Diagnostic and prognostic biomarkers in immune checkpoint inhibitor-related encephalitis: a retrospective cohort study

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Summary

Background Immune checkpoint inhibitor-related encephalitis (ICI-encephalitis) is not well characterised and diagnostic and prognostic biomarkers are lacking. We aimed to comprehensively characterise ICI-encephalitis and identify diagnostic biomarkers and outcome predictors.

Methods This retrospective observational study included all patients with ICI-encephalitis studied in the French Reference Centre on Paraneoplastic Neurological Syndromes (PNS) and Autoimmune Encephalitis (2015–2023). ICI encephalitis was considered definite in case of inflammatory findings at paraclinical tests and/or well-characterised neural antibodies. Predictors of immune-related adverse event (irAE) treatment response, defined as a Common Terminology Criteria for Adverse Events v5.0 grade < 3 at any time after therapeutic intervention, were assessed by logistic regression analysis, and predictors of mortality by Cox regression analysis. Neurofilament light chain (NfL) was measured by enzyme-linked immunosorbent assay.

Findings Sixty-seven patients with definite encephalitis were identified (median age, 69 years; 66% male). A focal syndrome was observed in 43/67 patients (64%; limbic encephalitis, cerebellar ataxia, and/or brainstem encephalitis), while 24/67 (36%) had meningoencephalitis, a non-focal syndrome with altered mental status (22/24 patients, 92%) and pleocytosis (24/24 patients, 100%). Patients with focal encephalitis more frequently had abnormal brain MRI (26/42, 62% *versus* 8/24, 33%, p = 0.025), PNS-related antibodies (36/43, 84% *versus* 1/24, 4%, p < 0.001), and neuroendocrine cancers (22/43, 51% *versus* 1/24, 4%; p < 0.001) than patients with meningoencephalitis. Focal encephalitis patients had a lower rate of irAE treatment response (7/39, 18%) and higher mortality (27/43, 63%) compared to meningoencephalitis patients (12/22, 77% and 5/24, 21%, respectively, p < 0.001 each). PNS-related

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antibodies were associated with less irAE treatment response, independently of age, sex, and baseline severity (adjusted OR 0.05; 95%CI [0.01; 0.19]; p < 0.001) as well as higher mortality, independently of age and cancer type (adjusted HR 5.07; 95% CI [2.12; 12.12]; p < 0.001). Serum NfL discriminated patients with definite ICI-encephalitis (n = 27) from cancer-matched controls (n = 16; optimal cut-off >273.5 pg/mL, sensitivity 81%, specificity 88%, AUC 0.87, 95% CI [0.76; 0.98]) and irAE treatment responders (n = 10) from non-responders (n = 17, optimal cut-off >645 pg/mL, sensitivity 90%, specificity 65%; AUC 0.75, 95% CI [0.55; 0.94]).

Interpretation **ICI-encephalitis corresponds to a set of clinically-recognisable syndromes. Patients with focal encephalitis, PNS-related antibodies, and/or higher serum NfL have low irAE treatment response rates. Research is needed on the underlying immunopathogenesis to foster therapeutic innovations.**

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Keywords: **Encephalitis; Autoimmune encephalitis; Immune checkpoint inhibitors; Cancer immunotherapy; Adverse events; Neurological immune-related adverse events; Paraneoplastic neurological syndromes; Neural antibodies; Biomarkers; Prognostic factors; Cancer**

Introduction

The use of immune checkpoint inhibitors (ICI) in the oncological clinical practice is rapidly expanding.[1] These monoclonal antibodies elicit clinically effective and often durable anti-tumour responses by targeting key negative regulators of T cells, such as cytotoxic T-lymphocyte antigen-4 (CTLA4), programmed death-1 (PD1), and programmed death ligand-1 (PDL1).[2] However, ICI often induce immune-related adverse events (irAEs),[3,4] including, in 1–3% of patients, neurological adverse events (n-irAEs), which may affect both the central and/or peripheral nervous system.[5–8] ICI-encephalitis is the most frequent central nervous system (CNS) n-irAE; it is usually severe and associated with a high mortality rate (around 20%).[5,9,10] Previous reports mentioned patients with meningoencephalitis, paraneoplastic-like limbic encephalitis, non-limbic encephalitis, encephalopathy, and encephalitis without a distinctive syndrome,[10–16] suggesting clinical heterogeneity. Systematic clinical descriptions however, are lacking. The difficult recognition of specific clinical syndromes, along with the lack of diagnostic biomarkers and the broad list of alternative diagnoses (e.g., metabolic encephalopathy, cancer dissemination) hinder the diagnosis of ICI-encephalitis and likely prevent or delay essential therapeutic interventions, such as ICI discontinuation and initiation of immunosuppressive treatments.[17] Furthermore, data on ICI-encephalitis outcomes and prognostic factors are limited and conflicting, which complicates decisions regarding immunological treatments, or ICI rechallenge.[9–11,14] With the aim to facilitate the recognition of ICI-encephalitis, improve diagnostic procedures, and identify prognostic factors, we characterised the encephalitis syndromes and assessed outcomes in a large cohort of patients with ICI-encephalitis. We also explored the possible association of axonal and astroglial injury biomarkers with the diagnosis, syndromes, and outcomes of ICI-encephalitis.

Methods

Patient selection and antibody detection

The database of the French Reference Centre on Paraneoplastic Neurological Syndromes (PNS) was screened to identify all patients whose serum and/or cerebrospinal fluid (CSF) was tested for neural antibodies between July 2015 and March 2023 (n = 31,228) and who had received ICI treatment. In this centre, information on ICI treatments is collected systematically through the test request forms, and, additionally, when our opinion is requested on a case (by email, phone, or during the bimonthly national multicentre interdisciplinary meetings). After a first screening of all the available medical charts, all patients with new-onset symptoms of brain parenchyma dysfunction within 12 months from the last administration of any ICI[17] not explained by alternative causes were included in the study; patients with other CNS syndromes (e.g. meningitis, myelitis) were excluded; Supplementary Fig. S1). The diagnosis of ICI-encephalitis was considered definite in case of CSF pleocytosis (>5 cells/mm3), and/or oligoclonal bands, and/or brain magnetic resonance imaging (MRI) abnormalities suggesting neuroinflammation (increased T2/FLAIR signal and/or contrast enhancement), and/or positive well-characterised neural antibodies[18,19]; otherwise the diagnosis was considered possible. Demographic, clinical, and paraclinical data were retrospectively extracted from the available medical charts. Treating physicians were contacted in case of missing information. Whenever possible, neurological symptoms were classified according to previously defined encephalitis syndromes.[19,20] Symptom progression was classified as acute (<24 h), subacute (24 h to 3 months), and chronic (>3 months). Neural antibodies were tested by immunohistofluorescence (IHF) on rat brain sections and a confirmatory test consisting of line-blot analysis on recombinant proteins (Euroimmun,

Research in context

Evidence before this study

We searched PubMed for articles published from May 1, 2017 to January 10, 2024. We used the MeSH terms "neurological immune-related adverse event", "n-irAE", "encephalitis", "neurotoxicity", alone or in combination with "immune checkpoint inhibitor", "anti-PD1", "anti-PDL1", "anti-CTLA4", "pembrolizumab", "ipilimumab", "nivolumab", "atezolizumab", "avelumab", "durvalumab", "tremelimumab", or "cemiplimab". We prioritised original research articles or case series (at least five cases), but we also included systematic reviews. Few observational retrospective studies and two independent systematic reviews have investigated the clinical presentation and the outcome of immune checkpoint inhibitor-(ICI) related encephalitis. Taken all together, published patients are described as having meningoencephalitis, paraneoplastic-like limbic encephalitis, non-limbic encephalitis, encephalopathy, or encephalitis without a distinctive syndrome, suggesting important clinical diversity, which has not been entirely captured by previous cohorts. Outcomes and prognostic factors have been assessed in small-sized cohorts with limited follow-up information. One study found that lung cancer was associated with poorer outcome, and that a subgroup of patients with an otherwise unexplained encephalopathy without inflammatory changes at paraclinical tests carried a higher risk of mortality. Predictors of irAE treatment response have not been identified and diagnostic biomarkers are also lacking. A recent retrospective study including 9 patients with central nervous system (CNS) immune-related adverse events (irAEs) found that serum concentration of brain injury biomarkers (NfL and S100B) may discriminate between ICI-treated patients who develop CNS irAEs and those who do not, with good sensitivity and specificity.

