Brain and Ocular Carcinomatosis Diagnosed Ante-Mortem by Cerebrospinal Fluid and Aqueous Humor Cytology in a Dog

Rapoport, K.,[1]* Aroch, I.,[1] Brenner, O.,[2] Srugo, I.,[3] Chai, O.[1] and Shamir, M.H.[1]

[1] The Hebrew University Veterinary Teaching Hospital, Koret School of Veterinary Medicine, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 7620001, Israel.
[2] Veterinary Resources, Weizmann Institute of Science, Rehovot, 7610001, Israel.
[3] Dr. Itay Srugo' current address is Vetneuro, P.O. Box 44905, Beit Berl, Israel.

* **Corresponding author:** Dr. Kira Rapoport, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 761001, Israel. Email: kira.rapoport@mail.huji.ac.il

ABSTRACT

A 9-year old female cocker spaniel dog was presented with uveitis, blindness and neurological signs, with a history of mixed malignant mammary tumor that had been excised two months prior to presentation. Brain and ocular metastasis of mammary adenocarcinoma, with meningeal carcinomatosis (MC) were diagnosed by both cerebrosspinal fluid (CSF) and the aqueous humor (AH) cytology, which revealed numerous cells, with overall morphology compatible with epithelial secretory origin, demonstrating malignant characteristics. The morphologic diagnosis was a secretory cell carcinoma, most probably of mammary gland origin. The dog was euthanized at its owners' request. Gross pathology showed multiple nodular masses of variable diameter (3 to 20 mm) on examination of the right ventral thalamus, inguinal mammary lymph nodes and lungs. Microscopic examination revealed neoplastic epithelial cell infiltrating the leptomeninges, brain parenchyma, inguinal mammary lymph nodes, eyes and lungs. The neoplastic cells were cytokeratin-positive, vimentin-negative, supporting their epithelial origin. The definitive diagnosis was malignant mixed mammary tumor with distant metastases to various organs, and MC. In this case, both CSF and AH cytological analyses were useful for the ante-mortem diagnosis of mammary carcinoma, MC and ocular metastases. These finding along with brain computed tomography suggested brain metastases.

Key words: Canine; Metastasis; Central Nervous System; Ocular; Mammary Gland Carcinoma.

INTRODUCTION

Mammary gland carcinoma (MGCA) is the most common malignant tumor in intact female dogs (1). Brain MGCA metastasis is not uncommon, and accounts for approximately 11% of secondary intracranial neoplasia cases (2). Meningeal carcinomatosis (MC), also termed neoplastic meningitis, is a type of carcinomatous central nervous system (CNS) metastasis. MC consists of diffuse leptomeningeal cancer cell infiltration and can be caused by both metastatic and primary CNS tumors (3). In humans, 5% to 8% of cancer patients develop MC, with breast, lung and hematologic malignancies, and melanoma being the most prevalent primary tumors involved (3,4). MC, although uncommon, has been previously reported in six dogs, three of which had MGCA, while two had choroid plexus carcinomas (5-10). Neurological signs of MC can be focal, multifocal or diffuse, and may occur months to years following the primary tumor excision (2, 5-8, 10).

In humans, MC is diagnosed based on the clinical signs, advanced imaging and cerebrospinal fluid (CSF) analysis. Its diagnosis may be challenging, since the lesions may not be apparent early in the course of the disease, while CSF analysis

