Sequential Treatment of a Large Pituitary Corticotroph Neoplasm and Associated Neurological Signs in a Dog

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ABSTRACT

No standardized treatment guidelines are reported in veterinary medicine for dogs with large pituitary corticotroph neoplasms causing neurological signs, and such dogs usually have a short overall survival. When these dogs undergo pituitary surgery and the tumor regrows there are few reports of subsequent treatments.

A 7 yr old male Maltese diagnosed with pituitary-dependent hypercortisolism developed seizures in conjunction with a large pituitary corticotroph adenoma and underwent transsphenoidal hypophysectomy. After 3 yr of clinical remission, hypercortisolism recurred, and trilostane therapy was initiated. One year later, the dog developed new neurological signs and computed tomography revealed regrowth of a large pituitary mass that was then treated with radiation therapy. The dog lived disease-free for 3 more yr. At postmortem examination, a more aggressive pituitary neoplasm than the one examined at the time of surgery was found, which is suggestive of malignant transformation into a carcinoma despite the absence of convincing metastasis. (*J Am Anim Hosp Assoc* 2019; 55:e552-02. DOI 10.5326/JAAHA-MS-6831)

Introduction

Pituitary adrenocorticotropin (ACTH)-secreting adenomas are the most commonly diagnosed pituitary neoplasms in dogs.[1] The most frequent clinical signs are polyuria, polydipsia, polyphagia, enlargement of the abdomen, and truncal alopecia associated with hypercortisolism (Cushing's syndrome) in response to the increased level of ACTH. Neurological signs occur in 10–20% of cases and are caused by compression of cerebral structures around the fossa hypophysialis by a very large and/or a fast-growing adenoma.[1]

The reported therapeutic options for large ACTH-secreting pituitary adenomas (often termed "macroadenomas") causing neurological signs are surgical removal or radiation therapy.[2–5] However, for both treatments, the size of the tumor has an impact on the prognosis for survival time and recurrence of hypercortisolism after surgery.[4–7] There is no consensus in veterinary medicine with respect to the best treatment modality for large pituitary adenomas (surgery versus radiation therapy) or what treatment should be used after recurrence of the tumor. In human medicine, surgery is the first line of treatment for Cushing's disease, with radiation therapy used when surgery is not feasible, when hypercortisolism persists after surgery (residual disease), after recurrence of the disease, or as adjuvant therapy following primary or repeated surgeries for pituitary macroadenomas.[8] Radiation therapy in humans results in good local control of the pituitary mass;

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ACTH (adrenocorticotropin); CT (computed tomography); P/B (pituitary height/brain area); PO (*per os*); UCCR (urine cortisol/creatinine ratio)

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however, medical therapy is usually required to regulate hypercortisolism at least in the initial period after treatment.[9]

This case report describes the long-term management and the histopathological evolution of a large ACTH-secreting pituitary adenoma in a dog.

