Toxic leukoencephalopathy caused by chemotherapeutic drugs other than methotrexate

Sung-Hye Park (shparknp@snu.ac.kr)
Seoul National University College of Medicine
Ka Young Lim
Seoul National University College of Medicine
Seong-Ik Kim
Seoul National University College of Medicine
Hyunhee Kim
Seoul National University College of Medicine
Jeongwan Kang
Seoul National University College of Medicine
Jin Woo Park
Seoul National University College of Medicine
Jae Kyung Won
Seoul National University College of Medicine
Dong-Yeop Shin
Seoul National University College of Medicine

Research article

Keywords: autopsy, chemotherapy, chemo brain, leukoencephalopathy, axonal spheroids

Posted Date: April 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-36667/v1

Abstract

Background & Objective Chemotherapy-induced toxic leukoencephalopathy is clinically characterized by progressive cognitive loss, often resulting in sudden death. The objective of this study is to share distinctive clinicopathological features of chemotherapy-induced brain change.

Methods The brains of a 64-year-old woman and a 63-year-old man who suffered from rapid deterioration of consciousness were autopsied. The initial clinical impressions were central nervous system (CNS) graft versus host disease (GVHD), infectious or autoimmune encephalitis. Both patients had been treated with multidrug chemotherapy, including cytarabine arabinoside, daunorubicin, fludarabine, azacitidine, and busulfan, and allogeneic peripheral blood stem cell transplantation because of hematological malignancy (acute myelogenous leukemia and myelodysplastic syndrome).

Results The autopsies revealed a vacuolar change of the white matter with axonal spheroids, reactive gliosis, and foamy macrophage infiltration in the brain, predominantly in the visual pathway of the occipital and temporal lobes. There was no lymphocytic infiltration in the brain tissue, which is characteristic of CNS-GVHD or encephalitis, suggesting these syndromes were not the cause of brain illness.

Conclusion The leukoencephalopathy found in our cases is often occur after methotrexate treatment, but our autopsy cases showed that it can also be caused by a regimen of chemotherapeutic drugs other than methotrexate.

Background

Chemotherapy-induced toxic leukoencephalopathy, or “chemo brain,” is clinically characterized by progressive cognitive disorder, often resulting in sudden death. Chemotherapy-related cognitive dysfunction has become an increasing concern as long-term cancer survivors are increasing dramatically. However, the exact incidence is unknown.

In 1972, Kay et al. reported eight patients with acute leukemia who developed encephalopathy associated with methotrexate therapy [1]; they were children or young adults. One of the patients was a 22-year-old man who presented with tremor, confusion, disorientation, irritability, and drowsiness after intrathecal methotrexate infusion. Some of them showed diminished irritability or improved neurotoxic symptoms with folic acid therapy [1]. One deceased patient showed multiple brain infarcts upon necropsy, but histology for disease confirmation was not conducted in the remaining cases [1].

In 1975, Rubinstein et al. described disseminated necrotizing leukoencephalopathy (DNL) after chemotherapy in four children with acute lymphoblastic lymphoma (ALL) and one child with Burkitt’s lymphoma [2]. The patients received courses of intrathecal methotrexate and whole-brain irradiation because of meningeal tumor cell infiltration. Various neurological complications had been presented, including chemical meningitis, motor and sensory losses, and encephalopathy characterized by

confusion, somnolence, ataxia, spasticity, and major seizures. These progressed to dementia, coma, and death. Rubinstein et al. reported characteristic pathological findings of DNL represented by multiple irregular foci of demyelination with spongiosis. In a severe case, acellular coagulative brain necrosis presented with reactive astrocytosis. The pathology was prominent in the white matter of the cerebrum, cerebellum, and brainstem. Furthermore, axonal spheroids were evident in the lesions [2].

In 2009, Matsubayashi et al. introduced a new type of chemotherapy-induced disseminated demyelinating leukoencephalopathy (DDL). Macroscopically, DDL is characterized by extensive fragility of the brain. Microscopically, it has widespread, demyelinated foci without significant axonal changes. These were distributed especially in the occipital lobe compared with other lobes [3].

In 2011, The European Association of Neuro-Oncology emphasized that neurological complications of chemotherapy need to be separated from central nervous system (CNS) dysfunctions caused by tumors themselves. This includes dysfunction caused by tumor metastasis, infection, or metabolic changes. Complete or partial recovery may occur after chemotherapy is completed; however, progression to irreversible damage and even death were possible outcomes as well. It is crucial to understand the toxic effects of chemotherapy on the brain and, subsequently, identify means to prevent them. The European association defined chemotherapy-induced neurotoxic complications and listed the respective causative agents. They also classified clinical syndromes caused by cytostatic drugs into 11 categories: acute (reversible) encephalopathy, subacute encephalopathy, chronic encephalopathy, posterior reversible (leuko-) encephalopathy syndrome (PRES), multifocal leukoencephalopathy, thrombotic microangiopathy, cerebral infarctions, cortical blindness, cerebellar dysfunction, seizure, and aseptic meningitis. PRES is clinically characterized by headaches, visual disturbances, confusion, seizures, and eventually coma.

We report two cases of autopsy-proven PRES that were irreversible and caused by various chemotherapy regimens other than methotrexate, including cytarabine, daunorubicin, fludarabine, and busulfan, and cytarabine arabinoside [Ara-C]. This study highlights the distinctive clinicopathological features which may help clinicians from misdiagnosing and mistreating patients with similar cases.

Material And Methods

The brain-only autopsies were conducted in two deceased patients with routine methods. The clinical differential diagnosis of the patients' brain illnesses included infectious or autoimmune encephalitis or central nervous system-graft versus host disease (CNS-GVHD), or toxic or metabolic encephalopathy. Hematoxylin and eosin (H&E) staining and immunohistochemical analysis were carried out on formalin-fixed, paraffin-embedded blocks. Primary antibodies for immunohistochemistry included NeuN (1: 100, DAKO, Glostrup, Denmark), synaptophysin (1: 200, DAKO), GFAP (1: 300, DAKO), neurofilament (NF, 1: 200, DAKO), CD3 (1: 200, DAKO), CD8 (1: 200, DAKO), CD20 (1: 200, DAKO), CD68 (1: 200, DAKO), and TMEM119 (1: 200, DAKO). Luxol fast blue (LFB) stain was carried out to determine the demyelination status. To rule out neurodegenerative disorders, immunohistochemical stains of β-amyloid (1: 200,

DAKO), phosphorylated tau (AT8) (1: 200, DAKO), α-synuclein (1: 200, DAKO), and TDP43 (1: 200, DAKO) were analyzed.

