Neuropathological Variability within a Spectrum of NMDAR-Encephalitis

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Objective: To describe the neuropathological features of N-methyl-D-aspartate receptor (NMDAR)-encephalitis in an archival autopsy cohort.

Methods: We examined four autopsies from patients with NMDAR-encephalitis; two patients were untreated, three had comorbidities: small cell lung cancer, brain post-transplant lymphoproliferative disease (PTLD), and overlapping demyelination.

Results: The two untreated patients had inflammatory infiltrates predominantly composed of perivascular and parenchymal CD3+/CD8+ T cells and CD79a+ B cells/plasma cells in basal ganglia, amygdala, and hippocampus with surrounding white matter. The hippocampi showed a significant decrease of NMDAR-immunoreactivity that correlated with disease severity. The patient with NMDAR-encephalitis and immunosuppression for kidney transplantation developed a brain monomorphic PTLD. Inflammatory changes were compatible with NMDAR-encephalitis. Additionally, plasma cells accumulated in the vicinity of the necrotic tumor along with macrophages and activated microglia that strongly expressed pro-inflammatory activation markers HLA-DR, CD68, and IL18. The fourth patient developed demyelinating lesions in the setting of a relapse 4 years after NMDAR-encephalitis. These lesions exhibited the hallmarks of classic multiple sclerosis with radially expanding lesions and remyelinated shadow plaques without complement or immunoglobulin deposition, compatible with a pattern I demyelination.

Interpretation: The topographic distribution of inflammation in patients with NMDAR-encephalitis reflects the clinical symptoms of movement disorders, abnormal behavior, and memory dysfunction with inflammation dominantly observed in basal ganglia, amygdala, and hippocampus, and loss of NMDAR-immunoreactivity correlates with disease severity. Co-occurring pathologies influence the spatial distribution, composition, and intensity of inflammation, which may modify patients’ clinical presentation and outcome.

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Encephalitis with IgG autoantibodies against the N-methyl-D-aspartate receptor (NMDAR) is associated with a characteristic clinical syndrome presenting with acute psychiatric symptoms, cognitive deficits, epileptic seizures, movement disorders and autonomic dysregulation. In vivo and in vitro experiments demonstrated that the antibodies bind to an extracellular region of the NMDAR causing internalization of the receptor and neuronal dysfunction. NMDAR-encephalitis is characterized by prominent intrathecal production of pathogenic autoantibodies and the reversibility of neuronal dysfunction is reflected by good response to immunotherapy. Due to the frequent recovery of patients, neuropathological studies on NMDAR-encephalitis are rare and mainly based on isolated cases with overlapping pathologies or small biopsy specimens, lacking systematic assessment of immune cell infiltration and anatomic correlations. This study aims to characterize the spectrum of inflammatory changes in different brain areas in patients who have or have not been treated with immunotherapy. Moreover, we describe the overlapping neuropathology of NMDAR-encephalitis with multiple sclerosis (MS)-type demyelination and post-transplant lymphoproliferative disease (PTLD).

Methods

Patient Identification and Inclusion Criteria

Patients were identified at the Neurobiobank of the Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Austria (3 autopsies) and the Neurological Tissue Bank of the Hospital Clinic - IDIBAPS, Barcelona (1 autopsy). Inclusion criteria were: (1) positive NMDAR-IgG in CSF determined by cell-based and tissue-based assays, and (2) sufficient archival tissue for pathological analysis. A total of 4 autopsies were included in the study. The study was approved by the Institutional Review Board of the Medical University of Vienna (EK 1123/2015), and the Ethics Committee of Hospital Clinic, Barcelona (R091217-12). Patient demographics, clinical data, comorbidities and cause of death are presented in Table 1. Patient 2 was briefly reported in a series of patients with autoimmune encephalitis.

Neuropathology and Immunohistochemistry

Neuropathological analysis was performed either on double hemispheric brain sections or small sections. Formalin-fixed and paraffin-embedded tissue blocks representing frontal, temporal, parietal and occipital cortices and adjacent white matter, thalamus, hippocampus, basal ganglia, amygdala, midbrain, pons, medulla oblongata, and cerebellum were regions of interest. All sections were stained with hematoxylin and eosin (H&E), and a panel of primary antibodies was used for immunohistochemistry techniques. The primary antibodies and type of antigen retrieval used are summarized in Table 2. Sections stained in the absence of primary antibodies and using isotype-matched control antibodies served as controls. Image acquisition was performed on a NanoZoomer 2.0-HT digital slide scanner C9600 (Hamamatsu Photonics, Hamamatsu, Japan). Double hemispheric brain sections were scanned using a custom slide scanner as described previously. In situ hybridization for Epstein Barr Virus (EBV) was performed using the EBER pNA detection kit (DAKO Y5200), including an Epstein–Barr virus+ (EBV) cerebral lymphoma as a positive control.

