Journal of Neuroimmunology 306 (2017) 25–30

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

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

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ARTICLE INFO

Article history:
Received 21 October 2016
Received in revised form 2 February 2017
Accepted 28 February 2017

Keywords:
Autoimmune neurological syndrome
Paraneoplastic neurologic syndrome
Non-paraneoplastic
Autoimmune encephalitis
Neurofilament light chain
Tau protein

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.

1. Introduction

Autoimmune neurological syndromes may be paraneoplastic [related to systemic malignancies and henceforward called paraneoplastic neurological syndromes (PaNS)] or non-paraneoplastic (non-PaNS), not related to malignancy. In PaNS a systemic malignancy sometimes expressing onconeuronal antigens triggers autoimmune processes which are responsible for the clinical symptoms (Dalmau and Rosenfeld, 2008; Graus et al., 2004). The clinical presentation of autoimmune neurologic syndromes is variable and not specific enough for identifying patients with malignancies. It comprises central nervous manifestations, including autoimmune encephalitis, and peripheral neurological syndromes. Detecting a malignancy is imperative for improving the long-term prognosis but may be exceedingly difficult, involving a large number of malignancy investigations which, when negative, need to be repeated over several years (Darnell and Posner, 2003; Graus and Dalmau, 2007; Gromadzka et al., 2013; Titulaer et al., 2011). Other available biomarkers such as cerebrospinal fluid (CSF) analysis, brain magnetic resonance imaging (MRI), and assessment of antibodies directed against neuronal surface and intracellular antigens, offer only limited additional help, as they often cannot differentiate patients with malignancies from those without (Psimaras et al., 2010; Graus et al., 2016; Dalmau et al., 2011). Increased CSF levels of neuronal damage markers [neurofilament light chain (NFL) and total tau (T-tau) protein] but not of glial cell damage [glial fibrillary acidic protein (GFAP)] were found in autoimmune encephalitis. However, they were not studied separately in patients with underlying malignancies and those without (Constantinescu et al., 2016) and, to the best of our knowledge, they have never been investigated in PaNS. There is a need for better and clinically accessible biomarkers to rapidly identify patients with an underlying malignancy.

The aim of this study is to investigate patients with paraneoplastic and non-paraneoplastic autoimmune neurological syndromes and

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http://dx.doi.org/10.1016/j.jneuroim.2017.02.018
0165-5728/

compare those with malignancies with those without, in an attempt to identify characteristic clinical and laboratory patterns.

2. Patients and methods

2.1. 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.

2.2. Patients

Patients with autoimmune neurological syndromes were identified by searching patient files from year 2000 to 2015 using combinations of search terms: (1) "autoimmune, limbic, malignant or paraneoplastic" and (2) "encephalitis, syndrome". Data of identified patients were then extracted and verified manually.

2.2.1. Symptoms

Following symptoms were recorded as present or absent: cognitive dysfunction (short-term memory loss), altered mental status (personality change, lethargy, decreased or altered level of consciousness), epileptic seizures, status epilepticus (defined as clinical and/or electrographic epileptic seizures either sustained or repeated without recovery in between), focal central neurological signs [cerebellar, brain stem, muscular stiffness consistent with stiff person syndrome], widespread central neurological signs [encephalopathy), peripheral neurologic signs [sensory or motor disturbance due to neuropathy or neuromuscular disorder, dysautonomia], and psychiatric symptoms (depression, psychotic symptoms). All symptoms had to be considered as subacute (< 3 months) and related to the neuroimmunological disorder, without any better underlying cause.

2.2.2. Diagnostic criteria for inclusion

For study inclusion, three diagnostic criteria were needed: (I) Definite or possible PaNS according to Graus et al., 2004 (Graus et al., 2004) [a combination of symptoms (a syndrome) and findings (onconeural antibodies or malignancy)] OR non-PaNS. All non-PaNS patients had to have any of following diagnoses: (1) possible or (2) antibody-negative probable autoimmune encephalitis; or (3) definite autoimmune limbic encephalitis; or (4) definite anti-NMDA receptor encephalitis according to Graus et al., 2016 (Graus et al., 2016) [a combination of symptoms (cognitive, mental, psychiatric) and findings (new focal central nervous system findings, seizures, CSF pleocytosis, MRI features, neuronal surface and/or synaptic antibodies)] and no onconeural antibodies or malignancy; and (II) Exclusion of other diagnoses with a similar clinical presentation (Asztely and Kumlien, 2012; Zuliani et al., 2012; Graus et al., 2008); and (III) Results from at least one CSF analysis.

2.3. Diagnostic workup

Investigations included brain MRI, EEG, CSF and blood analyses (including antibody analysis), and malignancy investigations.

2.3.1. Brain MRI

Brain MRI criteria suggesting a neurological autoimmune process/encephalitis were: (1) T2/FLAIR hyperintensity in mediotemporal areas; (2) gradual development of hippocampal atrophy; (3) other pathological brain findings not better explained by other etiology and considered by neuroradiologists to be caused by an autoimmune process.

2.3.2. Cerebrospinal fluid analysis

Details for routine CSF investigations, and CSF-NFL, T-tau and GFAP measurements were previously reported (Constantinescu et al., 2016) and are presented in the Supplementary material (section Laboratory analyses and Supplementary Table 5).

In this study, CSF immunopathy denoted immunological activation in the CSF and was defined as the presence of at least one of the following: (a) pleocytosis ($>3 \times 10^6$ mononuclear cells/L), (b) CSF-specific oligoclonal immunoglobulin G (IgG) bands (>1 CSF-selective band), (c) increased IgG index (>0.63), or (d) increased IgM index (>0.060).

Blood-brain barrier damage was defined as increased CSF-albumin (>320 mg/L) and/or increased CSF to serum albumin ratio (18–45 years: >6.8; 45–90 years: >10.2) (Blennow et al., 1993).

2.3.3. Neuronal surface antibodies and antibodies against intracellular antigens

Both serum and CSF were used and the panel included the following autoantibodies: (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). Details for antibody measurement have been published (Constantinescu et al., 2016) and are presented in the Supplementary material (section Laboratory analyses).

2.3.4. Malignancy investigations

The malignancy investigation 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 (^{18}FDG-PET), after 2007 combined with whole-body CT was performed. A negative initial malignancy investigation was repeated regularly, as recommended (Titulaer et al., 2011).

2.4. 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). Both scales include death as a possible outcome. Deaths were documented from hospital files. Patients lost to follow up were considered as alive, if not recorded as dead in the Swedish population registry as of December 1, 2016.

2.5. Last recorded visit and follow up

After the last recorded visit, patients could still have contact with our clinic or with our hospital, could be dead, or could be lost to follow up.

2.6. Statistical analysis

Symptoms, systemic malignancy, antibodies, MRI-brain abnormalities, CSF immunopathy, blood-brain barrier damage, were coded as present or absent, according to the definitions defined above. SPSS statistics package version 22 was used for analysis. The Independent-Samples Mann-Whitney *U* 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. Log-transformation of brain damage markers levels was performed as they were not normally distributed.