of variance for related samples was used. Associations were calculated with Spearman's rank correlation and with partial correlations with age adjustment. Due to the low numbers of patients, part of the data is presented descriptively.

Results

Biographical and clinical data
Details are presented in Tables 1 and 2. Twenty five patients, 17 females and 8 males, with a median age of 42 years (18-75 years) were treated for some form of autoimmune encephalitis between 2000 and 2014. All but one patient had epileptic seizures as part of the clinical presentation. 16 patients were treated for status epilepticus at the time for disease onset, most of the time partial, and one for epilepsia partialis continua (EEG-verified). A majority of the patients had also cognitive problems and several had psychiatric disturbances. Hypoventilation and hyponatremia were encountered in single individuals. CSF samples from all three timepoints, onset, 3±1 and 12±3 months (LP1, LP2 and LP3 respectively) were available from 12 patients; 8 patients performed LP just twice and 5 patients just once. At the time for disease onset, 20 patients were positive for CSF immunopathy and 5 negative. In 12 (48%) patients single or multiple antibodies (against neuronal surface, paraneoplastic or others) were found (2 Ma2, 3 GAD, 2 NMDA, 1 voltage gated kalium chanel (VGKC) later characterized as LGI1, 1 TPO, 1 TPO+GAD, 1 TPO+NMDA, 1 NMDA+GAD+TPO). In 13 patients, no antibodies were found. One of these patients had had a herpes 1 virus infection preceding with a few weeks the autoimmune encephalitis, but no signs of reactivation. It has been suggested previously that herpes encephalitis may trigger autoimmune processes (15). Three patients were treated before 2008 when the full antibody test battery was implemented. Malignancies were found in two patients (one mammary cancer and one neuroendocrine tumor). All but one patient were treated with at least one immunosuppressor.

CSF neuronal damage and glial cells markers
Results are shown in table 3.

NFL levels at LP2 were significantly higher than levels at LP3. T-tau levels at LP1 were significantly higher than levels at LP3. GFAP levels did not differ significantly between the three LPs. NFL, T-tau and GFAP levels were correlated to age and to each other at LP1 and 2 but not at LP3.

Increased CSF NFL, T-tau and GFAP levels at any time were present in 21, 19 and 14 out of 25 patients, respectively. Three patients had normal levels of all brain damage markers. In one patient only CSF T-tau was increased. These four patients, all females, underwent LP just once. Their outcomes at one year were good (mRS 1 and 2). Three of them had never had status epilepticus and one had had that but not at the time for LP. Their results were mixed in regard to CSF immunopathy and MR findings. Six patients (five females and one male) had at least one NFL value > 10000 ng/l. One of these had a good outcome at one year (mRS 1); the five others had bad outcomes (mRS 3-6), including patient 3 who was dead at one year.

CSF immunopathy: At LP3, out of 12 available CSF results, 4 still showed immunopathy. Of these, 2 showed still high NFL levels, one high GFAP, but T-tau was normal in all.

Antibodies: Eight of the 12 patients who were positive for neuronal surface and/or other antibodies had high and four had normal levels of neuronal damage and glial cells markers. However, in these patients only LP1 was performed.

MRI: At LP1, 15 patients had MRI abnormalities consistent with autoimmune encephalitis and 10 had normal MRIs. Seven of these had high CSF NFL and five had high T-tau. At LP3, 10 patients had no brain MRI abnormalities. None of these had increased levels of T-tau protein and GFAP but four had still elevated NFL levels. In 10 patients, the MRI abnormalities diminished but were not entirely gone at LP3 and could have been permanent rests after the disease process. In six of these cases, T-tau protein had normalized, in one case it remained high. CSF NFL and GFAP remained high in five cases.

Outcomes: At onset, 21 patients were severely disabled (mRS 3-6) and four were doing rather well (mRS 1-2). At three months, one patient deteriorated, 20 improved and two were unchanged. Data was missing for two of them. Outcomes at 12±3 months (LP3) varied from good (mRS 1-2) in 16 patients, to bad in seven patients (mRS 3-6). For two patients there was no outcome data at one year.

