Original Investigation

Abnormal Neurons in Teratomas in NMDAR Encephalitis

Gregory S. Day, MD, MSc, BScH; Simin Laiq, MD; David F. Tang-Wai, MD, FRCPC; David G. Munoz, MD, FRCPC

IMPORTANCE Ovarian teratomas are frequently described in patients with *N*-methyl-D-aspartate receptor (NMDAR) encephalitis, yet NMDAR encephalitis is rarely described in patients with ovarian teratomas. Understanding why a minority of patients with teratomas are seen with autoimmune encephalitis may improve the management of NMDAR encephalitis and other teratoma-associated autoimmune diseases.

OBJECTIVE To characterize the unique organization of neuroglial elements within ovarian teratomas resected from patients with NMDAR encephalitis.

DESIGN Case-control study comparing the pathological features of ovarian teratomas resected from consecutively accrued cases with NMDAR encephalitis between January 1, 2009, and December 15, 2013, and ovarian teratomas resected from controls between June 1, 2012, and June 30, 2013.

SETTING Pathology tissue database at a tertiary academic care center.

PARTICIPANTS Five cases with teratoma-associated NMDAR encephalitis and serum or cerebrospinal fluid autoantibodies against central nervous system (CNS) NMDAR and 38 controls (39 ovarian teratomas) without neurological symptoms or signs.

EXPOSURES Formalin-fixed, paraffin-embedded ovarian teratomas were examined for the presence of CNS tissue and inflammatory infiltrates using direct microscopy, enhanced with standard histopathological and immunological stains.

MAIN OUTCOMES AND MEASURES Frequency of detection of atypical (dysplastic) CNS neuronal elements in ovarian teratomas resected from cases vs controls, as well as characterization of the relationship between atypical neurons and immune infiltrates.

RESULTS Central nervous system neuronal elements were detected in 4 of 5 teratomas resected from cases with NMDAR encephalitis and in 20 of 39 controls ($P = .36$). Atypical neurons were seen within teratomas resected from 4 of 5 cases but not in 39 controls, reliably distinguishing teratomas associated with NMDAR encephalitis ($P < .001$). If found within the CNS, these histological abnormalities would have received the diagnosis of gangliogliomas (n = 3) and ganglioneuroblastoma (n = 1). Reactive changes were present in teratomas from controls, including ferruginated neurons and Rosenthal fibers. Abnormal neuroglial elements were closely related to immune infiltrates in teratomas resected from 4 of 4 cases. Inflammatory infiltrates were not associated with neuroglial tissue in 20 controls, further differentiating these populations ($P < .001$).

CONCLUSIONS AND RELEVANCE Abnormal neurons within teratomas distinguish cases with NMDAR encephalitis from controls and may promote the development of autoimmunity.

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Author Affiliations: Division of Neurology, University of Toronto, Toronto, Ontario, Canada (Day, Tang-Wai); University Health Network Memory Clinic, Toronto Western Hospital, Toronto, Ontario, Canada (Day, Tang-Wai); Division of Pathology, Department of Laboratory Medicine, Saint Michael's Hospital, Toronto, Ontario, Canada (Laiq, Munoz); Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario, Canada (Laiq, Munoz).

Corresponding Author: Gregory S. Day, MD, MSc, BScH, Division of Neurology, University of Toronto, 399 Bathurst St, Toronto, ON M5T 2S8, Canada (gregg.day@mail.utoronto.ca).

Ovarian teratomas are described in more than 50% of adult women with *N*-methyl-D-aspartate receptor (NMDAR) encephalitis, an autoimmune neurological disorder characterized by progressive psychiatric symptoms, hyperkinetic movements, autonomic compromise, and autoantibodies against the central nervous system (CNS) NMDAR.[1-3] The discovery of neuroglial elements (expressing the NMDAR)[2,4] within teratomas resected from patients with NMDAR encephalitis suggests that neuroglial tissue may be integral to autoantibody formation and disease pathogenesis. However, neuroglial tissue is observed in 30% to 50% of mature cystic teratomas resected from patients without CNS dysfunction[5] or NMDAR autoantibodies.[6] Therefore, neuroglial tissue alone does not trigger NMDAR autoantibody formation.

