Canine presumed glial brain tumours treated with radiotherapy: Is there an inferior outcome in tumours contacting the subventricular zone?

Canine intracranial glial tumors treated with radiotherapy: is there an inferior outcome in tumors contacting the subventricular zone?

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Abstract

Post-treatment outcome in canine glial tumors is described with a broad range of survival times between 2-28 months. After surgery or radiation therapy, the tumors may progress locally or spread within the central nervous system. It is unknown if tumor- or patient-specific factors influence prognosis. In humans, glioblastoma involving the subventricular zone have been found to recur distantly, with shortened time to progression and overall survival.

We included 32 dogs irradiated for a presumptive primary glial brain tumor in this retrospective cohort study. Tumors were grouped relative to subventricular zone contact and overt ventricular invasion assessing pre-treatment magnetic resonance images.

Median time to progression (TTP) for all cases was 534 days (95%CI, 310-758), with a significantly shorter TTP in dogs with lesions at the subventricular zone (median TTP, 260 vs. 687 days; $P = 0.049$). Tumors at the subventricular zone progressed more often ($P = 0.001$), and more likely as CNS-metastasis (52.9% vs. 13.3%, $P = 0.028$).

Median overall survival (OS) was 489 days (95%CI, 147-831) and median tumor-specific survival 609 days (95%CI, 382-835). Involvement of the subventricular zone was significantly associated with a shorter tumor-specific survival (median, 306 vs. 719 days; $P = 0.044$).

Glial tumors contacting the subventricular zone in dogs have a shorter tumor-specific survival and a higher rate of progression and CNS-metastasis. Despite of local tumor control, metastasis must be considered and should prompt further treatment approaches.

Key words: brain tumor, dog, drop metastasis, glioma, neuroepithelial tumors, radiation therapy

Introduction

Treatments for intracranial tumors in dogs have received a constant trickle of attention in the last decades, without massive improvement in outcome.[1-3] For glial tumors treated with radiation therapy, outcome is described with a rather broad range of median overall survival times between 8-23 months.[4-6] Median survival times after surgery range from 2-6 months[7,8], with more promising outcomes of 7-28 months[9] when experimental unspecified therapy (chemo-, immuno-, gene-therapy) was added. While radiation therapy and surgery remain the main pillars of brain tumor treatment in dogs, the inherent difficulties remain as well: Tumors are often not amenable to appropriately wide surgical excision[2] and the needed "extent" of treatment with radiation therapy is unknown. If the extent of treatment, the so-called treatment margins are too small, tumors will recur or continue their growth at this site after both types of therapy. In contrary to surgery, wide margins to include zones of invasion are easily attained in radiation treatment planning. But if the margins are too large, the delivered radiation dose could also lead to debilitating toxicities.

Furthermore, it is unknown if tumor- or patient-specific factors consistently influence prognosis. Non only are re-treatment biopsies rarely obtained, but also their interpretation can be difficult, and prognostic information is not well established.[10,11] In treated cases, standardized follow-up is often lacking, with times and pattern of tumor progression or relapse remaining unknown in these dogs.

One single study mentioned site of progression in irradiated glial tumors: of the 11 dogs that eventually succumbed to tumor-related death, 7/11 (64%) had local recurrence or progression and in 4/11 (36%) CNS-metastasis was suspected based on diagnostic imaging.[5] In humans, the anatomic relationship of glial tumors to certain brain regions predicts the pattern of progression and outcome. Lim et al., (2007) determined such a relation of glioblastoma multiforme to the subventricular zone, an area under the ependyma of the lateral ventricles (along the lateral aspects) (Figure 1) that contains the largest population of neural stem cells. Their classification system describes the contact of the contrast-enhancing lesion with this zone and in part predicts tumor recurrence pattern.[12] This system has since sparked the interest of radiation oncologists and has even raised the question of including this subventricular zone into

the radiation target, such as the clinical target volume (e.g. the margin around the gross disease). This matter, however, is still under investigation.[13,14] While it is well known that ventricular contact is a common finding in canine gliomas[15,16] and this stem-cell rich subventricular zone is comparable in dogs[17], a relationship between tumor location at any ventricle, or specifically at the subventricular zone and prognosis or outcome has not been investigated.

Classifying human glioma patients along this standardized spatial classification system revealed differences in outcome for tumors contacting the subventricular zone.[12] Hence, we were intrigued to have a second look at our dog glioma data in retrospect. We wanted to investigate whether a simple, imaging-based, non-invasive spatial anatomic feature could anticipate a similar pattern of failure (e.g., type of progression or relapse) or even be linked to an outcome variable such as overall survival in dogs undergoing radiation therapy.

We hypothesized that a clinically detectable difference of outcome for time to progression (TTP), overall survival (OS) and tumor-specific survival or both would occur between the presumed glial tumors contacting the subventricular zone compared to the non-contacting tumors.