Correlations between parameters

Spearman's correlation showed that CSF NFL levels were correlated with outcomes at LP1, 2 and 3 respectively. T-tau levels were correlated with outcomes at LP1, and GFAP levels at LP1 and 2. However, there was no association between outcomes and NFL, T-tau and GFAP levels, when correcting for age.

Levels of CSF NFL, T-tau and GFAP were correlated with status epilepticus at LP1 but the significance disappeared with age correction.

There was no correlation between NFL, T-tau and GFAP levels, and the presence of CSF immunopathy, antibodies to neuronal surface or intracellular antigens, or positive findings on MRI at LP1 and LP3. CSF immunopathy, antibodies and MRI findings were not correlated either at LP1 or at LP3.

Discussion

In this study we explored the pattern of CSF levels of two neuronal damage markers (NFL and T-tau protein) and a glial cell marker (GFAP) in patients with autoimmune encephalitis, over one year from disease onset. We also looked for associations between the levels of these markers and outcomes, the presence of CSF immunopathy, neuronal antibodies and MRI findings.

With some exceptions, CSF NFL was high at the time for disease onset but decreased and was in a third of cases normalized about one year later. At 3 months, levels were overall high and significantly increased compared with LP3, but, compared with onset, they could be both higher and lower. This pattern suggests that there may be a delay between the onset of symptoms and changes in CSF NFL.

CSF T-tau levels were significantly higher at LP1 compared with LP3. At the time for LP2, CSF T-tau levels had decreased and were sometimes normalized, in contrast to NFL, and at LP3 all but one of the available T-tau levels were normal. This restricted data seems to indicate that CSF T-tau reacts in the same fashion but faster than CSF NFL which seems to have a more protracted time

course.

CSF GFAP seems to follow the same pattern as the neuronal markers, with high levels at onset and lower and normalized later on, but the differences over time were not statistically significant. These findings may indicate that the immunological process in autoimmune encephalitis is directed more specifically at neurons and not indiscriminately at the surrounding tissue including glial cells, as GFAP is a protein expressed mainly in fibrillary astrocytes.

In neurodegenerative diseases, high CSF NFL levels are associated with disease severity and short survival (17) and in Creutzfeldt-Jakob's disease, the increase of T-tau levels is more pronounced in more advanced disease (18). In this study, no certain relationship could be established between NFL and T-tau levels, and disease severity, measured as outcomes at three timepoints over one year, but there seems to be a trend for a direct association. The two patients (no. 3 and 4) with the highest CSF NFL levels at LP1 and LP2 were also the least responsive to treatment and, at one year, the most disabled (mRS 4 and 6). Patient no. 3 was the only one who died at one year and autopsy confirmed the diagnosis showing rests after encephalitis. Three other patients with early five digits CSF NFL (no. 8, 16, 18) had at one year relatively high mRS scores (3), but one (no. 5) recovered almost completely (mRS 1). At one year, 2 of the 3 patients with the highest initial T-tau (>4000 ng/l) showed a poor outcome (no. 3 and 16, mRS 3 and 5 respectively) but one had a good recovery (no. 5, mRS 1).

CSF NFL and T-tau are known to be increased in several of the most important mimics of autoimmune encephalitis, such as Creutzfeldt-Jakob's disease, acute disseminated encephalo-myelitis (ADEM), and brain infections (21, 22). Thus, although increased CSF-NFL and total T-tau protein levels are not useful in the differential diagnosis of autoimmune encephalitis, they seem to be sensitive markers of ongoing disease activity with axonal and neuronal injury.

There was no correlation between positive findings on brain MRI and increased levels of neuronal damage markers. These markers may be more sensitive for detecting disease activity as they were increased while the initial MRI in several patients was normal. In addition, neuronal damage and glial cell markers seem in several cases to normalize first, before brain MRI normalizes. The same argumentation seems to apply in regard to CSF immunopathy also. There is insufficient data in this study to conclude whether neuronal damage and glial cell markers are helpful in a still symptomatic patient when having to decide if symptoms and persistent abnormalities on brain MRI or CSF immunopathy indicate continued active disease or represent just sequels after a now suppressed disease processes. The available results may suggest that normalized CSF T-tau protein and NFL indicate an arrested disease process, that T-tau protein may react faster than NFL thus being more sensitive, and that both are better in this respect than brain MRI and CSF immunopathy, but the data is too limited to draw these conclusions.