Result

Case presentation and imaging study

Autopsy performed patients were two deceased adults (64y/female and 65y/male), who shared the history of treating various chemoregimen and peripheral blood transplantation. A 62-year-old woman with acute myeloid leukemia had been administered chemotherapy, including Adriamycin, cytarabine arabinoside (Ara-C), daunorubicin (AD 7 + 3) and Adriamycin, Ara-C, idarubicin (AI 7 + 3). However, leukemia recurred 20 months after cessation of treatment, and she was diagnosed with acute undifferentiated leukemia. She was treated with FLAG induction chemotherapy (fludarabine, Ara-C, granulocyte colony-stimulating factor) and had achieved a favorable response. Intermediate-dose Ara-C #1 (IDAC) was administered for consolidation. She received a full match allogeneic hematopoietic stem cell transplant from a male donor, being registered in Korea Marrow Donor Program (KMDP). She had taken tacrolimus as GVHD prophylaxis. One month after transplantation, she became drowsy.

Mood disorder and hypoactive delirium appeared initially. The patient progressed to a subacute and chronic altered mental state. Cerebrospinal fluid (CSF) examination revealed polymorphonuclear neutrophil dominant pleocytosis and increased protein level up to 135 g/dL (cf. normal range: 15–45 g/dL). Three months after transplantation, the patient's consciousness deteriorated rapidly, but light reflexes were still intact. However, she did not respond to painful stimulations and was unable to communicate.

T2-weighted magnetic resonance imaging (MRI) showed high signal intensity in the bilateral centrum semiovale, and optic pathway with slightly decreased size of bilateral basal ganglia (Fig. 1). GVHD, toxic or metabolic encephalopathy, or encephalitis was considered as the differential diagnosis. The patient was treated with methylprednisolone and intrathecal steroid for suggested CNS-GVHD. In addition to neurological symptoms, she developed respiratory failure and shock, which appeared to be caused by sepsis and aspiration pneumonia despite aggressive use of broad-spectrum antibiotics. X/Y fluorescence *in situ* hybridization of the peripheral blood revealed 0.2% of the XX chromosome of the host and 99.8% of the XY chromosome of the male blood donor. The short tandem repeat examination showed her remnant host blood cells was 5.6%. She rapidly deteriorated, and an autopsy was performed to determine brain pathology.

The second patient (64y/male) had been diagnosed with myelodysplastic syndrome (MDS) with alcoholic liver cirrhosis and histories of multiple transfusions for anemia at other hospitals. He had been treated with bridging azacytidine #3 for MDS for three months and received busulfan-fludarabine-anti-thymocyte globulin (BuFluATG) conditioning. He received a full match hematopoietic stem cell transplant from a male donor, being registered in the KMDP. He received tacrolimus (for 13 days) as CNS-GVHD

prophylaxis. One month after transplantation, he displayed general weakness, gait disability, and fecal and urinary incontinence. Forty days after transplantation, he became drowsy and rapidly lost consciousness. After 10 days, he was unable to maintain eye contact and had a cognitive disorder with a sleep tendency. Brain MRI revealed a restrictive lesion on the left splenium of the corpus callosum, multiple T2 high signal intensity lesions at the central pons, and bilateral parietal periventricular white matter (Fig. 1).

Based on MRI findings and increased CSF protein levels (81 g/dL), CNS-GVHD or CNS infection was suggested. Steroid (methylprednisolone 1g/day for 5 days), intravenous immunoglobulin (IVIg) (400 mg/kg for 5 days), and Keppra [500 mg bid (twice a day) IV] were administered, and he recovered the ability to speak his name. However, he soon fell into coma. CNS-GVHD was suggested, and physicians started tacrolimus IV 0.04 mg/kg/day, targeting a dose of 10–20 ng/mL. Thereafter, methylprednisolone, tacrolimus, mycophenolate mofetil, intrathecal steroid, rituximab, and ruxolitinib were administered to suppress immune reaction. His consciousness worsened, and he presented with coarse respiratory sounds. Blood culture tested positive for extended-spectrum beta-lactamases (ESBL) + Klebsiella. He was diagnosed with ESBL-positive Klebsiella pneumonia and septic shock with disseminated intravascular coagulopathy. Although vancomycin was added to the broad-spectrum antibiotics regimen, he succumbed to multiorgan failure four months after transplantation.

Autopsy and pathological findings

The primary clinical information and neuropathological findings are summarized in Table 1 and Figures.

Brain autopsy of case 1 revealed symmetric global atrophy, pale parenchyma, and pale substantia nigra (Fig. 2). The brain weighed 1060 grams, and CSF was clear. Atherosclerosis was found in a short segment of the basilar artery.
Spongiform (vacuolar) change of the white matter highly suggested chemotherapy-related brain damage. Axonal spheroids, foamy macrophage infiltration, and reactive gliosis in the white matter, especially in the optic pathway of the occipital and temporal lobes, all consistent with previously reported chemotherapy-induced brain change. The final diagnosis was chemotherapy-induced PRES (Fig. 3). CD68-positive and TMEM-negative foamy macrophages were infiltrated in the cerebral white matter and the optic pathway of the occipital and temporal lobes. However, TMEM119-positive microglia were markedly reduced in the gray matter and mildly reduced in the white matter of the occipital lobe.

Mild white matter rarefaction was identified in the centrum semiovale with LFB and MBP stains. Many β-amyloid-positive diffuse-type senile plaques, but no neuritic plaques, were found in the neocortex, putamen, subiculum, and entorhinal cortex [Thal phase 2, consortium to establishe a registry for Alzheimer's disease (CERAD) stage 0). H&E stain and synaptophysin immunohistochemistry of the globus pallidus showed severe neuronal loss. Focal loss of dopaminergic neurons was identified in the midbrain, but AT8, α-synuclein and p-TDP43 were totally negative in the brain tissue, suggesting no other neurodegenerative disorders except a primary age-related tauopathy, (PART) which was confirmed with AT8 immunohistochemistry in the entorhinal cortex. There was no evidence of cerebral amyloid

angiopathy. Iron stain revealed no iron deposit in the entire brain, including the basal ganglia. Gomori's methanamine silver and Periodic acid Schiff stains revealed no fungal organisms. There was no evidence of viral or autoimmune encephalopathy.

We carefully investigated possible donor-derived inflammatory cell infiltration. However, there was no inflammatory cell infiltration on H&E stain or CD3, CD8, and CD20 immunostaining in the entire brain, which suggested against CNS-GVHD.

