Four cases of progressive multifocal leukoencephalopathy in iatrogenic immunocompromised patients

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Introduction

Progressive multifocal leukoencephalopathy (PML) is a severe demyelinating disease of the central nervous system (CNS) caused by the reactivation of a Polyomaviridae family virus, the John Cunningham Virus (JCV). The disease occurs almost exclusively in immunocompromised individuals, being the most common predisposing factor in HIV-induced immunodeficiency [1-7], but it has also been described in non-HIV immunosuppressive diseases, in minimal immunodeficiency conditions, in patients with occult immunodeficiency, and in patients who undergo transplantations and/or immunosuppressive treatments [3-9]. Clinical, radiological, and laboratory findings, including magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) analysis, and/or biopsy with polymerase chain reaction (PCR) amplification of JCV-DNA, are necessary to establish the diagnosis of PML. No specific treatments are available.

In recent years, the highly active antiretroviral therapy (HAART) improved the immunological status of HIV patients, reducing the risk of opportunist infections [4, 7, 10-11]. Despite HAART effect on PML incidence remains controversial [4], the increasing diffusion of immunosuppressive treatments and transplantations [12] has produced a growth in the PML incidence among non-HIV patients [4, 7, 10]. We reported four cases of PML characterized by iatrogenic immunosuppression due to different treatments.

Case 1

A 51-year-old woman, affected by highly aggressive MS diagnosed in 1993, started treatment with Natalizumab in December 2010. She had previously been treated with Interferon beta-1a, Azathioprine, Mitoxantrone, and Metotrexate, none of which had demonstrated efficacy. In November 2011, JCV-DNA in urine sample resulted negative, however, in April 2012, she tested positive for antibodies against JCV. In September 2012, few days after the 21° Natalizumab

infusion, she presented an acute clinical worsening, characterized by dysarthria, diplopia, weakness in right limbs with difficulty in ambulation, and memory impairment, so she was admitted to our Neurological Clinic. Mini-mental state examination showed a minimal cognitive impairment (raw score 26, corrected score 24.89). The brain MRI detected neither new lesions nor active ones, and CSF analysis revealed a normal chemical-physical panel and tested negative for cultural and molecular analyses for bacteria, viruses, including JCV, fungi, and parasites. The clinical worsening was attributed to MS relapse and steroid therapy with Methylprednisolone 1 g daily for 5 days was started with clinical benefits. At the discharge, Natalizumab was suspended. One month later, the MRI detected a new frontal lesion involving U fibres, which appeared hyperintense on T2-weighted (T2w) imaging and showed diffusion restriction on diffusion-weighted imaging (DWI) (Figure 1). Simultaneously, the patient complained of a progressive clinical worsening so in December 2012 she was re-admitted to our Neurological Clinic. The patient presented sleep and behavioural disorders, cognitive impairment, diplopia, dysarthria, dysmetria, and spastic-ataxic gait with two walking aids (EDSS 7.5). Lymphocyte subpopulations were normal and brain MRI detected multifocal white matter (WM) lesions in temporal lobes, left cerebellar peduncle, and subthalamic region. The lesions had increased signal on T2w and fluid attenuated inversion recovery (FLAIR) sequences, diffusion restriction on DWI, and inhomogeneous contrast enhancement. The MR spectroscopy (MRS) revealed a decrease of N-Acetyl-Aspartate (NAA) and an increase in myo-inositol (mI) and lactates. Clinical and radiological findings were highly suggestive of PML, and a second CSF analysis confirmed the presence of JCV-DNA (294 genomic copies/mL; cut-off at 150 genomic copies/mL). An off-label therapy was started with Mirtazapine 30 mg daily and Mefloquine 250 mg/tid. Considering the radiological inflammatory pattern, the patient underwent 5-day steroid treatment with Methylprednisolone 1 g daily followed by oral steroid tapering and further MRI showed a regression of the inflammation. No clinical improvement was registered. At discharge the patient was admitted to a Neuro-rehabilitation Institute. In April 2013, the

behavioural disorders had improved, but the remaining neurological features were unchanged (EDSS 7.5). MRI showed an enlargement of the frontal lesion whose posterior border had diffusion restriction on DWI; contrast enhancement appeared at T1-weighted (T1w) imaging (Figure 2). Mirtazapine therapy was suspended in May 2013, while Mefloquine was interrupted in September 2013. In the same period, the patient suffered a generalized seizure and a follow-up MRI showed a reduction of the right frontal hyperintensity on T2w images and a severe atrophy; the remaining findings appeared unchanged. Since September 2012 she is not in immunoactive therapy and in May 2017 she presented an EDSS of 7.0.

Case 2

A 69-year-old woman affected by type 2 diabetes and hypertension was diagnosed with abdominal follicular lymphoma G2 stage IVB bulky in June 2015 and underwent chemotherapy with Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone (CHOP) every 21 days, and Rituximab 375 mg/m^2 before cycle 1, 2, 3, and 5. The last cycle of therapy was administered in March 2016. In July 2016, she started complaining of headache, paraesthesia, and motor deficit at left upper limb and was admitted to another Hospital. Brain MRI revealed multiple gliotic lesions without contrast enhancement. However, after the discharge the patient developed behavioural disorders and a second MRI showed a diffuse increase of signal in the juxtacortical WM in right temporal, occipital, parietal, and frontal lobes with minimal contrast enhancement. In August 2016, she was unable to maintain the upright position and presented a left hemiplegia, so she was admitted to our Neurological Clinic. The MRI revealed a bilateral increase of signal on FLAIR sequences with DWI restricted diffusion exclusively on the right hemisphere. The findings were suggestive of an active pathological process in the right hemisphere and a chronic progressive cerebrovascular disease on the contralateral hemisphere (Figure 3). The MRS showed a decrease of NAA and an increase of choline (Cho) (Figure 4). Further MRI revealed a

