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RESEARCH ARTICLE

Low prevalence of neural autoantibodies in perioperative cerebrospinal fluid samples of epilepsy surgery patients: A multicenter prospective study

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Abstract

Objective: Refractory epilepsy may have an underlying autoimmune etiology. Our aim was to assess the prevalence of neural autoantibodies in a multicenter national prospective cohort of patients with drug-resistant epilepsy undergoing epilepsy surgery utilizing comprehensive clinical, serologic, and histopathological analyses.

Methods: We prospectively recruited patients undergoing epilepsy surgery for refractory focal epilepsy not caused by a brain tumor from epilepsy surgery centers in the Czech Republic. Perioperatively, we collected cerebrospinal fluid (CSF) and/or serum samples and performed comprehensive commercial and in-house assays for neural autoantibodies. Clinical data were obtained from the

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patients' medical records, and histopathological analysis of resected brain tissue was performed.

Results: Seventy-six patients were included, mostly magnetic resonance imaging (MRI)-lesional cases (74%). Mean time from diagnosis to surgery was 21 ± 13 years. Only one patient (1.3%) had antibodies in the CSF and serum (antibodies against glutamic acid decarboxylase 65) in relevant titers; histology revealed focal cortical dysplasia (FCD) III (FCD associated with hippocampal sclerosis [HS]). Five patients' samples displayed CSF-restricted oligoclonal bands (OCBs; 6.6%): three cases with FCD (one with FCD II and two with FCD I), one with HS, and one with negative histology. Importantly, eight patients (one of them with CSF-restricted OCBs) had findings on antibody testing in individual serum and/or CSF tests that could not be confirmed by complementary tests and were thus classified as nonspecific, yet could have been considered specific without confirmatory testing. Of these, two had FCD, two gliosis, and four HS. No inflammatory changes or lymphocyte cuffing was observed histopathologically in any of the 76 patients.

Significance: Neural autoantibodies are a rare finding in perioperatively collected serum and CSF of our cohort of mostly MRI-lesional epilepsy surgery patients. Confirmatory testing is essential to avoid overinterpretation of autoantibody-positive findings.

KEYWORDS

CSF, epilepsy, epilepsy surgery, GAD65, neural antibodies

1 | INTRODUCTION

Innate and adaptive immunity has long been hypothesized to be involved in the pathogenesis of seizures and epilepsy.[1,2] In its classification of epilepsies, 2017 revision, the International League against Epilepsy (ILAE) included immune etiology among possible causes of epilepsy,[3] and N-methyl-D-aspartate receptor (NMDAR) and anti-leucine-rich glioma inactivated protein 1 (LGI1) encephalitis were mentioned as examples. It was later recognized that seizures occurring in autoimmune encephalitis (AE)—up to 80% of patients—should be regarded as acute symptomatic seizures secondary to the encephalitis.[4]

Independent of these symptomatic seizures, the term *autoimmune-associated epilepsy* (AEp)[4] was suggested for a considerable subgroup of patients with epilepsy of undetermined origin (not fulfilling criteria of AE) with detectable antibodies directed against neural tissue (NAbs).[5] Prevalence of NAbs in epilepsy patients was reported to be between 3.4% and 35%.[6,7] High prevalences reported in some studies could be explained partly by the inclusion of acute symptomatic seizures secondary to AE and also by the inclusion of nonspecific antibodies (e.g., anti-thyroid peroxidase antibodies).[4,8] For the diagnosis of AEp, the patient should have unprovoked seizures requiring the continuation of antiseizure medication. Currently, it is recommended to follow up a patient for at least 1 year before the diagnosis of AEp is assigned.[9] The probability of developing "true" epilepsy (in contrast to symptomatic seizures) after AE also depends on the type of AE; the more common AE associated with NAbs directed against surface antigens (e.g., NMDAR AE) rarely leads to epilepsy (<5%),[9] whereas the risk is considerably higher in paraneoplastic AE with NAbs directed against intracellular antigens.[10]

Key Points

- Neural autoantibodies in relevant titers were rare in a cohort of mostly MRI-lesional epilepsy surgery patients.
- Glutamic acid decarboxylase (GAD) antibodies in serum and CSF and CSF-restricted oligoclonal bands were observed in a few cases; the significance of these findings is unknown.
- Without confirmatory testing, serum and/or CSF positivity can be a nonspecific finding that should not be overinterpreted.

