The Canadian Journal of Neurological Sciences (2025), **52**, 846–851
doi:10.1017/cjn.2025.1

Canadian Journal of Neurological Sciences
Journal Canadien des Sciences Neurologiques

Clinical Neuropathological Conference

Clinical Neuropathology Conference: "Liquid Assets"

Amirti Vivekanandan[1,*], Shervin Pejhan[2,*], Michael Strong[1], Joseph Megyesi[1], Andrew Leung[3], Maria MacDonald[1], Cynthia Hawkins[4] and Robert Hammond[2]

[1]Department of Clinical Neurological Sciences, London Health Sciences Centre, Western University, London, ON, Canada, [2]Department of Pathology and Laboratory Medicine, London Health Sciences Centre, Western University, London, ON, Canada, [3]Department of Diagnostic Imaging, London Health Sciences Centre, Western University, London, ON, Canada and [4]Department of Laboratory Medicine and Pathobiology, Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Keywords: Diffuse midline glioma; Liquid biopsy

Case Presentation: Dr Amirti Vivekanandan

A 45-year-old female began experiencing numbness with a burning sensation and paresthesias over the left side of her lips and tongue. She was initially examined a few weeks later at which time the symptoms had progressed to numbness with "pins and needles" paresthesias over the entire left face and left tongue. She also reported feeling slightly off-balance.

A month later, she felt more off-balance and experienced dizziness and nausea when turning over in bed. Her sense of imbalance continued to progress, and she described her left side as "lagging behind." She also began drooling from the left corner of her mouth and biting the inside of her left mouth, lip and tongue when eating. She denied any constitutional symptoms.

The patient had a history of Crohn's disease, requiring bowel surgery 12 years earlier. She has been treated with long-term immunosuppression, beginning with adalimumab and azathioprine, followed by adalimumab alone until 3 years ago when she was switched to infliximab.

The patient had no recent travel but visited the Caribbean 10 months earlier. She smokes cigarettes (13 pack-years total) and has approximately one alcoholic beverage per month. She works as a nurse. Medications include infliximab (600 mg IV q 6 weeks), citalopram (20 mg daily), omeprazole (20 mg daily) and zopiclone (5 mg PRN). Her mother had breast cancer, and her father had Crohn's disease.

On initial examination, the patient was alert and oriented, with normal language function. Her pupils were equal and reactive to light with no RAPD or INO noted. Visual acuity was 20/20 bilaterally, and visual fields were full. Left-beating nystagmus was present on leftward gaze. Sensation to pinprick was decreased on the left side of the face. Her left corneal reflex was absent. Facial strength was intact with no droop. Masseter strength was equal bilaterally. Temporalis strength was not tested. Her uvula and palate raised symmetrically, and her tongue was in the midline. Muscle bulk, tone and strength were normal in all limbs. Reflexes were 2+ throughout with the patellar reflexes being brisk but not pathologically so. There was no ataxia on finger-to-nose or heel-to-shin testing, but there was mild dysdiadochokinesia on the left side with rapid alternating movements. There were no sensory deficits to pinprick or vibration in the extremities. There was mild difficulty with tandem gait. Romberg was negative.

When re-examined a month later, there were noticeable changes in her neurological exam. Nystagmus was now present in all directions of gaze, and she had developed a left facial droop. Weakness was evident in her right extremities with strength rated 4−/5 in shoulder abduction, 4+/5 in elbow flexion, 4+/5 in elbow extension, 4/5 in finger abduction and 3+/5 in hip flexion. She had also developed mild dysmetria on finger-to-nose testing and ataxia in heel-to-shin testing on the left side. There was continued mild dysdiadochokinesia in the left upper extremity. Her difficulty with tandem gait was more pronounced.

Discussion: Dr Michael Strong

I have a few key questions that include knowing the patient's ethnicity and the status of her Crohn's disease. Was there anything to suggest evidence of malignancy or lymphoma, in particular, lymphadenopathy, splenomegaly or cutaneous lesions? I note the absence of any systemic symptoms (fever – especially diurnal; night sweats; weight loss).

Response to Questions: Dr Amirti Vivekanandan

The patient is Caucasian of western European descent. Her Crohn's disease was stable. There were no signs of malignancy, lymphadenopathy, splenomegaly or cutaneous lesions. Breast and rectal exams were not performed.