For double immunostaining using primary antibodies derived from different species, the same antigen retrieval techniques were applied (Table 2). Visualization was performed by using (1) alkaline phosphatase-conjugated secondary antibodies for subsequent development with fast blue BB salt as well as (2) biotinylated secondary antibodies and peroxidase-conjugated streptavidin for subsequent development with aminoethyl carbazole (AEC). NMDAR Antibody Testing

CSF NMDAR antibodies were assessed using an in-house tissue-based assay as described elsewhere and a commercial cell-based assay (Euroimmun, Lübeck, Germany) according to the manufacturer’s protocol. Positivity was defined as a positive neuropil staining pattern in the tissue-based assay and positive labeling of transfected cells.

Quantitative Analysis

All quantitative data were obtained by one investigator blinded to the clinical data. All sections were overlaid with a microscopic grid and 17 randomly distributed fields in the region of interest were counted. In each ROI, 1 mm² was quantified. All values are expressed as cell counts per square millimeter. The number of T cells and B cells were counted separately in the perivascular and parenchymal areas. To assess the anatomic distribution of CD79a positive plasmablasts/plasma cells (cytoplasmic expression of CD79a in plasma cells) in NMDAR-encephalitis overlapping with PTLD, a bi-hemispheric section was stained with anti-CD79a, scanned and each immunolabelled cell was classified as a red dot, using the bioimage analysis platform QuPath.

Results

Patients

The median age at disease onset was 45 years, and two patients were female (Table 1). Two patients died in 2004 and 2006 before NMDAR-encephalitis was discovered or...
systematically tested, and did not receive immunotherapy. NMDAR antibodies were retrospectively tested in archival CSF samples. Two patients were diagnosed with NMDAR-encephalitis during their lifetime and treated with immunotherapy, but developed a co-pathology and died. A summary of the clinical information is shown in Table 1.

Neuropathology of NMDAR-Encephalitis without Immunotherapy

Patient 1 was a 33-year-old female patient with unremarkable past medical history or family history and without children, who presented with severe head and neck pain, auditory hallucinations, agitation, fever and generalized seizures. She was admitted to the hospital and was treated for a potential herpes encephalitis as well as bacterial encephalitis with antibiotics and antiviral medication (acyclovir, doxycycline, ceftriaxone). Lumbar puncture revealed 18 WBC/μl, normal protein concentration (37.3 mg/dl) and CSF-specific (serum unmatched) oligoclonal bands. Microbiological studies revealed positive serum IgM antibodies directed against mycoplasma pneumoniae antigen, microbiological cultures and PCR analysis for viruses (including HSV1 and HSV2) resulted negative. Over the ensuing days, she developed central hypoventilation and was transferred to the ICU. Brain MRI revealed bilateral temporal edema. Any attempt to taper the sedation resulted in uncontrollable seizures. Four months after transfer to the ICU, she developed multiple bilateral pulmonary embolisms and died. The patient never received any steroid or immunomodulatory treatment due to the suspicion of an infectious disease. Post-mortem examination did not reveal any malignancy or ovarian teratoma.

Patient 2 was a 76-year-old male patient with a history of smoking, arterial hypertension, chronic renal insufficiency, and chronic diverticulosis. His daughter noticed behavioral abnormalities, disinhibition, and confusion and brought him to the hospital. Toxicological tests, chest X-ray and cranial CT were normal. Over the ensuing days he developed severe anterograde amnesia, confabulations, and preservation of remote memory. CSF showed 20 cells/μl, 51 mg/dl protein, OCB+. Brain MRI showed small vessel arteriopathy but was otherwise unremarkable. The patient rapidly progressed, became mute without responding to commands, presented frontal

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### Table 1. Clinical Information of NMDAR-Encephalitis Patients

| Nr | Sex | Age at onset (yr) | Age at death (yr) | Symptoms | Disease duration (days) | Cause of death | Immuno-therapy | CSF | Concomitant disease | MRI features | Post-mortem interval |
|----|-----|--------------------|-------------------|-----------|------------------------|----------------|----------------|-----|--------------------|-------------|----------------------|
| 1  | f   | 33                 | 33                | Auditory hallucinations, agitation, fever, generalized seizures, central hypoventilation | 128 | Pulmonary embolism | None | 18 cells/μl, 37.3 mg/dl protein and OCB+ | None | Flair hyperintensities | 24 d |
| 2  | m   | 76                 | 76                | Confusion, memory deficits, agitation, SIAD | 31 | Cardiopulmonary arrest | None | 20 cells/μl, 51 mg/dl protein, OCB+ | SCLC | None | 8 h |
| 3  | f   | 38                 | 42                | Religious delusions, acoustic hallucinations, mutism, reluctance to eat | 1460 | Cardiopulmonary arrest | Steroids, IVIG | 0 cells/μl, 16.4 mg/dl protein, OCB+ | Demyelination | Flair hyperintensities | 12 d |
| 4  | m   | 33                 | 34                | Behavioral changes, confusion, memory deficits, hypersexuality | 357 | Cardiopulmonary arrest | Steroids, PLEX, IVIG, chemotherapy (prior: cyclosporine, mycophenolate-mofetil and steroids) | 45 cells/μl, 54 mg/dl protein, OCB+ | Renal transplantation, B-cell lymphoma, EBV+ | none | 18 h |