Case Reports

A 7 yr old male Maltese was presented with polyuria, polydipsia, and polyphagia of 4 mo duration. In the 2 wk before the clinical examination, the dog had experienced two episodes of generalized tonic-clonic seizures. On physical examination, bilateral symmetrical alopecia of the trunk was evident. Neurological examination was within normal limits. The results of hematology, serum biochemistry, and urinalysis were suggestive of hypercortisolism, which was confirmed by a positive low-dose dexamethasone[a] (0.01 mg/kg of body weight) suppression test (cortisol at 0 hr was 3.6 μg/dL, reference range 1–4.8 μg/dL; cortisol after 4 hr 3 μg/dL, cortisol after 8 hr 4.1 μg/dL, reference range 0–1.4 μg/dL). In addition, the urine high-dose dexamethasone suppression test was performed by measuring the urine cortisol (nmol/L)/creatinine (μmol/L) ratio (UCCR) in morning samples (UCCRs of the first and second morning [342.8 and 317.9 $\times$ 10^{-6}, reference range 0.3–8.3 $\times$ 10^{-6}], and third morning after administration of dexamethasone [0.1 mg/kg *per os* {PO} of body weight] [UCCR 302.9 $\times$ 10–6]).[10] Both tests showed resistance to suppression after dexamethasone, and in order to confirm the origin of hypercortisolism, ultrasonography of the abdomen and computed tomography (CT) of the skull and thorax were performed. On abdominal ultrasonography, bilateral enlargement of the adrenal glands and increased liver echogenicity were detected. Contrast-enhanced CT of the skull showed a mass in the pituitary region of 9.9 mm in height, 8.3 mm in width, and 9.3 mm in length, compatible with a pituitary tumor (**Figure 1A**). The pituitary height/brain area (P/B) value was 0.75 (reference range for enlarged pituitaries or "macroadenoma" >0.31).[3,11] Imaging findings confirmed the diagnosis of dexamethasone-resistant pituitary-dependent hypercortisolism. Transsphenoidal hypophysectomy was performed using a previously described microsurgical technique.[3] Briefly, the pituitary fossa was approached by the midline transoral and transnasopharyngeal route. Following burring of the basisphenoid bone, the dura mater was exposed. After incision of the dura, the mucoid adenomatous pituitary tissue was removed from the pituitary fossa and submitted for histopathology. The surgeon classified the surgery as a total hypophysectomy based on absence of pituitary remnants in the fossa and visual identification of the unaffected hypothalamic surface and the entrance to the third ventricle. Recovery from surgery was uncomplicated. After surgery, the dog received hormone supplementation with cortisone acetate[b] (1 mg/kg PO *q* 12 hr tapered down to 0.25 mg/kg *q* 12 hr over 4 wk), thyroxine[c] (15 μg/kg PO *q* 12 hr), and desmopressin[d] (1 drop, ~4 μg in the eye *q* 12 hr).[3]

Postsurgical samples of the excised pituitary tissue were submitted for histology and stained with hematoxylin and eosin. Sections under light microscopy revealed a proliferation of the pituitary cells showing a basophilic to more chromophobic cytoplasm, nuclei of various sizes, and prominent nucleoli (**Figure 2A**). No mitotic figures were seen. Immunohistochemical staining was performed for ACTH, alpha-melanocyte stimulating hormone and growth hormone. The cytoplasm of the pituitary adenoma cells immunostained diffusely positive for ACTH and alpha-melanocyte stimulating hormone and negative for growth hormone, confirming the diagnosis of a corticotroph cell adenoma (Figure 2B). Staining for Ki67 was performed using anti-Ki67[e] diluted 1:600 with blocking solution 10% normal goat serum in phosphate-buffered saline for 30 min at 37°C and incubated overnight at 4°C. The avidin-biotin complex kit plus secondary biotinylated anti-mouse antibody for 30 min was used as the detection system, using 3,3'-diaminobenzidine as the chromogen and Papanicolau hematoxylin as the contrast. The canine intestine was used as the positive control. Ki67 staining was considered negative.

To confirm effectiveness of surgery, plasma ACTH and cortisol concentrations were measured at 1, 2, 3, 4, 5, and 24 hr after surgery.[7] The plasma ACTH concentrations at 3–5 hr after surgery were slightly above the detection limit of the ACTH assay and were indicative of near-complete hypophysectomy.[7]

After surgery, the seizures and other clinical signs resolved. Clinical rechecks and UCCR tests (reference range 0.3–8.3 $\times$ 10^{-6}) 2 and 8 wk after surgery, and then every 6 mo, confirmed remission of the hypercortisolism.[6]