Discussion: Dr Michael Strong

Thank you. The history describes an individual with long-standing Crohn's disease with both surgical and immunomodulatory therapy and, in particular, TNF inhibitors since 2010 (12 years duration), who presents with a relatively rapidly evolving pathology over the course of 2.5 months. The initial symptom complex is informative and can be characterised as progressive

Corresponding author: Robert Hammond; Email: robert.hammond@lhsc.on.ca
*Contributed equally to this work.

Cite this article: Vivekanandan A, Pejhan S, Strong M, Megyesi J, Leung A, MacDonald M, Hawkins C, and Hammond R. (2025) Clinical Neuropathology Conference: "Liquid Assets". *The Canadian Journal of Neurological Sciences* **52**: 846–851, https://doi.org/10.1017/cjn.2025.1

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https://doi.org/10.1017/cjn.2025.1 Published online by Cambridge University Press

unilateral trigeminal sensory pathology, beginning with the lingual and inferior alveolar branches of the mandibular division, but then relatively rapidly progressing to all three sensory divisions. These initial findings suggest either a very proximal cranial nerve five (CN V) nerve pathology (extra-axial) or mid-pontine at the level of the CN V, where the sensory nucleus wraps around the motor nucleus. It is unusual to spare motor fibres of CN V if the lesion is intra-axial. At this point, I would be favouring (by history alone) an extra-axial pathology.

However, 1 month later, an extra-axial localisation starts to become untenable when she starts to note imbalance, vague as that may be. We now find ourselves muddling around in the brainstem as the patient begins to note positional vestibular dysfunction, which while most commonly thought of as being peripheral can be central in origin; central can fully mimic peripheral but can be due to pathology of the vestibular portion of the VIIIth cranial nerve. The examination is useful in confirming CN V sensory deficit, no evidence of ophthalmic pathology or bulbar dysfunction, but findings of left dysdiadochokinesis and left-beating nystagmus on left lateral gaze. It is this latter feature that begins to confirm that we will be dragging ourselves, however unwillingly, into the brainstem. The most parsimonious localisation becomes pontine tegmentum, laterally, involving the CN V sensory nuclei on which sits the lateral and superior vestibular nuclei as the latter abuts against the floor of the 4th ventricle. Her physical exam supports this and might begin to suggest further progression to involve the superior cerebellar peduncle (now we are just rounding the edge of the 4th ventricle with a reasonably well-described anatomy of CN V sensory, CN VIII vestibular and superior cerebellar peduncle).

We get some help here when the patient presents with left-sided drooling and a sense of the "left side dragging." Her exam at that point shows nystagmus in all directions (is this resting and gaze evoked; is there any evidence of a skew deviation; any evidence of a Horner's – the absence of all of which further supports the now seemingly secure intra-axial localisation), a complete sensory CN V, left facial droop (CN VII), right-sided weakness suggestive of pyramidal involvement (I will come back to this) with left appendicular cerebellar dysfunction and tandem gait impairment.

Response to Questions: Dr Amirti Vivekanandan

Nystagmus was gaze evoked. No skew deviation was noted. There was no evidence of Horner's syndrome.

Discussion: Dr Michael Strong

Thank you. Everything is hanging together at this point for an intra-axial pontine tegmental pathology, localised to the lateral tegmental pons, except for the weakness. How is this happening, since at that level, it is a long way from my localisation to the descending supraspinal tracts? Herein lies the importance of both flexor and extensor weakness without reflex changes. I will postulate that the observed weakness is secondary to the pathology of the superior cerebellar peduncle, which can indeed produce weakness through involvement of its efferent pathways – the so-called primitive motor pathways. The only thing left is to confirm this with MR imaging.

The differential seems straightforward enough. This is a 45-year-old female on long-term immunosuppression secondary to her Crohn's disease with a rapidly progressive focal pontine tegmental pathology that abuts against or involves the superior cerebellar peduncle. There are three broad options: it's either related to the Crohn's, to another systemic pathology, or not.

If Crohn's related, we should consider lymphoma and atypical infections (e.g. mycobacterial or fungal).[1,2,3,4] This is not vasculitic for any number of reasons, but specifically the very focal nature of the brainstem pathology with progression, and not sarcoidosis (for which her therapies for Crohn's would have sufficed as treatment). If not Crohn's related, then metastatic disease is a consideration (recall that she is a smoker, so I do want to see a chest X-ray). A breast exam would also be reasonable. There was no history of skin lesions. A urinalysis for red blood cells (RBCs) would be reasonable, and we could consider a pelvic exam or ultrasound.