Other factors associated with AEp or acute symptomatic seizures secondary to AE include, among others, subacute onset, unusually high seizure frequency, intraindividual variability of seizure semiology or multifocality, history of a neoplasia, personal or family history of autoimmunity, abnormal cerebrospinal fluid (CSF), delayed initiation of immunotherapy, and history of status epilepticus (SE).[11,12] Scoring systems have been developed for the selection of patients with epilepsy for NAbs testing. Antibody prevalence in epilepsy before surgery (APES) was specifically designed for patients with drug-resistant epilepsy (DRE).[13] The most common structural lesions in DRE patients undergoing epilepsy surgery are hippocampal sclerosis (HS)[14] and focal cortical dysplasia (FCD).[15] In both HS and FCD, histopathological studies suggested involvement of innate immunity.[16,17] Furthermore, HS may occur as result of previous AE (up to 64% anti-LGI1, 20% anti-contactin associated protein 2 [CASPR2] encephalitis patients). In glutamic acid decarboxylase 65 (GAD65) autoimmunity, HS occurs in up to 62% of patients.[18,19] Patients with temporal lobe epilepsy (TLE) undergoing epilepsy surgery were found to have a worse outcome if NAbs were present.[20,21] In summary, the true prevalence of underlying autoimmune epilepsy in selected cohorts of patients with different epilepsy syndromes remains unknown.

The aim of our study was therefore to evaluate the prevalence of NAbs in our prospective, selected cohort of DRE patients undergoing epilepsy surgery and compare it to the reported frequency of at least 3.4%.[22] In contrast to previous studies of DRE,[20,21] we chose to include only patients with CSF available for testing, performed a very comprehensive autoantibody screening panel, and thoroughly confirmed imaging findings with histology. To overcome the obstacle of performing lumbar puncture exclusively for research purposes, we decided to evaluate the CSF taken during epilepsy surgery.

2 | MATERIALS AND METHODS

2.1 | Patients, design, and sample acquisition

The design of our study was multicenter and prospective. All patients undergoing resective epilepsy surgery were included in the study providing (1) they consented to participate and (2) their epilepsy was not caused by a brain tumor. None of the patients had a history or clinical diagnosis of limbic encephalitis. We included adult patients from three epilepsy surgery centers in the Czech Republic: Epilepsy Center of Motol University Hospital, Prague (MC) from February 2019 until September of 2022, Epilepsy Center of Na Homolce Hospital, Prague (HC) from November 2019 until September of 2022, and Epilepsy Center of St. Anne's University Hospital, Brno (BC) from December 2020 until September of 2022. We analyzed medical history, electroencephalography (EEG), video-EEG, neuropsychology, brain magnetic resonance imaging (MRI), and a predefined set of items: age at disease onset, age at surgery, family history of epilepsy, initial precipitating injuries (perinatal hypoxia, febrile seizures, history of head trauma or central nervous system [CNS] infection), developmental milestones, type(s) and frequency of all seizures, history of tonic–clonic seizures and SE, occurrence and type of auras, and occurrence of multimodal auras (i.e., at least two types of auras, in same seizure or as different seizure types).[23] Furthermore, detailed treatment history and neuropsychological evaluation were available.

For the purposes of this study, interictal EEG was evaluated only regarding interictal epileptiform activity and classified as follows: (1) unilateral, that is, strictly unilateral spikes/sharp-waves or clearly (>90%) predominating on one side; (2) bilateral; and (3) multifocal pathology, that is, bilateral with three or more independent sources.

Brain MRI was classified as follows: (1) nonlesional (including nonspecific gliosis) and (2) lesional, such as HS or FCD types II and III, vascular malformation, or gliosis (including subtle findings, i.e., slightly enlarged amygdala, atrophy of hippocampus without signal change).[24]

The resected tissue was processed and reviewed by an experienced pathologist according to current recommendations for a comprehensive neuropathologic workup.[25]

Patients with a tumor not identified by imaging but later confirmed by histopathology were excluded from the final analysis cohort.