Distinctive reactive lymphoid elements have been observed in close approximation to neuroglial elements in teratomas resected from patients with NMDAR encephalitis but not in teratomas resected from individuals without neurological symptoms.[7] Following the histological findings observed in one case, we hypothesized that the organization of neuroglial elements within teratomas resected from patients with NMDAR encephalitis may underlie the selective immune response that characterizes teratoma-associated disease.

Methods

Study Recruitment

The institutional research ethics board at Saint Michael's Hospital, Toronto, Ontario, Canada, approved the study methods and procedures. All cases and controls provided written consent to the use of resected tissue for research.

The pathological features of ovarian teratomas resected from 5 consecutively accrued cases with NMDAR encephalitis were compared with those resected from controls without neurological dysfunction. All cases had symptoms and signs consistent with NMDAR encephalitis,[1-3,8,9] with NMDAR autoantibodies detected in serum or cerebrospinal fluid (using established techniques[2,8]) between January 1, 2009, and December 15, 2013. Controls were derived through retrospective review of ovarian teratomas resected at Saint Michael's Hospital between June 1, 2012, and June 30, 2013. Review of medical records confirmed a diagnosis of NMDAR encephalitis in one individual (included as a case). No evidence of neurological disease was reported in the remaining 38 controls (39 ovarian teratomas). Clinical information for cases was obtained through review of health records. Clinical information concerning controls was limited to that specified on the pathology requisition accompanying tissue for review (demographic information and indications for resection).

Histopathological Examination

Tissues from cases and controls were fixed in formalin, embedded in paraffin, and sectioned according to standard laboratory techniques by laboratory technologists blinded to case-control status. On average, a mean (SD) of 3.0 (1.9) (maximum of 5 and minimum of 1) sections per case and 4.7 (2.0) (maximum of 10 and minimum of 1) sections per control were prepared ($P = .08$). Five-micrometer-thick sections were stained with hematoxylin-eosin or processed for immunohistochemistry using an automated staining system (BenchMark XT; Ventana) at 37°C, developed with diaminobenzine, and counterstained with hematoxylin. The antibodies used, concentrations, and antigen retrieval procedures are listed in **Table 1**.

Hematoxylin-eosin-stained sections were examined for the presence of nervous system tissue and inflammatory infiltrates by unblinded study authors (S.L. and D.G.M.). The presence of CNS tissue was confirmed using specific stains for CNS neurons and glial cells.[10] Ki-67 immunostaining was used to

Table 1. Antibodies Used in Immunostaining and Cell Types Stained

Antibody	Source	Catalog No.	Pretreatment[a]	Dilution	Specific Cell Types Stained
Neuron-specific enolase	Dako	M0873	HIER for 32 min	1:200	Neurons (perikaryon), neuroendocrine cells
Synaptophysin	Zymed	18-0130	HIER for 32 min	1:40	Neurons (terminals), neuroendocrine cells
Microtubule-associated protein 2	Invitrogen	13500	None	None	Neurons (dendrites), oligodendrocytes
Hu	Molecular Probes	A21271	HIER for 32 min	1:2000	Neurons (perikaryon)[b]
Neurofilament	Dako	M0762	Protease 1 for 4 min	1:50	Neurons (axons)
Chromogranin A	Roche	760-2519	HIER for 30 min	Prediluted	Neurons (perikaryon), neuroendocrine cells
Glial fibrillary acidic protein	Dako	2003H	Protease 1 for 4 min	1:4000	Astrocytes
Ki-67	Thermo Scientific	RM-9106-2	HIER for 32 min	1:200	Proliferating cells
CD3	Thermo Scientific	RM-9107-S1	HIER for 64 min	1:200	T lymphocytes
CD20	Dako	M0755	HIER for 32 min	1:200	B lymphocytes
CD45	Dako	M0701	HIER for 32 min	1:200	Leukocytes
CD79	Ventana	790-2932	HIER for 32 min	Prediluted	B lymphocytes
CD163	Cell Marque	163M-15	HIER for 64 min	1:100	Histiocytes, macrophages

[a] Heat-induced epitope retrieval (HIER) with Cell Conditioning 1 (Ventana) or pretreatment with Protease 1 (Ventana).

[b] Pan-neuronal marker.