If it is neither Crohn's-related nor metastatic, it is most likely a primary central nervous system (CNS) lesion, which is what I favour. It is unusual for primary glial tumours at this age to be in the brainstem, but the rate of progression supports that.[5,6,7] There is one caveat here. Over the years, we have been shown massive spinal cord and brainstem lesions with impressive oedema and neuroanatomic distortion, clearly long-standing, with the history being one of rapid progression. And we always use the analogy of the candle burning brightly until the very last moment. So, rapid progression of symptoms here does not mean rapid progression of pathology. Other neoplastic considerations seem much less likely but could include hemangiopericytoma, meningioma, schwannoma and hemangioblastoma.

In the end, I think that the underlying pathology here is localised to the left tegmental pons at the level of the superior cerebellar peduncle with involvement of the vestibular nuclei, CN V sensory nuclei, eventually CN VII as well and that the most likely pathology, although rare in this age group, is a highly aggressive primary glial tumour.

What do I want after all of this? Imaging and likely a biopsy.

Neuroradiology: Dr Andrew Leung

There are two MRIs, performed one month apart. From the first MRI (Figure 1), on the T2-weighted (A) and FLAIR (B) images, there is an infiltrative intra-axial abnormality centred on the left brachium pontis. There are small areas of extension into the pons itself. Anteriorly, there is focal extension into left CN V. Along the lateral margin of the fourth ventricle, there is involvement of the left superior cerebellar peduncle.

On the T1-weighted image with contrast, there is focal nodular enhancement within the lesion. On the DWI image, there is a mild high signal but no corresponding low signal on the ADC map, so it is not an acute stroke.

One month later (Figure 2), there has been a few millimetres of growth in every direction. The enhancement is fuller. There is also more mass effect with slight distortion of the fourth ventricle. There is no hydrocephalus.

As for the imaging differential diagnosis, tumour is at the top. Brainstem glioma is uncommon but most likely with this appearance. Another tumour on the differential would be lymphoma, which is a mimicker. It often has significant enhancement, but this can be variable.

With the story of Crohn's disease and treatment, we worry about immunocompromise. Tuberculosis is another mimicker. One other consideration in an immunocompromised patient is progressive multifocal leukoencephalopathy. Its classic description involves little to no mass effect and little to no enhancement.

The lesion involves white matter and has a slight mass effect, raising the possibility of tumefactive demyelination as a consideration. There is no diffusion restriction for an acute infarct, and myelinolysis is typically centred in the pons.

https://doi.org/10.1017/cjn.2025.1 Published online by Cambridge University Press