progressive widen of WM lesions with a punctate pattern on T2w (Figure 5) and FLAIR sequences, and the appearance of necrotizing areas. Minimal mass effect and contrast enhancement were observed. Clinical and radiological findings were suggestive of PML. The CSF analysis showed hyperproteinorrachia (754 mg/L) and hyperglycorrhachia (121 mg/dL) and was weakly positive for JCV-DNA; the remaining cultural and molecular analyses and PCR amplification for viruses, fungi, and parasites were negative. The autoantibody screening was uninformative. Lymphocyte subpopulation analyses were abnormal, showing a reduction in CD4+ T-cell (20%) and CD19+/CD20+ B-cells (0%) percentage and an increase in CD8+ T-cells (58%; CD4+/CD8+ ratio 0.34); the absolute value was considerably lower due to the previous oncological therapy. The patient referred reduction in visual acuity, so visual evoked potentials and ophthalmologic examination were performed, which revealed a severe increase of latency in P100 and a bilateral visual acuity of 1/10 in the absence of ophthalmological disease. These findings confirmed the neurological aetiology of the disorder. Several electroencephalographic exams recorded a bilateral no-specific slowing-down activity. Radiological and serological findings excluded a neoplastic relapse, so diagnosis of PML was confirmed and the patient underwent treatment with Mirtazapine 30 mg daily. No further treatment was introduced considering the comorbidity. The patient showed a progressive worsening and, at discharge, she was almost blind, unable to maintain the upright position, and affected by a left hemiplegia. She died in November 2017 due PML-related sequelae.

Case 3

A 57-year-old woman was diagnosed with Hodgkin’s lymphoma in April 2010 and underwent chemotherapy with Adriamycin, Bleomycin, Vinblastine, and Dacarbazine (ABVD) from June 2010 to December 2010. In September 2011, she developed a neoplastic relapse and was treated with Ifosfamide, Gemcitabine, and Vinorelbine (IGEV) and autologous stem-cell

transplantation (February 2012). In May 2012, a new neoplastic relapse was diagnosed, so in July 2012 the patient underwent radiotherapy and chemotherapy with Bleomycin, Etoposide, Adriamycin, Cyclophosphamide, Vincristine, Procarbazine, and Prednisone (BEACOPP). Chemotherapy was suspended in October 2012 for lymphopenia (lymphocytes 300/mmc). In June 2013, the patient started complaining of postural instability and visual disorders, with an arciform superior and inferior scotoma that progressively worsened to bilateral blindness, behavioural disorders, and a minimal right upper limb motor deficit. Brain MRI performed in August 2013 revealed abnormal bilateral lesions in the WM of the occipital lobes, which appeared hyperintense on T2w imaging and hypointense on T1w ones, with neither mass effect nor contrast enhancement. The patient was so admitted to our Neurological Clinic. A second MRI showed an enlargement of the lesions involving the occipital and parietal lobes bilaterally, the appearance of cortical atrophy in primary visual area, and mass effect on pre-central and post-central gyri (Figure 6). The lesions showed diffusion restriction on DWI (Figure 6) and apparent diffusion coefficient (ADC) imaging. The MRS revealed decreased NAA with a peak of Cho. In September 2013, the lesions appeared enlarged and characterized by necrotic processes (Figure 7). A total-body computerized tomography scan tested negative for neoplastic relapse. Clinic, anamnestic, and radiological data were suggestive of PML and the diagnosis was confirmed through the detection of JCV-DNA both in plasma (19,100 genomic copies/mL) and urine (15,000,000 genomic copies/mL) samples. Treatment with Mirtazapine was started. No further treatment was introduced considering the severe comorbidity of the patient. At discharge the neurological examination registered postural instability, bilateral blindness, behavioural disorders, and a minimal motor deficit at the right upper limb. PML-sequelae worsen the global clinical condition of the patients, who died few months later due to diabetes-related complications.

Case 4

This case has been previously published in 2010 [13]. We reviewed clinical, laboratory and radiological data considering the possibility of a PML. A 57-year-old Caucasian man, affected by chronic HCV-related hepatitis, underwent living-donor liver transplantation in February 2006 and then started Tacrolimus 3 mg daily. In August 2006, he started complaining of mild paraesthesia and hyposthenia in the left limbs, so he was admitted to another Hospital where MRI scan showed two small no-specific lesions that were diagnosed as recent ischemic strokes. Therapy with Warfarin was started, but the patient progressively worsened and in September 2006 he was admitted to our Neurological Clinic. Physical examination revealed a severe left pyramidal hemiparesis, moderate global hypoesthesia on the left side of the body, and attention disorders with confusional episodes. Brain MRI detected a large lesion in the right hemisphere WM, extending to the lateral and medial borders of the right lateral ventricle and involving the corpus callosum. The lesion was hyperintense on T2w (Figure 8A) and FLAIR imaging, and was characterised by restricted DWI coefficient (Figure 8C) and peripheral contrast enhancement (Figure 8B). A low count of platelets and leukocytes and mild anaemia were detected. Lymphocyte subpopulation analyses showed a higher proportion of CD8+ T-cells (43%, absolute value 671/mmc) and a low CD19+/CD20+ B-cells ratio (4.4%, absolute value 69/mmc). The CSF chemistry panel was normal and both cultural and molecular analyses and PCR for viruses, fungi, and parasites were negative. Serum analysis revealed low anti-Toxoplasma Gondii IgM levels, high specific-IgG levels, and a toxo-avidity rate of 45%. Despite clinical and radiological data were not suggestive of toxoplasmosis, Tacrolimus was stopped and Sulfadiazine 500 mg/qid plus Daraprim 25 mg/qid and Ambisome 3 mg/kg/die were started, producing no improvement over the next 3 weeks. In further MRI studies (Figure 8D, 8E, and 8F), the lesion appeared progressively expanded within right frontal and parietal lobes, reaching the cortical convolutions. This enlargement was particularly evident on DWI (Figure 8F) and was

characterized by peripheral contrast enhancement (Figure 8E). Small lesions appeared in the left hemisphere and in the right pons. 18F-FDG positron emission tomography (PET) revealed a severe hypometabolism in the right hemisphere and MRS showed a peak of Cho and a reduction of the other metabolites. PCR on peripheral blood showed a low positivity for JCV-DNA, but CSF analysis tested negative. In November 2006, the patient presented left hemiplegia, hallucinations, cognitive impairment, and awareness disorder. Cerebral biopsy was performed to test the two different speculated hypotheses: PML or a primitive CNS tumour. The histological analysis showed a widespread subacute necrotising process without lympho-monocytic infiltrates, a finding consistent with the immunodeficiency. Cultures and viral PCR analysis for adenoviruses, Herpes, HIV, CMV, EBV, and Coxsackie were negative; no JCV-DNA copies were detected at two consecutive analysis of biopsied material. Valganciclovir 450 mg/tid was started and a mild improvement of mental status was registered over the following weeks. Six months after, a brain MRI showed no change in the previously described lesions but neither abnormal enhancement nor restricted protons diffusion. About 12 months later, cognitive performances had improved and the patient showed a mild strength impairment in left leg although he remained unable to walk.