Materials and Methods

Patient selection

For this retrospective cohort study, we reviewed medical records of patients with an imaging diagnosis suspicious of a primary glial brain tumor, treated with radiation therapy at XXX between 2015 and 2020. For study inclusion all patients needed to have an MRI performed either at our or at the individual referring institution. As a minimum dataset of MRI-images, post-contrast T1-weighted images (T1W), T2-weighted images (T2W) and T2-weighted fluid attenuated inversion recovery images (T2-FLAIR) in at least one plane each were required. Additional sequences such as diffusion-weighted imaging (DWI) or sequences sensitive for hemorrhage such as susceptibility-weighted imaging (SWI) or T2*-weighted images were desired, but not required for inclusion.[18] As a minimum standard in this retrospective study, however, the imaging study must have been interpreted by a board-certified veterinary radiologist, who

assigned at least the superclass "glial"[19] to the suspected tumor diagnosis. Primary tumors of glial origins are usually suspected in a contrast-enhancing and non-contrast-enhancing single intra-axial lesion with mass effect. In cases of hemorrhagic and/or cystic lesions, an additional solitary part is usually present. Usually there are no further signs of inflammation in MRI and CSF.[15]

This diagnosis was then used for treatment decision, as common practice in our Animal Hospital. Further, the patient must have been followed-up for a minimum of three months after imaging, preferably until death of any cause.

Tumor topography and grouping

Anatomical location relative to the subventricular zone as well as size (gross tumor volume, GTV) of each tumor and contrast enhancement were recorded. The dog's subventricular zone lines the ventricular wall of the lateral ventricles and is laterally limited by the caudate nucleus, medially by the interventricular septum and dorsally by the corpus callosum (Figure 1).[17] Dogs were grouped to have their lesion either contacting the subventricular zone (GroupSVZ+) interpreted as tumor possibly infiltrating the subventricular zone[17]) or not (GroupSVZ-). As an additional factor, presence or absence of overt ventricular invasion on the MRI images was reported. All MR images were performed blinded by the authors, and cases of disagreement were discussed among the authors until consensus was reached. All images were reviewed on Horos Version 3.3.6.

Treatment

Dogs were treated based on owner's choice with either 20x2.5 Gy, 10x4 Gy or a 10x4 Gy protocol with a 15% boost to the GTV. The former two protocols had been found to yield non-different outcomes, even if the lower biologically effective dose (BED) in the 10x4 Gy-fraction protocol implies a lower efficacy and tumor control probability.[6,20] The third protocol is currently offered to owners with the intent of increasing the total dose, thereby improving outcome (ongoing project).

Target volumes and organs at risk (OAR), such as whole brain, were contoured in a facility internal standardized manner as previously published, allowing for volumetric measurements.[20] In brief, the gross tumor volume (GTV) was delineated using co-registered contrast-enhanced CT images or CT and MRI images, in tumors with no contrast uptake T2 sequences were used for delineation of GTV. Clinical target volume (CTV), accounting for subclinical microscopic disease extension of 4-8 mm was defined. The CTV-margin was then extended three-dimensionally by 2 mm to define the planning target volume (PTV), accounting for setup uncertainties in daily image-guided photon treatment. OAR were segmented as described previously by our research team.[xx] The recommendations for specifying dose and volumes were adhered to as in the ICRU reports 50 and 62 for 3DCRT and ICRU report 83 for IMRT plans.[21-24]All dogs were treated with a Varian Clinac iX 6MV linear accelerator (Varian Medical Systems, Palo Alto, USA) with a four degree-of-freedom couch, a 120 leaf multi-leaf-collimator and the treatment planning system ECLIPSE (version 10.0.28 or 15.1.25, Varian Oncology Systems, Palo Alto, USA) with AAA-algorithm. Treatment was delivered with photons as 3-dimensional conformal radiation therapy (3DCRT) or intensity-modulated radiation therapy (IMRT).

Daily positioning verification with kilovolt (kV) orthogonal radiographs and occasional kV-cone-beam CTs were performed with the on-board imaging system (Varian On-Board Imager, Varian, Palo Alto, USA) and matched by an experienced radiation therapist. Quality assurance of on-board imager and linear accelerator was performed as required by institutional and federal guidelines.

Treatment was delivered with definitive-intent, on a daily Monday to Friday schedule, over 2 to 4 weeks, depending on the protocol.

Additional medical treatment before and after radiotherapy was not standardized but adapted to the individual needs of the dogs. Medication was usually started at the day of diagnosis or beginning of neurological signs and adapted according to the improvement of clinical signs and consisted mostly of antiepileptic drugs and corticosteroids.