Brain autopsy of case 2 showed no gross abnormaltity, and the brain weighed 1310 grams. Atherosclerosis and arteriosclerosis were not identified. However, H&E, GFAP, NF, LFB, CD68, and TMEM119 stains revealed vacuolization of the white matter with diffuse axonal spheroids, reactive gliosis, and foamy macrophage infiltration in the cerebral white matter and the optic pathway of the temporal and occipital lobes. This was identical to that of case 1. GFAP revealed reactive gliosis of the entire neocortex. In CA1 and CA2 of the hippocampus and entorhinal cortex, many pTau positive neurofibrillary tangles and tau neuropil threads (Braak stage 2) were found, which was consistent with PART, but there was no evidence of other neurodegenerative disorders when analyzed immunohistochemically with AT8, α-Synuclein, β-amyloid, and TDP43.

TMEM119 was positive in the host microglia. Similar to case 1, there was no lymphocytic infiltration in the entire brain seen with CD3, CD8, and CD20 immunostaining, which suggested against a GVHD diagnosis. The final autopsy diagnosis was Chemotherapy-induced toxic leukoencephalopathy with predominant optic pathway involvement.

Discussion

Systemic chemotherapy, often with multidrug combinations, is a treatment of choice for oncological malignancy, especially for patients with hematopoietic cell malignancy. "Chemotherapy-induced toxic leukoencephalopathy" is currently a rising clinical syndrome characterized by progressive cognitive disorder and detrimental effects on the quality of life, which may lead to sudden death. However, a few data have been accumulated and most are methotrexate-induced leukoencephalopathy.

Here, two cases of autopsy-proven toxic leukoencephalopathy caused by chemotherapy regimens other than methotrexate were reported. In this way, we are able to share distinctive clinicopathologic findings and caution other physicians to make accurate diagnosis and treatment when meeting similar patients. Both patients were administered multiple courses of various chemotherapy regimens, in which fludarabine was included. Clinically, both patients presented with a neurological disorder, including cognitive disorder and motor and sensory loss, which rapidly progressed to coma and death. Brain autopsies depicted cerebral white matter spongiform change, axonal spheroids, and foamy macrophage infiltration. Infiltrating macrophages were CD68-positive but TMEM119-negative in both cases. CD3, CD8, and CD20 stains revealed no lymphocytic infiltration in both cases. No apoptotic cells were identified. Pathologic findings of these two cases were consistent with Chemotherapy-induced toxic leukoencephalopathy.[4–10]

Methotrexate is the most common drug associated with chemo brain, characterized by acute (reversible) encephalopathy, subacute encephalopathy, chronic encephalopathy, cerebral infarctions, seizure, or aseptic meningitis.[3] However, other chemotherapy drugs are also associated with toxic encephalopathy symptoms that present with the above-mentioned symptoms. Chronic encephalopathy and PRES are related to high dose multi-chemotherapies, including cyclophosphamide, Ara-C, cis-platinum, ifosfamide, vincristine, gemcitabine, and other immunosuppressants.[9, 11] Chronic encephalopathy usually develops after a latency of some months to years, often presenting progressive and irreversible clinical manifestations. PRES is clinically characterized by headaches, visual disturbances, confusion, seizures, and eventually coma.

In 2004, Lai et al. reported an autopsy-proven, methotrexate-based, chemotherapy-induced leukoencephalopathy in primary CNS lymphoma [12]. Unfortunately, treatment-related leukoencephalopathy is the leading brain pathology after the successful treatment of primary CNS lymphoma (PCNSL). Lai et al. reviewed five more autopsied patients who died of leukoencephalopathy [12]. Neurological symptoms developed at a median of 1 month after treatment completion. The median survival was 30 months (range, 22–68 months) after neurotoxicity onset. All had hyperintensity on T2-weighted MRI, and two patients presented with enhancing lesions that were observed 5 and 14 months after the treatment, respectively. The autopsy revealed no residual PCNSL. Common pathologies were myelin and axonal loss, reactive gliosis, spongiosis, and rarefaction of the white matter. Two patients had brain necrosis, which correlated to the enhancing lesions seen on MRI. Interestingly, all had small vessel disease, and four had atherosclerosis of large cerebral vessels in the circle of Willis; two had recent strokes that were discovered during autopsy. The authors concluded that Chemotherapy-induced toxic leukoencephalopathy is not always a late or delayed consequence of chemoradiation therapy but also can develop very early in some patients. They also suggested that vascular disease may be a component of this injury [12].

Our cases might be due to fludarabine, Ara-C, busulfan, and cytarabine treatment. High dose fludarabine may cause cortical blindness, which has been associated with the administration of immunosuppressive drugs, antibodies, and other substances [4]. Ara-C can induce cerebellar dysfunction and aseptic meningitis [9, 12]. Busulfan, cyclosporine, vincristine, cis-platinum, methotrexate, and paclitaxel may cause seizures [4]. The frequency and severity of CNS toxicity depend on the drug, cumulative doses, the duration of treatment, and additional risk factors such as coexisting neurological morbidity [13]. Well-known factors that increase the risk are dose escalation, combination therapy, stem cell transplantation, and irradiation of the brain [9].

In 1994, Cheson et al. reviewed the neurotoxicity of purine analogs that are widely used in indolent lymphoid malignancies, including fludarabine, cladribine, and pentostatin [14]. They compared the adverse drug effects of fludarabine in chronic lymphocytic leukemia and cladribine and pentostatin in hairy cell leukemia. The neurotoxicity spectrum of these drugs was similar, which include myelosuppression, immunosuppression, and sporadic neurotoxicity [14]. All three drugs lead to life-threatening or fatal neurotoxicity at higher-than-recommended doses. Each agent-induced neurologic

complication occurs in approximately 15% of patients at the recommended doses, mostly mild and reversible. However, severe neurologic deficits were encountered. They were occasionally delayed, often at least partially reversible, or sometimes fatal [14].

In 1994, Zabernigg first reported about late-onset fatal neurotoxicity induced by low dose fludarabine monotherapy in patients with B-cell chronic lymphocytic leukemia (CLL) [10]. A 55-year-old man developed a severe neurological disorder six months after finishing six cycles of fludarabine monotherapy. He presented with aphasia, apraxia, acalculia, hemihypesthesia, and spastic hemiparesis. A computed tomography scan of the brain showed multiple low-density areas involving the subcortical white matter. MRI showed subcortical white-matter abnormalities compatible with demyelination. Bizarre astrocytes and swollen oligodendrocytes were noted in the subcortical area. Multinuclear inclusion bodies were identified in some oligodendrocyte nuclei. The pathologic diagnosis was progressive multifocal leukoencephalopathy. Finally, he succumbed to coma and death [10]. Although a low dose of fludarabine was used, the spectrum and severity of neurotoxicity were not different from that of a high dose.

