My Oh Maya

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Introduction

In canine patients, primary brain tumors (PBT) account for 2-5% of all neoplasia cases. The most common neoplasms are meningiomas, gliomas, and choroid plexus tumors (CPT). Gliomas account for 50% of intracranial neoplasia cases with specifically astrocytomas having an incidence of 20% of all gliomas. Glioma neoplasms may occur at any age, though most presentations are patients over five years old. A locus on canine chromosome 26 has been mapped to show variation in multiple genes associated with increased glioma susceptibility. This variation spans many breeds but may be a genetic cause of overrepresentation in brachycephalic breeds (boxers, bulldogs, Boston terriers, etc.).[9] The glioma pathology board created by The National Cancer Institute-led multidisciplinary Comparative Brain Tumor Consortium (CBTC) determined from 2011-2014 statistical reports that there may be a male sex predilection, a predilection that has also been shown in humans. Via the same statistical reports, glioma formation has also been documented to develop most commonly within the frontal, temporal, and parietal lobes of the brain. A specific region of development has not been associated with astrocytomas, though masses located within the frontal lobe are commonly described.[8]

As terminology implies, astrocytomas develop from astrocytes within mammalian central nervous systems (CNS). Astrocytes are widely known as supportive cells of the CNS and play a large role in neuroelectrical transmission, nervous tissue repair, and the formation of the blood-brain barrier.[7] Tumors may be characterized from Grade I-Grade IV, with Grades III and IV considered malignant. Presentation of clinical signs generally occurs with advanced tumor progression. Higher grades generally have lesser survival times, but appropriate therapies can provide an enriched lifespan and greater quality of life.[8]

History and Presentation

Maya was an approximately eight-year-old, spayed female king shepherd who presented to the Animal Emergency & Referral Center (AERC) in Flowood, MS on May 19th, 2019 for the occurrence of three seizure episodes. On May 18th, during initial seizure activity, Maya was described to assume sternal posture and hypersalivate prior to progressing to lateral recumbency with loss of consciousness. No urination, defecation, or paddling was noted during the event. The initial seizure event on May 18th lasted approximately three minutes, and after she was ataxic and lethargic. Two shorter episodes with similar actions followed within the next 24 hours.

At AERC, Maya presented quiet but alert. All vital parameters were found to be within normal limits with a rectal temperature of 102.8 degrees Fahrenheit (not found to be clinically significant), a heart rate of 128 beats per minute, and a respiratory rate of 60 breaths per minute. The rest of her physical examination was unremarkable. She presented in the evening and was maintained overnight on diazepam (0.2mg/kg IV PRN), levetiracetam (20mg/kg IV q8hr), and dexmedetomidine (0.15mls IV PRN). No seizure activity or other clinical signs were noted overnight. The next morning, a complete blood count and chemistry panel revealed no significant findings. Intravenous medications were discontinued, and Maya was discharged from AERC with a referral to the Mississippi State University College of Veterinary Medicine Veterinary Specialty Center (MSU-CVM, VSC). She was continued on orally administered levetiracetam (1250mg q12hr) until her evaluation at VSC. Her only other medications included a glucosamine supplement for joint health. After discharge, Maya's levetiracetam dose was lowered to 1000mg orally every 12 hours due to the owner's concern for excessive sedation.

Diagnostics and Treatment

At VSC, a full neurological examination was performed which revealed normal mentation, posture, gait, and intact cranial nerves. Postural reactions and reflexes were normal. While ambulating, Maya circled widely towards the right. An MRI of Maya's brain was performed and revealed a large irregularly shaped and marginated heterogenous mass measuring 1.6 x 2.5 x 2.0cm in the right forebrain of the frontal and piriform lobes. The mass displayed T2 hyperintensity, T1 hypointensity, and was minimally enhanced by contrast. These characteristics are frequently associated intracranial gliomas, and the presence of peritumoral edema or contrast enhancement can commonly be indicative of tumor grade.[1] In addition to the MRI, a cerebrospinal fluid sample was submitted for analysis though no abnormalities were noted. Based on MRI characteristics and presentation, the top differential for Maya at this time was a high-grade glioma, though an ischemic event could not be ruled out. She was continued on levetiracetam and additionally prescribed an anti-inflammatory dose of prednisone. Maya's owners were instructed to return for a follow-up in roughly one month.