Table 2. Clinical Features and Results of Investigations in Cases[a]

Case/ Age, y	Symptoms at Presentation	Movement Disorder	Seizures	Autonomic Instability	Cerebrospinal Fluid at Presentation	Treatment (Duration of Admission)	Outcome MRS Score	Teratoma Pathological Findings
A/19	Mania, hallucinations, seizures	Myoclonus, prolonged posturing of arms with waxy flexibility	Early generalized tonic-clonic	Early tachycardia, hypertension, pyrexia, apneic episodes	WBC 3/μL, protein 2.8 g/dL	ECT, methylprednisolone, IV immunoglobulin (1 mo)	0 at 10 mo	Cystic 1.5 × 1.2 × 1.0-cm mature teratoma, ganglioglioma[b]
B/38	Agitation, amnesia, hallucinations	Orofacial dyskinesia	Late development of medically refractory nonconvulsive status epilepticus	Early tachycardia progressing to periods of asystole, apneic episodes	WBC 161/μL, protein 2.8 g/dL	Methylprednisolone, IV immunoglobulin, PLEX, rituximab (14 mo)	2 at 2 y	Cystic 4.5-cm-diameter immature teratoma, ganglioneuroblastoma[b]
C/36	Amnesia, hallucinations	None	Early generalized tonic-clonic	Early hypoventilation, apneic episodes	WBC 19/μL, protein 2.7 g/dL	Methylprednisolone (6 mo)	0 at 3 y	Cystic 4.3 × 4.1 × 3.5-cm mature teratoma, ganglioglioma[b]
D/27	Confusion, agitation, delusions, fluctuations in level of consciousness	Orofacial dyskinesia	Early convulsive status epilepticus progressing to medically refractory nonconvulsive status epilepticus	Early hypoventilation, bradycardia, hypotension, pyrexia	WBC 422/μL, protein 5.8 g/dL	Methylprednisolone, IV immunoglobulin, PLEX, rituximab, cyclophosphamide (ongoing)	5 at 4 mo	Cystic 4.5 × 2.5 × 2.0-cm mature teratoma, ganglioglioma[b]
E/21	Agitation, hallucinations, suicidal ideation	Orofacial dyskinesia, choreoathetoid movements of upper extremities	Early generalized tonic-clonic	Early hypoventilation, apneic episodes, late development of third-degree heart block with asystole	WBC 50/μL, protein 2.5 g/dL	Methylprednisolone, IV immunoglobulin (14 wk)	6 at 14 wk	Cystic 2.9-cm-diameter mature teratoma, no central nervous system tissue identified

Abbreviations: ECT, electroconvulsive therapy; IV, intravenous; MRS, Modified Rankin Scale[14]; PLEX, plasma exchange (apheresis); WBC, white blood cell count.

SI conversion factors: To convert white blood cell count to $\times 10^9$/L, multiply by 0.001; to convert protein level to grams per liter, multiply by 10.0.

[a] Early findings are within 2 weeks of presentation, and late findings are more than 2 weeks after presentation.

[b] Histopathological classification if the tumor was found within the central nervous system.

quantify rates of cellular proliferation. Inflammatory cell lineages were characterized using stains specific for leukocyte common antigen, B cells, T cells, and histiocytes. Pathological diagnoses and classification or staging of ovarian teratomas were based on accepted critiera.[11] Abnormal neuronal elements within teratomas were described using World Health Organization terminology for neuroglial tumors,[12,13] acknowledging that this terminology was developed for the diagnosis of CNS tumors.

Statistical Analysis

Prespecified clinical and pathological information was captured for cases and controls and was summarized using descriptive statistics. Continuous numerical variables were compared using the *t* test (2 sample, assuming equal variance). Categorical data were compared using the Fisher exact test. Statistical significance was defined as $P < .05$. Statistical analyses were performed using available software (SPSS Statistics 20; IBM Corporation).

Results

Clinical Findings

The clinical features, results of selected clinicopathological investigations, and disease course are summarized for all cases with NMDAR encephalitis in **Table 2**. Cases ranged in age from 19 to 38 years. No cases had a known history of autoimmune disease or gynecological disorders. All cases reported nonspecific flulike symptoms, which preceded behavioral or personality changes and psychiatric symptoms by days. Cases C and E were initially admitted under psychiatry services for the management of psychoses. Case A developed waxy flexibility of the upper extremities with pyrexia and nonresponsiveness, for which she received 7 courses of electroconvulsive therapy, without improvement. All cases developed symptoms and signs typical of NMDAR encephalitis, including movement disorders (4 of 5), seizures (5 of 5), and autonomic instability (5 of 5), requiring management in the intensive care unit. All cases had normal structural magnetic resonance imaging of the brain. Most cases (4 of 5) had an isolated lymphocytic pleocytosis within cerebrospinal fluid, without microbiological evidence of bacterial or viral infections (herpes simplex, varicella-zoster, or Epstein-Barr). A presumptive diagnosis of NMDAR encephalitis was established in 4 of 5 of cases within 4 weeks of presentation, with cerebrospinal fluid autoantibodies against CNS NMDAR confirmed in all cases. Unilateral cystic ovarian masses were identified on magnetic resonance imaging of the pelvis and were resected early in the disease course.