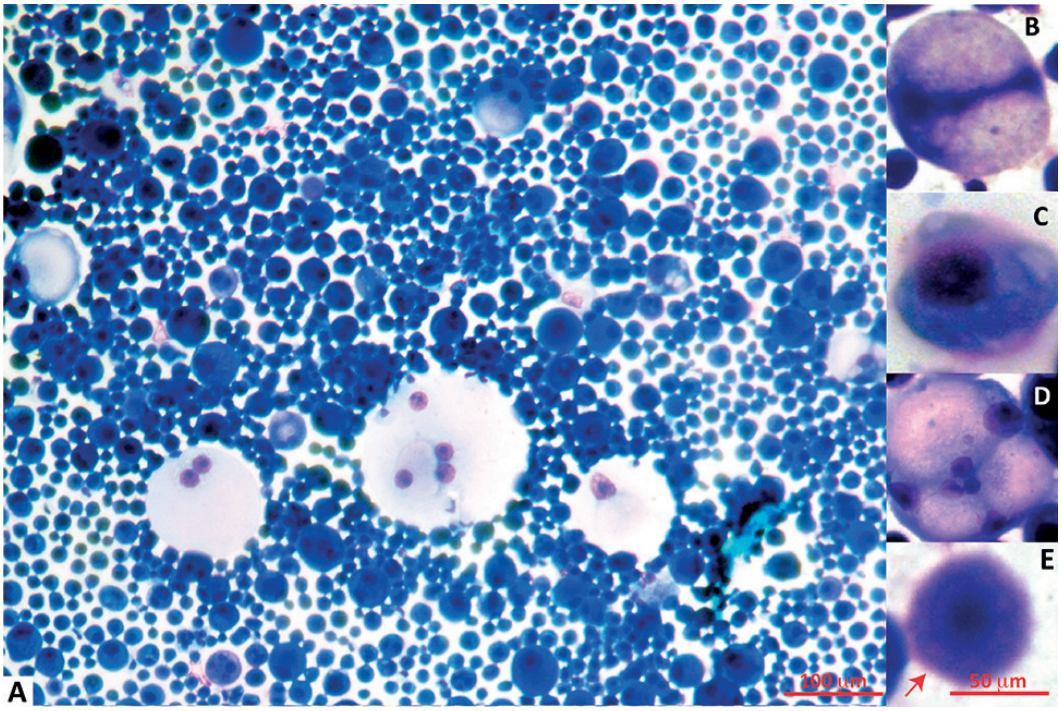

Figure 1: Cerebrospinal fluid cytology of a dog with meningeal carcinomatosis, brain and ocular metastases of mammary gland adenocarcinoma (Modified Wright's stain). Note the pleocytosis with round cell predominance, demonstrating marked anisocytosis, anisokaryosis, basophilic cytoplasm and increased nucleus to cytoplasm ratio, some of which contain amorphous, often unstained secretory product (A-D). The cytoplasm of these cells is basophilic, with markedly prominent nucleoli (B). Tumor cell with purplish to pinkish halo surrounding the cell perimeters, resembling epithelial cell brush borders (C; E, red arrow).

may be unremarkable (11). We present a case of CNS MC secondary to MGCA, where cytological analysis of both the CSF and aqueous humor (AH) led to the early ante-mortem definitive diagnosis.

CASE REPORT

A 9-year old spayed female cocker spaniel was referred to the Koret School of Veterinary Medicine Teaching Hospital (KSVMTH) with uveitis, blindness and circling to the left, of one week duration and weakness and anorexia of two days duration. The dog had undergone surgical excision of mammary tumors twice in the past, two years and two months prior to the onset of the current clinical signs. The histopathological diagnosis of the latter tumor was of a mixed malignant mammary tumor. At presentation, the dog was lethargic with pale mucous membranes, tachypnea and melena. Neurological examination showed dementia, tetraparesis, circling, head turn and head tilt to the left, bilaterally absent menace response and pupillary light reflex, hypermetria of all four limbs and ambulatory tetraparesis with mild conscious proprioception deficits. Ophthalmic examination showed bilateral blindness, fixed dilated pupils, severe corneal edema and uveitis. The intra ocular pressures (IOPs) were 35 and 45mm Hg in the right and left eyes, respectively (reference interval [RI], 12-25mm Hg), indicating bilateral glaucoma, likely secondary to the uveitis. The neuroanatomical lesion localization was multifocal, involving the forebrain, vestibular system and both eyes.

Complete blood count, serum biochemistry and chest survey radiographs were unremarkable. CSF was collected from the cerebello-medullary cisterna, cyto-centrifuged, and slides were stained by a modified Wright's staining solution. The CSF total nuclear cell count (TNCC) was 15,000 cell/µL, (Reference Interval (RI), <5 cells/µL), and the protein concentration was 49 mg/dL (RI, <25mg/dL).

Cytological analysis showed two cell populations. The majority (95%) were round to cuboidal cells, showing marked