Due to Maya's signalment, suspected glioma, and lack of co-morbidities, she was a candidate for a Phase I clinical trial utilizing M032, an attenuated herpes simplex virus (HSV), to treat malignant cells.[3] This trial is ongoing with a partnership between The University of Alabama at Birmingham and other southern universities including Auburn University, University of Georgia, and Mississippi State University. The study is funded by the National Institute of Health (NIH) and approved by the Institutional Animal Care and Use Committee (IACUC).

Between initial presentation to VSC and follow-up, Maya had no noted seizure activity or other clinical signs. A second MRI performed on June 12th, 2019 confirmed the previously noted characteristics of a likely glioma. Prior to surgery, complete staging including a complete blood count, neurochemistry panel, coagulation profile, thoracic radiographs, and abdominal

ultrasound was performed with no clinically significant abnormalities noted. On June 17th, Maya underwent a craniectomy for tumor debulking and biopsy submission. During the procedure, a Rickham reservoir and catheter was placed at the base of the tumor bed (placement confirmed by CT). The craniectomy and recovery was uneventful. The next day, June 18th, the modified HSV (M032) was administered via the reservoir catheter. Maya was discharged three days post-operatively with levetiracetam (1000mg PO q12hr), metronidazole (500mg PO q12hr), Gabapentin (600mg PO q8hr), and capromorellin (Entice) (5ml PO q24hr). At the time of discharge, she was mildly ataxic and displayed decreased conscious proprioception (CP) deficits in all four limbs. Her owners were instructed to return in one month for re-evaluation.

Maya's biopsy report was finalized on June 19th, 2019. Two sections of tissue core were submitted as biopsy samples, obtained using the Shores-Little biopsy device[a] and a Cavitron Ultrasonic Surgical Aspirator (CUSA). The samples displayed a densely cellular neoplasm that infiltrated and replaced surrounding neuroparenchyma. The neoplasm was composed of pleomorphic spindaloid to polygonal cells with indistinct borders and hypereosinophilic cytoplasm. The cells were noted to blend into a vacuolated neuropil. Cell nuclei were noted to be irregularly round to elongate with coarsely stippled chromatin. There were 28 mitotic figures noted in ten 400x fields of view with occasional bizarre mitoses present. Multifocal areas of hemorrhage were present with lymphocytes and plasma cells scattered throughout. Moderate anisocytosis and anisokaryosis was noted as well. Some single cell necrosis was described with shrunken cells also with present pyknosis or karyorrhexis. Lastly, neoplastic cells were immunoreactive for glial fibrillary acidic protein (GFAP). The histopathologic characteristics are consistent with an astrocytoma classified by the CBTC glioma pathology board. Cellular

pleomorphism and high mitotic rate, indicative signs of malignancy, led to the pathologist's determination that Maya's brain neoplasm was a grade III astrocytoma.[8]

Two weeks post-op, Maya's Rickham reservoir catheter was removed. Bloodwork (complete blood count and neurochemistry panel) and swabs were collected as part of the Phase I glioma clinical trial, but no complications or concerns were described at this time. Maya returned to MSU-CVM VSC on July 15th, 2019 for her one-month re-evaluation. At this time, she was receiving only 1000mg levetiracetam orally twice daily and a daily glucosamine supplement. Upon recheck presentation, Maya was bright, alert, and responsive. She had normal vital parameters with a rectal temperature of 100.5 degrees Fahrenheit, a heart rate of 100 beats per minute, and a panting respiratory rate. Her complete neurological examination was within normal limits as well. No seizure activity had been observed since surgery, and her owner reported that Maya was doing well at home. Instruction was given to return for a three-month re-evaluation in September. However, despite Maya's positive response to treatment, she was found dead inside her home at approximately 2am on July 29th, 2019. Her owner promptly transported her to the MSU-CVM Necropsy service that same morning.