Approximately 3 yr after surgery, the dog presented with recurrence of polyuria, polydipsia, polyphagia, and alopecia. Physical examination again showed truncal alopecia and marked atrophy of the testicles. The UCCR was elevated (52 $\times$ 10^{-6}, reference range 0.3–8.3 $\times$ 10^{-6}) and the plasma ACTH concentration (46.2 pg/mL, reference range 0.4–12.8 pg/mL) confirmed the recurrence of pituitary-dependent hypercortisolism.[3,6] CT revealed an asymmetrically elongated sellar mass of 4 $\times$ 5 mm in size, homogenously enhancing after intravenous administration of a contrast medium (Figure 1B). These findings were considered as regrowth of the pituitary tumor. Hormonal supplementation with cortisone acetate was stopped; oral treatment with trilostane[f] (1.5 mg/kg of body weight PO *q* 12 hr) was started and resulted in good control of the clinical signs. The administration of thyroxine and desmopressin was continued.

One year after recurrence of hypercortisolism (i.e., 4 yr after surgery), marked neurological signs appeared, including severe obtundation and recurrence of generalized tonic-clonic seizures.

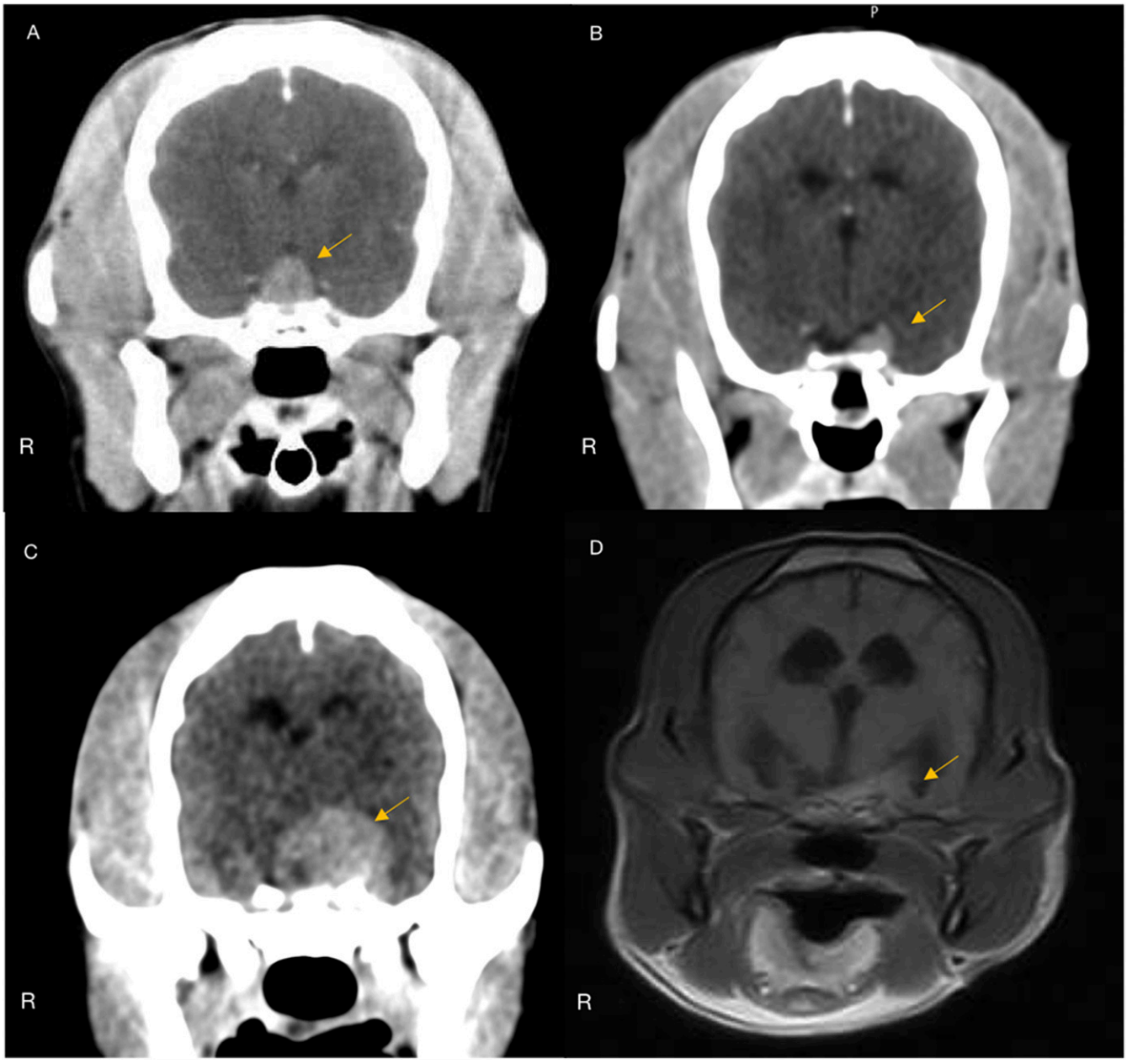

FIGURE 1 *Sequential transverse contrast-enhanced CT images* (A–C) *and transverse contrast-enhanced T1-weighted MRI* (D) *of the cranium over a 6 yr period in a Maltese with PDH.