Added value of this study

We retrospectively assessed the clinical presentations and outcomes of 76 patients with ICI-related encephalitis; brain injury biomarkers were studied in 30 of them (definite encephalitis, n = 27; possible encephalitis, n = 3). We found that ICI-encephalitis presents with clinically-recognisable clinical patterns, consisting of either focal syndromes (limbic encephalitis, rapidly progressive cerebellar ataxia, brainstem encephalitis) or meningoencephalitis, a non-focal syndrome consisting in new-onset alteration of the mental status and/or other neurological signs, such as seizures, aphasia, or decreased level of consciousness. In most patients the diagnosis is supported by inflammatory findings at paraclinical tests, including brain MRI abnormalities, CSF pleocytosis, or well-characterised neural antibodies. Nevertheless, in this study nearly 40% of the patients with definite encephalitis received 1 or more ICI infusions after the onset of the neurological symptoms, suggesting that the suspicion of ICI encephalitis is often delayed in the clinical practice. Additionally, some patients (12% in the present cohort) have no inflammatory changes at paraclinical tests or antibodies, but an immune-related pathogenesis is nevertheless suggested by other CSF findings (e.g., mild elevation of neopterin or IL-6 levels, and/or uncharacterised neural antibodies) and/or improvement after immunological treatments. PNS-related antibodies, which were mostly found in focal encephalitis patients, were independently associated with a lack of irAE treatment response and mortality. Serum NfL in ICI-encephalitis is high, to levels comparable to HSV encephalitis, and can efficiently discriminate patients with definite ICI-encephalitis from cancer-matched controls (with a sensitivity of 81% and a specificity of 88% in the present cohort), as well as irAE treatment responders from non-responders (with a sensitivity of 90% and a specificity of 65% in the present cohort).

Implications of all the available evidence

The study finds that ICI-encephalitis manifests as clinically recognisable syndromes and frequently associate with positive neural antibodies. Nevertheless, we also show that ICI-encephalitis is often overlooked, leading to delays in diagnosis and treatment. The results indicate that serum NfL levels, a marker of brain damage, could be used to identify the patients requiring urgent interventions, such as withdrawal of the offending ICI treatment and implementation of second-level procedures (e.g., lumbar puncture, brain MRI, neural antibody testing) necessary to confirm the diagnosis and initiate immunosuppression. Additionally, a large subset of focal ICI-encephalitis shares the clinical presentations, antibody and cancer associations, and poor prognosis with spontaneous paraneoplastic encephalitis, suggesting common pathogenic mechanisms. Critically, guidelines should be established for the diagnosis and classification of ICI-encephalitis, and research is needed to identify novel therapeutic targets and strategies.

Lubeck, Germany and/or Ravo Diagnostika, Freiburg, Germany), and/or cell-based assays, and/or western blots (in-house techniques) as reported elsewhere.[21] The definition of PNS antibodies was based on the frequent (>70%) epidemiological association with cancer, according to the updated PNS criteria[18]; anti-tripartite motif-containing protein 9 and 46 (Trim9/46)[22,23] and anti-Dachshund Family Transcription Factor 1 (Dach1) were also defined herein as PNS antibodies.[24] Antibodies targeting unknown antigens were defined by a positive IHF result without any antigenic specificity identified on the available tests. Anti-myelin oligodendrocyte glycoprotein (MOG) antibodies were tested as reported previously[25] in all available sera not already positive for PNS antibodies. Disease severity was evaluated at the first hospitalisation, at 3, 6, 9, and 12

months, and at last follow-up, and classified according to the Common Terminology Criteria for Adverse Events (CTCAE) v 5.0: grade 1 = asymptomatic or mild; grade 2 = minimal, non-invasive intervention indicated; grade 3 = severe, not immediately life-threatening; grade 4 = life-threatening, urgent intervention indicated; grade 5 = death.[17,26] IrAE treatment response was defined as the registration of a CTCAE grade <3 at any time after therapeutic intervention. N-irAE relapse was defined as the recurrence of the neurological symptoms following a sustained period of improvement lasting at least 4 weeks. For the analysis of demographic features, CSF data, and brain MRI results, three available retrospective cohorts were used as disease controls: 32 patients with herpes simplex virus (HSV) encephalitis, diagnosed in the *Hospices Civils de Lyon* (Lyon, France) and *Centre Hospitalier Universitaire de Grenoble* (Grenoble, France) between March 2012 and November 2023, as well as 179 patients with anti-LGI1 encephalitis[27] and 114 patients with anti-Hu encephalitis,[28] diagnosed in the study centre between July 2015 and January 2023.

Biomarker analysis

Levels of S-100 calcium-binding protein (S100-B), neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP) were measured in serum and CSF samples collected within 3 months of the neurological onset or relapse. The sera of 16 other patients sampled at the time of first ICI infusion and matched for age and cancer type were used as controls (cancer-matched controls; Supplementary Table S1) in addition to 26 patients randomly selected from the disease-control cohorts (6 HSV-encephalitis, 10 anti-LGI1 encephalitis, and 10 anti-Hu encephalitis), who had available sera sampled within 3 months after the neurological onset. Analyses were performed in one batch and with a maximum of two freeze–thaw cycles for each sample using commercially available kits (Human S100-B ELISA kit Millipore®, R-PLEX Human Neurofilament L Meso Scale Discovery®, and R-PLEX Human GFAP Meso Scale Discovery®) according to the respective standard operating procedures and ISO 15189:2012 standard recommendations.

Statistical analysis

Continuous data were expressed as median (range) and categorical data as count (percentage). Groups were compared by Fisher's exact test for categorical variables and by Kruskal–Wallis H test for continuous variables. Post-hoc analyses with correction for multiple tests (Holm-Bonferroni method) were used when significant results were obtained between >2 groups. The performance of serum and/or CSF levels of S100B, NfL, and GFAP for discriminating between patients with definite encephalitis and controls, as well as irAE treatment-responders and non-responder, was evaluated and graphed as receiver operating characteristic (ROC) curves; the empirical method by DeLong et al.[29] was used to estimate the area under the curve (AUC) and its 95% confidence interval. The optimal cut-off value for each biomarker was estimated using the Youden index and presented with its 95% confidence interval and its corresponding sensitivity and specificity. A multivariable logistic regression analysis was performed to assess the adjusted effect of the presence of PNS antibodies on irAE treatment response, considered as the dependent variable. The model was adjusted on age, sex, and severity (CTCAE grade at baseline). Odds ratios and Wald 95% confidence intervals are reported. The covariates of this model were selected because they were of clinical interest. The Kaplan–Meier method was used to estimate the median survival time and a Cox model was used to assess the effect of age, cancer type, and the presence of PNS-related antibodies on mortality. Hazard ratios and Wald 95% confidence intervals are reported. Schoenfeld residuals were assessed to verify the proportional hazards assumption for each covariate. The covariates of this model were selected because they were of clinical interest. All p-values were two-tailed and p values < 0.05 were considered statistically significant. Statistical analyses were performed using Rstudio, version 3.4.0 (R foundation for Statistical Computing, Vienna, Austria).