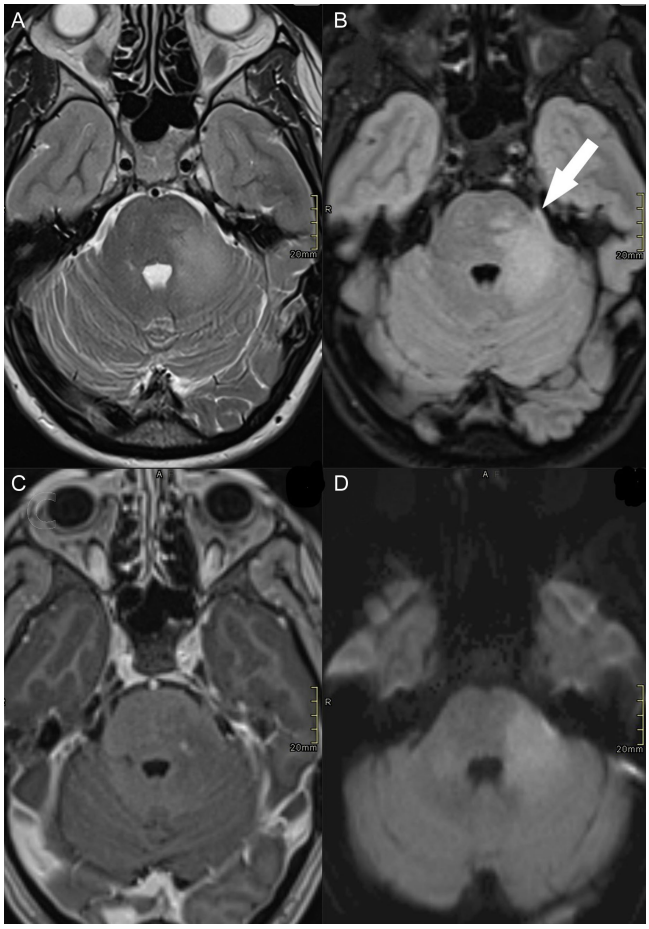


Figure 1. Initial MRI. Axial MR images demonstrate an ill-defined lesion centred in the left pons and brachium pontis. This is hyperintense on the (A) T2-weighted and (B) FLAIR sequences, with subtle infiltration of the left 5th cranial nerve (arrow). There is focal enhancement centrally on the (C) post-gadolinium T1 sequence. This has increased signal on the (D) DWI trace sequence, but no corresponding low signal on the ADC map (not shown).

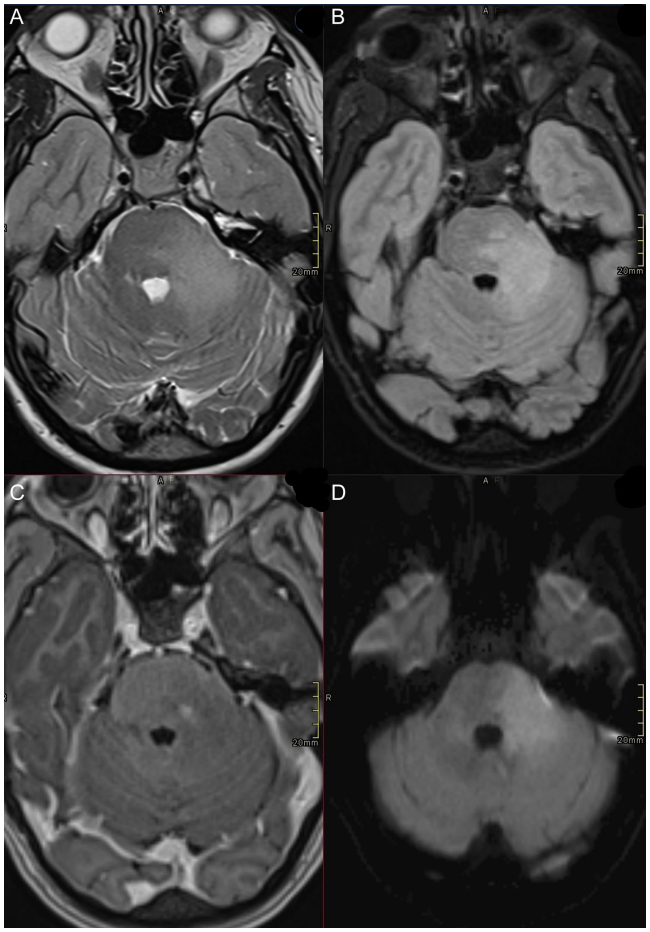


Figure 2. Follow-up MRI 1 month from initial presentation. Corresponding MRI images from the 1 month follow-up exam show mild enlargement of the lesion of the T2-weighted and FLAIR sequences. The enhancing focus has enlarged as well. The DWI trace signal has the same hyperintensity as before.

MR perfusion and MR spectroscopy were also acquired at the last exam. MR perfusion was negative. That said, the area of enhancement is rather small, and even if there was a perfusion abnormality, MRI may not be able to resolve it. MR spectroscopy (Figure 3) was very helpful. Going from left to right, the three main peaks represent choline, creatine and N-acetylaspartate (NAA). Normally, we would see "Hunter's angle," where the peaks are progressively taller from left to right. In this case, the choline peak is tallest, and creatine and NAA peaks are relatively small. This is a worrisome pattern on MR spectroscopy, compatible with a high-grade glioma.