* (A) *A pituitary mass was evident, measuring 9.9 (height) × 8.3 (width) × 9.3 mm (length) with a P/B value of 0.75 at time of diagnosis.* (B) *Three years after surgery, hypercortisolism recurred and CT revealed a small (4 × 5 mm) contrast-enhancing lesion* (yellow arrow) *on the left side of the fossa hypophysialis.* (C) *At 4 yr after surgery, the dog developed neurological signs and CT showed a large (14 × 21 mm) contrast-enhancing, asymmetric mass* (yellow arrow) *in the pituitary region.* (D) *Transverse contrast-enhanced T1-weighted MRI of the brain 6 yr after hypophysectomy and 2 yr after radiotherapy for the treatment of the ACTH-secreting adenoma. A remnant mass (5 × 14 mm) is present ventral to the caudal portion of the diencephalon* (yellow arrow). *ACTH, adrenocorticotropin; CT, computed tomography; P/B, pituitary height/brain area; PDH, pituitary-dependent hypercortisolism.*

Neurological examination revealed recumbence and stuporous mentation, bilaterally decreased menace response, and unresponsive miotic pupils. Mannitol[g] (1 g/kg of body weight) was administered, and the neurological condition improved. CT revealed a significant increase in the size (14 × 21 mm) of the sellar mass, with an asymmetric appearance on the left side (Figure 1C). Radiotherapy was prescribed and delivered with a 6 megavoltage linear accelerator[h] using photons and CT-based, three-dimensional conformal radiation