Follow-up

147 Results of follow-up examinations were retrieved from the medical files. At our institution we
148 recommend clinical re-checks 3 weeks and 3 months after the end of radiation therapy, and at three-
149 monthly intervals thereafter. In cases where clinical examination is not feasible, we usually contact the
150 owners by phone. Further, we recommend follow-up MRI 6 and 12 months after radiation therapy for
151 patients in clinically stable or improved condition and at any time in the case of neurologic deterioration.
152
153 *Statistical analysis*
154 Descriptive statistics were used in the analysis of dogs and tumor characteristics. When appropriate, data
155 were tested for normality by use of the Shapiro-Wilk normality test. Values were expressed as mean ± SD
156 in case of normal distribution, or as median with a range in case of non-normal distribution. Follow-up
157 time was defined as the time from the first radiation treatment until death, loss to follow-up or time of
158 data analysis. The time to progression (TTP) was defined as the interval between start of RT and
159 discovery of new or progressive neurological signs or evidence of disease progression based on MRI.[25]
160 Dogs dying without clinical or image-based evidence of disease progression were censored for TTP
161 analysis. The overall survival (OS) was defined as the interval between first RT until death of any cause;
162 for the disease-specific survival dogs that died of other causes were censored at the time of death. Dogs
163 still alive at the time of data evaluation or lost to follow-up were censored. Time to progression, overall
164 and disease-specific survival were coded and analyzed with Kaplan-Meier product-limit estimator,
165 accompanied by the log-rank or Breslow-Gehan-Wilcoxon tests. Survival estimates and median survival
166 time are reported with the corresponding 95% confidence intervals (95%CI). Cox's regression analysis
167 was used to determine whether co-variates showed an influence on TTP, OS and disease-specific survival.
168 The following variables were investigated for prognostic significance: sex, age (median used as cut-off),
169 weight (median used as cut-off), head conformance (brachycephalic or dolichocephalic), RT protocol
170 (10x4 Gy vs. 10x4Gy with 15% boost and 20x2.5 Gy), contact to SVZ, ventricular invasion, GTV
171 (median used as cut-off), brain volume (median used as cut-off) and GTV/brain volume ratio (median
172 used as cut-off).

The relationship between SVZ contact and metastatic rate and death prevalence was investigated by means of Mann-Whitey *U* test or Chi-square test.

Data were analyzed with SPSS (IBM® SPSS® Statistics, Version 26, IBM Corp., Armonk, New York). Results of statistical analyses with p-value <0.05 were considered statistically significant.

Results

Patient and tumor characteristics

Thirty-two dogs were included into this retrospective analysis and are presented in Table 1. All but one patient had pre-contrast, all patients had post-contrast T1W images in at least one plane (29/32 had the post-contrast T1W images in more than one plane). All patients had T2W images in at least two planes (14/32 transversal + sagittal, 18/32 in all three planes) and T2W-FLAIR images in at least one plane (29/32 in transversal or dorsal plane, 3 in different combinations of two orthogonal planes). DWI- and SWI- or T2*-sequences were available in 15/32 patients. The majority of tumors (30/32) were localized in the forebrain, with one each in brainstem and cerebellum. The majority of the tumors (25/32, 78.1%) were in contact to the ventricular system, with 17/32 (53.1%) at the subventricular zone. Eight tumors had ventricular contact, but not in the area of the SVZ. In 6/32 (18.8%) ventricular invasion was suspected based on the MRI images. Overall, 22/32 (68.8%) of dogs had contrast-enhancing lesions, 15 (46.9%) being in contact with the SVZ. In 10/32 (31.3%) dogs no contrast was detected within the lesion and 2 lesions (6.3%) were in contact with the SVZ.

Median tumor size GTV was 4.0 cm^3 (range, 0.2-16.1 cm^3). Tumors in GroupSVZ+ were significantly larger (median, 6.1 cm^3, range, 0.5-16.1) than tumors in GroupSVZ- (median, 3.0 cm^3; range, 0.2-9.2 cm^3; $P = 0.008$). Median brain volume was 90.4 cm^3 (range, 46.9-120.9 cm^3) corresponding to a median GTV/BV-ratio of 5.4% (range, 0.2-14.1%). A significant difference between the two groups was noted in GTV/BV ratio (6.6% vs. 3.4%; $P = 0.006$), but not in brain volume (Table 1).

Treatment

All dogs were irradiated with curative intent. Seventeen (53.1%) were treated with 10x4 Gy (8 GroupSVZ+, 9 GroupSVZ-), 8 with 10x4Gy and a 15% simultaneously integrated boost to the GTV 10x4 Gy (3 GroupSVZ+, 5 GroupSVZ-) and 7 were irradiated with 20x2.5 Gy (6 GroupSVZ+, 1 GroupSVZ-). Applying an EQD2 calculation with an alpha/beta value of 10 for protocol comparison, the 10x4 Gy protocol equals 46.7 Gy_{EQD2} (biologically effective dose, BED 56.0 Gy_{10}), the 10x4Gy with 15% boost dose protocol equals 53.6 Gy_{EQD2} (biologically effective dose, BED 64.3 Gy_{10}) and the 20x2.5 Gy protocol equals 52.8 Gy_{EQD2} (biologically effective dose, BED 62.5 Gy_{10}), rendering the two latter the more effective protocols from a calculation point of view. The 17 tumors contacting the SVZ were equally distributed between the BED levels, with 8/17 tumors in the lower BED group (10x4Gy) contacting the SVZ, and 9/17 tumors in the higher BED group (10x4Gy +15% boost and the 20x2.5Gy) contacting the SVZ.