The underlying cellular mechanism is still unclear. However, some models based on clinical and animal experiments help us speculate the possible mechanism of chemo brain. Peripheral cytokines initiate the development of chemo brain [15–17]. This cytokine-mediated signaling cascade induces persistent epigenetic alterations. These epigenetic changes alter gene expression, metabolic activity, and neuronal transmission that ultimately affect cognitive function. Chemotherapy drugs cause cellular stress and injury [15–17]. This induces an inflammatory response in the periphery, releasing cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-8, IL-10, and monocyte chemoattractant protein-1 (MCP-1). Peripheral cytokines can access the brain through leaky lesions in the blood-brain-barrier or can be transported via active mechanisms.[18, 19]

It is assumed that peripheral cytokines communicate with the cytokines of the CNS through the local inflammatory network.[20] Peripheral cytokines stimulate endothelial cells and perivascular macrophages, monocytes, and T cells in the brain to produce similar local cytokines and chemokines.[18] Microglia, astrocytes, oligodendrocytes, and neurons respond by releasing additional cytokines and chemokines in the brain. As a consequence of this cascade, oxidative stress increases, neurogenesis and neuroplasticity decreases, and neuronal excitotoxicity increases.[16] These factors ultimately influence neurotransmitters and neuronal alteration [16, 21, 22]. Cytokines in the CNS are known to be mainly derived from microglia.[16, 19] Along with the strong evidence of correlating peripheral cytokines and cognitive dysfunction, microglial activation has been identified as a key factor in the reactivation of astrocytes and dysfunctional oligodendrocyte precursor cells in the previous work.[23] TMEM119 is a reliable microglial marker, as it distinguishes microglia from circulating macrophages that flow into the brain [24]. Recently, neuroimaging studies have broadened our understanding of the structural and functional changes in progressive cognitive dysfunction, showing a reduction in the frontoparietal white and gray matter [13, 25].

Conclusion

We reported two cases of autopsy-proven leukoencephalopathy caused by chemotherapy other than methotrexat. The limitation of our study is that we do not know exactly which drugs cause significant change in the brain, and have not found the underlying mechanism of chemobrain. In this way, we were able to share distinctive clinicopathologic findings and caution other physicians from misdiagnosing and mistreating patients with similar cases. Autopsies revealed a vacuolar change of the white matter with many axonal spheroids and reactive gliosis. Scattered foamy macrophage infiltration was found predominantly in the visual pathway of the occipital and temporal lobes. Staining for CD68, TMEM119, CD3, CD8, and CD20 revealed no lymphocytic infiltration in the entire brain tissue. However, exogenous foamy macrophage infiltration was present. The clinicopathologic features of these two cases were identical to previously published chemotherapy-induced leukoencephalopathy cases. Autopsies are a medical research method to determine the uncertain cause of death. However, if no evidence-based report is garnered, the cause of death could be a mystery, even though an autopsy has been conducted. Hence, our report of chemo brain is valuable.

Abbreviations

AD: Adriamycin, ara-C, daunorubicin

AI: Adriamycin, Ara-C, idarubicin

FLAG: fludarabine, Ara-C, granulocyte colony-stimulating factor

IDAC: Intermediate-dose Ara-

CNS : central nervous system

GVHD: graft versus host disease

DNL: disseminated necrotizing leukoencephalopathy

ALL: acute lymphoblastic lymphoma

PRES: posterior reversible (leuko-) encephalopathy syndrome

Ara-C: cytarabine arabinoside

GVHD: graft versus host disease

H&E: Hematoxylin and eosin

LFB: Luxol fast blue

MDS: myelodysplastic syndrome

KMDP: Korea Marrow Donor Program

CSF: Cerebrospinal fluid

CERAD: Consortium establish to registry for Alzheimer's diseasa

PART: primary age-related tauopathy

IVIg: intravenous immunoglobulin

CLL: chronic lymphocytic leukemia

TNF-α: tumor necrosis factor-α

IN: interleukin

MCP-1: monocyte chemoattractant protein-1

Declarations

Ethics approval and consent to participate

These two deceased patients donated their brain to SNUH brain bank for the medical and basic research under anonymization and the informed consents were obtained from the next of kin The institutional review board of our hospital (SNUH) approved this study (IRB No: 1808-087-966). The authors kept the privacy policy and Helsinki declaration.

Consent for publication

Not applicable

Availability of data and materials

Not appicable

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by a fund (2018-ER6202-01) by Research and Development of Korea Centers for Disease Control and Prevention.

Authors' contributions

Ka Young Lim reviewed the cases and wrote the manuscript, Seong-Ik Kim and Jin Woo Park participated the autopsies, Hyunhee Kim and Jeong Wan Kim have collected and summarized clinical data, Jae Kyung Kim reviewed the pathology, Dong-Yeop Shin had treated the patients as hemato-oncologist, and Sung-Hye Park made pathologically made pathologic diagnoses, designed and edited the manuscript.

Acknowledgements

This research was supported by a fund (2018-ER6202-01) by Research and Development of Korea Centers for Disease Control and Prevention.

Author information

Ka Young Lim[1], Seong-Ik Kim[1], Hyunhee Kim[1], Jeongwan Kang[1], Jin Woo Park[1], Jae Kyung Won[1], Dong-Yeop Shin[2], and Sung-Hye Park[1,3]

[1]Department of Pathology, Seoul National University College of Medicine, Seoul 03080, Republic of Korea

[2]Internal Medicine, Seoul National University College of Medicine, Seoul 03080, Republic of Korea

[3]Institute of Neuroscience, Seoul National University College of Medicine, Seoul 03080, Republic of Korea