d = days; EBV = Epstein–Barr virus; h = hours; IVIg = Intravenous immunoglobulin; OCB = oligoclonal bands; PLEX = plasma exchange; SCLC = small-cell lung carcinoma; SIAD = syndrome of inappropriate antidiuresis.
release reflexes and an increased muscle tone in all 4 limbs. EEG showed diffuse slowing without epileptic features. The patient developed a syndrome of inappropriate anti-diuretic hormone secretion (SIADH) with hyponatremia as well as atrial fibrillation. The patient died 31 days after disease onset due to cardiac arrest. He did not receive any steroid or immunomodulatory treatment. Postmortem examination revealed small cell lung carcinoma with mediastinal and hepatic metastasis. A tumor in the testes was not found.

Postmortem brain studies of both patients demonstrated mild to moderate inflammatory infiltrates within the basal ganglia, amygdala, and the surrounding white matter. Moreover, mild inflammation was found in the CA4 sector of the hippocampus and in the Schaffer collateral and perforant pathway in the white matter. The brainstem showed only scattered T cell and plasma cell infiltrates (schematic drawing Fig 1A–F). Plasma cells were IgG1+ and IgG4− (Fig 1G, H). Meninges were predominantly affected in the frontal lobe with focal infiltrates of CD79a+ and CD138+ plasma cells, CD3+/CD4+ T cells, and only scattered CD20+ B cells. No ectopic lymph follicles were found (data not shown). In the amygdala, perivascular inflammation was composed of 62.5% T cells (44.9% CD3+/CD8+ and 55.1% CD3+/CD8−), 16.7% CD20+ cells, and 20.8% CD79a+ plasmablasts/plasma cells.

### TABLE 2. Primary Antibodies and Antigen Retrieval for Immunohistochemistry

| Antibody | Origin | Target | Dilution | Antigen retrieval | Source |
|----------|--------|--------|----------|------------------|--------|
| APP      | Mouse (mAB) | Amyloid precursor protein | 1:1,000 | St(C) | MAB348; Chemicon, |
| c9neo    | Rabbit (pAB) | Complement C9neo antigen | 1:2,000 | Prot | RM-9107-S, P. Morgan; Cardiff, UK |
| CD3      | Rabbit (mAB) | T cells | 1:200 | St (C) | RM-9107-S; Thermo Fischer scientific |
| CD4      | Mouse (mAB) | MHC Class II-restricted T cells | 1:100 | St (E) | 4B12; Dako |
| CD8      | Mouse (mAB) | MHC Class I restricted T cells | 1:100 | St (C) | M7103; Dako |
| CD20     | Mouse (mAB) | B cells | 1:400 | St (C) | M0755; Dako |
| CD68     | Mouse (mAB; IgG1) | Lysosomal glycoprotein | 1:100 | St (E) | M0814; Dako, |
| CD79a    | Mouse (mAB) | B cells | 1:50 | ST(E) | M7050; Dako |
| TPPP/p25 | Mouse (mAB) | Oligodendrocytes | 1:2,000 | None | Mouse clone 6C10; Prof. Kovacs |
| HLA-DR   | Mouse (mAB; IgG1) | MHC Class II antigen | 1:100 | St (C) | M0775; Dako, |
| Iba-1    | Rabbit (pAB) | Ionized calcium-binding adaptor molecule 1 | 1:3,000 | St (E) | 019-19741; Wako |
| p22phox  | Rabbit (pAB) | Subunit of NADPH oxidase | 1:100 | St (C) | sc-20781; Santa Cruz |
| P2RY12   | Rabbit (pAB) | Purinergic receptor | 1:2,500 | St (E) | Harvard, Dr. Butovsky |
| GFAP     | Goat (pAB) | Astrocytes | 1:100 | St (E) | Sc-6170, Santa Cruz |
| IL-18    | Rabbit (pAB) | Interleukin 18 | 1:2,000 | St (E) | Ab191152; Abcam |
| TMEM119  | Rabbit (pAB) | Transmembrane protein 119 | 1:500 | None | HPA051870; Sigma-Aldrich |
| IgG1     | Mouse (mAB) | Human IgG1 | 1:100 | St(E) | B6775, Sigma-Aldrich |
| IgG4     | Mouse (mAB) | Human IgG4 | 1:500 | St(E) | HP6025, Bio-Rad |
| SMI31    | Mouse (mAB) | Anti-phosphorylated neurofilament | 1:25,000 | St (E) | SMI-31; BioLegend |