Discussion

PML is a severe demyelinating disease of the CNS caused by JCV, an ubiquitous and worldwide diffuse virus whose seroconversion in adulthood is about 35–90% [3, 5, 7-8, 10]. JCV is transmitted through inhalation or ingestion, with the primary infection being predominantly asymptomatic and the virus remaining latent in the urinary tract, bone marrow, or spleen. In condition of immunosuppression [3-7], JCV reactivates, migrates to the CNS binding the surface of different blood cells, and attacks the myelin-producing oligodendrocytes, causing the lysis of glial cells and the subsequent demyelinating disease [3, 7, 10]. We reported four cases of PML

in patients with iatrogenic immunodeficiency subsequent to different immunosuppressive treatments. Case 1 was an MS patient in treatment with Natalizumab, a monoclonal antibody that prevents leukocyte trafficking through the blood-brain barrier (BBB) by binding to α4β1 and α4β7 integrin receptors. Case 2 had been previously treated with Rituximab, an anti-CD20 monoclonal antibody. Case 3 had undergone autologous stem-cell transplantation, different chemotherapies, and radiotherapy. Case 4 was being treated with Tacrolimus, a molecule which binds FK506 binding protein and inhibits calcineurin phosphatase blocking the interleukin-2 gene transcription, nitric oxide synthase, cell degranulation, apoptosis, and Type 1 T-helper cell proliferation.

Clinically, PML is a progressive, multifocal disease, characterized by a subacute onset and focal neurological deficits depending on the location of the lesions. The onset is usually characterised by visual symptoms, such as homonymous hemianopsia and cortical blindness, motor disorders, and cognitive impairments [1-3, 5]. All described cases presented motor, behavioural, and cognitive disorders at onset. In addition, case 3 presented cortical blindness at diagnosis and case 2 developed it during the first phases of the disease. Sensitive deficits, limb and gait ataxia, diplopia, and language disorders frequently develop during the first phases of the disease and gradually worsen due to the enlargement of the lesions [1-3, 5]. Patient 2 and 4 presented with sensitive disorders at onset and case 1 developed a severe cerebellar involvement, manifesting with dysarthria, spastic-ataxic ambulation, dysmetria, and diplopia. PML symptoms suggestive of a cortical disorder have been attributed to WM lesions that undercut relevant cortical areas [1-3, 5]. Patient 1 reported a generalized seizure 9 month after PML diagnosis and hallucinations appeared in patient 4 about 4 months after disease onset.

Neuroimaging represents the most useful diagnostic tool and typically show bilateral asymmetric, multifocal areas of demyelination involving the subcortical WM, more frequently in

the parieto-occipital and frontal lobes. Periventricular WM, brainstem, cerebellum, basal ganglia, thalamus, and cortical grey matter (GM) could be involved as well [7, 14-17]. MRI lesions appear hypointense on T1w images and hyperintense on T2w and FLAIR images, with borders less defined toward the WM [7, 14-18]. DWI characteristics are phase-specific: the advancing edge of the newer lesions shows diffusion restriction on DWI due to oligodendrocytes swelling and a low ADC, but progressively DWI signal decreases, ADC value increases, and Diffusion Tensor Imaging (DTI) shows an increased diffusivity with decreased anisotropy [15, 19-21]. PML lesions classically do not exhibit mass effect, nor contrast enhancement, although punctate and/or rim-like contrast enhancement has been described both in HIV-positive and HIV-negative PML patients [16-18, 22]. All our patients presented patchy and/or confluent areas hypointense on T1w images (figures 1, 6) and hyperintense on T2w and FLAIR images (figures 1, 3, 5, 6, 8). The lesions showed diffusion restriction at the advancing edge on DWI (figures 1, 2, 3, 6, 7, 8). Case 4 had contrast enhancing lesions at diagnosis (figure 8), while cases 1 (figure 1, 2) and 2 developed it subsequently, confirming that contrast enhancement could not be considered an exclusion criterion for PML.

MRS shows a decrease in NAA subsequent to neuronal loss, an increase in Cho due to cell membrane and myelin breakdown, elevated lactates, and variable mI levels, the latter generally increasing in inflammatory reconstitution syndrome (IRIS) as a marker of glial proliferation and inflammation [7, 15]. The few PET studies reported reveal a cortical hypometabolism of glucose [1, 15]. In all our patients, MRS findings were completely consistent with those previously reported. Patient 4 also underwent 18F-FDG PET that showed an hypometabolism in the right hemisphere.

CSF has generally a normal chemical-physical pattern and JCV-DNA detection through PCR amplification establishes the diagnosis [14]. In case 3 no CSF analysis was conducted, but a high-titer JCV-DNA was detected both in plasma and urine. Conversely, the presence of viral DNA in CSF confirmed the diagnosis in patients 1 and 2. Patient 4 underwent two subsequent

PCR analysis of CSF, but no viral DNA was detected despite the low-level JCV positivity in peripheral blood. Several studies have reported that a negative JCV PCR on CSF does not rule out PML [14, 23-25].

Brain biopsy remains the gold standard for the definite diagnosis [1, 14, 23-25]. It is characterised by the cytolytic destruction of glial cells in the WM resulting in multiple, bilateral, asymmetric lesions of demyelination that progressively enlarge and coalesce [1-2, 10]. The borders are characterised by JCV-infected swollen astrocytes with multilobulated hyperchromatic nuclei and oligodendrocytes enclosing enlarged densely basophilic nuclei and eosinophilic bodies containing virion particles [1-2]. Inflammation is rarely observed, except for few swollen macrophages containing lipids from myelin degradation [1-2, 10]. Nevertheless, inflammatory settings of PML have been described, in which the lesions are characterized by vasogenic edema and diffuse or focal perivascular infiltrates of mononuclear cells, predominantly CD8+ T-cells [5, 7, 10-11]. Patient 4 underwent cerebral biopsy, which showed a widespread subacute necrotising process without lympho-monocytic infiltrates. PCR amplification on cerebral tissue did not detect JCV-DNA. The presence of necrotic tissue is suggestive of no JCV active replication and a subsequent decrease of PCR sensibility could be hypothesised [2]. Moreover, JCV replication has been associated with genome mutation and sequence polymorphisms and the available test at the time of the investigation has been demonstrated to produce false negativity due to a limited set of primers and probes [14, 23-25]. Considering the clinical and radiological findings were highly suggestive of PML, the patient fulfilled the diagnostic criteria for “presumptive PML” [14].