Ethical considerations

This study was approved by the institutional review board of the *Université Claude Bernard Lyon 1* and *Hospices Civils de Lyon* (69HCL21-474), and registered with the national data protection agency (*Commission nationale de l'informatique et des libertés*, CNIL, 21–5474). According to the Declaration of Helsinki and its later amendments, patients' informed consent was obtained. Serum samples and clinical data from controls were provided by the *Hospices Civils de Lyon* as part of the "IMMUCARE-BASE" study, which was approved by the *Comité de Protection des Personnes* and registered with the CNIL (19–171).

Role of the funding source

The study funder had no role in study design, data collection, analysis, and interpretation, writing of the paper, and decision to submit it for publication.

Results

Demographic and oncological features

A total of 76 patients with ICI-encephalitis, referred from 39 tertiary hospitals in France, were identified. Among them, 67/76 (88%) had a definite diagnosis, and 9/76 (12%) had a possible diagnosis and were analysed separately (Supplementary Fig. S1). In patients with definite ICI-encephalitis (median age, 69 years, range 33–89; 44/67 males, 66%), cancer types were non-small cell lung cancer (NSCLC, 23/67, 34%), small cell lung

cancer (SCLC, 21/67, 31%), urological malignancies (7/67, 10%), melanoma (6/67, 9%), and other cancers (10/67, 15%; Supplementary Table S2); 23/67 were neuroendocrine tumours (34%). While brain metastases were reported in 20/67 patients (30%), these had regressed (n = 13) or were stable (n = 7) at neurological symptom onset and their localisation could not explain the neurological symptoms. Encephalitis occurred in a median of 2.5 months (range 0.1–23.3) after the first ICI dose (median of 4 ICI cycles, range 1–46; Supplementary Table S2). All patients had received PD1 or PDL1 inhibitors, alone (59/67, 88%) or in combination with CTLA4 or LAG3 inhibitors (7/67, 10%). One patient was in a double-blind randomised controlled trial comparing anti-PD1 monotherapy *versus* combination with a TIGIT inhibitor. Two patients (3%) had received a previous ICI treatment before the offending ICI drug (pembrolizumab or nivolumab, 1 patient each) and 2/67 patients (3%) had a pre-existent encephalitis that worsened after ICI administration (acute cerebellar ataxia with anti-Hu and anti-Ma2, respectively). None had pre-existent peripheral nervous system disorders. Forty-six of 67 patients (69%) tested positive for well-characterised neural antibodies.

Focal encephalitis syndromes

Forty-three patients (64%) had a focal encephalitis syndrome, of which the most frequent was definite autoimmune limbic encephalitis (25/67, 37%), which could be either isolated (18/25, 72%) or associated with brainstem (5/25, 20%), diencephalic (4/25, 16%), and/or cerebellar (3/25, 12%) dysfunction. The remaining patients with focal encephalitis had rapidly progressive cerebellar ataxia (RPCA; 12/67, 18%), brainstem encephalitis (3/67, 4%), or a combination of RPCA and brainstem encephalitis (3/67, 4%). Among the patients with focal encephalitis, 6 had concomitant peripheral neuropathy, including sensory neuronopathy (3 patients), polyradiculoneuropathy (1 patient), and a combination of sensory neuronopathy with polyradiculoneuropathy or enteric neuropathy (2 patients). Twenty-six of 40 patients (65%) had increased CSF cell counts (median 20 cells/mm3, 9–83) and 22/42 (52%) had abnormal brain MRI T2/FLAIR hyperintensity (gadolinium-enhanced in 5/22 cases, 23%) in the mesial temporal lobes (20/25, 80%), brainstem (3/42, 7%), cerebellum (2/42, 5%), and/or other regions (5/42, 12%; Supplementary Table S3). Pachy- and/or leptomeningeal enhancement was observed in 3 patients with limbic encephalitis and in 1 patient with RPCA, and focal atrophy was observed in 6 patients (hippocampal in 3 limbic encephalitis; cerebellar in 3 RPCA; Fig. 1). In patients with focal ICI-encephalitis, CSF pleocytosis (white blood cell count >5/mm^3; 26/40, 65%) was more frequent than in the anti-LGI1 cohort (16/130, 12%, $p < 0.001$) and less frequent

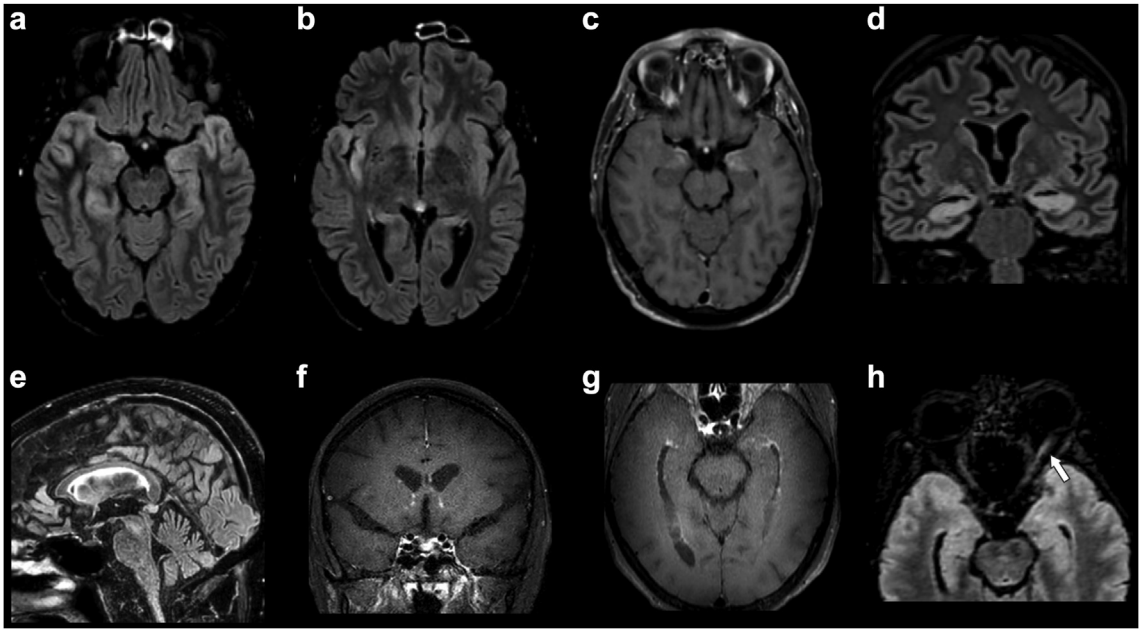

Fig. 1: **Representative brain MRI findings.** Bilateral, left predominant temporo-insular T2/FLAIR hyperintensity (**a** and **b**) and temporo-mesial contrast enhancement (**c**) in a 44-year-old man with antibody-negative limbic encephalitis. Bilateral temporo-mesial T2/FLAIR hyperintensity in a 63-year-old woman with anti-Hu limbic encephalitis (**d**). Moderate cerebellar atrophy and T2/FLAIR superior cerebellar peduncles hyperintensity in a 76-year-old man with anti-Dach1 cerebellitis (**e**). Leptomeningeal (perivascular spaces) (**f**) and epdendymal (**g**) contrast enhancement, and T2/FLAIR left optic nerve hyperintensity (arrow, **h**) in a 70-year-old patient with anti-GFAP meningoencephaltis.