mAB = monoclonal antibody; pAB = polyclonal antibody; Prot = protease digestion; St (C) = steaming of tissue sections in citrate buffer; St (E) = steaming of tissue sections in EDTA buffer; according to 17.
Parenchymal infiltration consisted of 94.4% T cells (56.3% CD3⁺CD8⁻ and 42.7% CD3⁺CD8⁺), 0% CD20⁺ cells, and 5.6% CD79a⁺ plasmablasts/plasma cells. In the basal ganglia, perivascular infiltration was composed of 47.8% T cells (51.5% CD3⁺CD8⁻ and 48.5% CD3⁺CD8⁺), 11.1% CD20⁺ cells, and 41.1% CD79a⁺ plasmablasts/plasma cells. Parenchymal infiltration consisted of 84.4% T cells (47.6% CD3⁺CD8⁻ and 52.3% CD3⁺CD8⁺), 0% CD20⁺ cells, and 15.6% CD79a⁺ plasmablasts/plasma cells.

In the hippocampus, perivascular inflammation was composed of 90.1% T cells (49.3% CD3⁺CD8⁻ and 50.7% CD3⁺CD8⁺), 0.4% CD20⁺ cells, and 9.5% CD79a⁺ plasmablasts/plasma cells. Parenchymal infiltration consisted in the CA4 area of 91.8% T cells (60.5% CD3⁺CD8⁻ and 39.5% CD3⁺CD8⁺), 0% CD20⁺ cells, and 8.2% CD79a⁺ plasmablasts/plasma cells, in the CA1 area of 96.4% T cells (28.3% CD3⁺CD8⁻ and 71.7% CD3⁺CD8⁺), 3.6% CD20⁺ cells, and 0% CD79a⁺ plasmablasts/plasma cells, and in the adjacent parenchymal white matter of 93.7% T cells (78.6% CD3⁺CD8⁻ and 21.4% CD3⁺CD8⁺), 0% CD20⁺ cells, and 6.3% CD79a⁺ plasmablasts/plasma cells (Fig 1E, F, I–M).

Lymphocytic infiltration was accompanied by pronounced microglial activation characterized by shortened and thickened processes expressing the activation markers HLA-DR and CD68 (Fig 1N). Immunohistochemical staining of the hippocampus in one of the two patients showed a significant decrease of NMDAR-expression compared to an age-matched control (Fig 1O, P). This reduction was more pronounced compared to those two patients, who were treated with immunotherapy and did not need intensive care support (clinical information see below; data not shown). We did not detect specific complement C9neo deposits. T cells in the vicinity of neurons did not express granzyme B. The H&E and immunohistochemistry for neurofilament, GFAP, and HLA-DR showed no obvious neuronal loss, reactive scar-forming astrocytes or microglial nodules in the pyramidal cell layer of the hippocampus, cortical regions, brainstem, and cerebellum.

Neuropathology of NMDAR-Encephalitis with Overlapping CNS Comorbidities

NMDAR-Encephalitis and PTLD. A 33-year-old man with past medical history of renal transplantation under immunosuppressive therapy (cyclosporine, mycophenolate mofetil, and steroids) presented 13 years after transplantation to the emergency department with behavioral changes, confusion, memory deficits, and hypersexuality over the previous 2 weeks. Brain MRI was unremarkable. The CSF showed pleocytosis (45 WBC/μl), increased protein concentration (54.0 mg/dl), CSF-specific (serum unmatched) oligoclonal bands, and positive EBV-PCR. NMDAR antibodies were positive in serum and CSF. The patient received steroid pulse therapy, plasma exchange, and intravenous immunoglobulins (IVIGs), which lead to partial neurological improvement. Treatment escalation with rituximab was initially avoided due to the risk of EBV-encephalitis. Six months later, he was admitted to the emergency department with altered mental status. Laboratory findings showed hyponatremia, and brain MRI revealed a 1.4 cm mass in the left globus pallidus. CSF showed mild pleocytosis (16 WBC/μl) and continued to be EBV-PCR positive (1.12 × 10⁵ copies/ml) and NMDAR-antibody positive (titer 1:8). Brain biopsy showed a monomorphic PTLD (EBV-associated diffuse large B cell lymphoma, DLBCL). The patient was treated with immuno- and chemotherapy (rituximab, vincristine, methotrexate, and procarbazine) and radiation; however, he died 4 months later due to cardiopulmonary arrest.

Post mortem examination of the brain showed a resection cavity in the left frontal lobe and a necrotic tumor lesion in the left globus pallidus (Fig 2A, B) accompanied by a small residual area of PTLD with cells positive in the in situ hybridization for Epstein–Barr virus latency-associated RNA (EBER) (Fig 2C).