Final outcome was assessed at 2 years from surgery, using ILAE classification. In patients with shorter follow-up, data were recorded as well. ILAE 1 and 2 outcomes were rated as favorable, ≥3 as unfavorable outcome.[26]

CSF and serum were acquired during surgery. CSF was sampled from subdural space prior to brain tissue resection. This had no impact on the procedure itself. Cytology analysis (leukocyte and erythrocyte count) and biochemistry (albumin, immunoglobulins, IgG index, oligoclonal bands [OCBs]) were performed in patients from MC and HC. Due to transportation delay, only the biochemical analysis of centrifuged CSF was performed on samples from BC. All samples were then kept in aliquots frozen at −80°C until further analysis.

2.2 | Antibody testing

Antibodies were tested with four methods as recommended earlier[27,28]: (1) line blot (PNS 11 Line assay, Ravo Diagnostika), which detects anti-Hu, anti-Yo, anti-Ri, anti-CV2/Collapsin Response Mediator Protein 5, anti-amphiphysin, anti-Ma1, anti-Ma2, anti-SOX1, anti-GAD65, anti-Tr, and anti-Zic4 antibodies; (2) cell-based assay (CBA; Autoimmune Encephalitis Mosaic 1, EUROIMMUN), which detects anti-NMDAR, anti-CASPR2, anti-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) 1/2, anti-LGI1, and anti-γ-aminobutyric acid (GABA) B receptor antibodies; (3) fixed tissue-based assay (TBA) to detect of NAbs against intracellular antigens (Neurology Mosaic 8 IIFT, EUROIMMUN); samples with positive or borderline results were sent to a second laboratory (Labor Stöcker, Lübeck, Germany) for confirmation; and (4) tissue-based indirect immunohistochemistry on rat brain slices suited for detection of neural surface antibodies as previously described by Dalmau and colleagues, performed at a reference laboratory.[29] Positivity was always rated by two examiners (H.M., F.L., or J.D., Institute for Clinical Chemistry, Kiel, Universitätsklinikum Schleswig-Holstein, Germany). All samples testing positive on rat brain immunohistochemistry underwent further testing using (1) human embryonic kidney 293T cells transfected with plasmid coding for GABA A receptor, glycine receptor, dopamine receptor 2, metabotropic glutamate receptor 5, neurexin3α, and IgLON5 on fixed assays, and AMPA receptor on a live assay to search for characterized rare autoantibodies; and (2) indirect immunofluorescence assays using live, nonpermeabilized, rat hippocampal neuronal cultures as described previously to identify putative neuronal cell surface staining.[30,31] Additionally, samples positive for anti-GAD65 had titers determined by enzyme-linked immunosorbent assay (ELISA; Medizym antiGAD M, Medipan).

Cases with (1) findings in serum CBAs without confirmation in CSF and/or TBAs, (2) findings in CSF TBAs without confirmation in CBAs or live hippocampal neuron indirect immunofluorescence assay, or (3) borderline or low line-blot positivity in serum without tissue-based confirmation and considered unrelated to the clinical picture (e.g., borderline positivity of anti-Yo only on line blot) were labeled antibody-negative.[32]

2.3 | Statistics

Descriptive statistical analysis was performed using Minitab software (version 21.2, 2022). Data were expressed as mean (SD) when in normal distribution or as median (interquartile range) when the distribution was nonnormal for continuous variables, or as percentages or range for categorical variables. Comparison of two groups of epilepsy patients was done using Fisher exact test for categorical variables and Mann–Whitney test for continuous nonparametric variables where appropriate. Significance level was set to $p = .05$, with Bonferroni correction for multiple testing.

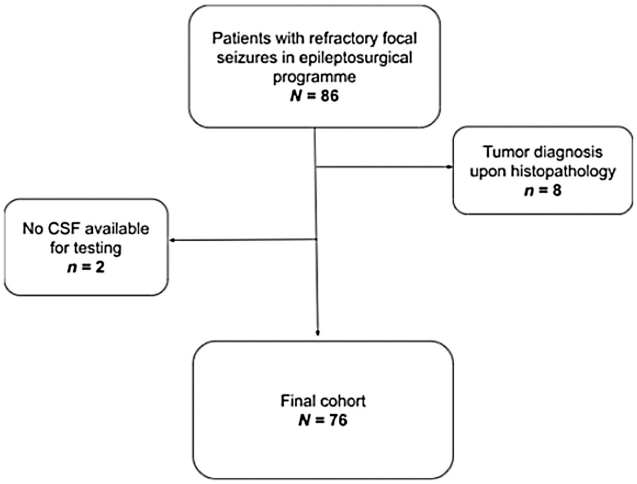

FIGURE 1 Patient selection flowchart. CSF, cerebrospinal fluid.