than in patients with HSV-encephalitis (29/32, 91%, p = 0.011); however, the proportion was not significantly different compared to the anti-Hu cohort (48/98, 49%, p = 0.087). The median cell count in patients with pleocytosis was also higher in focal ICI-encephalitis compared to anti-LGI1 encephalitis (8/mm3, range 6–50; p = 0.007), and it was lower than in HSV encephalitis (54/mm3, range 6–1158; p = 0.010); there was no significant difference when compared anti-Hu encephalitis (16/mm3, range 6–153; p = 0.097). Additionally, mesial temporal lobe T2/FLAIR hyperintensity (20/43, 48%) was less frequently observed in focal ICI-encephalitis than in anti-LGI1 and HSV-encephalitis (130/179, 75% and 30/32, 97%, respectively; p < 0.001 each), while there was no significant difference when compared to the anti-Hu cohort (26/114, 36%, p = 0.206). Meningeal enhancement as well as T2/FLAIR hyperintensity in other brain areas were also less frequent in focal ICI-encephalitis than in HSV-encephalitis (16/31, 52% and 27/31, 87%, respectively; p < 0.001 each; Supplementary Table S4). PNS antibodies were detected in most patients (36/43, 84%; in majority anti-Hu, Ma2, and Yo antibodies), with antigenic targets always consistent with the clinical syndrome (Fig. 2).[18] Antibody-cancer mismatch was observed in 5/36 patients (14%; anti-Ma2 with mesothelioma, spleen liposarcoma, or renal clear cell carcinoma, 3 patients; anti-Trim46 with mesothelioma and anti-Yo with lung adenocarcinoma, 1 patient each). The sera of 3 patients were sampled before ICI administration and all tested positive for PNS antibodies in retrospect (anti-Ma2,[9,30] anti-Yo,[9] anti-Hu, 1 patient each). All patients with focal encephalitis (100%) fulfilled the diagnostic criteria for PNS (definite 29/43, 67%; probable, 11/43, 26%; possible, 3/43, 7%; median PNS-Care score[18] 10, range 4–10).

Meningoencephalitis

The remaining 24/67 patients (36%) were classified as meningoencephalitis, a non-focal syndrome characterised in most cases by altered mental status (22/24, 92%) and increased CSF white blood cells (100%), variably accompanied by language impairment (10/24, 42%), seizures (6/24, 25%), fever (6/24, 25%), coma (6/24, 25%), gait impairment (5/24, 21%), hyperkinetic movement disorders (6/24, 25%), behavioural changes (4/24, 17%), psychiatric symptoms (3/24, 12%), or others (headache, dysautonomia, or memory impairment; 1 patient each). The onset of seizures was focal or unknown (3 cases each), awareness was impaired in 5 patients, bilateral motor activity was observed in 3, and EEG findings suggested temporal lobe involvement in 1 patient. In addition, 4/24 (17%) had concomitant polyradiculoneuropathy and 3/24 (12.5%) had myelitis. In ICI-meningoencephalitis, CSF pleocytosis (24/24, 100%) was more frequent than in focal ICI-encephalitis (p = 0.001), anti-Hu encephalitis (p < 0.001), and anti-LGI1 encephalitis (p < 0.001). The median cell count in patients with pleocytosis was also higher in ICI-meningoencephalitis (median 30 cells/mm3, range 6–261) compared to anti-LGI1 encephalitis (p = 0.004) and anti-Hu encephalitis (p = 0.006), while it was similar to HSV encephalitis (p = 0.428). Of note, the time to lumbar puncture was significantly longer in patients with focal ICI-encephalitis (5 weeks, range 0–45) when compared to patients with meningoencephalitis (1 week, range 0–31, p = 0.020). Brain MRI was unremarkable in the majority of patients with meningoencephalitis

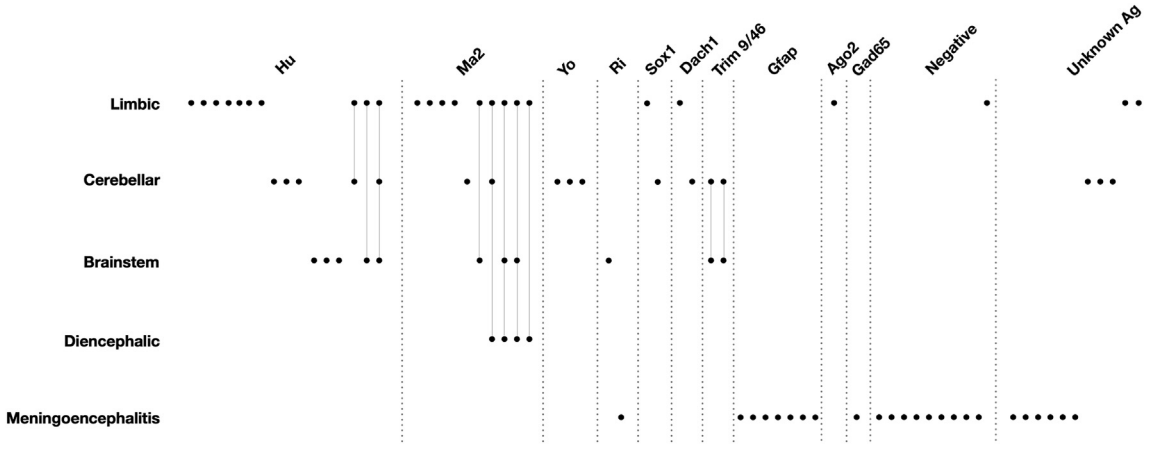

Fig. 2: **Syndrome-antibody correlations in patients with definite immune checkpoint inhibitor-encephalitis.** Syndromes observed in each antibody-defined group are represented at the individual level. The intersections between syndromes and antibodies in each patient are represented by dots, connected by a line in case of overlapping syndromes. Anti-MOG antibodies were negative in all 16 patients tested (12 meningoencephalitis, 4 focal encephalitis).

(16/24, 67%), contrasting with focal ICI-encephalitis (16/42, 38%, p = 0.025), anti-LGI1 encephalitis (130/174, 25%, p < 0.001), and HSV encephalitis (0/31, 0%, p < 0.001; Supplementary Table S5). Only 4/24 patients with meningoencephalitis (17%) had T2/FLAIR hyperintensities, all in non-limbic areas, and 5/24 (21%) had contrast enhancement (Supplementary Table S3).

The vast majority of the patients with meningoencephalitis were antibody-negative (9/24, 37%) or tested positive for either non-PNS antibodies (8/24, 33%; anti-GFAP, 7 patients and anti- GAD65, 1 patient) or brain-reactive antibodies of unknown specificity (6/24, 25%; Fig. 2). Most patients with meningoencephalitis had cancers without neuroendocrine histology (23/24, 96%), in contrast with the patients with focal encephalitis (22/43 neuroendocrine, 51%; p < 0.001; Table 1). Most patients with meningoencephalitis (22/24, 92%) fulfilled the Graus criteria for possible autoimmune encephalitis and none fulfilled the criteria for definite autoimmune limbic encephalitis[19]; the majority (17/24, 71%) were defined as non-PNS (median PNS-Care score[18] 3, range 3–9). Of note, the 7 anti-GFAP patients had presentations highly similar to previously reported cases of ICI-naïve GFAP encephalitis: all had a subacute alteration of the mental status along with fever, myelitis, optic neuritis, and/or polyradiculoneuropathy, with elevated CSF cell counts (median 136 cells/mm3, range 15–210) and protein levels (median 166 mg/dl, range 67–510), and 4 had periventricular white matter T2/FLAIR hyperintensities, brainstem T2/FLAIR hyperintensity, and/or leptomeningeal gadolinium enhancement on brain MRI.[31,32]