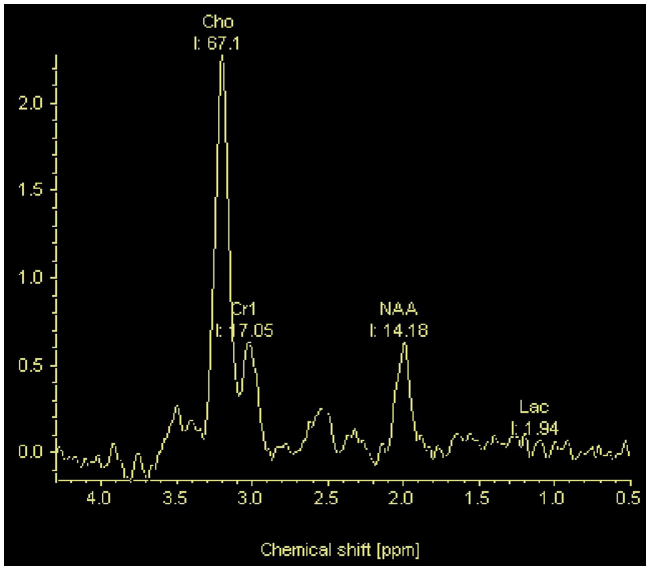


Figure 3. MR spectroscopy. Single-voxel MR spectrum (TR/TE, 1500 ms/136 ms) of the lesion from the 1 month follow-up MRI. This shows marked elevation of the choline peak relative to creatine and slightly lower N-acetylaspartate relative to creatine. This pattern is consistent with high-grade glioma.

Neurosurgery: Dr Joseph Megyesi

Performing a biopsy for a brainstem lesion is not common, although in neuro-oncology, it is becoming requested more often. I think that's because of the advances in molecular characterisation of tumours. Now that we have more information about the molecular structure of these tumours, there are more frequent requests to biopsy them.

During the work-up of this patient, there was uncertainty if this was a tumour. The concept of a liquid biopsy had been discussed at neuro-oncology conferences more frequently leading to the

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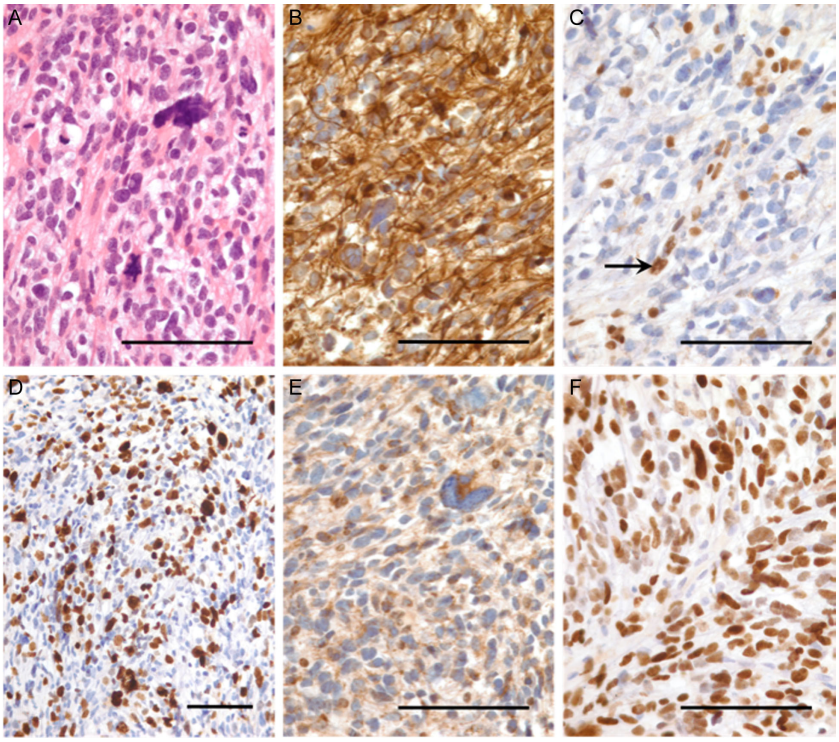


Figure 4. Neuropathology. Selected photomicrographs demonstrate the following features on routine stains and immunohistochemistry (IHC) (scale bars = 100 um). (A) A hypercellular, pleomorphic tumour with mitotic activity (H&E). (B) Abundant GFAP expression (anti-GFAP IHC). (C) Loss of ATRX nuclear expression (anti-ATRX IHC), note preserved expression among endothelial nuclei (arrow). (D) The tumour's proliferative index was high (anti-Ki67 IHC). (E) H3K27me3 nuclear labelling is reduced or absent (anti-H3K27me3 IHC). (F) H3K27M mutation is expressed (anti-H3K27M IHC).

question, "Can we come up with a diagnosis using molecular markers found in CSF or blood, so we don't have to biopsy these tumours?" CSF was ultimately sent for molecular analysis. We had a discussion with the neuro-oncologist about performing a biopsy for this lesion and decided to schedule the patient for biopsy.

We performed a stereotactic biopsy with the patient prone in a frame passing the needle into the lesion through a small craniectomy. Postoperatively, the patient had no untoward effects from the operation.