References

1. Kay HE, Knapton PJ, O'Sullivan JP, Wells DG, Harris RF, Innes EM, Stuart J, Schwartz FC, Thompson EN. Encephalopathy in acute leukaemia associated with methotrexate therapy. Arch Dis Child. 1972;47(253):344–54.
2. Rubinstein JL, Herman MM, Long TF, Wilbur JR. Leukoencephalopathy following combined therapy of central nervous system leukemia and lymphoma. Acta Neuropathol Suppl. 1975;Suppl 6:251–5.
3. Matsubayashi J, Tsuchiya K, Matsunaga T, Mukai K. Methotrexate-related leukoencephalopathy without radiation therapy: distribution of brain lesions and pathological heterogeneity on two autopsy cases. Neuropathology. 2009;29(2):105–15.
4. Beitinjaneh A, McKinney AM, Cao Q, Weisdorf DJ. Toxic leukoencephalopathy following fludarabine-associated hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2011;17(3):300–8.
5. Crombe A, Alberti N, Gilles M, Guy A, De Roquefeuil E, Dousset V, Tourdias T. Extensive acute toxic leukoencephalopathy induced by fludarabine: two months follow-up on brain MRI. J Neuroradiol. 2015;42(2):127–30.
6. Mahadeo KM, Dhall G, Panigrahy A, Lastra C, Ettinger LJ. Subacute methotrexate neurotoxicity and cerebral venous sinus thrombosis in a 12-year-old with acute lymphoblastic leukemia and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: homocysteine-mediated methotrexate neurotoxicity via direct endothelial injury. Pediatr Hematol Oncol. 2010;27(1):46–52.

7. Ozutemiz C, Roshan SK, Kroll NJ, Benson JC, Rykken JB, Oswood MC, Zhang L, McKinney AM. Acute Toxic Leukoencephalopathy: Etiologies, Imaging Findings, and Outcomes in 101 Patients. AJNR Am J Neuroradiol. 2019;40(2):267–75.
8. Peddi PF, Peddi S, Santos ES, Morgensztern D. Central nervous system toxicities of chemotherapeutic agents. Expert Rev Anticancer Ther. 2014;14(7):857–63.
9. Schlegel U. Central Nervous System Toxicity of Chemotherapy. European Association of NeuroOncoly Magazine. 2011;1(1):25–9.
10. Zabernigg A, Maier H, Thaler J, Gattringer C. Late-onset fatal neurological toxicity of fludarabine. Lancet. 1994;344(8939–8940):1780.
11. Cifci Sunamak E, Ozdemir N, Celkan T. Posterior reversible encephalopathy syndrome in children with acute lymphoblastic leukemia: Experience of a single center using BFM protocols. Pediatr Blood Cancer. 2019;66(6):e27711.
12. Lai R, Abrey LE, Rosenblum MK, DeAngelis LM. Treatment-induced leukoencephalopathy in primary CNS lymphoma: a clinical and autopsy study. Neurology. 2004;62(3):451–6.
13. Scherling CS, Smith A. Opening up the window into "chemobrain": a neuroimaging review. Sensors (Basel). 2013;13(3):3169–203.
14. Cheson BD, Vena DA, Foss FM, Sorensen JM. Neurotoxicity of purine analogs: a review. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 1994;12(10):2216–28.
15. Wigmore P. The effect of systemic chemotherapy on neurogenesis, plasticity and memory. Curr Top Behav Neurosci. 2013;15:211–40.
16. Wang XM, Walitt B, Saligan L, Tiwari AF, Cheung CW, Zhang ZJ. Chemobrain: a critical review and causal hypothesis of link between cytokines and epigenetic reprogramming associated with chemotherapy. Cytokine. 2015;72(1):86–96.
17. Pearre DC, Bota DA. Chemotherapy-related cognitive dysfunction and effects on quality of life in gynecologic cancer patients. Expert Rev Qual Life Cancer Care. 2018;3(1):19–26.
18. Ramesh G, MacLean AG, Philipp MT. Cytokines and chemokines at the crossroads of neuroinflammation, neurodegeneration, and neuropathic pain. Mediators Inflamm. 2013;2013:480739.
19. Hanisch UK. Microglia as a source and target of cytokines. Glia. 2002;40(2):140–55.
20. Felger JC, Lotrich FE. Inflammatory cytokines in depression: neurobiological mechanisms and therapeutic implications. Neuroscience. 2013;246:199–229.
21. Nguyen LD, Ehrlich BE. **Cellular mechanisms and treatments for chemobrain: insight from aging and neurodegenerative diseases**. *EMBO Mol Med* 2020:e12075.
22. Gibson EM, Nagaraja S, Ocampo A, Tam LT, Wood LS, Pallegar PN, Greene JJ, Geraghty AC, Goldstein AK, Ni L, et al. Methotrexate Chemotherapy Induces Persistent Tri-glial Dysregulation that Underlies Chemotherapy-Related Cognitive Impairment. Cell. 2019;176(1–2):43–55. e13.

23. De Luca SN, Miller AA, Sominsky L, Spencer SJ. Microglial regulation of satiety and cognition. J Neuroendocrinol. 2020;32(3):e12838.
24. Satoh J, Kino Y, Asahina N, Takitani M, Miyoshi J, Ishida T, Saito Y. TMEM119 marks a subset of microglia in the human brain. Neuropathology. 2016;36(1):39–49.
25. Simo M, Rifa-Ros X, Rodriguez-Fornells A, Bruna J. Chemobrain: a systematic review of structural and functional neuroimaging studies. Neurosci Biobehav Rev. 2013;37(8):1311–21.
26. Legends.

Tables

Table 1. Primary antibodies used in our two autopsy cases for diagnosis.

Antibodies	Dilution	Company	Findings in Case 1
GFAP	1:300	DAKO, Glostrup, Denmark	+ in reactive astrocytes
NeuN	1: 500	Millipore, Temecula, USA	+ in neurons
Neurofilament (NF)	1: 2000	DAKO, Glostrup, Denmark	+ in axons and axonal spheroids
Phosphorylated NF	1: 10,000	Millipore, Temecula, USA	+ in axons and axonal spheroids
Synaptophysin	1: 100	Novocastra, Newcastle, UK	+ in gray matter
TMEM	1: 500	ABCAM, Bristol, UK	+ in intrinsic microglia
CD163	1: 200	ABCAM, Bristol, UK	+ in pigmented microglia
CD68	1: 2000	DAKO, Glostrup, Denmark	+ in exogenous macrophages
CD3	1: 100	DAKO, Glostrup, Denmark	Negative in entire brain
CD20	1: 100	DAKO, Glostrup, Denmark	Negative in entire brain
a-synuclein	1: 200	ABCAM, Bristol, UK	Negative in entire brain
β-amyloid	1: 500	Covance, Dallas, USA	Negative in entire brain
3 repeat (3R) tau	1: 100	Millipore, Ontario, Canada	Negative in entire brain
4 repeat (4R) tau	1: 1000	Millipore, Ontario, Canada	Negative in entire brain
p-Tau (AT8)	1: 100	ThermoFisher, Waltham, USA,	Negative in entire brain
p-TDP43	1: 1,000	Cosmobio, Tokyo, Japan	Negative in entire brain