3 | RESULTS

3.1 | Patient cohort

We recruited 86 patients. Ten patients were excluded, based on (1) lack of CSF samples ($n = 2$) or (2) histopathological diagnosis of a tumor ($n = 8$; see Figure 1). This resulted in a total of 76 patients included in the final cohort. Of note, in two of these patients, the CSF was acquired prior to surgical resection by a standard lumbar puncture; both were antibody negative, and CSF was thus not sampled again perioperatively. Patient age at seizure onset was 14 ± 11 years, and average time from diagnosis to epilepsy surgery was 21 ± 13 years. Sixty-nine percent of patients had TLE, 61% had a history of tonic–clonic seizures, and 11% had a history of SE during the course of their disease. Almost half of the patients had prior intracranial procedures (intracranial EEG exploration, thermocoagulation, previous resective surgery) performed before the surgery.

Demographics, epilepsy characteristics, and CSF findings are indicated in Tables 1–3. Seventy-four percent of patients had lesional MRI. Inflammatory CSF changes (e.g., pleocytosis) were difficult to evaluate due to blood contamination, but presence of CSF-restricted OCBs was found in 6.6.%.

Upon histology, an unequivocal underlying structural etiology of refractory epilepsy was observed in 69 (90.8%) cases. Of these, 53% had FCD and 36% had HS. Gliosis was

TABLE 1 Clinical characteristics, MRI, electroencephalography, and histopathology.

Variable	Value
Sex, M:F, *n*	34:42 (45% female)[a]
Age at diagnosis, years, average ± 95% CI	14 ± 2
Age at surgery, years, average ± 95% CI	35 ± 2
MRI, *n* (%)	
Nonlesional	19/74 (26%)
Lesional	55/74 (74%)
Histopathology	
Malformations of cortical development, *n* (%)	34 (45%)
FCD, *n* (%)	31/34 (91%)
Type I	13/31 (42%)
Type II	13/31 (42%)
Type III	5/31 (16%)
Hippocampal sclerosis, *n* (%)	25 (33%)[b]
Type[40]	Type I, 73%; II, 23%; III, 4% (available in 22)
Dual pathology, *n* (%)[c,d]	2 (3%)
Glial scar, *n* (%)	7 (9%)
Negative histology, *n* (%)	7 (9%)
Vascular malformation, *n* (%)	1 (1%)
Concomitant autoimmune disease, *n* (%)	6 (8%)
Immunotherapy, *n* (%)	2/75 (3%)
Extra-CNS tumor, *n* (%, type)	1/74 (1%, carcinoid of appendix)
Other symptoms: behavior changes; dysautonomia; language problems; cognitive deficit, *n* (%)	36 (47%); 1/75 (1%); 7/75 (9%); 11/75 (15%)

Abbreviations: CI, confidence interval; CNS, central nervous system; F, female; FCD, focal cortical dysplasia; HS, hippocampal sclerosis; M, male; MRI, magnetic resonance imaging.

[a]One female patient underwent a sex conversion from male.

[b]Initial precipitating injury was present in 14 of 25 patients.

[c]One patient had HS and focal gliosis of temporal pole, and one patient had HS and mild malformation of cortical development with oligodendroglial hyperplasia.

[d]In the temporal lobe, the presence of hippocampal sclerosis and associated FCD I was not regarded as dual pathology and was classified as FCD IIIa.[24]

found in 10% of patients. None of the patients had prominent leukocytic infiltrates in brain parenchyma, perivascular cuffing of lymphocytes, or granulomatous or infiltrating vessel destruction. Eight percent of patients had a comorbid autoimmune disease, and one had an extra-CNS tumor.

Overall, outcome at 2 years after surgery classified by ILAE was favorable in 24 of 35 (69%) assessable patients.

3.2 | Clinical characteristics and findings of investigations

Clinical characteristics, MRI, EEG, and CSF analysis results are presented in Tables 1–3 and Figure 2.