An important question which this study cannot definitely answer is whether the high levels of neuronal damage and glial cell markers are a result of the primary pathological mechanisms responsible for the autoimmune encephalitis, or merely a reflection of diffuse neuronal damage caused by status epilepticus. Supporting the first alternative is the fact that, although a majority of our patients had status epilepticus, there were a few with high NFL and T-tau levels without concomitant status epilepticus. In addition, we know of several unpublished cases of patients with status epilepticus as exacerbation of severe but not inflammatory or autoimmune chronic epilepsy who do not show any abnormalities in regard to CSF neuronal damage markers.
The same considerations could also be raised regarding the cause of the MRI abnormalities which a large proportion of but not all patients with autoimmune encephalitis show: were they caused by the

inflammatory autoimmune processes or by status epilepticus? In this respect, it is generally assumed that MRI abnormalities are seen in a substantial proportion of patients with autoimmune encephalitis (8).

This study does not inform on whether there is any relationship between levels of neuronal brain markers and the presence or the levels of neuronal surface and/or other antibodies in limbic encephalitis. Patients both positive and negative for antibodies have shown increased levels of neuronal damage and glial cell markers.

The presence of specific antibodies directed against neuronal cell membranes or intracellular antigens is to a certain degree useful in the practical management of autoimmune encephalitis. Thus, assuming a credible clinical presentation, positive titers of antibodies directed at NMDA-receptors make a diagnosis of NMDA-receptor encephalitis highly plausible, offer a consistent model for the disease processes, trigger prompt investigations for teratomas, and are expected to correlate to some degree with disease outcomes and relapses (7). However, their mere presence does not necessarily imply active disease as NMDA receptor antibodies can also be present in healthy individuals (23), in patients who have recovered from encephalitis (7) and also in Creutzfeldt-Jakob's disease (24). The same applies to other antibodies, as recent studies have found serosprevalence of several brain directed antibodies such as NMDAR1, amphiphysin, CASPR2, Ma1 and 2, GAD 65, in healthy and neuropsychiatrically ill subjects (25). Being entirely non-specific, CSF neuronal damage and glial cell markers cannot be interpreted in the same way as the specific neuronal antibodies. They have no diagnostic value outside a specific clinical context and do not contribute with any etiological guidance. Nevertheless, their high sensitivity for indicating brain damage can be regarded as an asset in the context of autoimmune encephalitis, especially in those not very infrequent cases (52% of all patients, in this study) in which no specific antibodies can be detected, or in the treated but still symptomatic patient. In those cases, levels of neuronal damage and glial cell markers may be to some degree a substitute for, or a complement to titers of specific antibodies, and may offer support in the clinical management by informing on disease activity. However, more studies are needed before this can be established. Future work regarding the usefulness of these markers in autoimmune encephalitis should probably not primarily focus on differential diagnosis aspects but rather on assessing disease activity.

The available data from this study cannot be used to investigate whether there is a correlation between immunosuppression and the levels of neuronal damage and glial cell markers. All but one patient received at least one immunosuppressor during the disease course. Generally, treatment intensity was escalated according to the clinical situation, with more aggressive immunosuppression given to more treatment unresponsive, disabled patients.

There are limitations to this study. First, the amount of data is restricted due to a low number of patients. Second, the available data is incomplete as three consecutive CSF samples and outcome measures at LP3 were not available from all patients. In addition, there is a risk for bias in this respect, as patients doing well were generally less prone to undergo repeated LP-s. Third, some patients with autoimmune encephalitis may have been missed by the selection process especially in the first years of this century as awareness regarding autoimmune encephalitides has markedly increased during the last 10 years. For several years, our clinic had a program for managing acute limbic encephalitis, and not autoimmune encephalitis in general, and this may have influenced the selection process to a certain degree in favor of patients with symptoms of limbic encephalitis. Fourth, the management of autoimmune encephalitis has changed during the period covered by this study, which makes more difficult a direct comparison of all patients. There may be differences between how patients were investigated and treated during the first half of the study period