Necropsy Findings

Postmortem examination revealed marked, diffuse vascular congestion of the organ systems. Advanced postmortem autolysis was present in the form of tissue discoloration and degradation. There were multiple areas of petechial and ecchymotic hemorrhages, especially noted within the myocardium. These findings are consistent with an elevated body temperature. The elevated body temperature could be a result of seizure activity, an alteration in thermoregulation, or an environmental influence. Due to the history of Maya's care and the time of death, it is unlikely that the cause of death is environmentally related.

Histopathology of the brain revealed densely packed polygonal to spindaloid cells with indistinct borders forming a poorly demarcated mass within the right forebrain and spanning into the left forebrain. The cells were noted to have moderate eosinophilic cytoplasm, and they blended into adjacent neuropil in multiple regions. Further descriptions were similar to Maya's initial brain biopsy report. There were noted to be 35 mitotic figures in ten 400x views. Multiple large areas of necrosis and hemorrhage were present. The majority of the mass was present within the frontal cortex; however, neoplastic cells were described to infiltrate normal nervous tissues extending in large effect around the lateral ventricle and to the level of the thalamus.

Pathophysiology and Diagnosis

Ongoing research is still determining how and why neoplastic cells develop, especially in canine patients. Previous literature has identified mutations within the p53 (tumor suppression) pathway, the cyclin-dependent kinase 4 (CDK4) gene as well as the inhibitor gene, and the platelet-derived growth factor (PDGF) genes. PDGF is known to be expressed within neural stem cells and play a role in neural cell malignancy development, though the path to gliomagenesis isn't yet determined. PDGF has documented overexpression in all grades of human astrocytomas and is overexpressed in as high as 25% of Grade IV astrocytomas. This particular gene set has documented overexpression in canine glioma presentation, but the consistency and tumor type associations have not been fully explored.[6] Within the p53 pathway, mutations in the TP53 gene result in loss of cell division regulation and disruption of tumor suppression. Though TP53 mutations are noted to be rare in canine gliomas, this pathway was affected in 2/3 of the astrocytoma cases (whereas it was not consistently noted in oligodendroglioma cases) presented in a study at the University of California, suggesting it may play a role in specifically astrocytoma development. Additionally, documented in this study, increased phosphorylation of

protein kinase b (AKT) and mitogen-activated protein kinase (MAPK) was associated with astrocytoma cases compared to oligodendrogliomas. Lastly, in humans, alterations of the retinoblastoma tumor suppressor (RB1) and tumor suppressor P16 were documented in as high as 80% of astrocytoma cases. When evaluated in canine patients, the expression was variable across all glioma types and could not be consistently linked to tumor formation.[2] Another widely recognized pathogenesis in humans is a co-deletion of 1p/19q genes (combined loss of chromosomal arms due to translocation) leading to glioma formation, but this deletion is not recognized in the dog.[9] Throughout the years, publications on canine glioma development show a wide array of heterogenicity in associated proteins and altered pathways.

Though the catalyst(s) for development of gliomas is yet to be determined, multiple clinical signs associated with brain tumors have been established. Clinical signs usually correlate to the Monro-Kellie doctrine which describes the relationship between volume and pressure within the calvarium. The osseous calvarium is the least compressible factor of the system involving skull, brain, blood, and cerebrospinal fluid (CSF). According to the doctrine, as one factor increases in volume, the other factors must compensate to maintain homeostatic conditions, with the most compressible factors (blood and CSF) usually being the compensatory factors. As neoplastic cells spread, normal brain tissue, CSF flow, and blood flow all are compromised. Compensatory mechanisms like increased blood flow and decreased cerebrospinal fluid production become exhausted. After homeostatic exhaustion is reached, secondary clinical signs begin to develop, commonly including epilepsy.[10] Tumorigenic epilepsy is likely due to disrupted neuronal activity, impaired glial cell function, and altered vascular supply to the brain as mentioned above. Seizure activity and behavioral changes are especially associated with lesions of the forebrain. Secondary effects from continued growth include edema surrounding the

tumor site, inflammation, and intracranial hemorrhage. As the neoplasm progresses, ischemic events and herniation is possible. Non-specific signs such as lethargy, inappetence, and weight loss without the presence of behavior changes or seizures may make a brain lesion difficult to diagnose. Differentiation of gliomas cannot be definitively determined by clinical signs or imaging modalities.