GFAP; Glial fibrillary acidic protein, NeuN; Neuronal nuclei, CD; Cluster of differentiation, p-Tau; phospho-Tau, p-TDP43; phosphorylated TAR DNA binding protein

Table 2 Clinicopathological findings of two cases with autopsy-proven chemobrains

	Case 1 (A18-19)	Case 2 (A18-29)
Age/Gender	64/F	63/M
Active problem	neurological dysfunction, type I respiratory failure - d/t aspiration pneumonia, sepsis	disorientation, sepsis, & liver cirrhosis
Underlying disease	Acute myelogenous leukemia, M7 <- M4	Myelodysplastic syndrome, Refractory Anemia with Excess Blasts (RAEB-1), del (20q)
Chemtherapy-Regimen	AD (cytarabine, daunorubicin) 7+3 (2016.3.31) AI (cytarabine, idarubicin) 7+3 (16/5/3-5/9) * recur (18/2/6): acute undifferentiated leukemia FLAG (fludarabine, Ara-C) #1 (2018.2.8) – Comprete remission IDAC (intermediate dose Ara-C) consolidation #1 (18.04.30)	Bridging VIDAZA (=azacitidine) #3 (18.2.23~18.5.14.) Busulfan-fludarabine-antithymocyte globulin (BuFluATG) conditioning and alloPBSCT (D0; '18.07.17)
Autopsy finding	Leukoencephalopathy with numerous axonal spheroid and exogenous macrophage (CD68+/TMEM119-) infiltration	Leukoencephalopathy with diffuse axonal spheroids with vacuolation of white matter with exogenous foamy macrophage (CD68+/TMEM119-) infiltration
Main involving area	White matter of occipital lobe	White matter of occipital lobe
T- or B-cells	Absent in the entire brain parenchyma	Absent in the entire brain parenchyma
Survival (months)	31 from diagnosis of leukemia	9.1 from diagnosis of myelodysplasia

AD (cytarabine, daunorubicin); AI (cytarabine, idarubicin); FLAG (fludarabine, Ara-C); G-CSF (granulocyte colony-stimulating factor); IDAC, intermediate dose Ara-C (Cytarabine); PBSCT: peripheral blood stem cell transplantation

Figures

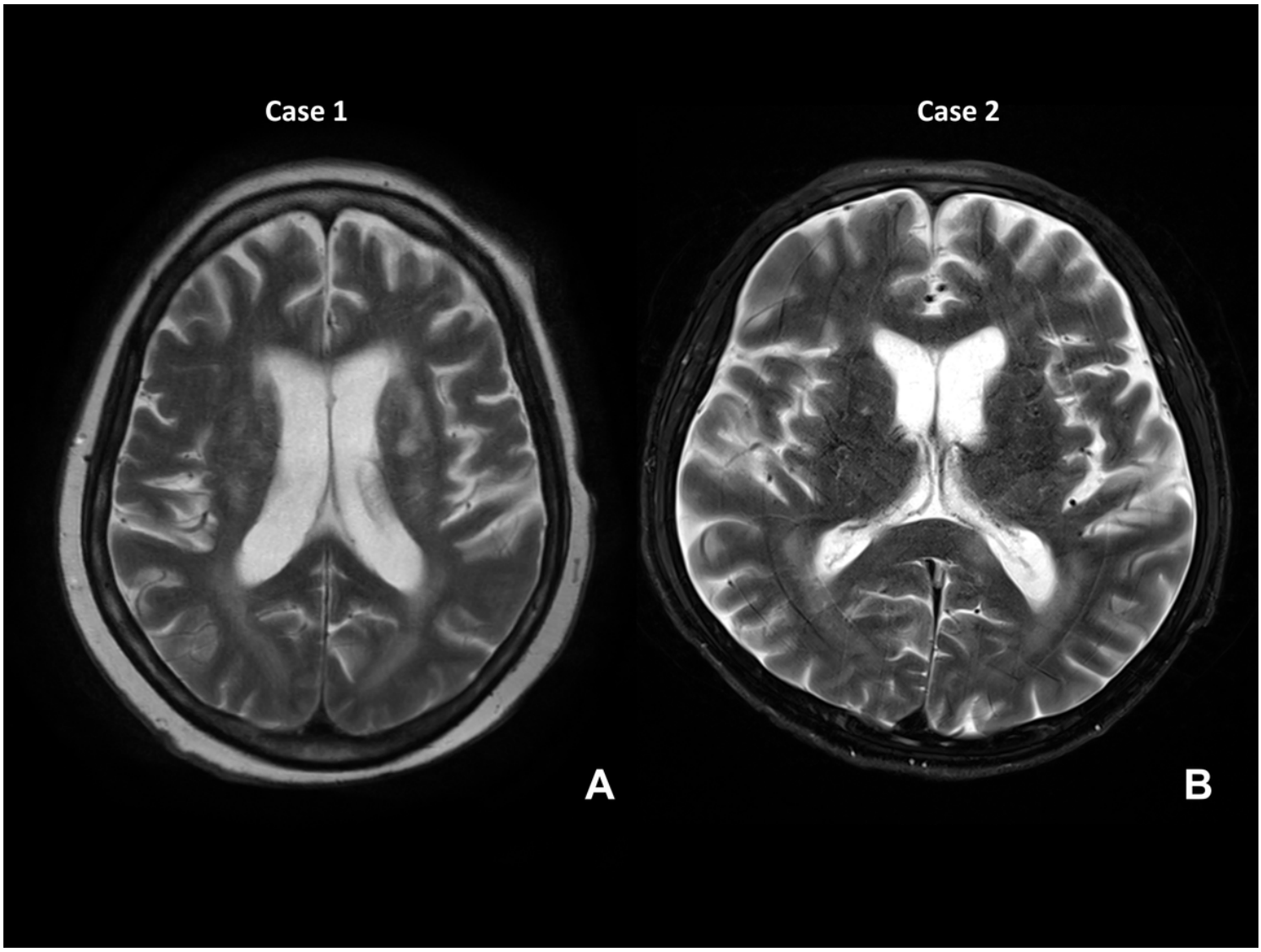

Figure 1

A) T2-weighted brain magnetic resonance imaging of case 1 reveals multiple high signal intensity lesions (arrows) in the centrum semiovale with diffuse high signal intensity of the optic pathway and central pons . B) T2-weighted image of case 2 shows high signal intensity lesions (arrows) in the centrum semiovale, in both the parietal periventricular white matter, and in the splenium of the corpus callosum (arrow).