Neuropathology: Dr Shervin Pejhan

Lumbar puncture was performed before surgery after her initial presentation. The CSF contained 316 mg/L of protein, with no nucleated cell or RBCs as assessed by the cell counter. Cytological assessment of the CSF was reported as indeterminate with scattered small-appearing lymphocytes and monocytes and occasional degenerated cells. 7 mL of CSF was sent to the Hospital for Sick Children (courtesy of Dr Cynthia Hawkins) for assessment of cell-free DNA.

Cell-free DNA was extracted from CSF before surgery for examination at the Hospital for Sick Children, courtesy of Dr Cynthia Hawkins. The DNA-derived next-generation sequencing (NGS) libraries were enriched and sequenced on an Illumina NextSeq. The detected oncogenic single nucleotide variant was H3F3A (Lys28Met). The identified copy number variations (CNVs) included partial gain of 2 and 4; partial loss of 4, 5, 9, 10, 11, 13, 14, 16 and 22; and loss of 8. Platelet-derived growth factor receptor alpha amplification was also present. No fusion was detected by the automated caller.

Microscopic examination (Figure 4) of the biopsied tissue showed a hypercellular, fibrillary tumour. The tumour cells displayed marked atypia. Mitotic activity was present but no endothelial hyperplasia or necrosis. Tumour cells expressed GFAP, Olig-2 and P53. IDH1-R132H was not expressed. ATRX and H3K27 me3 nuclear expression were lost in many tumour cells. Tumour cells expressed H3 K27M.

Mutation screening was performed by NGS using the Ion AmpliSeq™ technology on the Ion GeneStudio S5 System (ThermoFisher). Library preparation was performed as per manufacturer's instructions using the Ion Torrent™ Oncomine™ Comprehensive Assay v3 (ThermoFisher). ATRX mutation ([ATRX:c.6185_6191delCAGAAGA, p.[Thr2062Ilefs*23]) was noted in 51.3% of tumour cells. Two different mutations were noted in TP53 (TP53:c.817C>T, p.[Arg273Cys] and TP53: c.743G>A, p.[Arg248Gln]) in 49% and 47.5% of the tumour cells, respectively. Finally, in line with the findings from molecular assessment of circulating tumour DNA from CSF liquid biopsy, H3K27M mutation (H3F3A:c.83A>T, p.[Lys28Met]) was noted in 40.9% of tumour cells.

Conclusion and Review of Topic: Dr Amirti Vivekanandan

Diffuse midline gliomas (DMG) are infiltrative gliomas that have a loss of H3K27me3. Usually, they have a H3 p.K28M (K27M) substitution in one of the histone H3 isoforms, aberrant overexpression of EZHIP or an EGFR mutation. There are four subtypes of DMG: H3.3 K27 mutant, H3.1 or H3.2 K27 mutant, H3 wildtype with EZHIP overexpression and EGFR mutant.

DMGs are WHO grade 4.[8] As the name implies, these tumours are located in or near the midline.[9] These occur primarily in paediatric patients but can also occur in adults.[9]

The estimated incidence of diffuse intrinsic pontine glioma (DIPG) is 0.54 cases per 1 million person-years with 2.32 cases per 1 million person-years in patients 20 years old and younger.[10] DIPG accounts for 10%–15% of paediatric brain tumours and 75% of paediatric brainstem tumours. DMG can also occur in the thalamus, but account for only 1%–5% of paediatric brain tumours

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but 25% of thalamic paediatric brain tumours.[8] Finally, DMG can also occur in the cord accounting for approximately 40% of spinal astrocytomas in children and adults.[8]

In an autopsy series, leptomeningeal involvement was found in 40%.[11] Patients usually present with a short period of symptoms, typically less than 2 months. The triad involved with DMG includes multiple cranial nerve palsies (82%), long tract signs (51%) and ataxia (62%).[10] Patients can also present with obstructive hydrocephalus.

The role of surgical intervention is limited given tumour location and the morbidity and mortality that could occur with surgical resection. However, we can obtain a biopsy that can help with diagnosis and molecular characterisation.

Imaging characteristics include T1 hypointensity or isointensity and T2 hyperintensity. Appearance on FLAIR imaging can vary. These tumours tend to have minimal enhancement. As pontine examples grow and infiltrate, progressive distortion of the brainstem, exophytic growth, encasement of the basilar artery and compression of the fourth ventricle are common sequelae. Thalamic tumours can be bilateral (typical in children) or unilateral (typical in adults). Bilateral thalamic DMGs are more frequently encountered in the EGFR-mutant subtype.[8] It is recommended to image the entire craniospinal axis as leptomeningeal spread is common.