3.3 | Antibodies and CSF-restricted OCBs

Next, we analyzed the subgroup of patients who had either detectable neural autoantibodies or inflammatory findings in CSF. We only considered presence of CSF-restricted OCBs and not pleocytosis as indicative of underlying inflammation, because perioperative elevated leukocyte count was observed in most samples due to high blood contamination. We only considered results of autoantibody testing as specific if confirmed by testing (TBAs when positive on CBAs and vice versa); for example, findings on CASPR2 CBAs would only be considered specific if confirmed on TBAs. Among all investigated patients, one (1.3%) had anti-GAD65 antibodies in the CSF

TABLE 2 Epilepsy characteristics.

Variable	Value
Temporal lobe epilepsy, *n* (%)	53/75 (67%)
Frontal lobe epilepsy, *n* (%)	15/75 (20%)
Posterior cortex epilepsy, *n* (%)	8/75 (11%)
Previous intracranial procedure, *n* (%)[a]	36 (47%)
ASMs at surgery, median (range; IQR)	2 (1–5; 1) [evaluated in 75]
ASM tested in total, median (range; IQR)	5 (1–14; 5) [evaluated in 69]
History of tonic–clonic seizures, *n* (%)	46/75 (61%)
History of status epilepticus, *n* (%)	8 (11%)
Seizure days per month, median (range; IQR)	6 (1–30; 22) [evaluated in 74]
IPI or positive family history, *n* (%)	38/75 (51%)

Abbreviations: ASM, antiseizure medication; IPI, initial precipitating injury (febrile seizures, perinatal injury, meningitis/encephalitis, traumatic brain injury); IQR, interquartile range.

[a]Previous invasive procedure: intracranial electroencephalographic exploration, thermocoagulation, previous resective surgery.

TABLE 3 CSF analysis results.

Variable[a]	Result [number of samples available]
Leukocyte count, per 3 mm^3, median (range; IQR)	14 (0–960; 52) [35]
Erythrocyte count, per 3 mm^3, median (range; IQR)	8960 (2–124 000; 19 840) [29]
Albumin, mg/L, median (range; IQR)	587 (83–4240; 682) [71]
Oligoclonal bands, pattern II, *n* (%)	5/71 (7%)
Oligoclonal bands, pattern IV, *n* (%)	3/71 (4%)

Note: Pattern II: two or more oligoclonal bands in CSF that are absent in serum. Pattern IV: two or more identical oligoclonal bands in CSF and serum.Abbreviations: CSF, cerebrospinal fluid; IQR, interquartile range.

[a]CSF cytology and biochemistry results were frequently contaminated by blood (sampling during surgery) and precluded us from analysis of the presence of pleocytosis or elevated protein.

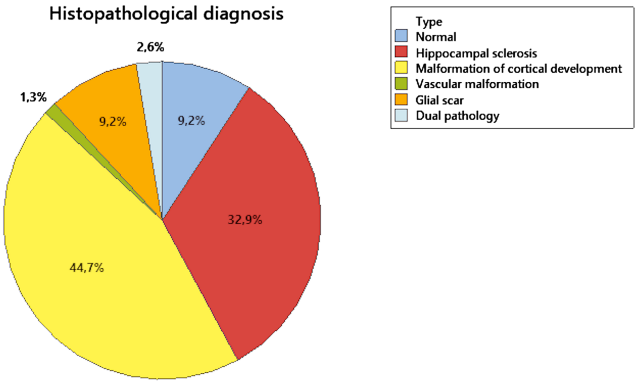

FIGURE 2 Histopathology results.

and serum detected by both commercial line-blot assay and TBA; the titer was determined by ELISA 7166 IU/mL in CSF and 439 140 IU/mL in serum. These two samples were obtained (due to technical reasons) several months apart so antibody index (AI) was not counted (see Table 4). This patient was 26 years old at seizure onset and had a 13-year history of the disease. MRI was described as nonlesional, but FCD IIIa (FCD I with HS) was found upon histopathology. Final outcome was favorable in this patient (ILAE outcome scale score of 1).

Of note, eight patients had positive CBAs or TBAs not confirmed by other technique (three CASPR2, one NMDAR, four unknown tissue staining with detection of primary neuron cultures); as mentioned before, these were excluded, besides the one that was also part of the CSF-restricted OCB group.