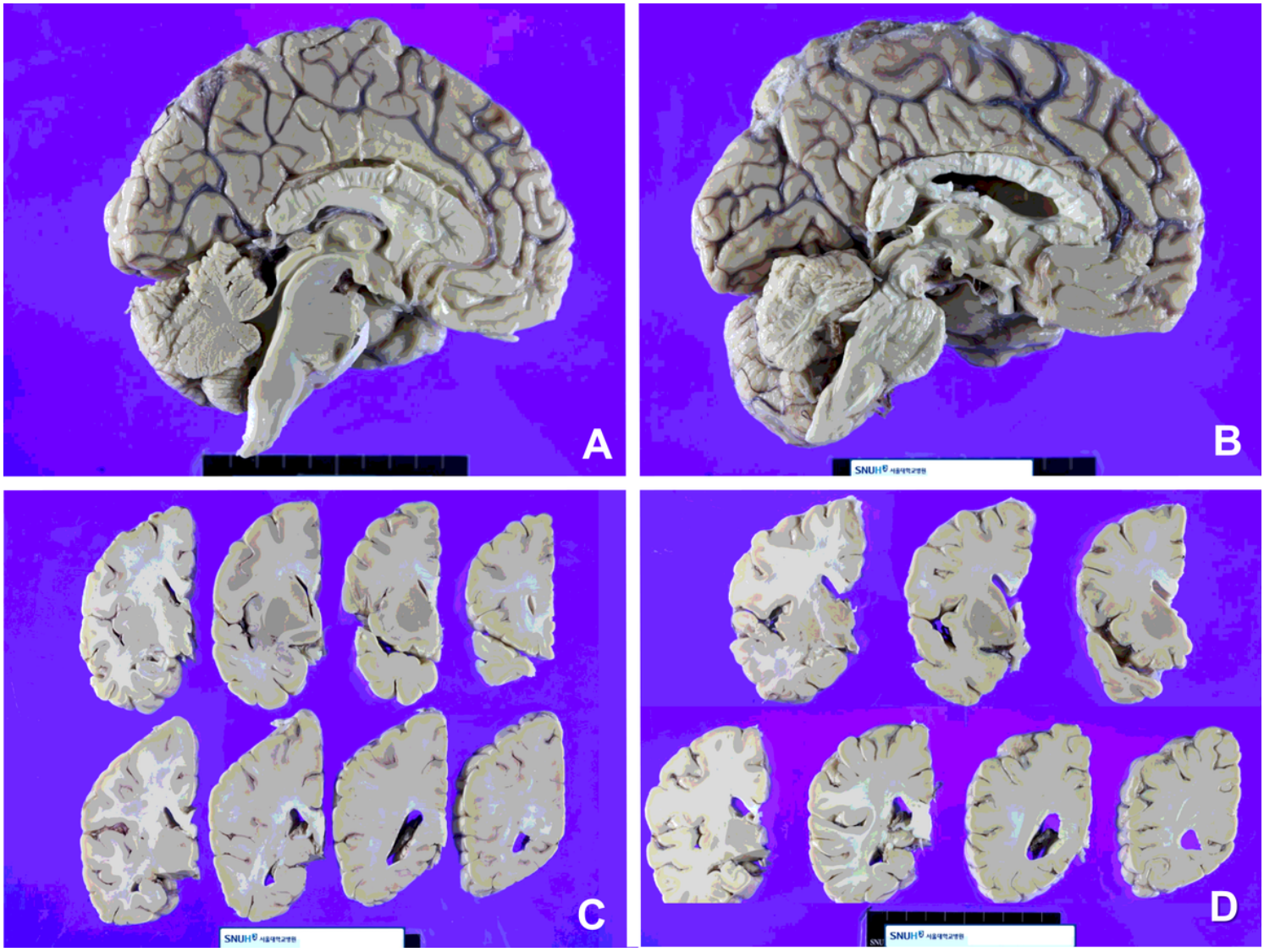

Figure 2

A-D) Gross histology of both cases reveals mild global atrophy. The brain in case 1 weighs 1060 grams and in case 2 weighs 1310 grams. The cut surface of the brain shows irregular discoloration of the white matter (arrows) (A, C: Case 1, B, D: Case 2).