Thirteen of 32 dogs (40.6%) were treated with a conformal photon plan (3DCRT), and 19/32 (59.4%) with an intensity-modulated radiation therapy plan (IMRT). At the start of radiation therapy, all but one patients received corticosteroids at a median dosage of 0.73 mg/kg sid (range 0-2.0 mg/kg) and all but two patients were treated at a median dosage of 0.49 mg/kg sid (range 0-1.4mg/kg) at the end of radiation therapy. All 26 dogs with reported seizures received antiepileptic medical treatment. At the end of radiation therapy, a total of 21 dogs received phenobarbital at a median dosage of 2.6mg/kg bid (range 1.1-3.8 mg/kg) and a total of 14 dogs received levetiracetam at a median dosage of 21.0 mg/kg tid (range 9.1-35.6 mg/kg). One dog received potassium bromide (8.9 mg/kg bid). As a monotherapy, 12 dogs received phenobarbital at a median dosage of 2.8 mg/kg bid (range 1.6-3.8 mg/kg) and 5 dogs received levetiracetam at a median dosage of 23.8 mg/kg tid (range 19.2-35.6 mg/kg), respectively. More than one antiepileptic drug (combinations of phenobarbital, levetiracetam and potassium bromide) was prescribed to 9 patients. In general, phenobarbital dose was titrated based on blood-levels to the upper recommended range (25-30 mg/L)[26] and levetiracetam was added if seizure control was insufficient, or if dogs did not tolerate phenobarbital.

Follow-up

Eighteen patients (56.3%) had at least one follow-up MRI after radiation therapy. With a total number of 30 MRIs performed, 9 were performed in patients that showed neurologic deterioration and 21 in dogs presented for a regular re-check without any suspicion of neurologic deterioration. Six patients had the MRI three months after radiation therapy (4/6 as routine re-check, 2/6 with neurologic deterioration), 13 at six months (11/13 as a routine re-check, 2/13 with neurologic deterioration) 4 at 12 months (all as a routine re-check), two at 18 months (one as a routine re-recheck, one with neurologic worsening) and one 24 months (routine re-check) after radiation therapy. In four patients an MRI was repeated 1-3 months after a routine MRI re-check without any signs of tumor progression or metastases, because of sudden onset of signs of neurologic worsening/suspected tumor progression.

Outcome

The median follow-up time was 341 days (range, 102-861 days). For a subset of patients (10/32), outcome was already published in an earlier study.[xx] At the time of writing, 8/32 dogs (25%) were still alive.

Because of deterioration of neurological signs or evidence of disease progression based on MRI in 22/32 (68.8%) dogs during the follow-up period, tumor progression was suspected (8/32) or confirmed (14/32) (Table 1). Hence, in 8 dogs (25.0%) tumor progression was clinically suspected but not imaging-confirmed, but not imaging-confirmed. In 3 dogs (9.4%), only local progression was found. In 11 dogs (34.4%) CNS-metastasis was part of the progressive pattern, with 8 patients (25%) exhibiting CNS-metastasis despite of local tumor control. In 4/11 (36%) of dogs with CNS-metastasis, lesions were limited to the spinal cord, and in 6/11 (54%), lesions were found to affect both, spinal cord and brain. In 6/11 (54%) cases, metastases were found within the ventricular system and 2 of these 6 were in contact with the subventricular zone (in only two of these 6 patients, the intraventricular lesion was a single lesion, in the other 4 dogs multifocal subarachnoid or diffuse meningeal lesions were present. In one of these cases, an additional intra-axial lesion was present). Diffuse meningeal infiltration at the level of the

brain stem and cranial spinal cord was found in 1/11 (9%) of dogs with metastasis. Overall, 9 dogs developed neurological signs again consistent with the clinical signs or neuroanatomical localization at initial presentation. Clinical signs included behavioral changes and pacing (2), uncontrolled seizures (5) or status epilepticus (2) (after prior medical seizure control). In the 9 dogs with neurological deterioration consistent with the initial neuroanatomical localization, CNS-metastasis were confirmed by MRI in 3 cases (one with additional histopathology) and progression of the initial tumor by MRI or postmortem in 2 cases. Other 9 dogs were presented with additional neurological deficits consistent with an additional neuroanatomical localization. Of these 9 dogs 4 were presented with spinal cord localization (C1-C5 (2), Th3-L3 (2)), 3 with multifocal neurological signs, 1 with forebrain signs and 1 with central vestibular signs. In 3 dogs, progression was reported by the owner or referring vets, but detailed neurological examinations were not available. In all but 1 dog with additional neurological deficits MRI confirmed CNS-metastasis.

Of the 22 dogs (68.8%) with progressive disease, 16 dogs were in the GroupSVZ+ (94.1%, thereof) and 6 dogs in the GroupSVZ- (37.5%) (P = 0.001). Also, dogs with the GroupSVZ+ were more likely to succumb to CNS-metastasis (52.9% vs 13.3%, P = 0.028).