Five patients had presence of CSF-restricted OCBs in their CSF samples. Patients were aged 2, 12, 15, 15, and 25 years at diagnosis and had 27-, 7-, 19-, 19-, and 9-year intervals between diagnosis and epilepsy surgery. Three had underlying FCDs suspected upon MRI and confirmed in histopathology; all had unfavorable outcomes. One patient had HS, and one patient had negative histopathologic findings; outcome in both was favorable.

We compared clinical data of patients with CSF-restricted OCBs and patients with unconfirmed antibodies

TABLE 4 Patients with neural autoantibodies or CSF-restricted oligoclonal bands.

Pt/sex	Age, years, at dg; at surg	Findings (CSF, serum)	MRI finding	Surgery localization	PIP	Histology	AI (IT); tumor	Seizure type	Risk factor	ASMs at surg (total)	Outcome (ILAE)[a]
1/M	26; 39	GAD65 (CSF 7166 IU/mL, serum 439 140 IU/mL)[b]	Nonlesional	T left	Yes	FCD IIIa	0 (0); 0	FIAS, FAS	0	2 (4)	Favorable (1)[c]
2/M	15; 34	OCB (2, 0)	FCD	T left	Yes	Negative; previous surg: FCD Ib	0 (0); 0	FIAS	0	4 (8)	Unfavorable (4)
3/F	2; 29	OCB (1, 4)	FCD	Fr right	No	FCD IIb	0 (0); 0	FAS, FIAS	FS	3 (10)	Unfavorable (5)
4/M	25; 34	OCB (3, 0)	FCD	T left	No	FCD Ia	Skin mastocytosis (0); 0	FAS, FIAS	0	2 (6)	Unfavorable (4)
5/F	15; 34	OCB (2, 0)	Nonlesional	T right	Yes	Negative	0 (0); 0	FAS, FBTCS	0	3 (7)	Favorable (1)
6/F[d]	12; 19	OCB (6, 0)	Enlarged amygdala	T right	Yes	HS	0 (0); 0	FIAS, FBTCS (12)	0	2 (2)	Favorable (1)

Abbreviations: AI, autoimmunity; ASM, antiseizure medication; CSF, cerebrospinal fluid; dg, diagnosis; F, female; FAS, focal aware seizures; FBTCS, focal to bilateral tonic–clonic seizures; FCD, focal cortical dysplasia; FIAS, focal impaired awareness seizures; Fr, frontal; FS, febrile seizures; GAD65, glutamic acid decarboxylase 65; HS, hippocampal sclerosis; ILAE, International League against Epilepsy; IT, immunotherapy; M, male; MRI, magnetic resonance imaging; OCB, oligoclonal bands; PIP, previous invasive procedure (resective surgery, stereotactic EEG monitoring, thermolesion); pt, patient; surg, surgery; T, temporal.

[a]Favorable outcome was defined as ILAE outcome scale of 1–2 points, unfavorable when the score was 3–6 points.

[b]Measured by enzyme-linked immunosorbent assay.

[c]Scored after <2 years.

[d]Also had contactin associated protein 2 antibodies in titer 1:10 in serum.

to the rest of the group (unequivocally nonautoimmune group); they differed in none of these parameters significantly (see Tables S1 and S2).

4 | DISCUSSION

Our main findings are as follows. (1) In patients with long-standing refractory focal epilepsy, 90.8% had structural lesions related to the epileptic syndrome identified on histopathology. More than 50% of these had FCD, and approximately one third had HS. We did not observe inflammatory changes suggestive of active inflammation in any of these patients. (2) The overall prevalence of underlying specific neural autoantibodies indicative of autoimmune epilepsy syndromes was rare (one patient, 1.3%). This patient had CSF and serum glutamic acid decarboxylase (GAD) antibodies with titers specific for anti-GAD syndrome, with GAD antibody-associated TLE being a well-accepted autoimmune epilepsy syndrome.[33] However, we cannot exclude contamination of CSF with serum antibodies due to blood contamination, because the AI was not available (serum and CSF were taken months apart due to technical issues). Interestingly, this patient had FCD and HS (FCD IIIa) on histopathology, which was not identified by MRI. His final outcome was favorable, which would be more in line with the FCD being the "true" etiology of epilepsy and the GAD antibodies the "epiphenomenon." (3) A further five patients (6.6%) had CSF-restricted OCBs, a finding that might indicate a chronic inflammatory milieu in the CSF. Of note, three of these had an underlying FCD and all had an unfavorable outcome. It is tempting to speculate that ongoing chronic inflammation in the CSF might be a negative predictor of outcome following epilepsy surgery, yet further work is needed to confirm this tentative observation. (4) We found eight patients (10.5%) who had positive findings in some antibody tests that, using rigorous testing strategies, could not be unequivocally reproduced in confirmatory tests. Although it is possible that our strict criteria have led to misclassifying some findings as false negatives, we chose to err on the side of sensitivity to get a conservative estimation of prevalence. Moreover, they did not significantly differ in any of the clinical data from the unequivocally nonautoimmune group. Without confirmatory testing and histology, our estimate would have been 17.1% underlying autoimmune epilepsy, a figure reminiscent of prior observations in other epilepsy cohorts using less stringent criteria.