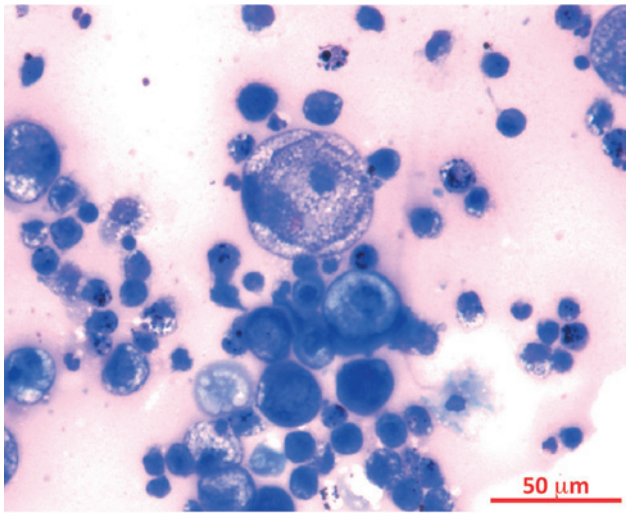

Figure 2: Aqueous humor cytology of a dog with meningeal carcinomatosis, brain and ocular metastases of mammary gland adenocarcinoma (Modified Wright's stain). Note the marked anisocytosis and anisokariosis, large nucleoli and bizarre atypical giant cells. Some cells contain cytoplasmic secretory vacuoles and dark secretory product.

anisocytosis and anisokaryosis, high nucleus to cytoplasm ratio and prominent, large, sometimes multiple nucleoli (Figure 1 A-E). The nuclei in the larger cells were displaced eccentrically and composed of course chromatin. Nuclear molding, binucleation and multiple nuclei (up to 8 nuclei) were observed. The cytoplasm was basophilic, occasionally containing amorphous secretory product of variable amounts. The medium sized cells contained small clear vacuoles, while the larger ones showed diffuse cytoplasmic vacuolation. The cell perimeter sometimes showed purple to pink halo resembling brush borders. Mitotic figures, sometimes of abnormal forms, were noted. The overall morphology of the cells was compatible with an epithelial, secretory origin. The morphologic diagnosis was carcinoma of secretory cells, most probably MGCA or anaplastic carcinoma. The other cell population (5%) consisted of mononuclear cells, including monocytes and moderately reactive macrophages, suggestive of chronic granulomatous inflammation.

Aqueous humor (AH) was obtained with a 25 gauge hypodermal needle during anesthesia. Cytological analysis of the aqueous humor showed high cellularity. The overall cellular morphology and proportions were similar to cerebrospinal fluid (CSF) cytological findings (Figure 2). Some of the AH neoplastic cells contained cytoplasmic dark-greenish to black amorphous aggregates, highly resembling active secretory material of mammary gland cells during lactation (12). The