The median TTP for all cases was 534 days (95%CI, 310-758). TTP was significantly shorter in dogs with lesions at the SVZ compared with GroupSVZ- (median TTP, 260 vs. 687 days; P = 0.049) (Figure 2). Also was median TTP significantly shorter for the dogs with clear ventricular invasion (median TTP, 203 vs. 609 days; P = 0.013)

At the time of analysis 24/32 (75.0%) dogs were dead. Of these, 21/24 (87.5%) died of tumor-specific causes, including 15 in GroupSVZ+ (88.2%) and 6 in GroupSVZ- (40%) (P = 0.008). Of the 8 patients still alive, 7 (87.5%) had tumors not in contact to the SVZ (P = 0.013). Median OS was 489 days (95%CI, 147-831). Median tumor-specific survival was 609 days (95%CI, 382-835). Involvement of the subventricular zone was significantly associated with a shorter tumor-specific survival (median, 306 vs. 719 days; P = 0.044) (Figure 3) and influenced OS (median, 226 vs. 609 days; P = 0.066). OS was

significantly shorter in dogs with clear ventricular invasion (171 vs. 536 days; P = 0.010) and so was tumor-specific survival (224 vs. 609 days; P = 0.037).

The variables significantly associated with an increased risk of tumor progression included a larger GTV (HR = 2.8; 95% CI = 1.1-7.3; P = 0.037) and ventricular invasion (HR = 3.6; 95% CI = 1.2-10.5; P = 0.019), whereas a tendency was observed for SVZ contact (HR = 2.5; 95% CI = 0.9-6.6; P = 0.057). The only variable significantly associated with an increased risk of disease-specific death was ventricular invasion (HR = 3.1; 95% CI = 1.1-8.8; P = 0.038) and a tendency was observed for SVZ contact (HR = 2.3; 95% CI = 0.9-6.1; P = 0.081).

Ventricular invasion was the only variable significantly associated with an increased risk of death for any cause (HR = 2.3; 95% CI = 0.9-6.1; P = 0.081).

Discussion

In humans, glial tumors involving the subventricular zone have been found to recur at locations distant from the initial lesion, with shortened time to progression and overall survival. Hence, it was suggested that tumors involving this zone are more invasive and migratory than tumors originating from other regions.[12,14] Here, we investigated the outcomes of a series of 32 dogs based on this described spatial classification system. In 78.1%, the majority of our convenience sample, the tumors were in contact with the ventricular system, with 53.1% at the subventricular zone. Ventricular contact of canine glioma is common, described in up to 90% of dogs[16] and tumors frequently involve the subventricular zone. Disruption of the ependyma of the wall of the lateral ventricles has sporadically been associated with cerebrospinal fluid (CSF) drop metastasis[27], but systematic investigations of progression are lacking. In our case sample, dogs with tumors located at the subventricular zone had a tendency for earlier progression and a significantly shorter tumor-specific survival. Comparable to 36% in the prior small sample[5], 11/32 (34.4%) of all tumors progressed distantly as CNS-metastases. Furthermore, the 6 dogs with obvious ventricular invasion, had a shorter TTP and OS. Five of these 6 tumors progressed into CNS-metastasis. In many of the cases with known site of progression, however, radiation therapy

provided a long-term local control with primary tumors in remission or stable after radiation therapy and only in three patients simultaneous local *and* distant progression was found.

In humans, one of the main reasons for glial cell tumors to recur is the high migratory capacity of a small subpopulation of tumor cells, the so-called brain tumor stem cells.[28,29] These brain tumor stem cells are more resistant to treatment and share common features with neuronal stem cells, indicating potential transformation of neurogenic stem cells into brain tumor stem cells: both possess self-renewal and multipotential capabilities and are highly migratory. Gliomas can be induced from subventricular zone cells in animal models, supporting this theory.[29] In the adult brain, neuronal stem cells reside within specialized neurogenic niches. The largest of these niches in mammals is the subventricular zone. This subventricular zone is therefore not only a source of the neuronal stem cells, but potentially also of brain tumor stem cells and its microenvironment supports tumorigenesis.[30] Neoplastic glial tumors, however, can not only arise from stem cell regions, but also be initiated by neoplastic transformation of non-subventricular progenitor cells or mature glial cells that have undergone dedifferentiation.[29] And while many malignant brain tumors arise distant to the subventricular zone, findings support the more invasive nature of tumors arising from this zone, with such tumors occurring multifocally at initial diagnosis and recurring distantly from the initial tumor.[12] A second pattern of recurrence beside the parenchymal infiltration described above, is dissemination via CSF. This type of recurrence has not been investigated as deeply as the parenchymal migration. Localization near the CSF pathways seems to be more important than histological grade in CSF spread of human gliomas.[31,32]

Glial tumors in dogs commonly recur or progress after surgical or radiation treatment.[2,5,6,9] When considering the published literature, the site of progression was only mentioned for a few dogs and was either local, distant but within the CNS (intraventricular or spinal spreading, "drop metastasis"), or both.[5,6,27] In our cohort, CNS-metastasis based on MRI findings were diagnosed in 10 cases. Of these 10 cases, only 3 dogs experienced additional local progression. MR features are commonly the only access to information on canine intracranial tumors treated with radiation therapy, as pretreatment histopathologic evaluation is uncommon.[4-6,33-35] MRI is the preferred modality for the evaluation of intracranial disease