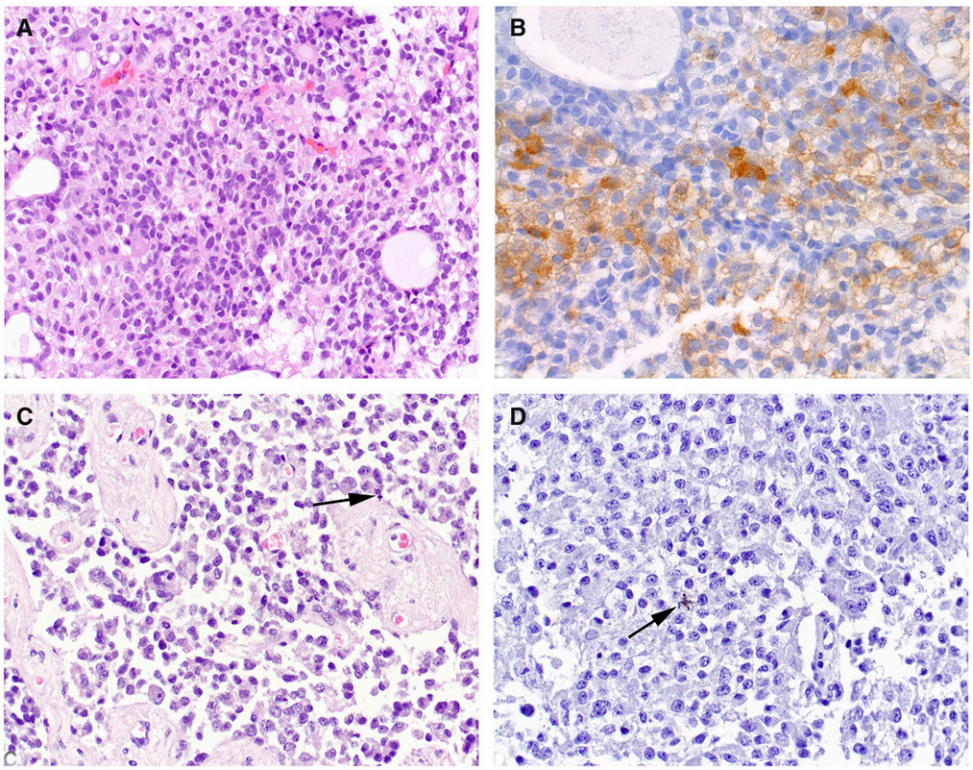

FIGURE 2 *Histology and immunohistochemistry of the pituitary adenoma over time. (A) Photomicrograph of the initial neoplasm showing a moderately pleomorphic population of basophilic to chromophobic neoplastic cells using an H&E stain. (B) Some of the neoplastic cells had clear immunoreactivity for ACTH. (C) The recurrent pituitary neoplasm 6 yr after surgery showed neoplastic cells with a similar morphology to the initial neoplasm but with a higher number of mitotic figures* (arrow; *H&E). (D) Ki67 immunoreactivity was noted in the mitotic figure in the recurrent neoplasm* (arrow). *All histological pictures were taken with a 40× objective. ACTH, adrenocorticotropin; H&E, hematoxylin and eosin.*

therapy. A total dose of 45 Gy was delivered, divided into 20 fractions of 2.25 Gy given over 4 consecutive wk. Neurological signs regressed 3 wk after starting the radiotherapy, and trilostane therapy was continued to control hypercortisolism. The dog remained free of clinical signs thereafter.

Two years later (i.e., 6 yr after surgery), the dog developed paraparesis, decreased tail movement, difficulties during defecation, and urinary incontinence. The neurologic examination localized the problem to the cauda equina (L4–S3 spinal cord segments). MRI was performed, and a hypointense lesion of the seventh lumbar vertebral body was identified on T1- and T2-weighted sequences. In the area included between L5 and S1, the lumbosacral epidural space, the meninges, and the nerve roots, which were isointense on T1- and T2-weighted sequences, had marked T1 contrast enhancement. The differential diagnosis included neoplastic and inflammatory infiltrative diseases. MRI of the brain revealed the presence of a mass (5 × 14 mm) located ventral to the caudal portion of the diencephalon. The mass was hyperintense on T2-weighted sequences and hypointense on T1-weighted sequences, with paramagnetic contrast enhancement on T1. The lesion extended to the left without defined margins and slightly displaced adjacent brain structures (Figure 1D). It was considered compatible with regrowth of the pituitary neoplasm.

Two weeks later, after the progression of the neurological signs and development of paraplegia, the dog was euthanized at the owner's request. The owner gave permission for cosmetic necropsy, and only the brain was removed from the skull and formalin fixed. Macroscopic examination of the ventral surface of the brain revealed grey tissue in the pituitary region. Subsequent histological evaluation of the pituitary tissue identified a nonencapsulated neoplastic proliferation contiguous with the meninges and infiltrating the brain parenchyma. Neoplastic cells were arranged in islands and cords, were embedded in a rich amorphous eosinophilic extracellular matrix, and had a basophilic cytoplasm. The nuclei of affected cells were vesicular with marginated chromatin and had a prominent nucleolus. Anisocytosis and anisokariosis were moderate. Three mitotic figures were counted in 10 randomly selected fields at 400× magnification (Figure 2C). Cytoplasmic granules of the neoplastic cells stained intensely periodic acid-Schiff–positive. The sections were submitted for immunohistochemistry similar to the surgical samples. Multifocally, pituitary neoplastic cells displayed ACTH-immunoreactivity. Ki67 immunoreactivity was evident in neoplastic cells, occasionally even in neoplastic cells with a mitotic figure in the recurrent neoplasm (Figure 2D), whereas the surgical sample had shown negative Ki67 immunostaining. The postmortem histological diagnosis was recurrent, infiltrating, malignant, ACTH-secreting pituitary neoplasm, probably a carcinoma.