Empirical immunosuppressant treatments were started at the time of clinical diagnosis of NMDAR encephalitis. Cases A and C responded to first-line treatments (methylprednisolone and/or immune globulin intravenous pentetate) and made an excellent recovery. The clinical course of case B was complicated by medically refractory nonconvulsive status epilepticus. Case E died of progressive autonomic collapse (third-degree heart block with hypotension and eventual cardiac

arrest) early in the course of illness.[9] At the time of publication, case D was continuing to receive treatment for NMDAR encephalitis in a tertiary care center. No case experienced a clinical relapse.

Pathological Findings

Teratomas resected from cases with NMDAR encephalitis and from controls without neurological dysfunction were characterized by the presence of nervous system tissue, abnormal CNS neurons, and inflammatory infiltrates (**Table 3**). Cases and controls did not differ significantly in age or the frequency with which nervous system tissue was identified (**Table 4**). Four mature and one immature cystic teratomas were resected from cases. All teratomas resected from controls were mature teratomas. Central nervous system tissue was identified within teratomas in 4 of 5 cases.

The CNS tissue found within the 3 mature teratomas (cases A, C, and D) contained mature but dysplastic neurons, exhibiting binucleation or multinucleation, with dysmorphic shape and clustering (**Figure 1**, A-D and I-P). Mitotic activity was absent in case A, rare in case D, and moderately elevated in case C, with a Ki-67 proliferative index of 10%. Abnormal neurons were observed within clusters as small as 1 mm (case A) or as large as 5 mm (case D) in diameter. These aggregates of neural tissue would have been diagnosed as gangliogliomas if found in the CNS. The immature teratoma (case B) contained a focal area of CNS tissue (Figure 1, E-H) composed of astrocytes and neural cells in various stages of maturation. Primitive cells characterized by dark nuclei surrounded by scant cytoplasm (neuroblasts) congregated around small blood vessels. These cells showed high mitotic activity, including numerous atypical mitotic figures, and were labeled by neuron-specific enolase and chromogranin A but not markers of mature neurons (Hu or synatophysin). Increasingly mature neurons were observed moving outward from the central blood vessels; accordingly, the high Ki-67 proliferative index characteristic of the inner ring of primitive cells dropped to 2% in the peripheral area of mature neurons. This focal area of neural tissue would have been diagnosed as a glioneuroblastoma if found within the CNS.

Inflammatory cell infiltrates composed of CD163-positive histiocytes and lymphocytes closely approximated dysplastic neurons in all cases. The lymphoid cells were usually organized in follicles with CD20-positive and CD79a-positive B cells at the center and CD3-positive T cells at the periphery. The neural tissue often formed a rim at the periphery of the inflammatory infiltrates (eg, case D; and Figure 1, O), suggesting that neural tissues were infiltrated and obliterated by immune infiltrates.

Reactive abnormalities were detected in 13 of 20 neuropil-containing teratomas, including ferruginated neurons (3 of 20), Rosenthal fibers (6 of 20), pigmented epithelium (5 of 20), and eosinophilic granular bodies (1 of 20) (**Figure 2**). However, no dysplastic neurons, mitotic figures, or glial nuclear atypia were identified in control teratomas. Therefore, the presence of atypical CNS neurons reliably distinguished teratomas resected from cases ($P < .001$). Central nervous system neuronal elements were closely approximated by inflammatory infiltrates in all cases containing CNS tissue (4 of 4) but not in controls (0 of 20), further distinguishing these 2 populations ($P < .001$).