328 with a 70% accuracy of predicting the type of primary brain tumors.[36-38] While MR features provide a
329 multitude of valuable information, no features allow to reliably differentiate tumors on a cellular level,
330 such as astrocytoma from oligodendroglioma or provide a tumor grade.[15,16] No prognostic factors on MRI
331 have been consistently identified in canine glioma patients.[5,6] One group, however, recently described
332 cystic tumors of presumed glial origin to have shorter OS.[4] We found an increased risk for tumor
333 progression in larger tumors. This was in line with findings in dogs with glioma treated with radiation
334 therapy, where the risk of death was increasing with relatively larger tumors.[4]
335 The correlation described in Lim et al. that tumor location relative to the SVZ predicts the pattern
336 of progression and outcome focused on glioblastoma multiforme, while in our study histopathology is
337 lacking and only imaging was available to characterize the brain tumors. The tumors described herein
338 most likely represent a heterogenous glioma population. Possibly, tumors contacting the subventricular
339 zone could represent less migratory tumor types in dogs, and thus glial tumors of lower aggressive
340 histotypes. Three quarters (76-80%) of glial tumor in dogs with survival data, however, were described of
341 grade III or IV (high grade).[5,9] Median survival time after surgery is not well described and found to be
342 low with 2-6 months[7,8], but one group found surgery and additional unspecified adjuvant immunotherapy
343 to result in 6.7 months for grade IV and 10 months for grade III tumors. The most common cause of death
344 was tumor recurrence (50/86, 58.1%), without specification of the site.[9] This survival time is comparable
345 to the 7.5 months we described after radiation therapy earlier, where in 5/12 (41.6%) glial tumors local
346 recurrence or progression was observed, whereof 4/12 (33.3%) had suspected CNS-metastasis
347 (unpublished result).[6]
348 With 26-40% of glial tumors recurring or progressing after therapy[5,6,9], and – at least after
349 radiation therapy – one third with CNS-metastasis[5,6], it would be clinically relevant to better identify this
350 subpopulation of patients in the future. Canine glioma is often marketed as a model for the human disease
351 counterpart.[9,11,39,40] With this in mind, backward extrapolations from human glioblastoma therapy to dogs
352 could be made as well: glioblastoma treatment usually includes maximal safe resection, followed by
353 radiation therapy and temozolomide chemotherapy (reviewed in Nam and de Groot, 2017).[41] Several

radiation oncologists have now even started exploring the subventricular zone as a potential target for therapeutic intervention with radiotherapy in humans. While some found improved outcome when including the ipsilateral subventricular zone into the high-dose area, others have not seen any associations and currently findings remain inconclusive.[14]

Apart from the lack of consensus on how to operate or irradiate dogs with glioma, adjuvant chemotherapy has only been very sporadically used. No survival advantage could be found with lomustine at maximally tolerated doses of 90 mg/m^2 every four weeks[42] or with subtherapeutic doses of either lomustine (mean 60.6 mg/m^2, every 3 to 6 weeks) or temozolomide (65 mg/m^2 daily in a five-day cycle).[5,43] The maximally tolerated dose for temozolomide for dogs is now established as 150 mg/m^2 daily in a five-day cycle and should serve as a future reference dose.[44] Whether the addition of temozolomide is useful for dogs with glioma remains to be explored in parallel with tumor-specific markers from biopsies. For example, the methylation status of the repair protein O^6-alkylguanine DNA alkyl-transferase, encoded by the O-6-methylguanine-DNA methyltransferase (MGMT) gene is of highest relevance, when treating with temozolomide.[45-47] In glioblastoma patients with methylated (epigenetically silenced) MGMT promoter and therefore incomplete DNA-repair capacity, chemoradiation treatment is more damaging and leads to a survival advantage.[48-50] In dogs, the possible importance of the MGMT methylation has neither been investigated on an *in vitro* nor *in vivo* level up to date, presenting a future outlook for research.

We acknowledge limitations to this study: 1) All patients were treated based on an imaging diagnosis only, hence there is a lack of histopathological information such as type or grade. Yet, we deem our findings of relevance as many of our pet owners do not consider a biopsy due to associated risks and/or costs. 2) In two dogs of our sample, the tumors were not located in the forebrain, but in the cerebellum and in the brainstem area, respectively. Tumor location in dog's glioma has not been described to be of prognostic impact, and hence we decided to include these patients, even they were very unlikely to invade the SVZ for anatomical reasons. 3) The retrospective nature of the study precludes standardized imaging protocols at initial diagnosis and standardized follow-up (imaging and postmortem examination). Including advanced imaging in the initial work up (such as diffusion and perfusion sequences),

performing standardized imaging follow-up and availability of postmortem examinations could add essential information about the involvement of the subventricular zone in progression of canine glioma. 4) the three factors, "tumors located at the subventricular zone", "ventricular contact" and "ventricular invasion", partially overlap. Furthermore, based on the available imaging quality and to the general resolution of MR-images, ventricular invasion can't be excluded in many cases with ventricular contact. We believe it is important to further investigate possible prognostic factors, as a future approach to canine glioma treatment will include a shift in treatment or the addition of adjuvant therapies.