compared with the second half, considering the evolution of the neurological community's understanding of these disorders. Fifth, there is a risk of bias in the selection of patients for this study, as all patients, at onset, were severely disabled and were hospitalized. Although hospitalization was an inclusion criterion, we are not aware of any out-patients with a suspected diagnosis of autoimmune encephalitis who did not pass through the neurological ward. Sixth, the inability of this study to show a significant association between the neuronal damage markers and outcomes may also be due to the heterogeneity of the time for disease onset. This time marked only the point in time when symptoms were severe enough for the patient to be hospitalized, and not the real time when the disease process started. Analysis of case histories showed that some patients developed epilepsy or other more dramatic symptoms from the beginning, which prompted them to seek medical care very early, while others had more subtle symptoms and come later to medical attention. Finally, retrospective retrieval of patient data always lowers its quality.

In conclusion, CSF NFL and T-tau levels seem to reflect the disease course in autoimmune encephalitis with high early levels which decrease and sometimes normalize one year after disease onset, in parallel with amelioration of symptoms. This restricted data cannot confirm an association between the levels of these markers and disease severity but it suggests a trend for higher levels in more severe disease. Due to its limitation, particularly in respect with data quantity and quality, the findings of this study need replication by larger and longer studies.

Table 1 Demographics and clinical data. Modified Rankin Scale (mRS) score at onset (LP1), at 3±1 month (LP 2) and at 12±3 months (LP3) (unless stated otherwise).

Patient	Sex	Age	Symptoms	Status epilepticus (at onset)*	Immuno-suppressants	mRS at LP1 (onset)	mRS at LP2	mRS at 12±3 months
1	M	75	Ep, Cog	Yes	I	5	3	4 (hydrocephalus)
2	F	74	Ep, Cog	Yes	C	5	2	2
3	F	68	Cog, hVent, Diz	No	C P	3	4	6
4	F	68	Ep, Cog	Yes	C	5	5	5
5	M	69	Ep, Cog, hNa	Yes	C	5	2	1
6	F	66	Ep, Cog	Yes	C	5	3	3
7	M	46	Ep	Yes	None	5	2	2
8	F	55	Ep, Cog	Yes	C	5	3	3
9	M	48	Ep, Cog	Yes	C	5	2	2
10	M	43	Ep, Cog, Psy	Yes	C I P	5	3	3
11	M	45	Ep, Cog	Yes	C A	5	3	2
12	F	42	Ep	Yes	C I P M R	5	2	2
13	F	39	Ep, Cog, Psy	No	C I P A	1	1	1
14	F	37	Ep, Psy	Yes	C I	5	3	2
15	F	36	Ep, Psy	Yes	I	5	-	2
16	F	29	Ep, Cog, Psy	Yes	C P	5	3	3
17	F	31	Ep, Psy	No	C	2	1	1
18	F	31	Ep, Psy	Yes	C I P	5	4	3
19	F	28	Ep, Cog, Psy	Yes	C Cy	5	3	2
20	F	25	Ep, Cog, Psy	No	C I P	3	2	2
21	M	27	Ep, Cog, Psy	No	C P	5	4	2
22	M	19	Ep, Cog	No	C	3	1	1
23	F	20	Ep, Cog, Psy	Yes	C I P R	5	1	1
24	F	18	Ep, Cog, Psy	No	C	2	1	1
25	F	43	Ep, Cog	No	Cc	1	-	-

Symptoms
C = Cognition
Diz = Dizziness
Ep = Epilepsy (including status epilepticus, epileptic seizures)
hNa = Hyponatremia

hVent = Hypoventilation
Psy = Psychiatric symptoms

Immunosuppressants
A = Azathioprin
C=Intravenous corticosteroid pulse
c = Peroral corticosteroid cure
I = Intravenous high dose immunoglobuline
M = Mycophenolat mofetil
P = Plasmapheresis
R = Rituximab

LP = lumbar puncture; mRS = modified Rankin Scale score

* Unless stated otherwise

Table 2 CSF immunopathy and MRI findings at onset (LP1) and at 12±3 months (LP3) (unless stated otherwise). Antibodies and results of malignancy workup.