Anatomic distribution of inflammation in the tumor-affected left hemisphere showed maximal inflammation around the necrotic tumor lesion, with macrophages and activated microglia that strongly expressed pro-inflammatory activation markers HLA-DR, CD68, and IL18 (Fig 2D) along with numerous EBER-negative plasma cells (Fig 2E). Plasma cells were also abundant in the medial temporal lobe, including the amygdala (Fig 2F) and hippocampus, as well as inferior and medial temporal gyrus. The right hemisphere did not show any tumor lesions and type of inflammatory infiltrates and distribution was compatible with NMDAR-encephalitis (Fig 2B, G). In both hemispheres, the inflammation was predominantly composed of CD20⁻/CD79a⁺ plasmablasts/plasma cells and only single CD20⁺ B cells and T cells (Fig 2H–K). Plasma cells showed polypartic staining for kappa and lambda light chain and were EBER-negative. Immunohistochemistry for NMDAR only revealed a mild reduction of immunoreactivity in the hippocampus compared to a healthy control (data not shown). No neuronal loss was observed except around the necrotic tumor lesion.

NMDAR-Encephalitis and Demyelination. A 42-year-old woman with past medical history of borderline personality disorder presented with religious delusions, acoustic hallucinations, mutism, and refusal to eat. Brain MRI was unremarkable; CSF showed normal cell count, positive oligoclonal bands, and NMDAR antibodies, which were
FIGURE 1: Neuropathology of untreated NMDAR-encephalitis. Topographic distribution of inflammation in the brain of both patients shows a prominent involvement of basal ganglia and amygdala (A), and CA4 sector of the hippocampus (B) while pons and medulla oblongata (C, D) display only scattered T cells and plasma cells (A–D; red: CD79a⁺ plasma cells; blue: CD3⁺ T cells). The inflammatory infiltrates were characterized by perivascular CD3⁺ T cells (E) and CD79a⁺ plasmablasts/plasma cells (F). Parenchymal lymphocytes were dominantly composed of CD3⁺ T cells and CD79⁺ plasmablasts/plasma cells (G, H). Plasma cells were IgG1 positive (I) and IgG4 negative (J). Double immunostaining revealed that perivascular T cell inflammation was composed of equal CD3⁺CD8⁻ (red) and CD3⁺CD8⁺ (brown) cells (K). Parenchymal lymphocytes were dominantly composed of CD3⁺CD8⁻ T cells (red) (L) and CD79⁺ plasmablasts/plasma cells (blue) (M). HLA-DR staining revealed pronounced microglial activation (N). Immunohistochemical staining of the hippocampus showed a significant decrease of NMDAR-expression compared to an age-matched control (O, P). (E–H) taken from patient 2; (I–O) taken from patient 1; Scale bars: 25 μm.
also present in serum. Myelin oligodendrocyte glycoprotein (MOG)- and aquaporin-4 (AQP4)-antibodies were negative in serum and CSF. Studies for an infectious process were negative. The patient received initial steroid pulse therapy, followed by IVIGs that lead to fast improvement. CT and MRI of the pelvis were normal. Four years later, she returned because of agitation, hemiparesis and a complex pain syndrome. NMDAR antibodies were again identified in serum (1:20) and CSF (1:8); the CSF revealed 0 WBC/μl, protein concentration (16.4 mg/dl) and CSF-specific (serum unmatched) oligoclonal bands. Brain MRI showed T2 hyperintense white matter abnormalities suggestive for multiple sclerosis. The patient again received steroid pulse therapy and IVIGs, which lead to improvement of the hemiparesis; however, she refused other treatments. Five months later, she was found dead at home; a forensic autopsy suggested that the cause of death was sudden cardiopulmonary arrest.

Analysis of whole brain hemispheric sections showed predominantly periventricular demyelinating plaques with perivenous finger-like extensions into the adjacent white matter (Dawson Fingers) (Fig 3A). In addition, multiple
FIGURE 3: Neuropathology of NMDAR-encephalitis overlapping with MS-type demyelination. Bi-hemispheric topographic evaluation showed several demyelinated plaques in the periventricular and deep white matter (A, B green: white matter demyelination, C, D, LFB; rectangle in D enlarged in K). Some demyelinated lesions showed a perivenous accentuation (E, G MBP) and were characterized by early active demyelination with LFB-positive degradation products within the macrophages (F inset in E, arrows; LFB/PAS). The plaque-like periventricular demyelinated lesions (H, MBP) showed a rim of activated macrophages and microglia at the edge and profound microglia activation in the surrounding white matter (I, CD68; rectangle enlarged in J; arrow in I marks vessel enlarged in N–Q). Oligodendrocytes were well preserved at the lesion edge and are lost in the inactive lesion center (L, rectangle enlarged in M). Inflammatory perivascular cuffs contained high numbers of CD20⁺ B cells (N), CD79a⁺ plasmablasts/plasma cells (O), CD3⁺ T cells (P), and less CD8⁺ T cells (Q). Scale bars: 25 μm (except for: E, H, I, K, L: 100 μm).
### TABLE 3. Neuropathological Findings in NMDAR-Encephalitis