Outcomes and prognostic factors

At baseline, encephalitis was severe or life-threatening (CTCAE grade ≥3) in all patients (Fig. 3). ICI were withdrawn in all of the patients, although 26/66 (39%, including 21 with focal encephalitis) received 1 or more infusions after the onset of the neurological symptoms. The majority (59/65, 91%) were treated with steroids (58/65, 89%), intravenous immunoglobulins (IgIV, 34/65, 52%), and/or plasma exchange (PLEX, 7/65, 11%; Supplementary Fig. S2). The patients with focal encephalitis were more likely to receive second-line immunosuppressants (18/41, 44%) compared to those with meningoencephalitis (2/24, 8%, p = 0.003). Of 61 patients with available follow-up information, 24 (39%) improved to CTCAE grade <3 in a median of 1.5 months after therapeutic intervention (range 0–8), including 10/61 patients (16%) who recovered without sequelae (CTCAE grade = 1; Table 1). Patients with meningoencephalitis had higher rates of both irAE treatment response (17/22, 77%) and full irAE recovery (9/22, 41%) than patients with focal encephalitis (7/39, 18% and 1/39, 3%, respectively; p < 0.001 each; Table 1). During follow-up (median 7.5 months; range 0.5–51), 32 patients (48%) died (median survival, 15.7 months, 95%

	Focal encephalitis (N = 43)	Meningoencephalitis (N = 24)	p
Age, median (range)	66 (33–82)	70 (56–89)	0.122
Male, N (%)	28 (65)	16 (67)	0.898
Cancer organ, N (%)			0.029[a]
Lung	33 (77)	11 (46)	
Melanoma	2 (5)	4 (17)	
Urological	2 (5)	5 (21)	
Other	6 (14)	4 (17)	
Neuroendocrine tumour, N (%)	22 (51)	1 (4)	**<0.001**
Combination of 2 ICI classes, n/N (%)	2/42 (5)	5/24 (21)	0.088
Time from first ICI to onset, median, months (range)	2.4 (0.1–23,3)	3.1 (0.3–20.6)	0.424
Type of onset, N (%)			0.300
Acute	2 (5)	4 (17)	
Subacute	40 (93)	19 (79)	
Chronic	1 (2)	1 (4)	
Peripheral neuropathy	6 (14)	4 (17)	0.737
Cranial neuropathy	3 (7)[b]	1 (4)[c]	1
Myelitis	0 (0)	3 (12)	**0.042**
Non-neurological irAEs, N (%)	5 (12)	8 (33)	0.051
Well-characterised neural antibodies, N (%)	37 (86)	9 (38)	**<0.001**
PNS antibodies	36 (84)	1 (4)	**<0.001**
Non-PNS antibodies	1 (2)	8 (33)	**<0.001**
Antibodies *versus* unknown neural antigens, N (%)	5 (12)	6 (25)	0.182
Neural antibody-negative, N (%)	1 (4)	9 (38)	**<0.001**
Cerebrospinal fluid analysis, n/N (%)			
White blood cell > 5/mm3	26/40 (65)	24/24 (100)	**0.001**
White blood cell count, median (range)	10 (0–83)	30 (6–261)	**<0.001**
Protein level > 60 mg/dL	24/42 (57)	20/22 (91)	**0.006**
Protein level, mg/dL, median (range)	68 (30–191)	106 (48–510)	**0.016**
Oligoclonal bands	16/20 (80)	6/11 (55)	0.217
Interleukin-6 > 4 pg/mL	6/12 (50)	1/4 (25)	0.585
Weeks from onset to lumbar puncture, median (range)	5 (0–45)	1 (0–32)	**0.020**
Missing data	6	1	
Abnormal brain MRI, n/N (%)	26/42 (62)	8/24 (33)	**0.025**
Weeks from onset to brain MRI, median (range)	3 (0–17)	2 (0–10)	0.060
Missing data	4	2	
Diagnostic level for PNS, N (%)			**<0.001**
Definite (PNS-Care Score ≥8)	29 (67)	1 (4)	
Probable (PNS-Care Score 6–7)	11 (26)	6 (25)	
Possible (PNS-Care Score 4–5)	3 (7)	0 (0)	
Non-PNS (PNS-Care Score ≤3)	0 (0)	17 (71)	
CTCAE grade at baseline, N (%)			0.525
3	33 (77)	20 (83)	
4	10 (23)	4 (17)	
Corticosteroids, n/N (%)	38/41 (93)	20/24 (83)	0.408
Intravenous immunoglobulins, n/N (%)	28/41 (68)	6/24 (25)	**<0.001**
Plasma exchange, n/N (%)	4/41 (10)	3/24 (12)	0.703
Second-line immunosuppressants, n/N (%)	18/41 (44)	2/24 (8)	**0.003**
Cyclophosphamide, n/N (%)	7/41 (17)	1/24 (4)	0.240
Rituximab, n/N (%)	7/41 (17)	0/24 (0)	**0.041**
Tocilizumab, n/N (%)	7/41 (17)	0/24 (0)	**0.041**

(Table 1 continues on next page)

	Focal encephalitis (N = 43)	Meningoencephalitis (N = 24)	p
(Continued from previous page)			
Natalizumab, n/N (%)	0/41 (0)	1/24 (4)	0.369
Tofacitinib, n/N (%)	1/41 (2)	0/24 (0)	1
Follow-up duration, months, median (range)	6 (1–48)	10 (0–51)	0.305
CTCAE grade <3 reached during follow-up, n/N	7/39 (18)	17/22 (77)	**<0.001**
Resolution without sequelae	1/39 (3)	9/22 (41)	**<0.001**
Time from therapeutic intervention to improvement, median, months (range)	2.5 (0.5–6.9)	1.1 (0.7–1.1)	0.100
Death	27 (63)	5 (21)	**<0.001**
Cause of death			0.185
Encephalitis	12 (28)	1 (4)	
Cancer progression	6 (14)	2 (8)	
Other causes	3 (7)	2 (8)	
Unknown cause	6 (14)	0 (0)	

Significant p-values are bolded. CTCAE, Common Terminology Criteria for Adverse Events; ICI, immune checkpoint inhibitor; irAE, immune-related adverse event; MRI, magnetic resonance imaging; PNS, paraneoplastic neurological syndrome. [a]Post-hoc test non-significant. [b]Vestibulocochlear neuropathy in 2 patients with limbic encephalitis, and abducens neuropathy in 1 patient with brainstem and cerebellar encephalitis. [c]Optic neuritis.

Table 1: **Comparison between focal encephalitis and meningoencephalitis.**

CI [9.6; 27.5], Supplementary Fig. S3; time to death, median 5.8 months, range 0.5–27.5), 13 of them from the neurological toxicity (41%) and 8/32 (25%) from cancer progression (other causes, 16%; unknown, 19%). Mortality was higher in patients with focal ICI-encephalitis (27/43, 63%) than in patients with ICI-meningoencephalitis (5/24, 21%, p < 0.001). Accordingly, PNS antibodies were associated with less chance of achieving CTCAE grade <3 (adjusted OR 0.05; 95% CI [0.01; 0.19]; p < 0.001; Supplementary Fig. S4a) and with mortality (adjusted HR 5.07; 95% CI [2.12; 12.12]; proportional hazards assumption met; p < 0.001; Supplementary Fig. S4b). Of note, performing these analyses using data not only from definite, but also from possible ICI-encephalitis patients, yielded similar results (not shown). Seven patients with focal encephalitis, including 4/5 with elevated CSF interleukin-6 (IL-6) levels (CSF sampled in a median of 27 days after onset, range 16–39), were treated with tocilizumab based on previous experience[33]; only 1 of them (14%) reached CTCAE grade <3 (Table 1). Three patients were rechallenged with ICI (2 meningoencephalitis, 1 anti-Ma2 limbic encephalitis with diencephalic and brainstem