Discussion

Four of five ovarian teratomas resected from cases with NMDAR encephalitis contained abnormal CNS neurons, including dysplastic neurons or proliferating neuroblasts. Inflammatory infiltrates approximating neural elements were observed more frequently in cases than controls, consistent with prior evidence.[7] However, in the cases reported herein the inflammatory infiltrates were closely related to abnormal CNS neurons. The colocalization of dysplastic neurons and inflammatory infiltrates may have important implications for the pathogenesis of teratoma-associated NMDAR encephalitis and may explain why a minority of teratomas are associated with autoantibodies against NMDAR encephalitis.

Organized dysplastic neurons have not previously been described within ovarian teratomas or in patients with NMDAR encephalitis. Had these tumors been resected from the CNS, the neuronal abnormalities would have met World Health Organization[12,13] criteria for classification as gangliogliomas (n = 3) and a ganglioneuroblastoma (n = 1). A pathological hallmark of gangliogliomas and ganglioneuroblastomas is the frequent association with peritumoral inflammation,[15] which may organize into follicles in well-differentiated ganglioneuroblastomas.[16] The greatest degree of inflammation and follicular organization is reported in patients with associated autoimmune phenomena (ie, opsoclonus-myoclonus syndrome).[17] Therefore, the abnormalities reminiscent of tumors observed within teratomas from cases may provide the nidus for an immune response causing antibody formation against tumor antigens, including the NMDAR, leading to the pathogenesis of NMDAR encephalitis. These findings build on previous evidence[7] by providing the first direct evidence that intrinsic tumoral abnormalities associate with inflammatory infiltrates in teratoma-associated NMDAR encephalitis. Our findings may also explain the preponderance of immature teratomas reported in patients with NMDAR encephalitis.[2,4,7]

In case E, neither abnormal neurons nor CNS tissue was found in the teratoma. While it is possible that abnormal neurons were present but not sectioned, we speculate that autoimmunity may have developed via alternate mechanisms. Epitopes recognized by NMDAR autoantibodies are described within the cytoplasm of normal human oocytes[18] and in many non-CNS areas.[8] Therefore, immune interactions with normal tissues could promote the development of autoimmunity. Such interactions could be further promoted by exposure to immunogenic agents, including viruses, explaining the frequently reported infectious prodrome[2,9] and the development of NMDAR encephalitis following herpes simplex encephalitis.[19-23]

Abnormal CNS cells were observed within teratomas resected from controls. However, these abnormalities were consistent with changes observed in normal neuroglial tissues

Table 3. Pathological Findings in Cases and Controls

Identifier	Age, y	Nervous System			Inflammatory Infiltrates	Abnormal Central Nervous System Neurons[a]	Comment[b]
		Central	Peripheral	Enteric			
Cases							
A	19	Yes	Yes	No	Yes	Yes	Abnormal neuronal clusters with multinucleated mature central nervous system neurons; consistent with gangliocytoma
B	38	Yes	No	No	Yes	Yes	Focal clusters of neural cells in various stages of maturation, including proliferating neuroblasts; consistent with ganglioneuroblastoma
C	36	Yes	No	No	Yes	Yes	Abnormal bands of mature central nervous system neurons, including an area with increased proliferation; consistent with ganglioglioma
D	27	Yes	No	No	Yes	Yes	Abnormal multinucleated ganglion and neurocytelike neurons, with rare mitotic figures; consistent with ganglioglioma
E	21	No	No	No	No	No	No neural tissue
Controls							
1	57	No	No	No	No	No	...
2	28	Yes	No	No	No	No	...
3	53	No	No	No	No	No	...
4	23	No	No	No	No	No	...
5	42	Yes	Yes	No	No	No	...
6	27	No	No	No	No	No	...
7	59	No	No	No	No	No	...
8	25	No	Yes	No	No	No	Normal-appearing peripheral nerve
9	31	Yes	No	No	No	No	Rosenthal fibers, eosinophilic granular bodies
10 (Right)	31	Yes	No	No	No	No	Rosenthal fibers
10 (Left)	31	No	No	No	No	No	...
11	30	Yes	Yes	Yes	No	No	...
12	27	No	No	No	No	No	...
13	21	No	No	No	No	No	...
14	32	Yes	No	No	No	No	Rosenthal fibers, pigmented epithelium
15	45	Yes	No	No	No	No	Rosenthal fibers, pigmented epithelium
16	40	No	No	No	No	No	
17	24	Yes	No	No	No	No	Abundant glial tissue with ferruginated neurons
18	33	Yes	Yes	No	No	No	...
19	38	No	No	No	No	No	...
20	32	No	No	No	No	No	...
21	29	Yes	No	No	No	No	Ferruginated neurons
22	26	Yes	Yes	No	No	No	...
23	27	No	No	No	No	No	...
24	31	No	No	No	No	No	...
25	33	Yes	No	No	No	No	...
26	38	Yes	No	No	No	No	Cerebellar-type neurons and pigmented epithelium
27	26	Yes	No	No	No	No	...
28	33	Yes	No	No	No	No	Rosenthal fibers
29	34	No	No	No	No	No	...
30	54	No	No	No	No	No	...
31	44	Yes	No	No	No	No	...
32	30	Yes	No	No	No	No	Retinal neurons and pigmented epithelium
33	38	Yes	No	No	No	No	Rosenthal fibers, pigmented epithelium, ferruginated neurons
34	41	No	No	No	No	No	...
35	29	No	No	No	No	No	...
36	35	Yes	No	No	No	No	...
37	54	No	No	No	No	No	...
38	24	Yes	No	No	No	No	...