The disease course is usually progressive and fatal, and severe neurological sequelae are described in the survivors [5, 26-27]. A low JCV burden (50–100 genomic copies/μL) in the CSF and its progressive decline, and the presence of cellular immune response mediated by CD8+ T-cells, which play a decisive role in containing the disease, are considered predictive factors of

longer survival [5, 10-11, 27]. These findings supported the hypothesis that the inflammatory immune response stabilises the disease, carrying to a better prognosis when the host immunity manages to burst an inflammatory response activating JCV-specific CD8+ cells and effectively preventing viral resurgence [3, 5, 7, 10-11, 26-27]. However, an overwhelming recovery of the immune system might lead to IRIS, a paradoxical deterioration in clinical status due to an hyper-activation of the immune system [5, 28-29]. IRIS could develop with a latency of 3-9 weeks both in HIV-patients treated with HAART and MS-patients who suspend Natalizumab: the restoration of the immune system might lead to a massive inflammation associated with the appearance of contrast enhancement, oedema, and mass effect leading to a worsen outcome [6, 11, 15]. Patient 1 developed IRIS with a long latency of about 4 months, likely due to the first steroid therapy she underwent in September 2012. A clinical improvement was observed in IRIS with the steroid therapy.

Several medications have been used to treat PML based upon anecdotal evidences of efficacy or hypothetical mechanisms of action, but none of those has shown clear clinical benefits in randomized trials or prospective studies [30]. 5HT2a receptors has been demonstrated to allow JCV internalization in glial cells in vitro [31] and Mirtazapine, a 5HT2a receptors blocker, has been used in PML determining clinical improvements in a small group of Natalizumab-associated PML patients [32]. Mefloquine is a quinolinemethanol administered in parasite infection and considered an inhibiting factor of JCV infection. Despite the small number of patients who benefit from Mirtazapine [32] and although an open-label, randomized, parallel-group study failed in detecting any efficacy in the Mefloquine-treated group [33], both the drugs are empirically used. We treated case 2 and 3 with Mirtazapine, while case 1 underwent therapy with both Mirtazapine and Mefloquine. No significant clinical improvement was observed. Patient 4 was treated with Valganciclovir and a minimal improvement was registered, but it was more likely due to the immune system reconstitution. Indeed, considering the lack of a specific

treatment for PML and its high mortality rate, restoring the host adaptive immune response remains the more efficient strategy [5, 10-11, 26-27]. We registered a better outcome in patient 1 and 4, whose immunosuppressive therapies were suspended on disease onset with a likely immune system recovery.

Conclusion

We reported four cases of PML in iatrogenic immunocompromised patients who had undergone different immunosuppressive treatments. Clinical presentations were highly suggestive of classical PML, while diagnostic findings were not completely consistent with the diagnosis: in three patients MRI showed contrast enhancement, considered atypical in PML, and in one patient both CSF analysis and biopsy failed in detecting JCV-DNA. These findings underline the importance of considering the diagnosis even in presence of atypical characteristics of disease when anamnestic and clinical data are highly suggestive of PML. We hypothesize that these atypical findings could be the results of the clinical condition leading to PML.

The disease course is usually progressive and fatal and survivors often develop severe neurological sequelae, however a recovery of the immune system seems to be the most effective treatment of PML. Indeed, patients 1 and 4 outcomes were likely due to the reconstitution of the immune system.

Concluding, several conditions could fail the diagnosis of PML and no treatment has demonstrated specific efficacy in the disease, but considering the widening diffusion of biological immunosuppressive therapies and stem-cell transplantation, and the subsequent increasing risk of PML, more studies should be conducted to improve the diagnostic and therapeutic management of patients.

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Images

Figure 1. Case 1. In October 2012, MRI showed a new frontal, hyperintense, small punctate or milky way–like lesions on T2w imaging (1A; red arrow), that appeared hypointense on T1w imaging (1C; red arrow). The lesion involved the U fibres and showed diffusion restriction on DWI (1B). There was no contrast-enhancement (1D).

Figure 2. Case 1. On April 2013, the frontal lesion had enlarged, diffusion restriction on DWI was limited at posterior border (2A), and contrast-enhancement appeared (2B).

Figure 3. Case 2. In August 2016, MRI revealed a bilateral increase of signal on FLAIR imaging (3A) with DWI restricted diffusion on the right hemisphere (3B), findings consistent with an active pathological process in the right hemisphere and chronic progressive cerebrovascular disease on the contralateral hemisphere.

Figure 4. Case 2. MRS showed a decrease of NAA and an increase of Cho in pathological areas (4A) compared to normal appearing tissue (4B).

Figure 5. Case 2. In September 2016, T2w imaging showed a punctate pattern in right hemisphere with disseminated hyperintense lesions that progressively widen.

Figure 6. Case 3. In August 2013, MRI revealed an enlargement of the lesions that involved the occipital and parietal lobes bilaterally, cortical atrophy in primary visual area, and mass effect on pre-central and post-central gyri (6A-6B-6D). The lesions presented diffusion restriction on DWI (6C).

Figure 7. Case 3. In September 2013, the lesions appeared further enlarged and characterized by necrotic processes. On DWI, diffusion restriction was produced by the swollen oligodendrocytes at the active margin: comparing MRI performed in August 2013 (7A) and September 2013 (7B), the active lesion border appeared expanded due to the diffusion of the infection (7B).