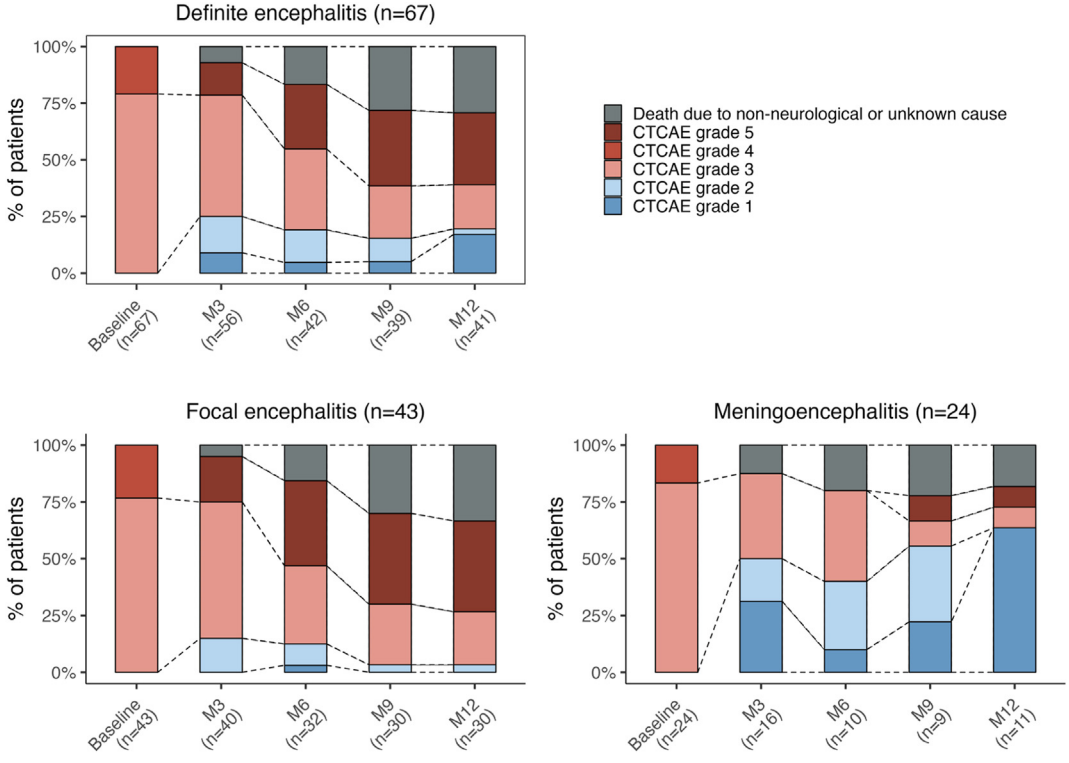

Fig. 3: **Outcome and prognostic factors.** CTCAE severity at baseline (M0) and at 3 (M3), 6 (M6), 9 (M9), and 12 months (M12) in all patients with definite encephalitis (n = 67), and in the subgroups of patients with focal encephalitis (n = 43) and with meningoencephalitis (n = 24). CTCAE v 5.0: grade 1 = asymptomatic or mild; grade 2 = minimal, non-invasive intervention indicated; grade 3 = severe, not immediately life-threatening; grade 4 = life-threatening, urgent intervention indicated; grade 5 = death. Abbreviations: CTCAE, Common Terminology Criteria for Adverse Events; M, month.

involvement), in a median of 4 months (range 1–37) after the last ICI dose, and did not experience any relapse (median follow-up after ICI reintroduction 16 months, range 3–17; Supplementary Table S6). Eight other patients, who were not rechallenged (6 focal encephalitis and 2 meningoencephalitis), had a neurological relapse (median 9 months after the first event, range 3–28), all similar to the initial event, and fatal in 3 patients (all with limbic encephalitis). Cancer progressed in 25/67 patients (37%), in a median of 5 months (range 0–39) after the last ICI dose.

Possible immune checkpoint inhibitor-encephalitis

The 9 patients with possible ICI-encephalitis (44% male; median age 76 years, range 40–83) had a subacute (7/9, 78%) or acute (2/9, 12%) onset, after a median of 5 ICI cycles (range 1–15). Two of them (22%) fulfilled the Graus criteria for possible autoimmune encephalitis[19] because they had a subacute alteration of the mental status with new-onset seizures or aphasia. Six patients (67%) had a clinical presentation similar to the patients with definite ICI-meningoencephalitis, and 3/9 (33%) had RPCA (Supplementary Table S7). All had a CTCAE grade ≥3, and 6/9 (67%) responded to treatment (ICI discontinuation, 9/9; corticosteroids, 7/9, IgIV 3/9, and/or PLEX 1/9). An inflammatory process was suggested in 5/9 patients (55%) by elevated CSF protein (4/9), neopterin (2/2), and/or IL-6 levels (1/3), and/or neural antibodies targeting unknown antigens (3/9). After a median follow-up of 6 months (range 2–49), 5/9 (55%) patients died, due to either cancer progression (3/5) or the neurological disease (2/5).

Brain injury biomarkers

S100B, NfL, and GFAP were assessed in the CSF (n = 19) and/or serum (n = 30) of 30/67 patients with clinical features and outcomes similar to the rest of the cohort (Supplementary Table S1). No significant correlation with age nor sampling time, and no association with tumour type, brain metastases, seizures, clinical severity (CTCAE grade 3 or 4), nor MRI findings were observed. The median serum NfL levels in patients with definite ICI-encephalitis (n = 27; 562 pg/mL, range 32–13,522) were not significantly different than in patients with HSV-encephalitis (660 pg/mL, range 119–3340, p = 0.910) but were significantly higher than in cancer-matched controls (108 pg/mL, range 14–647, p < 0.001) and patients with LGI1-encephalitis (237 pg/mL, range 56–2571, p = 0.034). Remarkably, patients with anti-Hu encephalitis had even higher levels of NfL (2879 pg/mL, range 181–32,320) than patients with definite ICI-encephalitis (p = 0.004; Fig. 4a). Moreover, serum NfL levels discriminated patients with definite ICI-encephalitis from cancer-matched controls (optimal cut-off >273.5 pg/mL, 95% CI [120; 431]) with a sensitivity of 81% and a specificity of 88% (AUC 0.87, 95% CI [0.76; 0.98]; Fig. 4b). Of note, the 3 patients with possible ICI-encephalitis and available serum had serum NfL below this threshold (109–182 pg/mL). Serum NfL levels also discriminated irAE treatment responders and non-responders (optimal cut-off >645 pg/mL, 95% CI [380.5–671.5], sensitivity 90%, specificity 65%; AUC 0.75, 95% CI [0.55; 0.94]), as did CSF NfL levels (optimal cut-off >22,064 pg/mL, 95% CI [3677; 100,400], sensitivity 88%, specificity 62%, AUC