Patient	CSF LP1 immuno-pathy	CSF LP3 immuno-pathy	MRI LP1 findings	MRI LP3 findings	Antibody	Malignancy
1	Yes	Yes	Yes	Rests	Neg	Neg
2	No	-	Yes	-	Neg	Mammar
3	Yes	-	Yes	Rests	Neg	Neg
4	Yes	-	Yes	Progress	Neg (post herpes)	Neg
5	Yes	No	Yes	Rests	Ma2	Neg
6	Yes	Yes	Yes	Rests	NMDAR, GAD, TPO	Neuro-endocrine
7	Yes	-	No	No	Neg	Neg
8	Yes	No	Yes	Rests	Neg	Neg
9	No	-	Yes	No (at LP2)	Neg	Neg
10	No	No	Yes	Rests	Neg	Neg
11	Yes	Yes	Yes	No	GAD	Neg
12	Yes	-	No	-	VGKC (LGI1)	Neg
13	No	-	Yes	Rests	Ma2	Neg
14	Yes	No	No	No	NMDAR	Neg
15	Yes	-	Yes	Rests	Neg	Neg
16	Yes	No	No	No	Neg	Neg
17	Yes	-	Yes	Rests	GAD	Neg
18	No	No	Yes	Rests	Neg	Neg
19	Yes	No	No	No	TPO	Neg
20	Yes	Yes	Yes	Yes	TPO, GAD	Neg
21	Yes	-	No	No	NMDAR, TPO	Neg
22	Yes	No	No	No	Neg	Neg
23	Yes	-	No	No	NMDAR	Neg
24	Yes	-	No	No	Neg	Neg
25	Yes	-	No	-	GAD	Neg

Antibodies: GAD = glutamic acid decarboxylase; NMDAR = anti-N-methyl-D-aspartate receptor; TPO = thyroid peroxidase; VGKC = voltage gated kalium chanel.
CSF = cerebrospinal fluid; LP = lumbar puncture

Table 3 CSF levels of neuronal damage markers at onset (LP1), 3±1 months (LP2) and 12±3 months (LP3). Levels marked with * are normal (for age), all the others are increased.

Patient	NFL 1	NFL 2	NFL 3	T-tau 1	T-tau 2	T-tau 3	GFAP 1	GFAP 2	GFAP 3
1	4469	-	2719	1230	-	290*	960*	-	2910
2	4063	-	-	2520	-	-	1400	-	-
3	54063	179688	-	-	5330	-	-	1480	-
4	32500	32600	-	2850	1950	-	1810	350*	-
5	5560	12200	870*	6910	500	353*	520*	560*	360*
6	6000	4240	1700*	434	168*	157*	1650	1430	1060*
7	5313	6031	-	520	310*	-	320*	530*	-
8	9530	44700	2760	2000	2930	328*	1300	930	430
9	3580	7920	-	3090	233*	-	600*	420*	-
10	< 781*	9094	3438	1120	1190	240*	570	620	280
11	1310	1330	480*	541	138*	155*	380*	340*	180*
12	-	410*	-	-	137*	-	-	290*	-
13	< 781*	-	-	140*	-	-	90*	-	-
14	High	960	720	-	100*	110*	-	580*	320*
15	2700	1520	940	420	250*	168*	7810	930	2560
16	2531	31875	5906	4290	640	240*	940*	520*	590*
17	1094*	-	-	360	-	-	180*	-	-
18	2550	19300	1220	1400	1230	408	250*	650	360
19	3510	1710	340*	840	200*	200*	280*	400*	420*
20	250	-	490	150*	-	108*	220*	-	110*
21	740	1800	-	211*	114*	-	50*	420	-
22	400	480	300*	635	243*	160*	460	400	330*
23	4810	4440	High	1130	227*	-	270*	310*	-
24	210*	390	-	295	136*	-	230	190	-
25	310*	-	-	188*	-	-	400*	-	-

CSF = cerebrospinal fluid; GFAP = glial fibrillary acidic protein; LP = lumbar puncture; NFL = neurofilament light chain; T-tau = total tau protein.

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