[a] All abnormal central nervous neurons stained positive with specific central nervous system neuronal markers, Hu, and neurofilament.

[b] Ellipsis indicates no comment.

Table 4. Summary of Findings in Cases vs Controls[a]

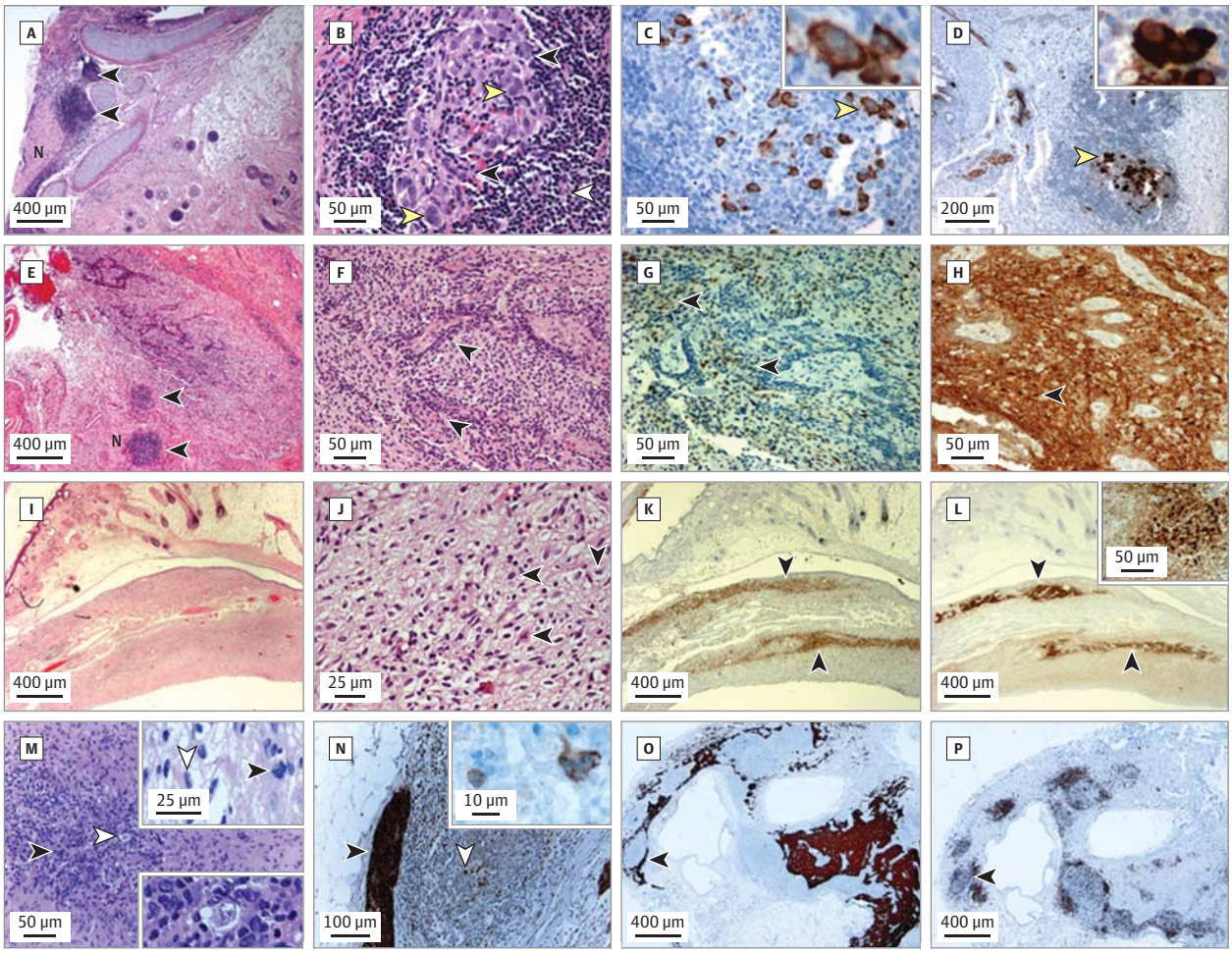
Variable	Cases (n = 5)	Controls (n = 39)[b]	*P* Value
Age, mean (SD), y	28.2 (8.6)	34.8 (10.0)	.17
Mature cystic teratoma	4/5	39/39	.11
Central nervous system tissue	4/5	20/39	.36
Peripheral nervous system tissue	1/5	5/39	.54
Enteric nervous system tissue	0/5	1/39	>.99
Abnormal central nervous system neurons	4/5	0/39	<.001
Inflammatory infiltrates[c]	4/4	0/20	<.001