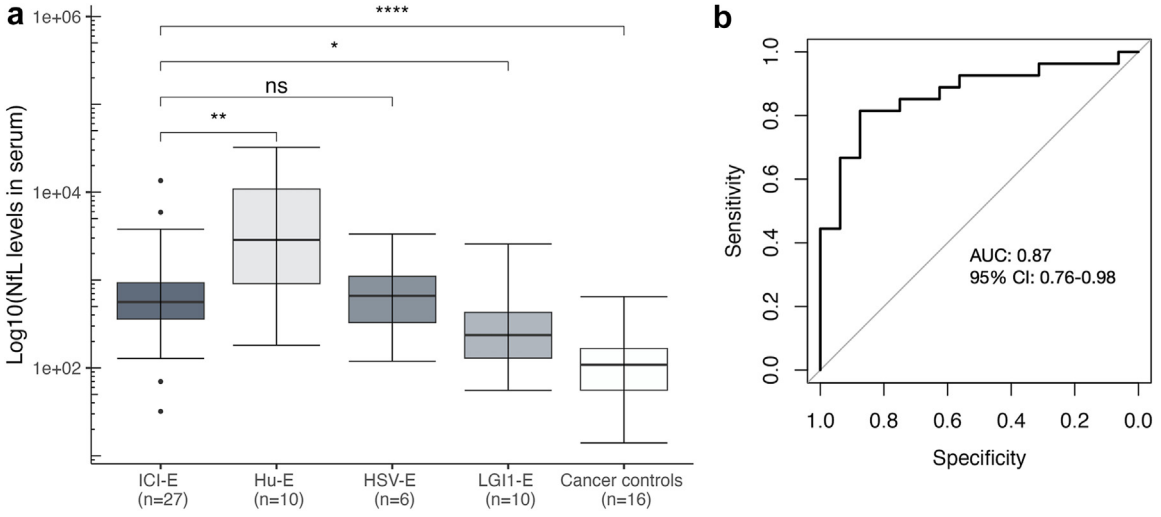

Fig. 4: **Light chain neurofilaments: diagnostic performance for definite immune-checkpoint inhibitor-encephalitis and comparison with Herpes Simplex Virus encephalitis, anti-LGI1 encephalitis, anti-Hu encephalitis, and cancer-matched controls.** Comparison of serum NfL in patients with definite ICI-encephalitis, Herpes Simplex Virus encephalitis, anti-LGI1 encephalitis, anti-Hu encephalitis and cancer-matched controls (**a**); ****p ≤ 0.0001; *p ≤ 0.05; ns, p > 0.05. Receiver operating characteristic (ROC) curves of serum NfL levels to discriminate patients with definite ICI encephalitis from cancer-matched controls (**b**). Abbreviations: AUC, area under the ROC curve; CI, 95% confidence interval; HSV-E, Herpes Simplex Virus encephalitis; Hu-E, anti-Hu encephalitis; ICI-E, ICI-encephalitis; LGI1-E, anti-LGI1 encephalitis.

0.77, 95% CI [0.51; 1.00]; Fig. 5a–b). Of note, serum S100B levels (which is a biomarker of both melanoma and brain damage) discriminated definite ICI-encephalitis from cancer-matched controls only after exclusion of the 3 patients with melanoma (median 84 pg/mL, range 3–386 *versus* 38 pg/mL, range 4–145, respectively, p = 0.022; optimal cut-off >75.7 pg/mL, 95% CI [42.63; 95.73], sensitivity 65%, specificity 87%, AUC 0.723, 95% CI [0.554; 0.892]; Supplementary Fig. S5); additionally, serum S100B levels in the patients with definite ICI-encephalitis were not significantly higher than in any of the disease-control cohorts (Supplementary Fig. S6). Serum GFAP levels did not discriminate any of the groups (Supplementary Fig. S6), and the serum and CSF levels of S100B, NfL, and GFAP were not significantly different between patients with

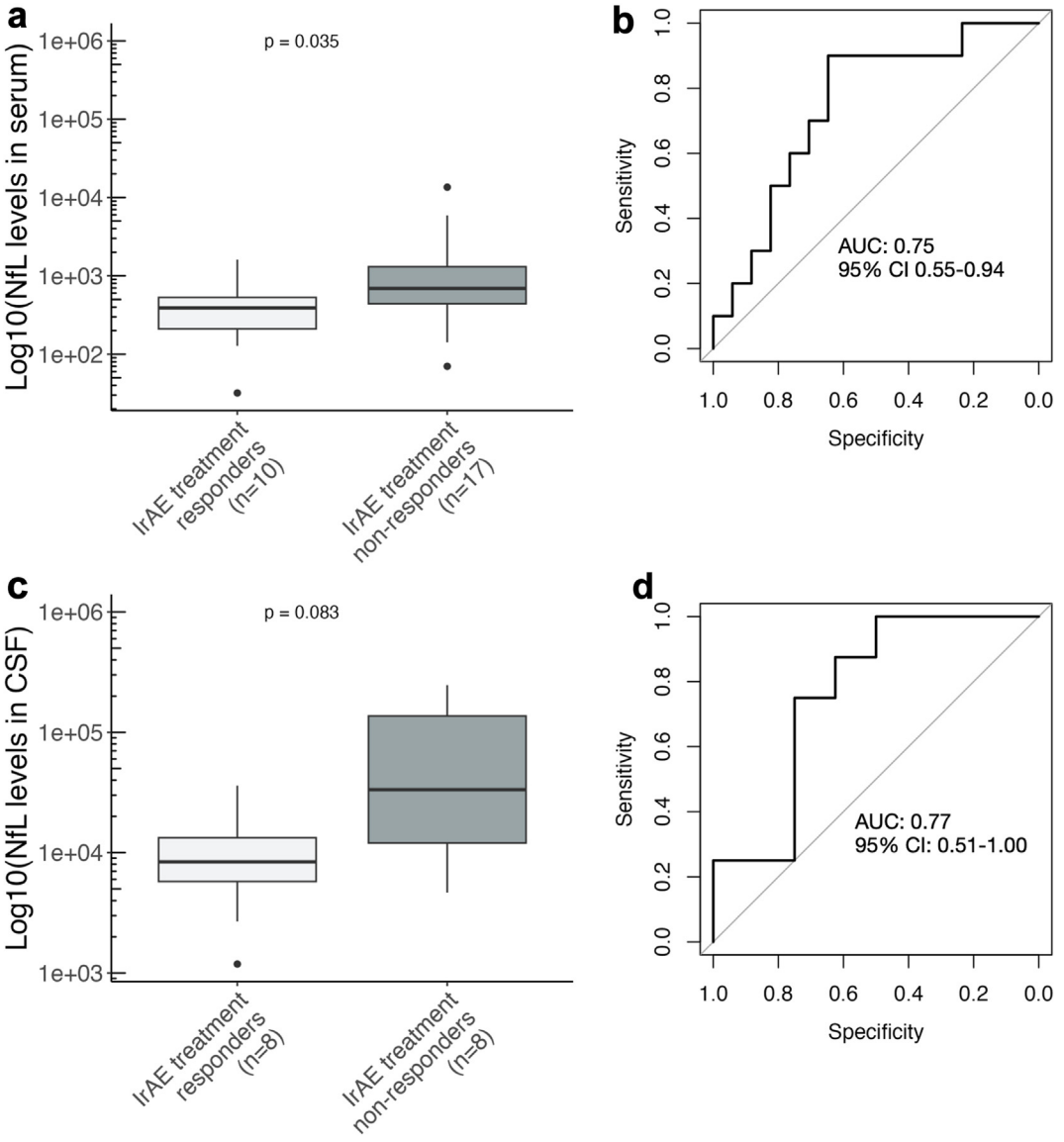

Fig. 5: **Light chain neurofilaments: comparison between irAE treatment responders and non-responders.** Comparison of NfL serum levels between irAE treatment responders and non-responders (**a**), and receiver operating characteristic (ROC) curves of serum NfL levels to discriminate irAE treatment responders and non-responders (**b**); irAE treatment responders (n = 10) had lower serum NfL levels (median 392.5 pg/mL, range 32–1606) compared to non-responders (n = 17, median 689 pg/mL, range 70–13522, p = 0.035). Comparison of NfL CSF levels between irAE treatment responders and non-responders (**c**), and receiver operating characteristic (ROC) curves of CSF NfL levels to discriminate treatment responders and non-responders (**d**); NfL levels were lower in the CSF of irAE treatment responders (n = 8, median 8400 pg/mL, range 1186–359,556) compared with non-responders (n = 8, median 34,200 pg/mL, range 4669–241,762), but this difference was not significant (p = 0.083). Abbreviations: AUC, area under the ROC curve; CI, 95% confidence interval; irAEs, immune-related adverse events.

focal encephalitis and those with meningoencephalitis (Supplementary Fig. S7).