| Number | Sex | Investigated areas | Findings | Ref |
|--------|-----|---------------------|----------|-----|
| 2 autopsies | 2F | Temporal lobe, hippocampus, cerebellum, basal ganglia, parietal cortex, frontal cortex, occipital cortex, spinal cord | Case 1: ammonshorn sclerosis (Sommer’s sector); neuronal degeneration and microgliosis in amygdala; mild lymphocytic infiltrates in meninges and moderate perivascular lymphocytic infiltrates in parahippocampi; few in thalami, insula and medulla; IgG deposits predominantly in hippocampus; case 2: minimal neuronal drop out in hippocampus but gliosis most severe in CA4; minimal changes (gliosis, rare swollen axons) in frontal and occipital cortex, basal ganglia; gliosis in arcuate nuclei of medulla and ventral horns in spinal cord; both cases: decrease in NMDAR immunostaining without affecting the number of synapses; | 2,6,9 |
| 1 biopsy | F | Right frontal-lobe biopsy | Marked leptomeningeal and perivascular infiltrates mainly composed of CD20+ B cells, few CD138+ plasma cells, CD3+ T cells and CD68+ macrophages; neuronal or axonal pathology was absent; | 49 |
| 3 autopsies, 2 biopsies | 3F/2M | Autopsies (one novel, two cases from Dalmau et al 2007): frontal, temporal lateral, temporal medial/hippocampus, and parietal; biopsies: 1 temporal, 1 frontal | No complement deposition (C9neo), abundant perivascular and meningeal CD138+ plasmablasts/plasma cells along with B and T cell lymphocytes; | 8 |
| 3 biopsies | 2F, 1M | 2 frontal and 1 temporal lobe | No neuro-axonal injury; perivascular CD8/CD3 ratio higher than in parenchyma; occasional CD20+ B cells and CD138+ plasma cells; no complement deposition, no appositions of CD3+/granzymeB+ T cells to neurons | 7 |
| 1 biopsy | M | Frontal lobe | Co-pathology (demyelination); demyelination with relative preservation of axons; foamy macrophages containing Luxol fast blue-positive particles of myelin, reactive astrocytes, perivascular inflammatory infiltrates | 37 |
| 1 autopsy | F | Cortex, basal ganglia, thalamus, midbrain, cerebellum, brainstem, spinal cord, eyes | No neuronal loss; prominent microgliosis (Iba-1); scattered T cells in parenchyma, B cells and plasmablasts/plasma cells were absent | 12 |
| 1 autopsy | M | Frontal lobe and basal ganglia | Microgliosis, intraparenchymal and perivascular CD3+ T cells in the basal ganglia and frontal lobe; CD4:CD8 ratio 1:1, C4d complement deposition | 50 |
| 1 autopsy | M | Fusiform gyrus, amygdala, insular cortex, superior temporal and precentral cortices, claustrum | Co-pathology (demyelination); mild neuronal loss and astrocytosis in CA4 and dentate gyrus of hippocampus; perivascular T cell dominated cuffs; demyelinating lesions in temporal, frontal, and insular cortex, caudate nuclei, claustrum; cortico-subcortical demyelination in fusiform gyrus | 14 |
| 1 autopsy | M | Frontal, temporal, parietal, and occipital cortex, hippocampus, basal ganglia, thalamus, amygdala, brainstem, cerebellum | Co-pathology (primary CNS lymphoma); perivascular CD79a+ mature plasma cells in tumor-free frontal region and hippocampus; no neuronal loss; | 10 |
| 4 autopsies | 2F, 2M | Frontal, temporal, parietal, and occipital cortex, basal ganglia, thalamus, amygdala, hippocampus, midbrain, pons, medulla, cerebellum, meninges/dura | Two untreated cases, 2 with co-pathology (1 PTLD, 1 demyelination); inflammatory infiltrates most prominent in clinically correlated regions: basal ganglia, amygdala, hippocampus: T cells (between 48–90% perivascular and 84–96% parenchymal) and B cells/plasma cells (CD20+ cells: between 0.4–17% perivascular and 0–3.6% parenchymal; CD79a+ cells: between 10–41% perivascular and 0–15.6% parenchymal); co-pathology influences spatial distribution and composition of inflammation; decrease of NMDAR-immunoreactivity in hippocampus; no complement deposition, no obvious neuronal loss; | Present manuscript |
smaller perivascular inflammatory demyelinating lesions throughout the deep white matter were seen (Fig 3A–G). The small perivascular lesions displayed active and demyelinating/post-demyelinating activity,20 characterized by LFB- and MBP positive myelin degradation products within macrophages (Fig 3F, G). The confluent periventricular lesions showed a rim of activated microglia (TMEM119+/P2RY12) at the border and an inactive lesion center (Fig 3H, J). The axons were relatively well preserved, with some axonal spheroids at the lesion margin. Few plaques were remyelinated and presented as shadow plaques (Fig 3K). TPPP/p25 positive oligodendrocytes were numerous at the margin of the lesion and periplaque white matter but were almost absent in the inactive lesion centers (Fig 3L, M). Cortical or deep grey matter demyelination was absent. None of the lesions showed deposition of activated complement (C9neo antigen). TUNEL staining revealed some cells with DNA fragmentation in the inactive lesion centers that were negative in the double-staining for MOG and TPPP/p25 (data not shown). Inflammatory reaction was prominent and consisted of perivascular B cells (CD20+) and plasma cells (CD79a+ IgG+) next to abundant CD3+ and CD8+ perivascular and parenchymal T cells (ratio CD8:CD4 was 1.38:1) (Fig 3N–Q). Overall, the demyelinating lesions showed the pathological hallmarks of typical MS with inflammatory demyelination and remyelination, without antibody or complement deposition, compatible with a pattern I demyelination.21 However, in contrast to classic MS, which is dominated by CD8+ T cell inflammation, the inflammatory infiltrates of this patient contained a relatively higher number of CD4+ T cells in addition to abundant infiltrating CD20+ B cells and plasma cells. We did not find features of pattern II demyelination with complement and immunoglobulin deposition, or pattern III demyelination defined by distal oligodendrogliopathy, oligodendrocyte apoptosis, or concentric type of demyelination in this patient. B cells and plasma cells were EBER-negative.