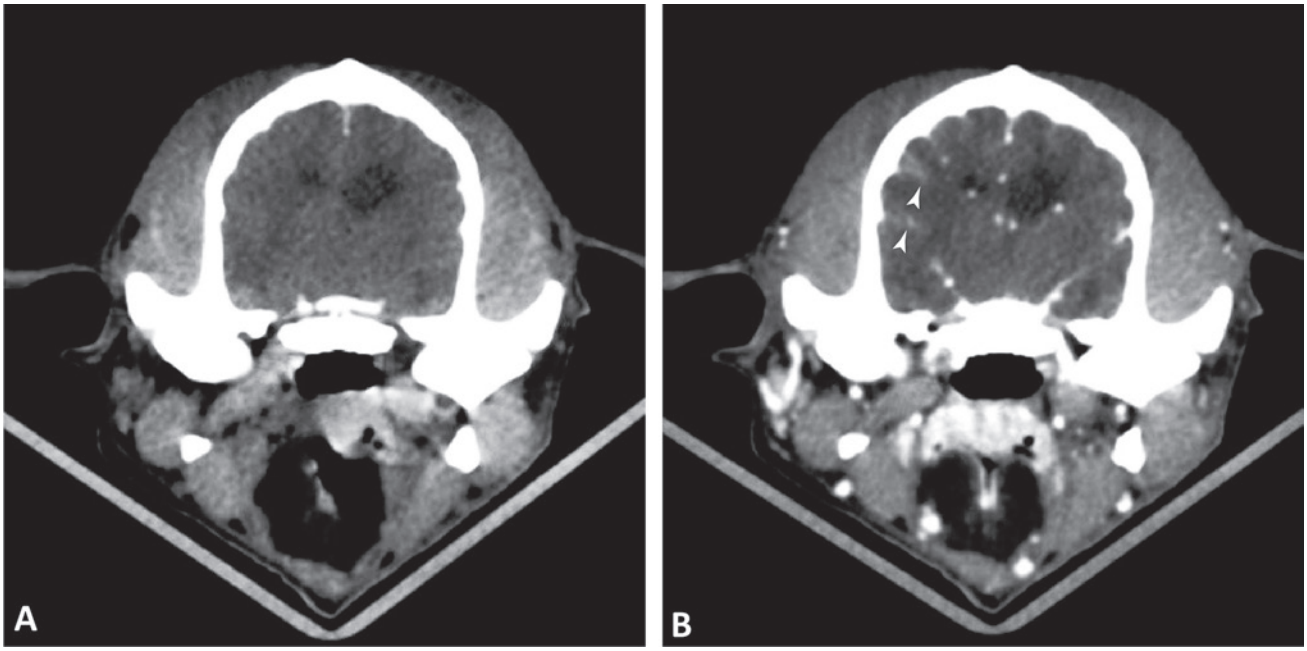

Figure 3: Computed tomography images of the brain at the thalamus level of a dog with meningeal carcinomatosis, brain and ocular metastases of mammary gland adenocarcinoma. Pre-contrast transverse section images at the thalamus level. Note the lateral ventricles asymmetry, with the left ventricle larger than the right one in the pre-contrast image (A). The post-contrast image demonstrates mild contrast meningeal enhancement (white arrow heads) (B).

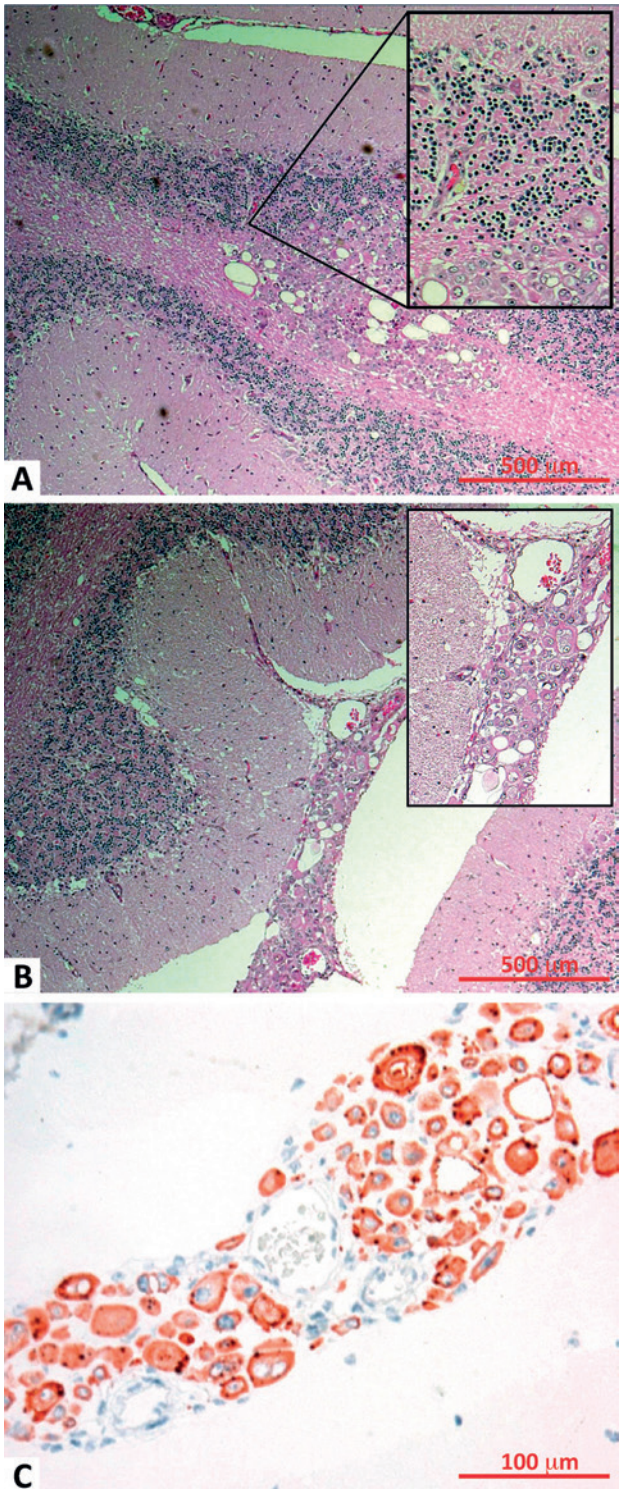

Figure 4: Microscopic findings in the cerebellum of a dog with meningeal carcinomatosis, brain and ocular metastases of mammary gland adenocarcinoma. Focal infiltration of neoplastic cells in the gray and white matter of the cerebellum. Inset: high magnification (A). Neoplastic cells in the leptomeninges. Inset: high magnification (B). The neoplastic cells are cytokeratin-positive (stained brown) (C). (All photos except C are of hematoxylin and eosin-stained slides).

cytological diagnosis of the AH findings was the same as the above-mentioned one for the CSF findings.