Liquid biopsy as in the present case can be used to detect tumour-derived analytes, such as cell-free DNA, released in biofluids such as blood or CSF. Increased levels of tumour DNA in CSF are associated with tumour progression, increased tumour burden and spread of tumour towards the ventricles or subarachnoid space.[12,13] Although obtaining tumour tissue remains the gold standard for diagnosis and prognostication for H3K27M DMG, this is not without risk. Liquid biopsy has great potential in the diagnosis and monitoring of CNS tumours such as DMG.

Several studies highlight the value of CSF for diagnosing CNS tumours, particularly when characteristic mutations are present as in glioma H3K27M or IDH1 mutated.[14] In most of these studies, circulating tumour DNA (ctDNA) analysis in CSF serves as a complementary tool to imaging. While not a replacement for traditional diagnostic methods at the centres that performed the studies, ctDNA analysis can aid in initial diagnosis, treatment planning and monitoring response to therapy.[15] Sequencing of ctDNA in CSF has demonstrated high efficiency for the differential diagnosis of DMG, H3K27-altered, with sensitivity of 85.7% and specificity of 100% in one study.[16] Similarly, droplet digital PCR, another techniques of assessing ctDNA, has proven adept at detecting 4 out 5 cases of DMG with no false positive among 34 brain tumour cases that were studied.[17] Despite high sensitivity and specificity of these methods shown in case series and small studies, accuracy of liquid biopsy for DIPG in isolation is not extensively validated in large cohorts, and more studies are needed to establish widespread clinical application.[14–19]

Resection is not feasible given the location of these tumours. A multidisciplinary approach to treatment is required including radiation and medical oncology. Despite current standard treatment of radiation and chemotherapy, the prognosis for H3K27M DMG is poor with a 2-year survival rate of less than 10%, regardless of location.[20] The prognosis for subtypes with H3.1 or H3.2 K27 mutation and EZHIP overexpression is better (16 months) than the subtype with H3.3 K27 mutation (11 months).[8] Predictors of longer survival include age 3 years and younger or 10 years and older, longer symptom latency (>24 weeks) and systemic therapy at diagnosis.[10] Tumours with TP53 mutations tend to be more radioresistant.[21] Surgery may improve symptoms in some cases and treat hydrocephalus but does not seem to affect survival.[22]

In regard to our patient, she underwent 6 weeks of radiation as well as treatment with temozolomide postoperatively. Unfortunately, the temozolomide had to be suspended due to thrombocytopenia. Her last assessment was approximately 4 months after her initial presentation. She complained of significant nausea and vomiting and used a wheelchair for mobility due to ataxia. Her examination showed left-sided dysmetria as well as nystagmus with lateral gaze. She ultimately decided to undergo medical assistance in dying, which was done shortly thereafter.

There have been studies looking into novel therapeutic agents for DMG, such as with immunotherapy. For example, chimeric antigen receptor (CAR) T-cell therapy against disialoganglioside 2 (GD2) has shown some promise. Three out of four patients who received an intravenous infusion of GD2-directed CAR-T cells showed both clinical and radiographic improvement.[23] Another agent that has been studied is ONC201, a dopamine receptor D2 antagonist. Patients who received ONC201 after radiation and before a recurrence showed a median overall survival of 2.1 months, compared to a median overall survival of 9.3 months in those who received the treatment after a recurrence.[24]

In summary, DMG (H3K27M-mutant) is a WHO grade 4 most common in a paediatric setting. It can be encountered in adults as exemplified by the present case. Surgical intervention is limited to biopsy given the location of these tumours. Surgical biopsy remains the gold standard for obtaining diagnostic material, but liquid biopsy offers a minimally invasive alternative with great potential. Though treatment is limited currently, there are preclinical and clinical studies underway to search for more effective treatment agents.

Author contributions. Case Presentation: AV, SP and RH
Discussion: MS
Neuroradiology: AL
Neuropathology: SP, RH, CH
Neuosurgery: JM
Conclusion and Review of Topic: AV, SP
Preparation and review of Manuscript Drafts: all authors.

Funding statement. None.

Competing interests. The authors claim no conflict of interest with the present publication.

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