In the normal-appearing white matter, prominent T and B-cell infiltration in the parenchyma and perivascular space was observed. The highest number of perivascular lymphocytes was identified in the basal ganglia, CA4 sector in the hippocampus, adjacent hippocampal white matter, periaqueductal grey matter and the red nucleus, tegmentum, and the formatio reticularis. Overall, the parenchymal cell distribution was dominated by T-cells with a substantial percentage of plasma cells: 72.2% CD3+ T-cells (72.4% of them were CD8+), 26.2% CD79a+ plasmablasts/plasma cells and 1.5% CD20+ B cells. In the perivascular compartment the overall cell distribution was 47.3% CD3+ (of these 71.8% CD8+), 16.5% CD20+ and 36.2% CD79a+ cells. The dura mater with sinus sagittalis was available and showed focal infiltrates of CD79a+/CD138+ plasma cells and T cells. No lymph follicles were found (data not shown). Immunohistochemistry for NMDAR revealed a mild reduction of immunoreactivity in the hippocampus compared to a healthy control.

Compared to the average number of lymphocytes in the two untreated NMDAR-encephalitis cases, we found intra-parenchymally 5.9 times more CD3+ T cells, 8 times more CD8+ T-cells, and 10 times more CD20+ B cells and CD79a+ plasmablasts/plasma cells. Perivascularly we observed 3.9 times more CD3+ T cells 5.6 times more CD8+ T cells, 7.2 and 6.3 more CD20/CD79a+ B cells, respectively.

Discussion

This is an extensive neuropathological study of NMDAR-encephalitis with and without overlapping brain pathologies. We observed a different qualitative, quantitative, and topographic distribution of inflammatory reaction, depending on the association with other disorders. In the two untreated NMDAR-encephalitis patients, we found that the areas with maximal inflammation were the amygdala, hippocampus, and basal ganglia, which correlated well with the clinical presentation of abnormal behavior, memory dysfunction, and movement disorders. The numerous plasma cells probably explain the intrathecal NMDAR antibody production reported in patients with this encephalitis.1,5 In addition, we found numerous CD3+/CD8+ helper T cells necessary for activating B cells and antibody production, whereas no cytotoxic T cells (CD8+ granzymeB+B T cells) were identified. Neurons were well preserved but had a reduced NMDAR-immunoreactivity as already shown in previous reports (current and previously reported pathological findings are summarized in Table 3).2,8,9 The extent of decreased immunoreactivity for NMDAR correlated with disease severity (eg, it was most reduced in the patient with intensive medical treatment), which supports the primary antibody-mediated mechanism in NMDAR-encephalitis. No complement deposition was found. Overall, the findings are clearly different from those associated with cytotoxic T cell mechanisms (with CD8+ granzymeB+B T cells and substantial neuronal loss).7,22,23