Figure 8. Case 4. MRI had a lower quality. On September 2006, MRI revealed a large active lesion extending to the lateral and the medial border of the right lateral ventricle and to corpus callosum, characterized by hyperintensity on T2w (8A), peripheral contrast enhancement (8B), and diffusion restriction on DWI (8C). In October 2006, the lesion appeared active in right frontal and parietal lobes, while the corpus callosum lesion appeared inactive, with an increased signal in T2w imaging (8D) but no diffusion restriction on DWI (8F). These findings were consistent with the necrosis revealed by brain biopsy.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Figure 1

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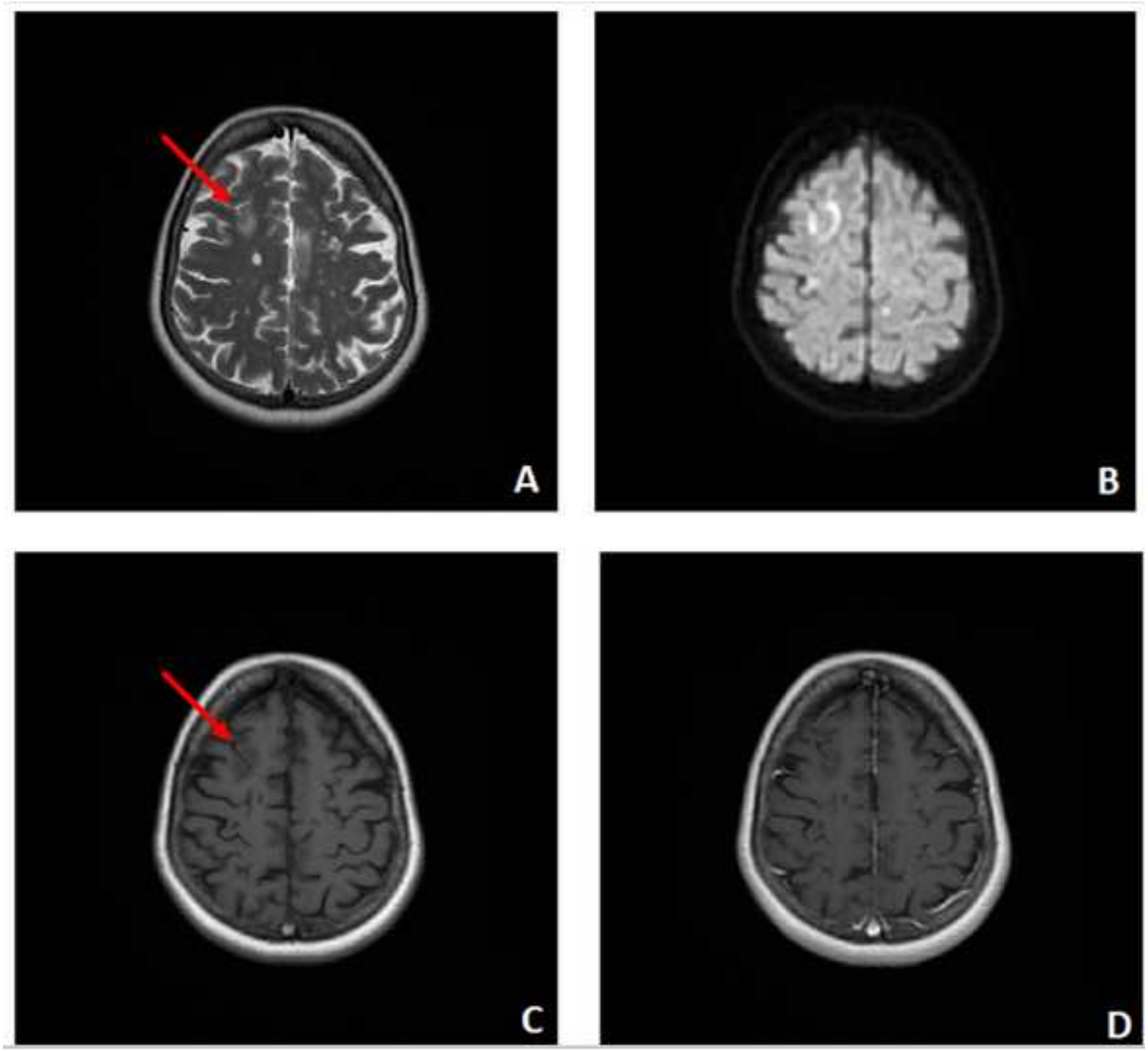

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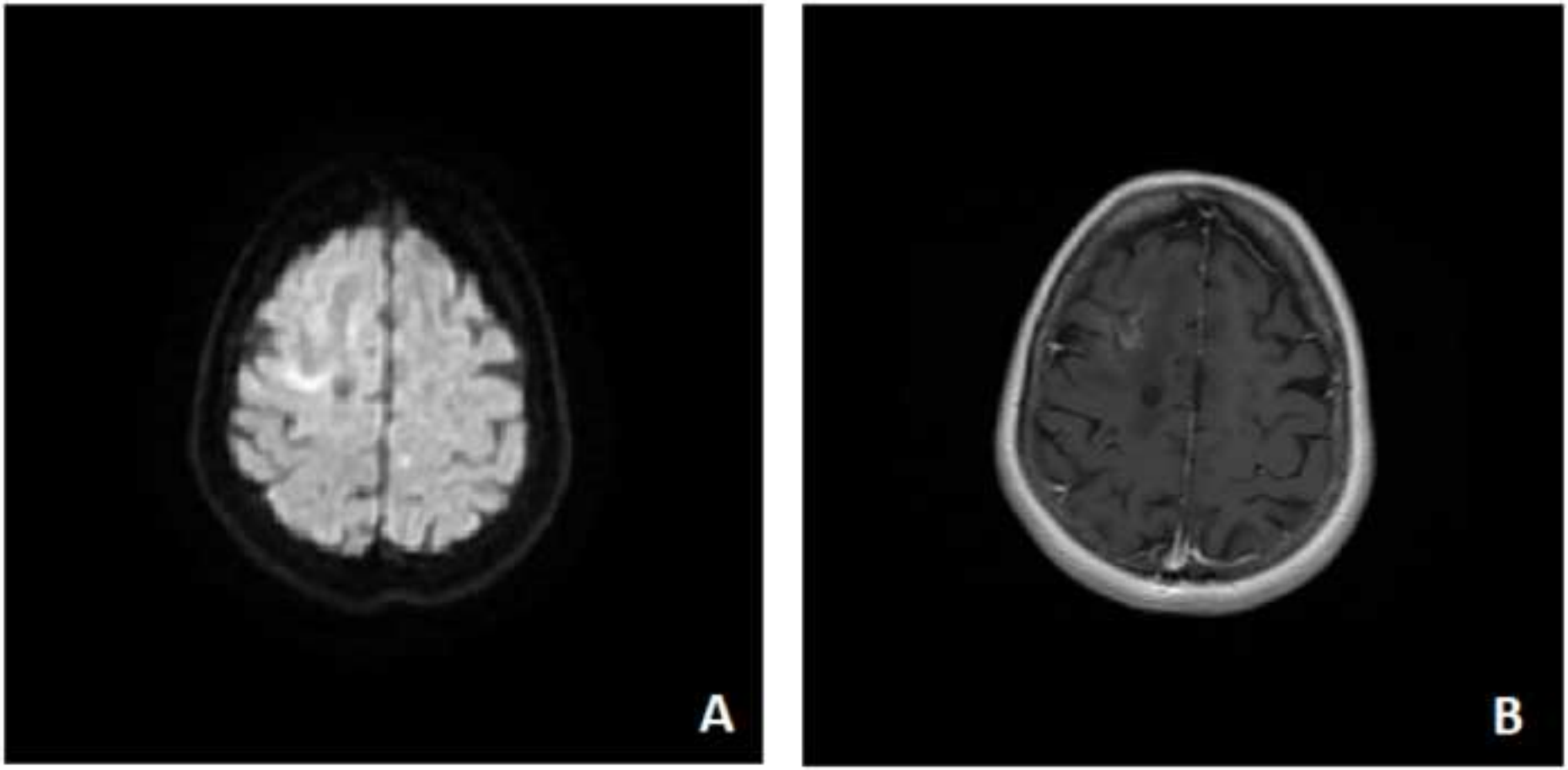