[a] Data are given as number/total number unless otherwise indicated.

[b] Thirty-nine teratomas in 38 controls.

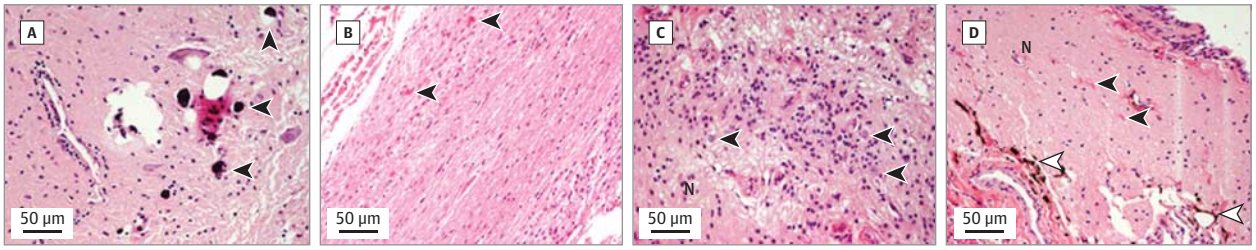
[c] Inflammatory infiltrates closely related to neuronal elements.

Figure 1. Pathological Findings in Teratomas Resected From Cases



A-D, Case A. A, Mature cystic ovarian teratoma (low power, hematoxylin-eosin) showing a small focus of neuroglial tissue (N) surrounded by chronic inflammation (black arrowheads). B, On higher power, clusters of dysplastic ganglion cells (black arrowheads) with binucleated forms (yellow arrowheads) are seen surrounded by chronic inflammation (white arrowhead). C and D, Neurons were labeled by antibodies to Hu (C) and neuron-specific enolase (D) (yellow arrowheads and insets delineate binucleated forms). E-H, Case B. E, Immature cystic ovarian teratoma (low power, hematoxylin-eosin) showing neuroglial tissue (N) surrounded by inflammation with lymphoid follicles (black arrowheads). F, On higher power, primitive neuronal elements were detected concentrated around small blood vessels (black arrowheads). G and H, Immunohistochemistry showed graded expression of Hu (G) and neuron-specific enolase (H) moving outward from the central blood vessels, labeling more mature neurons (black arrowheads). I-L, Case C. I, Mature cystic ovarian teratoma (low power, hematoxylin-eosin) showing skin (upper half) and neural tissue (lower half). J, Higher power revealed neoplastic neuroglial tissue with primitive neurons and abnormal mitotic figures (black arrowheads). K, CD163 immunostain on low power revealed a bandlike distribution of CD163-positive histiocytic infiltrates (black arrowheads) within the segment of neuroglial tissue. L, Neuron-specific enolase staining confirmed that neurons were closely approximated by the inflammatory infiltrates (black arrowheads). Higher-power view (inset) showing abnormal, primitive neurons with dendrites closely approximating one another. M-P, Case D. M, Mature cystic ovarian teratoma (medium power, hematoxylin-eosin) showing neuroglial tissue containing ganglion cells (white arrowhead and bottom inset), neurocytes, and mitotic figures (black arrowhead). The top inset shows astrocytes (white arrowhead) and mitotic figures (black arrowhead). N, On medium power, neuron-specific enolase staining identified neuropil (black arrowhead) and individual neurons (white arrowhead). The inset shows multinucleated neuronal cells labeled by Hu. O and P, On low power, comparison of glial fibrillary acidic protein immunostain (O) and CD3 immunostain (P) demonstrated the intimate association between the neuropil and the lymphocytic infiltrate. A rim of neuropil was observed surrounding the inflammatory infiltrates (black arrowheads in O and P).