One of the patients with kidney transplantation developed NMDAR-encephalitis while he was on immunosuppressive therapy. Together with the NMDAR-antibodies, he was found to be EBV-positive in CSF. Later, the patient developed an EBV-associated PTLD and was therefore treated with rituximab. Isolated cases of
NMDAR-encephalitis have been described in patients treated with immunosuppressants after solid organ transplantation or allogeneic hematopoietic stem cell transplantation. In our patient, it is unclear whether the encephalitis was related to the immunological dysfunction caused by the chronic immunosuppression or the destructive effect of the brain PTLD lesion similar to that seen after herpes simplex type I encephalitis. However, the development of the NMDAR-encephalitis when the brain MRI was normal does not support the latter possibility. Four of the reported 6 cases with NMDAR-encephalitis post-solid organ transplantation had a positive EBV PCR in CSF that initially suggested EBV-related encephalitis. Patients with long-term immunosuppressive therapies are typically at risk of infection or reactivation of EBV. A typical aspect of EBV infection is a polyclonal proliferation of B cells with accompanying antibody formation. It was suggested that in these 4 patients the polyclonal proliferation of B cells was accompanied by the synthesis of antibodies against the NMDAR. In this setting, rituximab is a reasonable option for the treatment of the polyclonal proliferation of B cells as well as for the treatment of NMDAR-encephalitis. In our patient, neuropathological investigations revealed a residual necrotic tumor in the basal ganglia that was surrounded by numerous plasma cells along with macrophages expressing HLA-DR, CD68, and IL18. Whether this plasma cell accumulation is a result of the pro-inflammatory microenvironment and/or correspond to a residual infiltrate of the treated PTLD is unclear, but the polypctic staining for kappa and lambda light chains and the prominent neurological symptoms along with the persistent NMDAR antibodies in the CSF make a component of the anti-NMDAR immune response more likely.

In our case, the morphology of the demyelinating lesions was consistent with classic MS with periventricular, plaque-like demyelination with radially expanding lesions, and signs of remyelination with shadow plaques. The absence of complement deposition in the demyelinating lesions may indicate other inflammatory mechanisms, such as microglia-driven tissue injury. Indeed, we found high numbers of activated microglia (TMEM119) in the normal-appearing white and grey matter expressing the pro-inflammatory markers HLA-DR, CD68, and loss of the homeostatic marker P2RY12. Of note was the intense inflammatory infiltrate composed of plasma cells and T cells that was 6 to 10-fold higher than in the two untreated NMDAR-encephalitis cases. The concomitant pathologies may have mutually reinforced the inflammation and microglia activation, which would explain previously published clinical observations that NMDAR-encephalitis concurrent with demyelination may require more intensive immunotherapy and result in more residual deficits compared with isolated NMDAR-encephalitis. In some patients, white matter changes may be caused by the NMDAR antibodies, although these patients usually do not develop clinical MRI features of demyelination. In fact, in these patients the alteration of myelin is better observed with advanced MRI than in routine MRI studies.

Two of our patients underwent a forensic autopsy, one to exclude medical error in the treatment of unclear encephalitis (the patient died before NMDAR-encephalitis was discovered), the other because she was found dead at home. Forensic (neuro-) pathologists are often confronted with challenging situations and should be aware of NMDAR- or other autoimmune encephalitides as a possible cause of unclear encephalitis. Particularly cases of acute psychosis and neuropathological findings of abundant plasma cells should prompt antibody testing in stored serum and CSF samples.

In conclusion, our study reveals several important findings: (1) In untreated patients, the clinical presentation of abnormal behavior, memory dysfunction, and movement disorders correlates well with the topographic distribution of the inflammation with plasma cells in amygdala, hippocampus, and basal ganglia, while disease severity correlates with the decrease of NMDAR-immunoreactivity, (2) overlapping pathologies with NMDAR-encephalitis may change the distribution and composition of inflammatory infiltrates, and the pro-inflammatory microenvironment may enhance the intensity of inflammation, which in turn may influence the clinical presentation and outcome of patients, and (3) pathogenic mechanisms of overlapping NMDAR-encephalitis and demyelination may be heterogeneous and the tissue injury...
may be driven by pro-inflammatory microglia and macrophages in MOG- or AQP4-negative patients.

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Author Contributions

T.Z., J.D., R.H contributed to conception and design of the study. T.Z., V.E., J.B., S.M., N.M., C.S., G.R., M.W., S.G., M.B., I.W., G.G.K., D.U.R., N.K., I.S.K, T.R., P.R., T.B., E.G., H.L., F.G., J.D., R.H contributed to acquisition and analysis of data. T.Z. and R.H. contributed to drafting the text or preparing the figures.

Potential Conflicts of Interest

F.G. holds a patent for the use of IgLON5 as an autoantibody test. J.D. receives royalties related to autoantibody tests from Athena Diagnostics and Euroimmun and holds patents for the use of Ma2, NMDAR, GABABR, GABAAR, DPPX and IgLON5 as autoantibody tests.

R.H. reports speaker’s honoraria from Novartis and Biogen. The other authors have no potential conflicts to report.

Data Availability

Data can be made available from the corresponding author upon reasonable request and after approval from the ethics review board at the Medical University of Vienna.

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