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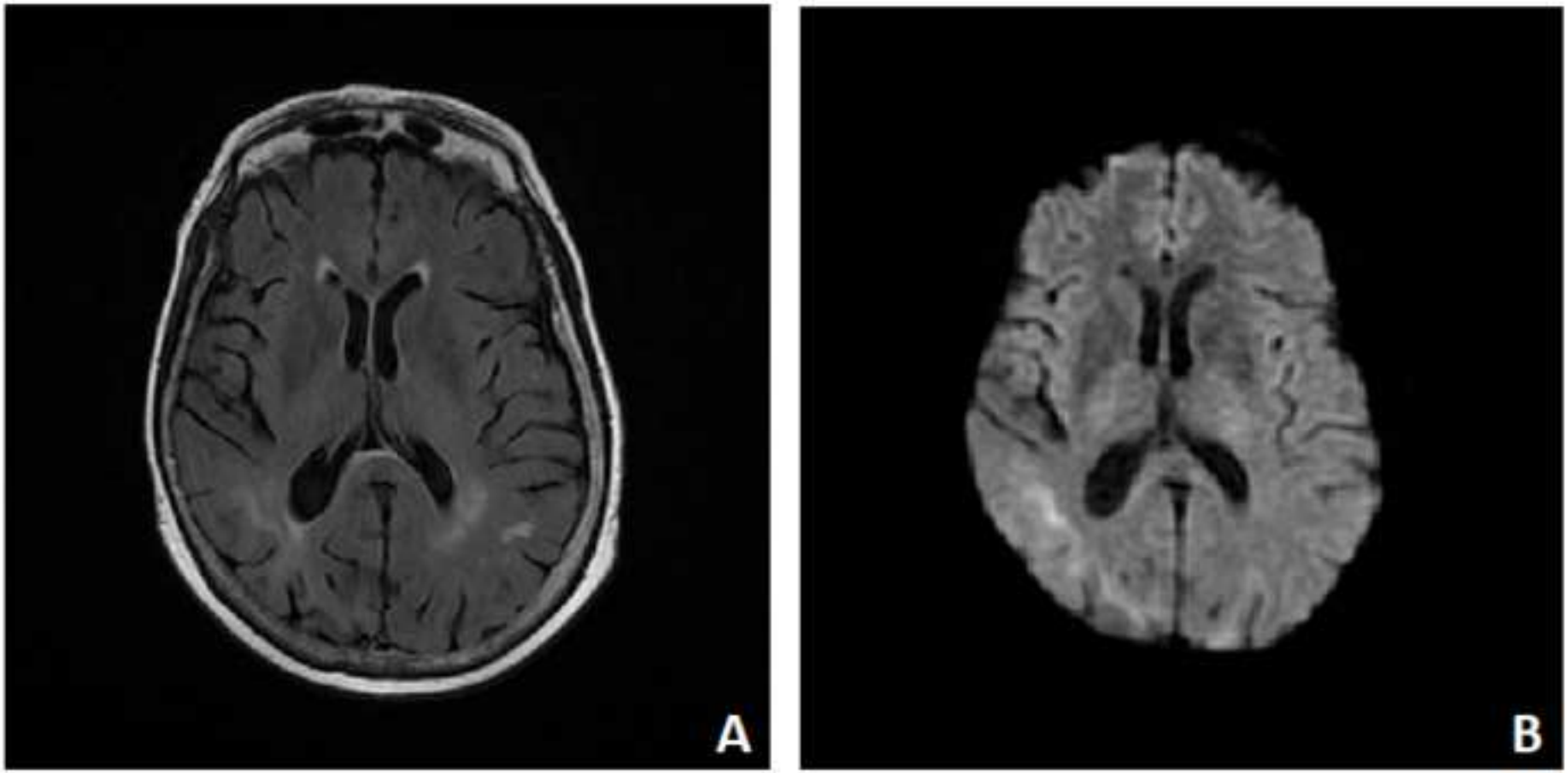

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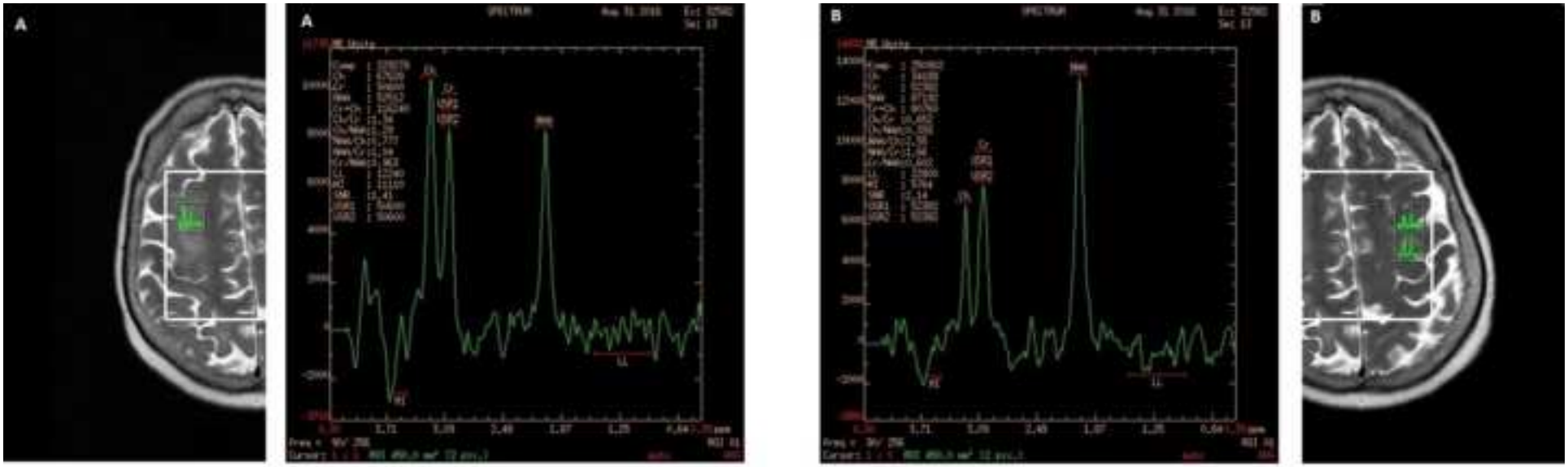