A minimum database consisting of a complete blood count, chemistry profile, and urinalysis is usually indicated during the evaluation of suspected brain lesion patients. In addition to the middle-aged to older presentation of these patients, a minimum database is also helpful in ruling out other causes of disease prior to moving forward with more financially demanding diagnostics. Thoracic or abdominal radiographs may be utilized to identify co-morbidities but are not particularly useful in defining a primary brain tumor. A cerebrospinal fluid (CSF) analysis can be pursued but should always be performed after imaging to assess the patient's risk for the procedure. Additionally, CSF analysis is variable and susceptible to degradation, so it should be utilized in conjunction with imaging. Computed tomography (CT) and magnetic resonance imaging (MRI) are the primary methods of diagnosis, with MRI being the gold standard. MRI is invaluable in diagnosing PBTs and hypotheses as to tumor type can even be made based off imaging characteristics by experienced personnel.

Advanced imaging may diagnose a mass, but biopsy analysis is needed for definitive tumor type and grade. As mentioned in Maya's histopathology results, common characteristics noted in astrocytoma histopathology include pleomorphism, angular nuclei, spindaloid cell morphology, and abundant eosinophilic cytoplasm. Anisocytosis and anisokaryosis are common in multiple tumor types and are a universal association with malignancy. Immunohistochemistry is beneficial for diagnosing an astrocytoma when evaluating GFAP, an intermediate filament

protein expressed within central nervous system cells. However, based on current literature, the use of GFAP immunoreactivity evaluation is limited and not adequate enough to determine the level of tissue infiltration by the mass.[8]

Traditional Treatment and Case Outcome

Treatment of primary brain tumors in companion animal patients pursues the main goals of extending the lifespan of the patient and improving quality of life. Combination therapy is associated with the most positive outcomes, meaning longer median survival times (MST) and reduction of neoplastic effects. Surgical excision is beneficial for reducing secondary effects of PBTs like increased intracranial pressure, seizures, hemorrhage, etc. but often wide margins for complete excision are not attainable. Surgical therapies may prove difficult to pursue for the owner due the financial demand of finding a skilled team to perform the procedure and monitor the patient. Radiation therapy is found to be beneficial as a monotherapy and can increase MST up to 16 months (specifically in cases of low grade oligodendrogliomas) when used alone, but it is best utilized in combination with another therapy. Chemotherapeutics usage is widely variable and has not demonstrated great results in increased MST compared to palliative care when used as a monotherapy, though some agents may be beneficial as adjuncts. Therapeutic drugs such as anticonvulsants, analgesics, and corticosteroids are commonly utilized to prevent seizure events, provide visceral or neuropathic pain relief, or reduce neuroinflammation and edema. These agents are commonly used during any protocol, but they are the mainstays of palliative treatments. Palliative care provides an average MST of three months, while combination therapy (most commonly surgical excision and radiation therapy) has been shown to lead to a median survival time of six months for higher grade gliomas (III and IV). [9]

Immunotherapies are being actively explored as a treatment for intracranial neoplasia, and human literature shows some benefits. Maya's case was invaluable in the information provided for HSV M032 and its effects as an immunotherapy. Despite surgical excision and M032 administration, Maya's tumor continued to infiltrate local tissues. Necropsy histopathology revealed large regions of necrosis to the level of the thalamus, indicating the level of malignancy present. Due to the lack of noted seizure activity since beginning levetiracetam therapy, it is likely that thermoregulation was affected, and she succumbed to the response associated with an increased internal body temperature. Her survival past presumptive diagnosis was approximately 2 months. Despite the outcome, ongoing studies and human literature results display the hope that in the future, immunotherapies may be an extremely beneficial tool for treating primary intracranial neoplasia.

[a]Shores-Little Cerebral Biopsy Device; MILA, International, Florence, KY.

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