Discussion

Transsphenoidal hypophysectomy is an effective treatment for Cushing's disease in dogs and humans, and in the subject of the

present report, remission of hypercortisolism was achieved for 3 yr.[3,8]

Large pituitary tumors may be associated with and responsible for seizures.[1,12] As in our case, seizures are the main neurological signs reported at initial presentation and disappear after hypophysectomy.

Published negative prognostic factors for clinical remission (disease-free interval) and survival rate after transsphenoidal hypophysectomy include large pituitary tumor size, high preoperative UCCRs, preoperative lack of suppression in response to high dose dexamethasone, and immediate postoperative plasma ACTH concentration above the detection limit.[6,7] Because all these factors were documented in the present case, the dog was closely monitored clinically and hormonally postoperatively. In humans, conflicting results have emerged from the literature about prognostic factors for recurrence. However, the consensus exists that patients with very large pituitary adenomas seem to have a lower chance of achieving remission and, therefore, a higher chance of recurrence after surgery, similar to what has been observed in dogs.[6]

Calculation of P/B ratio allowed normalization of the pituitary dimensions for the size of the dog. The P/B ratio had the highest discriminatory power in distinguishing enlarged from nonenlarged pituitaries.[3,11]

Mamelak et al. proposed a modification of the technique for transsphenoidal hypophysectomy using a high-definition video telescope for better inspection of the fossa hypophysialis at the end of surgery.[13] The use of the video telescope may reduce the chance of leaving neoplastic tissue behind and recurrence of the tumor.[13] Moreover, a new MRI classification system for pituitary neoplasia was developed by Sato et al. to improve the case selection of dogs for transsphenoidal hypophysectomy.[14] In the present case report, the preoperative diagnostic imaging was done with CT to better localize the pituitary gland in relation to the surgical landmarks. A direct comparison of our case with this MRI-based classification system was therefore not possible.

In the present case, initial recurrence of hypercortisolism was controlled by medical treatment with trilostane for 3 additional yr. However, the subsequent enlargement of the tumor made further treatment necessary. A second pituitary surgery was not considered the best option because of the asymmetric appearance of the large recurrent neoplasm. Radiation therapy was performed with the aim of reducing the tumor size and decreasing the neurological signs. The dog regained a normal neurological state 3 wk after radiation treatment was begun. According to the literature, radiotherapy is effective in the case of mild neurological signs in dogs with hypophyseal tumors, and it appears to have been helpful for our patient with pituitary disease.[4,5] In our case, radiotherapy produced a regression of severe neurological signs in a short time. In dogs, radiotherapy causes reduction in the pituitary mass size in a variable period of time and delays tumor enlargement, but it does not eliminate the hypersecretion of ACTH and hypercortisolism.[1] In a recent study of nine dogs with pituitary-dependent hypercortisolism, it was concluded that radiotherapy is effective to reduce pituitary size and the mass effect but does not appear to affect blood hormone concentrations, necessitating additional medical treatment against hypercortisolism.[15] For this reason, treatment with trilostane was continued in our patient.