Conclusion

Treatment of glial tumors in dogs can be recommended irrespective of tumor location, with the caveat of a possible shorter time-to-progression, tumor-specific survival and a higher rate of CNS-metastasis in tumors contacting the subventricular zone and in tumors with overt ventricular invasion. After radiation therapy, the pattern of failure seems to frequently involve CNS-metastasis. CNS-metastasis despite local tumor control should be addressed in future treatment approaches.

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522

Figure Legends

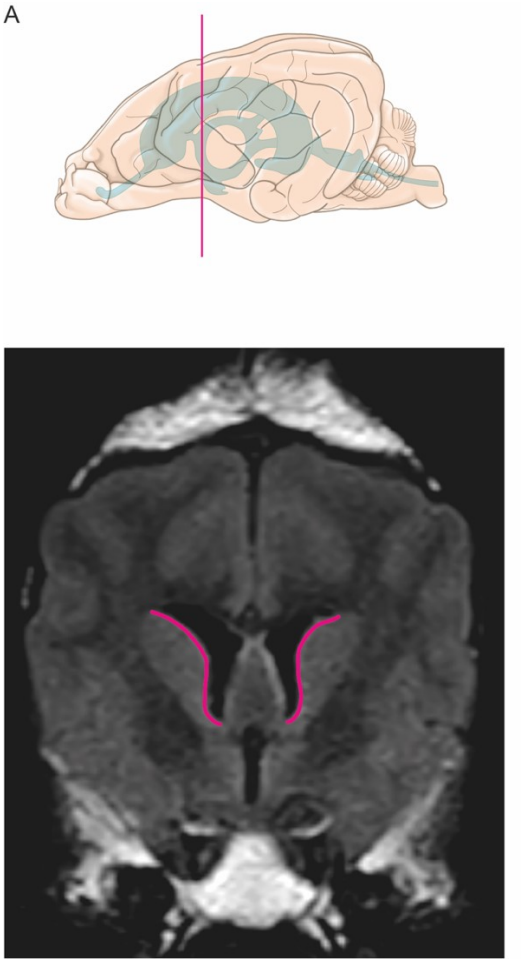
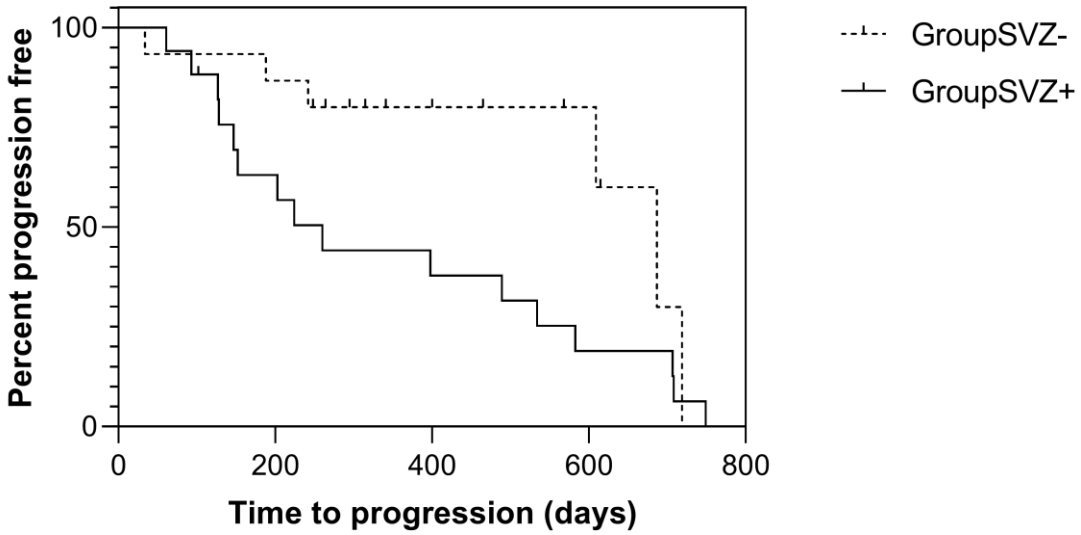


Figure 1: Transverse MR image of the brain. The transverse pink line indicates the rostrocaudal location. A, The pink curve between the nucleus caudatus and lateral aspect of the lateral ventricle on this FLAIR image illustrates the subventricular zone.[17]