Figure 2. Selected Pathological Findings in Teratomas Resected From Controls (High Power, Hematoxylin-eosin)



A, Ferruginated neurons with calcifications (black arrowheads). B, Neuroglial tissue with gliosis and Rosenthal fibers (black arrowheads). C, Neuroglial tissue (N) with unremarkable neurons (black arrowheads). D, Neuroglial tissue (N) with Rosenthal fibers (black arrowheads) and pigmented epithelium (white arrowheads). No inflammatory infiltrates were observed in association with neuroglial tissue in teratomas resected from controls.

subjected to chronic extrinsic stressors (ie, inflammation, ischemia, trauma, and toxins).[24-26] Extrinsic stressors are not known to induce dysplastic changes, including multinucleation, mitotic figures, or neuroblasts; such changes are associated with somatic or germline genomic abnormalities and are characteristic of tumors.[12,13]

This case-control study is subject to several limitations, notably the small number of cases enrolled. Despite this shortcoming, the detection of dysplastic neurons within teratomas in our sample predicted cases with NMDAR encephalitis with good sensitivity (80%) and specificity (100%). Future studies with larger cohorts are required to verify these findings and to evaluate the specificity of intratumoral neuronal abnormalities for the diagnosis of NMDAR encephalitis. One additional limitation is that study authors were unblinded to the teratoma source (ie, cases or controls) when reviewing the histopathological findings. Although suboptimal, this limitation is unlikely to have affected the histopathological interpretation because the samples were sectioned and prepared by blinded laboratory technologists before review. Furthermore, CNS neurons were observed with similar frequencies in teratomas resected from cases and controls, strengthening the assertion that abnormal neurons were characteristic of teratomas from cases and not merely overlooked in controls.

The specific abnormalities reported in this study have not previously been described in the literature. This may reflect the underrecognition of dysplastic neurons within teratomas from patients with NMDAR encephalitis. Our observations emphasize the importance of direct visualization of the cytoarchitectural organization of neuronal elements using basic tissue stains. Extrapolating from our experience, pathological assessment can be completed efficiently in teratomas resected from patients with unexplained neurological symptoms by adopting a 2-step strategy focusing first on areas containing CNS tissue and second on neurons closely approximated by inflammatory infiltrates. In centers without rapid access to autoantibody testing, it is plausible that direct histopathological assessment of teratomas could be completed before the autoantibody results are reported. In such scenarios, the colocalization of dysplastic CNS neurons and inflammatory infiltrates would support an autoimmune cause for the clinical presentation. Although the ability to formally evaluate clinicopathological associations was beyond the scope of this article, it is notable that the case with the teratoma containing the most dysplastic neurons (case B) experienced the longest inpatient admission (14 months) and the poorest recovery among cases surviving to discharge (Modified Rankin Scale[14] score of 2 at 2 years after disease onset). Autoantibody titers are highest in individuals with teratoma-associated NMDAR encephalitis[2,27] and may be highest in patients with teratomas containing markedly dysplastic neurons. The possible relationship between teratoma pathological findings and clinical and immunological findings is intriguing and worthy of prospective study among larger numbers of individuals with teratoma-associated encephalitis.

Conclusions

We report an association between dysplastic neuronal elements within teratomas resected from 4 cases with NMDAR encephalitis but not controls. Abnormal neurons were closely associated with prominent inflammatory infiltrates. Intrinsic abnormalities within teratomas may promote the development of autoimmunity in teratoma-associated NMDAR encephalitis.

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Study concept and design: Day, Laiq, Munoz.
Acquisition, analysis, or interpretation data: All authors.
Drafting of the manuscript: Day, Laiq, Munoz.
Critical revision of the manuscript for important intellectual content: All authors.
Study supervision: Tang-Wai, Munoz.
Statistical analysis: Day.

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