In general, two alternative (yet not mutually exclusive) hypotheses could address the role of inflammation in epilepsy and specifically in DRE. First, underlying AE might have led to secondary changes in various brain structures underlying increased excitability and epileptogenesis. Second, repeated seizure activity might itself induce—primarily innate and possibly secondary adaptive—inflammation/immunity in the CNS, which may in turn accelerate the epileptogenic mechanisms. Our observation of a patient with GAD antibodies in CSF and serum and FCD IIIa on histopathology would tentatively point more in the second direction. One could speculate that a primary epileptic syndrome caused by abnormal cortical development was causing long-standing refractory epilepsy and triggering secondary development of GAD-directed autoimmunity, which might again influence the course of the disease.

The prevalence of antibodies among patients with epilepsy was previously reported to be 3.4%–35%. Literature on the prevalence of NAbs in DRE is even more scarce. When applying strict criteria excluding older studies that included anti-LGI1/CASPR2 negative anti-VGKC, anti-TPO, and ANA as positive antibody findings, only two relevant studies were published focusing on this subgroup. One of these studies described a small cohort of eight cases; none had antibodies in serum or CSF.[34] The other included 27 patients with drug-resistant TLE of unknown etiology and reported 14 (51.9%) antibody-positive cases.[35] The percentage of antibody-positive patients with epilepsy including DRE may be exaggerated, due to inclusion of either irrelevant antibodies or patients with acute symptomatic seizures due to AE. In contrast, our cohort included all focal DRE patients considered for surgery, and patients were not selected regarding the type and etiology of epilepsy (apart from the exclusion of tumors). Patients with structural lesions such as malformation of cortical development were included as well, and this could have lowered the percentage of antibody-positive cases. However, many surgical cases of FCD are MRI-negative,[36] and thus it is probable that such patients were included in cohorts studying antibodies in epilepsy reported in the literature as having unknown etiology. In our cohort, 26% of patients were classified as nonlesional on MRI.

To identify antibody-positive patients among those with DRE who are candidates for epilepsy surgery, the APES score was designed. In our opinion, APES may be less useful in surgical patients with long-standing epilepsy, as it includes many components that are difficult to score with certainty in these patients (e.g., viral prodrome before the onset of epilepsy). In the original study that designed APES, the prevalence of antibody positivity among patients with DRE of unknown cause was 8%. For our cohort, this would mean that among 32 (45%) cases that fulfilled the strict criteria for DRE of unknown origin (i.e., no structural lesions other than HS), there should be two antibody-positive patients,

whereas we identified only one anti-GAD65-antibody positive case with unclear relation of anti-GAD antibodies to the course of epilepsy itself. Although this could be due to small numbers, it might also indicate overestimation of underlying autoimmunity using the APES score.

Due to increasing awareness of autoimmune-associated epilepsy, the evaluation of autoimmune etiology is increasingly performed during presurgical evaluation. In patients where such etiology is confirmed, the pursuit of surgery is often delayed or even stopped. This preselection could have contributed to the lack of antibody-positive patients in our cohort; however, in routine practice, we mostly tested neural autoantibodies in epilepsy patients only with commercially available methods that are known to have lower sensitivity.

In regard to CSF-restricted OCBs, earlier studies have confirmed higher prevalence of this finding in AEp in comparison with patients with other etiologies of epilepsy.[37] Of note, one patient with FCD type II had CSF-restricted OCB, and the outcome of the surgery was unfavorable (compared to 7/7 cases with FCD II without OCB with favorable outcome; data not shown). Multiple reasons, including incomplete resection, could have influenced these results, but it might be of value to explore OCBs in FCD II further. Investigation of CSF in epilepsy surgery candidates to check for signs of immunopathology (not only for presence of antibodies) could thus be encouraged. Importantly, almost half of the patients had an invasive procedure before the surgery, which could, in theory, have an effect on the presence of OCBs. Unfortunately, due to almost uniform high blood contamination, pleocytosis could not be evaluated in samples of our patients.