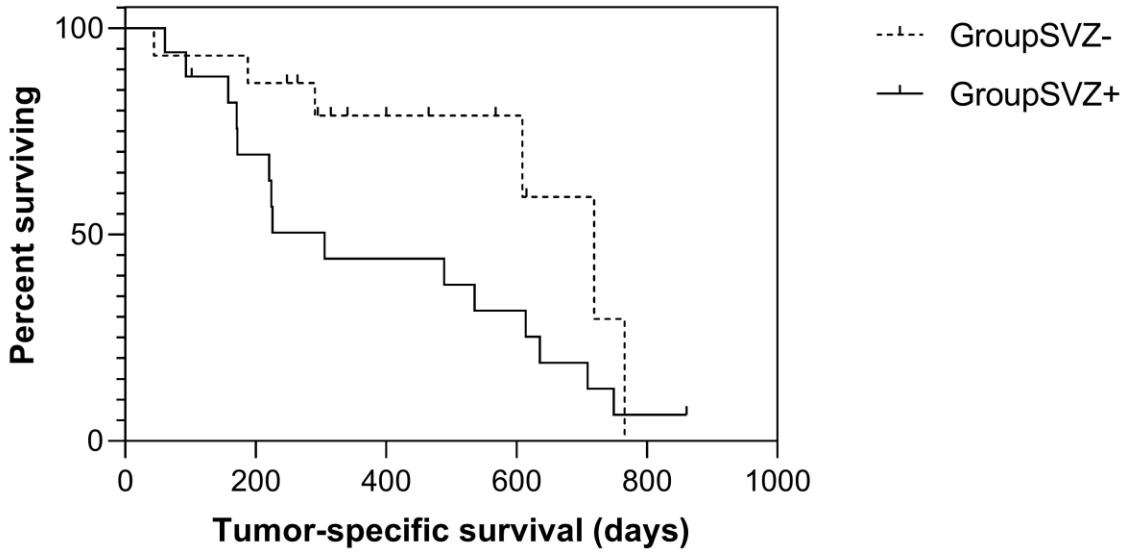


528

529 **Figure 2:** Kaplan-Meier plot showing time to progression for the 32 dogs with glial tumors after

530 radiotherapy: Median TTP for all cases was 534 days (95%CI, 310-758). TTP was significantly shorter in

531 dogs with lesions at the SVZ compared with GroupSVZ- (median TTP, 260 vs. 687 days; $P = 0.049$).



532

533 **Figure 3:** Kaplan-Meier plot showing tumor-specific survival for the 32 dogs with glial tumors after

534 radiotherapy: Median tumor-specific survival was 609 days (95%CI, 382-835). Involvement of the

535 subventricular zone was significantly associated with a shorter tumor-specific survival (median, 306 vs.

536 719 days; P = 0.044).

Table 1. Distribution of patient and tumor characteristics in 32 dogs with intracranial glial tumors. Tumors were either considered as a whole or stratified according to whether they contacted the subventricular zone (SVZ) or not.

	Total (n=32)	**GroupSVZ+** [lesion contacting subventricular zone] (n=17)	**GroupSVZ-** [lesion NOT contacting subventricular zone] (n=15)	**P**
Age (years)**	7.6 (1.5-13.5)	7 (1.5-10.8)	8.2 (5.8-13-5)	0.007
Weight (kg)**	15.6 (3.4-39.5)	26 (3.4-38)	14.2 (7.9-39.5)	0.417
Sex				0.526
female, intact	4 (12.5%)	2 (11.8%)	2 (13.3%)	
female, spayed	11 (34.4%)	7 (41.2%)	4 (26.7%)	
male, intact	10 (31.3%)	6 (35.2%)	4 (26.7%)	
male, castrated	7 (21.9%)	2 (11.8%)	5 (33.3%)	
Head conformance				0.678
brachycephalic	25 (78.1%)	14 (82.4%)	11 (73.3%)	
dolichocephalic	7 (21.9%)	3 (17.6%)	4 (26.7%)	
GTV (cm^3)**	4 (0.2-16.1)	6.1 (0.5-16.1)	3 (0.2-9.2)	0.008*
Brain volume (cm^3)**	90.4 (46.0-120.9)	95.9 (46.9-119.5)	86.6 (56.4-120.9)	0.115
GTV/brain volume (%)**	5.4 (0.2-14.1)	6.6 (0.8-14.1)	3.4 (0.2-10.2)	0.006*
Progression (any)				0.001*
yes	22 (68.8%)	16 (94.1%)	6 (40%)	
no	11 (34.4%)	1 (5.9%)	9 (60%)	
CNS metastasis				0.028*
yes	11 (34.4%)	9 (52.9%)	2 (13.3%)	
no	21 (65.6%)	8 (47.1%)	13 (86.7%)	
Dead, any cause				0.013*
yes	24 (75.0%)	16 (94.1%)	8 (53.3%)	
no	8 (25.0%)	1 (5.9%)	7 (46.7%)	
Dead, tumor-related				0.035*
yes	21 (65.6%)	15 (88.2%)	6 (40.0%)	
no	11 (34.3%)	2 (11.8%)	9 (60.0%)	
Median tumor progression (95% CI) (days)	534 (310-758)	260 (150-370)	687 (569-805)	0.049*
Median overall survival (95% CI) (days)	489 (147-831)	226 (112-340)	609 (274-944)	0.066
Median tumor-specific survival (95% CI) (days)	609 (382-835)	306 (148-464)	719 (552-886)	0.044*

* = significant; ** = values expressed as median and range; GTV = gross tumor volume; CNS = central nervous system.