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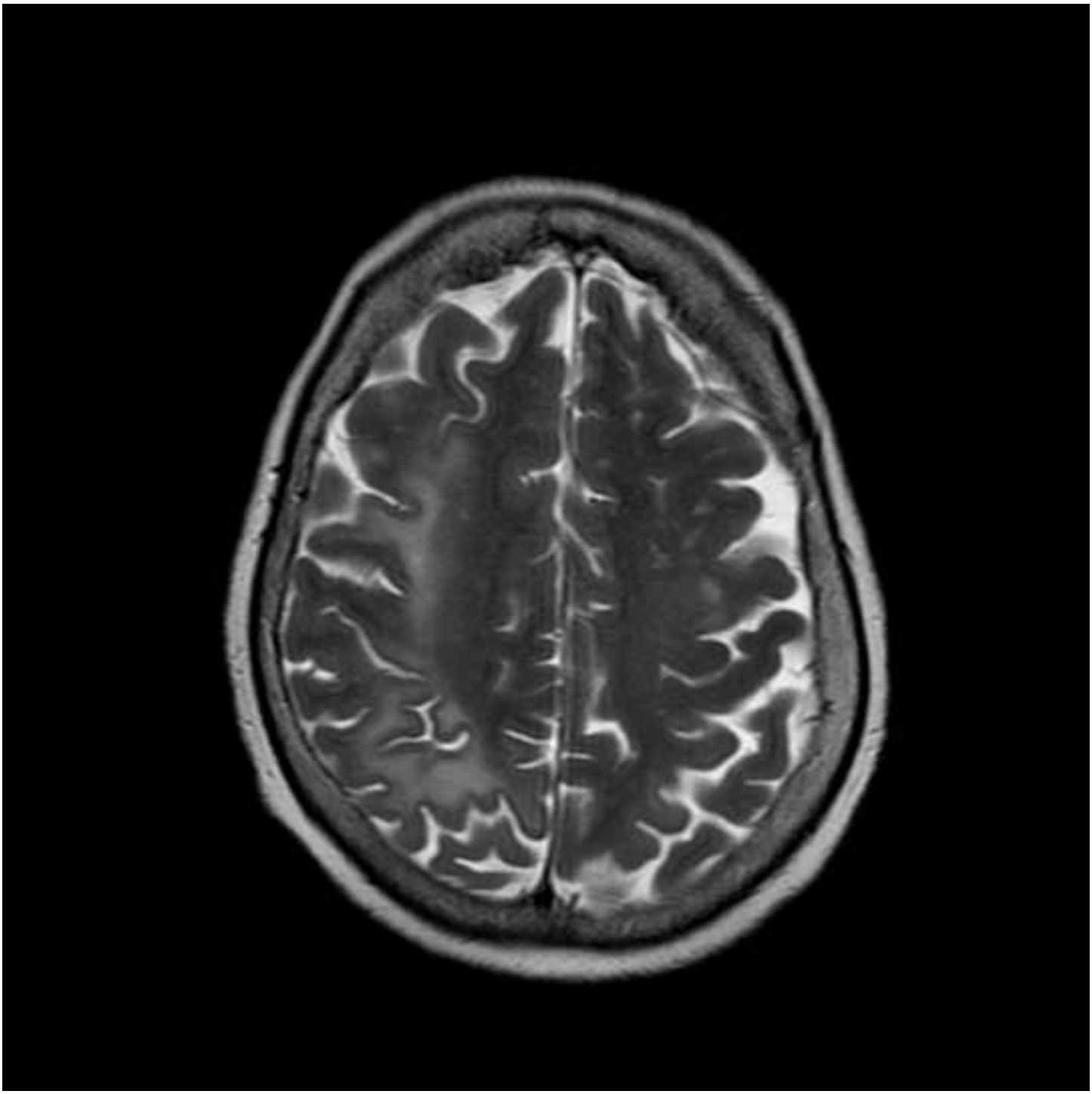