Regarding patients with HS (33% of all), we cannot rule out the possibility that some of these patients may have experienced AE in the past[38] with no residual antibodies present at the time of the sampling. On the other hand, our results suggest that antibodies confirmed by two techniques do not occur in the following months after a CNS invasive procedure (intracranial EEG exploration performed in many patients), implicating high specificity of antibodies. Also, if CSF immunopathology or antibody positivity would be the result of frequent seizures (as was suggested by Brenner et al.[39]), we would expect more antibody-positive cases in our group.

Our study has several limitations. One obvious limitation is the size of our cohort. The way the CSF and serum samples were collected has to be taken into account when interpreting the results. However, we believe that the method used for CSF sample collection did not influence the results regarding antibody positivity as well as robust findings such as presence of CSF-restricted OCBs. Special histopathological methods (specific immunostainings) outside of the standard protocol were not used. However, when designing the study, we expected, based on available data on epilepsy surgery in the Czech Republic, that our group would be too heterogeneous and thus too small to be able to address the differences in components of innate or adaptive immunity in different types of lesions.

In summary, no relevant antibody positivity was observed in this prospective cohort of surgically treated patients with DRE, besides one patient with anti-GAD65 positivity in both serum and CSF, with the presence of FCD in histopathology. We consider the finding to have an unclear relationship to DRE in this patient. Unlike many other studies, we did not include nonspecific antibodies like anti-TPO, nor those that were not confirmed by two methods.

Importantly, the low prevalence of antibody positivity in our cohort of mostly MRI-lesional epilepsy surgery patients should not be interpreted as evidence against performing an autoimmune diagnostic workup in patients with refractory epilepsy in general. In addition, on the basis of our data, we cannot conclude what the prevalence of antibody positivity would be in a different patient population, for example, in a strictly nonlesional cohort.

5 | CONCLUSIONS

Immune etiology was rare in our cohort of mostly MRI-lesional epilepsy surgery patients. Thorough confirmation of antibody findings, especially when detected in serum only, is needed to prevent misclassification of patients with underlying structural lesions as autoimmune epilepsy. Including CSF in presurgical examinations can help to prevent false-positive findings. Identifying CSF-restricted OCBs in presurgical patients needs further evaluation before it can be considered as a biomarker of unfavorable outcome.

AUTHOR CONTRIBUTIONS

Hana Mojžišová: Conceptualization; methodology, formal analysis; investigation; writing–original draft preparation; writing–review and editing. **Martin Elišák:** Conceptualization; methodology, writing–review and editing. **David Krýsl:** Writing–original draft preparation; writing–review and editing. **Jitka Hanzalová:** Investigation; writing–review and editing. **Adam Kalina:** Investigation; writing–review and editing. **Marko Petržalka:** Writing–review and editing. **Irena Doležalová:** Investigation; writing–review and editing. **Matěj Červenka:** Investigation; writing–review and editing. **Barbora Cvičková:** Investigation; writing–review and editing. **Robert Leško:** Investigation; writing–review and editing. **Jan Šroubek:** Investigation; writing–review and editing. **Daniela Sochůrková:** Investigation; writing–review and editing. **Jan Hemza:** Writing–review

and editing. **Eva Brichtová:** Writing–review and editing. **Justina Dargvainiene:** Investigation; methodology; writing–review and editing. **Zdeněk Vojtěch:** Writing–review and editing. **Milan Brázdil:** Writing–review and editing. **Klaus-Peter Wandinger:** Methodology, writing–review and editing. **Frank Leypoldt:** Methodology, investigation; writing–original draft preparation; writing–review and editing. **Petr Marusič:** Conceptualization; methodology, writing–original draft; supervision.

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CONFLICT OF INTEREST STATEMENT

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Appropriate ethics approval was obtained for the collection of serum and CSF samples and clinical information at all three centers, and written informed consent was obtained from all patients. Inclusion of patients in the study had no effect on the indications or the extent of the surgery. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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