Discussion

Early recognition of ICI-encephalitis is essential to promptly initiate treatments. In this large retrospective cohort, we found that all patients developed clinically recognisable encephalitic syndromes, including focal syndromes such as limbic encephalitis, RPCA, brainstem encephalitis, or a combination of them, usually few months after the first ICI infusion. The remaining cases, labelled meningoencephalitis herein and elsewhere,[11,13] mostly consisted in new-onset alteration of the mental status with seizures, aphasia, movement disorders, and/or decreased level of consciousness. The brain MRI and CSF analysis provided important diagnostic clues: for instance, the brain MRI in patients with ICI-meningoencephalitis was mostly unremarkable or showed meningeal enhancement, whereas about half of the patients with focal ICI-encephalitis had mesial temporal lobe hyperintensity reminiscent of paraneoplastic limbic encephalitis (e.g., associated with Hu antibodies) or autoimmune encephalitis (e.g., associated with anti-LGI1 antibodies).[18] These radiological patterns are clearly distinct from those observed in HSV-encephalitis, an important differential diagnosis in the post-ICI context.[17] Additionally, although the CSF cell count was elevated in all patients with ICI-meningoencephalitis at levels similar to HSV encephalitis, the patients with focal ICI-encephalitis less frequently had elevated CSF cell counts, and at lower levels than patients with HSV encephalitis. Altogether the results indicate that informed physicians could identify most cases of ICI-encephalitis soon after disease onset. Nevertheless, in more than one-third of the patients herein, ICI were not immediately withdrawn, suggesting delays in the clinical suspicion of ICI-encephalitis. As the present study, and others,[34] found that serum NfL levels are increased in most definite ICI-encephalitis patients (to levels comparable with HSV encephalitis), NfL measurement could represent a first step for quickly identifying patients in whom secondary procedures (brain MRI, lumbar puncture) should be prioritized. Moreover, the present results suggest that NfL could also help predicting irAE treatment response, and may be useful to identify patients who may require immunosuppressants in addition to steroids. Future studies are needed to verify these hypotheses.

Alongside NfL, the present study confirms the diagnostic importance of neural antibodies, especially PNS antibodies in focal ICI-encephalitis and anti-GFAP antibodies in meningoencephalitis. There are, however, shortcomings regarding neural antibody testing since more than half of patients with ICI-meningoencephalitis did not have well-characterised neural antibodies, the specificity of PNS antibodies is unknown in this context (they can be detected in neurologically asymptomatic cancer patients),[35] and there is a long delay (often several weeks) to obtain the results.[21] Nevertheless, we found that PNS antibodies were independently associated with a lack of response to irAE treatment, indicating they may be used not only for diagnosis, but also for the determination of prognosis, in conjunction with NfL levels. It is noteworthy that PNS antibodies were found almost only in patients with focal encephalitis syndromes (i.e., limbic or brainstem encephalitis and RPCA), which were clinically undiscernible from spontaneous PNS and likewise associated with neuroendocrine cancers,[35–37] in contrast with patients with meningoencephalitis, who mostly had non-neuroendocrine cancers and were negative for PNS antibodies. Accordingly, the focal encephalitis syndromes had highly unfavourable outcomes, mirroring spontaneous PNS, while ICI-meningoencephalitis patients responded well to steroids and other immunosuppressants, similarly to neuromuscular and non-neurological ICI toxicities.[3] Altogether, these observations support the hypothesis of shared pathogenic mechanisms between antibody-positive focal encephalitic syndromes and spontaneous PNS, where cancer-antigen driven autoimmune responses lead to the generation of autoreactive cytotoxic T cells and the irreversible destruction of neuronal populations.[36,38–40] Conversely, meningoencephalitis without PNS antibodies may be associated with less extensive involvement of CD8+ T cells and neuronal loss, in line with the higher chances of clinical reversibility we observed in these patients.[11]

Regardless of these pathophysiological considerations, the present results underscore that early immunosuppression is likely needed in patients with focal encephalitis, PNS antibodies, and/or high NfL levels. However, they also suggest that immunosuppressants commonly used in autoimmune encephalitis (i.e., rituximab, cyclophosphamide, and tocilizumab) are probably inadequate in most of these patients. Therefore, future research should focus on the immunological mechanisms of ICI-encephalitis, particularly the focal syndromes, in order to identify novel therapeutic targets (which may be also relevant in spontaneous PNS).[36] Additionally, only 3 of the patients included herein were rechallenged with ICI, and while none of them had a neurological relapse, the question of the safety of ICI rechallenge after resolution of the neurotoxicity remains an unsolved issue.[15,16,36]

Of note, we identified a subset of patients clinically similar to either meningoencephalitis or RPCA, yet without increased CSF cellularity, brain MRI abnormalities, or well-characterised antibodies, which is in line with other reports.[10] Some of these patients had CSF findings suggestive of inflammation (e.g., mild elevation of neopterin or IL-6 levels and/or uncharacterised neural antibodies) and/or improved with treatment, indicating a possible immune-related

mechanism. Although it is still unclear whether these patients do correspond to immune-related ICI neurotoxicities (they notably had lower serum NfL levels than the definite cases), in the absence of alternative diagnoses such as opportunistic infections, metabolic disorders, or meningeal carcinomatosis, the results suggest that these patients should be managed as patients with definite ICI-encephalitis. Further multicentre studies enrolling a larger number of patients are needed to assess the outcomes and prognostic factors, as well as the diagnostic utility of biomarkers of brain injury in patients with possible ICI-encephalitis.

The present study has limitations. First, our position as a national reference centre for PNS may cause a referral bias towards paraneoplastic-like syndromes, positive antibody results, and/or severe presentations. Notwithstanding, the under-representation of certain tumours, such as melanoma, is more likely to result from their preferential association with peripheral neurological toxicities of ICI, as shown elsewhere.[5,9,41] Second, the retrospective design of the study prevented the uniform assessment of response to individual irAEs treatments, which will need prospective studies, and the present analyses of irAE treatment response and mortality need to be interpreted with caution considering the relatively small number of included patients. Finally, the lack of validated diagnostic criteria for ICI-encephalitis required us to apply rigorous selection criteria for the inclusion of patients, which, while possibly limiting the generalizability of the present findings, has been essential to reduce the likelihood of misdiagnosis. The choice of including a possible ICI-encephalitis group was made to reduce as much as possible the loss of information. Importantly, future diagnostic guidelines for ICI-encephalitis will need to consider not only the clinical presentations reported herein, but also the possibility of rarer, not yet reported clinical syndromes, and to take into account both the possible and definite cases of ICI-encephalitis.

In conclusion, ICI-encephalitis corresponds to a set of clinically-recognisable syndromes, and the analysis of serum NfL likely facilitates diagnosis and prognostication. Focal encephalitis and PNS-related antibodies are associated with less response to irAE treatment, indicating a higher risk of long-term disability and mortality, and suggesting that the current treatment strategies are inadequate for these patients. Research is needed on the underlying immunological mechanisms in order to foster therapeutic innovation.

Contributors

AFa and BJ conceived and designed the study. AFa did the literature search, collected, analysed, and interpreted the data, drafted, revised, and approved the final Article. MVG participated in the data collection, analysis, and interpretation, critically reviewed, and approved the final Article. AFo, IQ, ALP, and VD had a major role in the brain injury biomarkers analysis and interpretation, critically reviewed, and approved the final Article. NT had a major role in the statistical analysis, critically reviewed, and approved the final Article. All other authors helped analyse and interpret the data, critically reviewed, and approved the final Article. AFa and BJ have accessed and verified the data of all patients. All other authors had access to the data if they wished. AFa and BJ were responsible for the decision to submit the manuscript. The corresponding author had full access to all the data and final responsibility for the decision to submit the Article for publication.

Data sharing statement

Data reported in this study are available within the article and/or the Supplementary material. More information is available from the corresponding author on reasonable request from any qualified investigator.

Declaration of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanepe.2024.101011.

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