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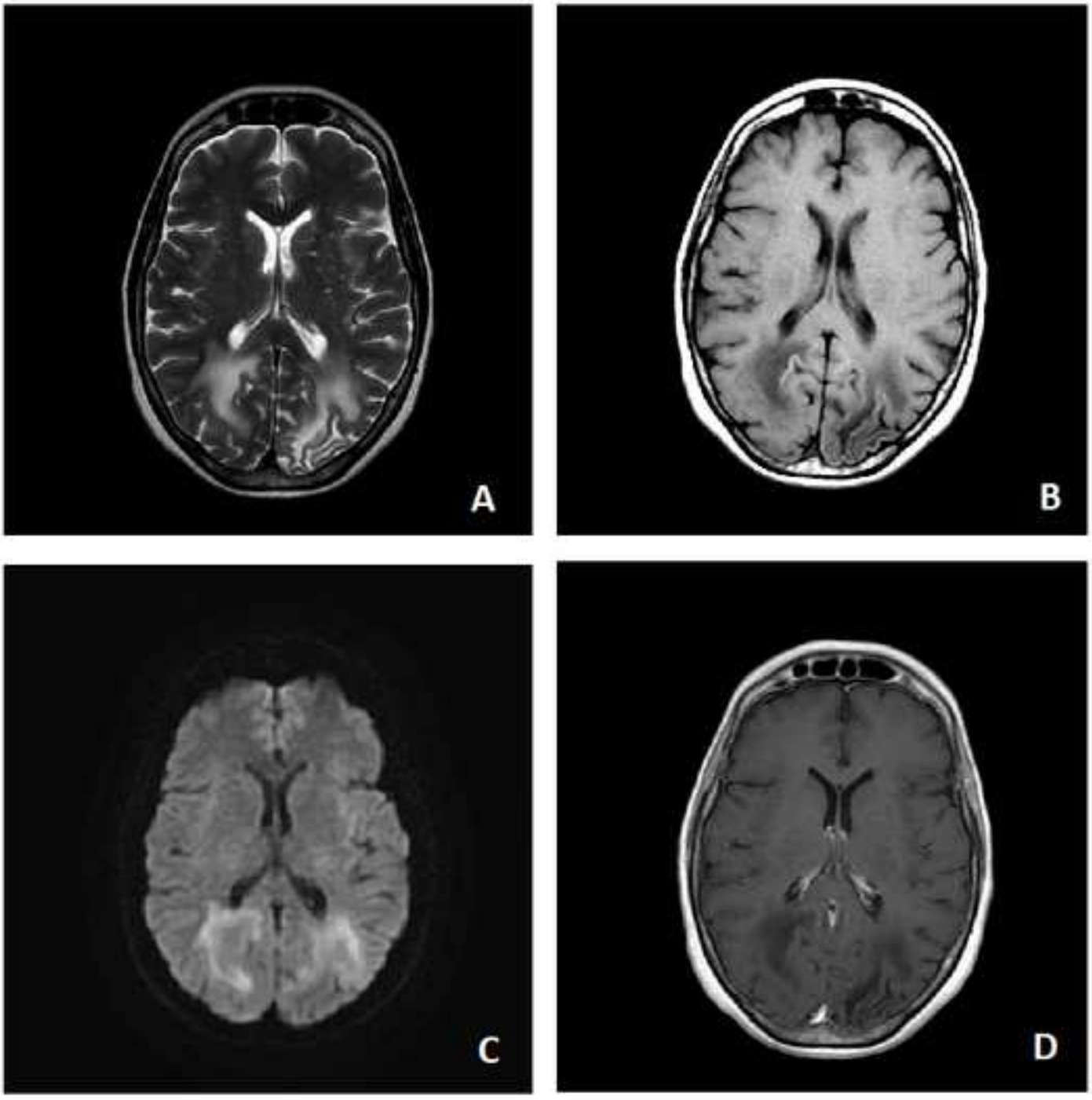

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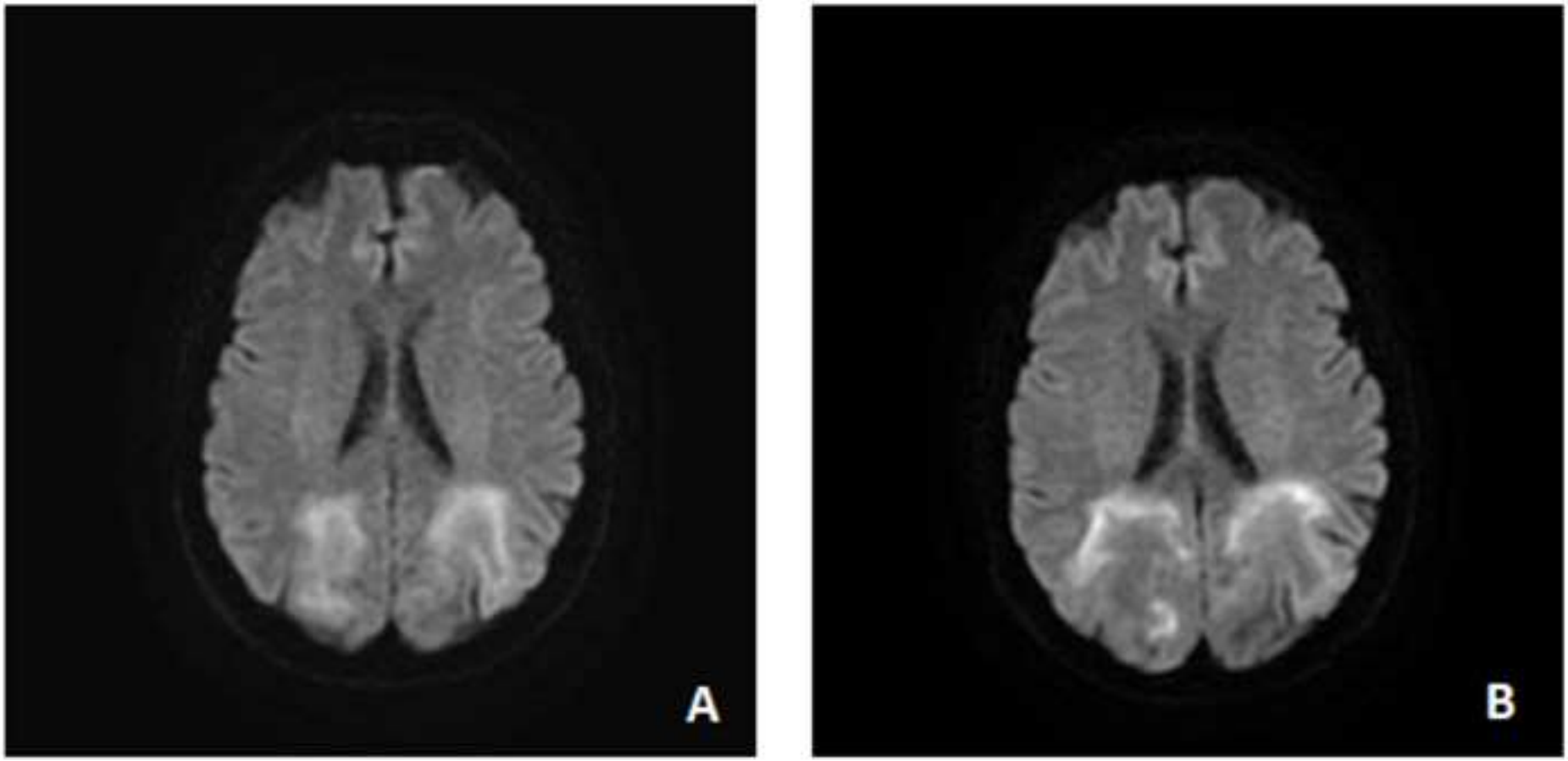

Figure 8

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