There is little information available about treatment in the case of recurrence or persistence of the disease after surgery. The combined use of surgery and radiation therapy was only recently and briefly reported in five dogs with a P/B ratio >0.74 by Owen et al. with survival outcomes ranging from 371 to 1190 days in three dogs, whereas two dogs were still alive at the time of the writing of the article.[16] A single case report of a dog who was operated on twice for a pituitary corticotroph adenoma was found in a search of the veterinary literature.[17] Further studies are needed to better understand the efficacy of a combination of therapy in dogs with hypercortisolism from large pituitary neoplasm. Nevertheless, the dog in the present report survived for 6 yr after the sequential therapies of surgery, medical therapy, and radiation therapy.

Pituitary tumors are usually found to be histologically benign neoplasms, but some very large adenomas may exhibit aggressive biological behavior because of their fast and invasive growth to the surrounding cerebral tissue.[18,19] To the best of our knowledge, there are no studies on the growth potential of these tumors over time, their invasiveness, and their potential transformation to carcinomas. It is unclear whether the pituitary neoplasm in our patient changed its biological behavior because of the natural progression of the disease, whether a new neoplasm arose, or whether radiation therapy played a role in the evolution of the neoplasm toward more malignant histologic features, a higher proliferation rate, and invasive behavior.

The Ki67 labeling index is a well-known marker of proliferation rate in human pituitary adenomas.[20] Studies on proliferation markers (Ki67 and minichromosome maintenance-7) in canine pituitary adenomas are limited to a few reports, and no significant differences in Ki67 labeling indexes between enlarged and nonenlarged pituitaries were found.[19,21] In humans, pituitary tumors with elevated Ki67 labeling are considered more aggressive.[20] For this reason, the Ki67 labeling was measured in the present case. There was negative Ki67 staining in the surgical sample, whereas Ki67 staining was evident in the postmortem pituitary tumor sample. However, a clear classification based on histopathological criteria for more malignant pituitary neoplasia, is missing in dogs and humans.[18–20,22]

The postmortem examination was limited and did not include the lumbar spinal lesion that caused the signs that resulted in euthanasia. This was especially unfortunate in the light of a recent case report that

described an 8 yr old border collie dog diagnosed with an invasive pituitary macrotumor who developed paraparesis and lumbosacral pain 5 mo after radiation therapy for the pituitary tumor. Necropsy revealed a pituitary carcinoma with meningeal drop metastasis to the cauda equina.[23] Pituitary carcinomas, classified by the presence of intracranial or extracranial metastatic lesions, are very uncommon in dogs. We, therefore, cannot conclude whether the caudal lumbar pathology seen on MRI in our case was a separate disease process or related (i.e., metastatic disease) to the pituitary tumor, but considering the rather extensive infiltration and destruction of adjacent neural tissue the progression of the disease could be considered to be malignant transformation of the neoplasm into a carcinoma.

Conclusion

This report demonstrated a 6 yr survival time in a dog who underwent surgery for a large pituitary tumor and, after recurrence of the clinical signs and regrowth of the tumor, was managed with medical and then radiation therapy. In addition, a change in morphological and biological behavior of the pituitary mass was detected over time, marked by an increase in proliferation rate and a tendency to invade the surrounding neural tissue, with at least possible metastasis. We found that sequential multimodal treatment of pituitary adenomas has the potential to prolong disease-free interval and survival time in dogs with large pituitary neoplasms, and further studies of combination and/or sequential multimodal therapies for this disease are warranted.

FOOTNOTES

[a] Dexadreson; Intervet International BV, Boxmeer, the Netherlands
[b] Cortisoniacetas; Genfarma, Maarssen, the Netherlands
[c] L-thyroxine; Aesculaap, Boxtel, the Netherlands
[d] Minirin; Ferring, Hoofddorp, the Netherlands
[e] Clone MIB1; Dako, Amstelveen, the Netherlands
[f] Vetoryl; Dechra, Skipton, United Kingdom
[g] Mannitolo FKI 18%; Fresenius Kabi Italy, Isola Della Scala, Italy
[h] Clinac DHX; Varian, Palo Alto, California

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