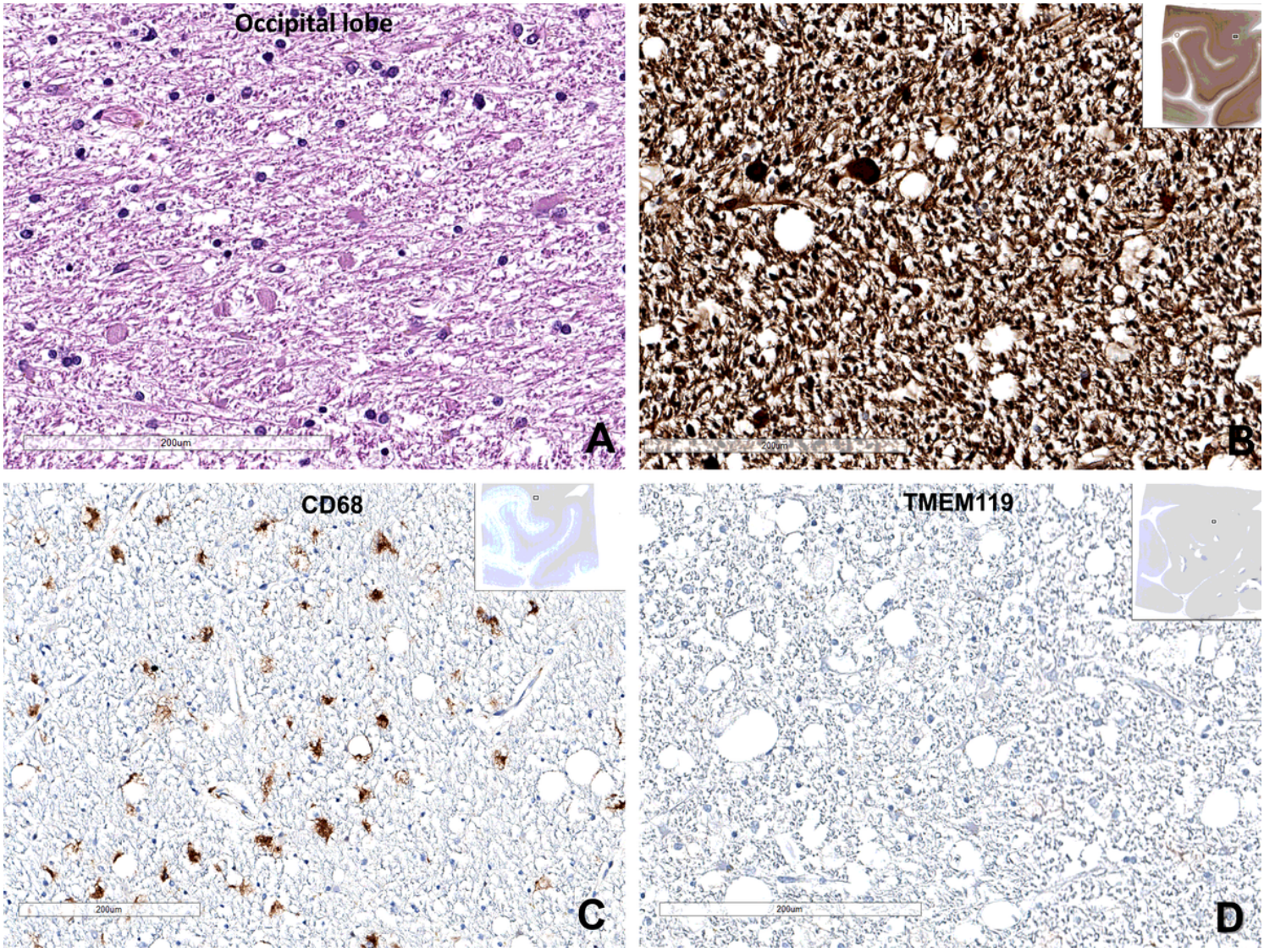

Figure 3

A) Hematoxylin and eosin stain of the optic pathway of the left occipital lobe in case 1 reveals multiple axonal spheroids (arrows). B) Neurofilaments stain reveals several oval-to-round axonal spheroids with the vacuolar change of the optic pathway of the left occipital lobe. C) CD68 stain reveals many CD68-positive foamy macrophages in the optic pathway of the left occipital lobe. D) TMEM119 stain reveals complete loss of microglia in the optic pathway of the occipital lobe (Bar A-D: 200 micrometers).

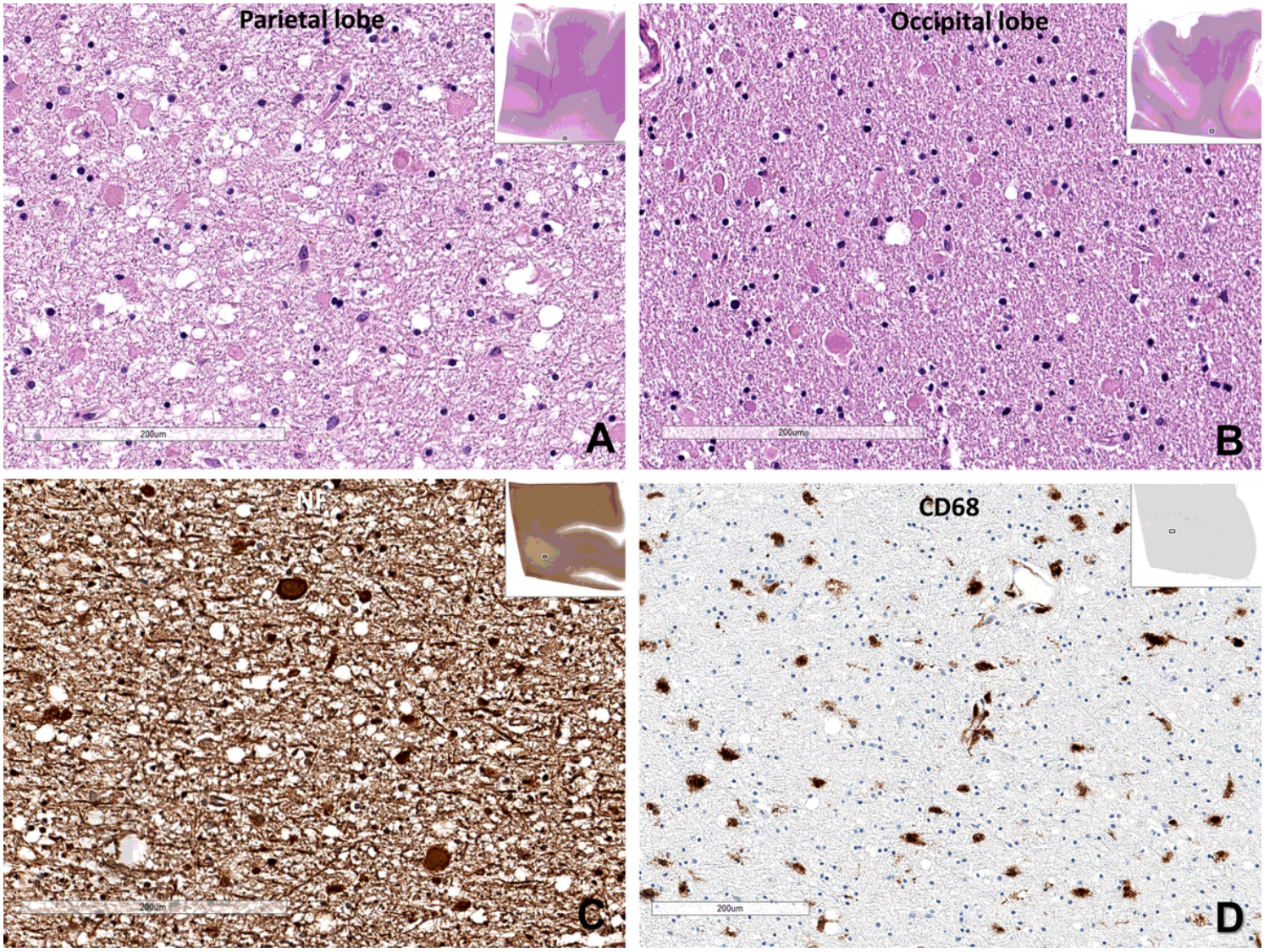

Figure 4

A) The left inferior parietal lobe and B) the left inferior temporal lobe show many variable sized axonal spheroids (arrows) with the vacuolar change of the white matter. C) The optic pathway of the left occipital lobe shows oval shape neurofilaments-positive axonal spheroids (arrows). D) The white matter of the left motor cortex shows scattered CD68-positive large macrophages (Bar A-D: 200 micrometers).