Brain computed tomography (CT; ElscintTwin, Haifa, Israel) revealed asymmetry of the lateral ventricles, with the left being larger than the right one, midline shift to the right and mild third ventricle enlargement. Few moderately contrast-enhanced meningeal areas were seen on transverse images at the level of the thalamus (Figure 3 A, B).

The overall tentative diagnosis was brain and ocular MGCA metastases and MC. At the owner's request, the dog was euthanized and sent for necropsy. Gross pathologic examination revealed multiple nodular masses of variable diameter (3 to 20 mm) in the right ventral thalamus, inguinal mammary lymph nodes and lung. Microscopic examination of these showed groups of anaplastic epithelial cell in the leptomeninges, cerebellar cortex (Figure 4 A, B), cerebral cortex and thalamus. Similar lesions were observed in the eyes, superficial inguinal mammary lymph nodes and lungs.

Immunohistochemical analysis demonstrated that the neoplastic cells were cytokeratin-positive and vimentin-negative, confirming their epithelial origin (Figure 4C). The neoplastic cells found in the CNS, eyes and lung showed similar morphology and immuno-staining characteristics to those in the mammary mass and mammary lymph nodes. The histopathologic and immunohistochemical results confirmed the tentative ante-mortem cytological diagnosis of malignant mixed mammary tumor with distant metastases to various organs, including the brain, eyes and lung, with MC.

DISCUSSION

We describe a case of brain and ocular metastases of MGCA, with MC that was diagnosed ante-mortem by cytological evaluation of both the CSF and AH. The morphology of the neoplastic cells, both in the CSF and AH, was compatible with epithelial cells of secretory origin, which led to the diagnosis of CNS and ocular metastatic secretory cell carcinoma, most likely of mammary gland origin. CT confirmed the presence of CNS lesions. The definitive epithelial origin of the cells was confirmed post-mortem by immunohistochemistry, but with the history of two excisional surgeries of mammary tumors, of which one was diagnosed as mixed malignant mammary tumor two months prior to presenta-

tion, metastatic MGCA was very high on the differential diagnosis list.

Immunocytochemistry of CSF cells has been used previously in dogs to diagnose histiocytic sarcoma (13), but to the best of our knowledge, not in cases of MC and metastatic CNS neoplasia. In human medicine, however, it has previously described in order to increase the sensitivity for identification of tumor cells and their origin (14, 15). Therefore, this method should be considered in future similar cases in dogs to reach a definitive ante-mortem diagnosis.

This report joins the two previous reports of neoplastic cells in the CSF that have originated from MGCA which have resulted in MC (5, 8). In one of these (8), CSF was sampled only post-mortem because of increased intra-cranial pressure (ICP). Furthermore, in all the previous reports, including the present one, where CNS neoplasia had been suspected based on CSF cytology, post-mortem histopathology confirmed the putative diagnosis of CNS neoplasia (5, 10, 13, 16-19). It therefore appears that when suspected neoplastic cells are present in the CSF based on cytological examination, CNS involvement in the neoplastic process is very likely.

Cerebrospinal tap might cause brain herniation in cases with increased ICP or brain displacement due to an intracranial lesion (20). Nonetheless, based on the present case, obtaining CSF, while taking appropriate precautions, should be considered, to enable definitive ante-mortem diagnosis of metastatic CNS involvement, even when increased ICP is suspected.

In humans, the neoplastic cell dissemination routes from primary tumor sites to the meninges include hematogenous spread, direct local brain tumor invasion, spread along nerve pathways and iatrogenic spread following surgical excision of brain metastases (4, 11). In the present case, metastases were present in both eyes, brain parenchyma, lungs and mammary lymph nodes, and therefore, the neoplastic cell spread to the meninges was likely hematogenous, although direct invasion of the brain metastases was also a possiblity.

Aqueocentesis has been recently proven diagnostically useful in dogs and cats with ocular neoplasia (mostly lymphoma), and in all such reported cases where this diagnostic measure had been used, the animals displayed ocular involvement (21, 22). In the present dog, cytology of the AH aspirate proved useful in the overall ante-mortem diagnostic process, as the AH neoplastic cell morphological characteristics were suggestive of secretory epithelial cells, and resembled those observed in the CSF. Although easy to perform, aqueocentesis is not a benign procedure, and may induce lens, iris or corneal endothelial cell injury and damage (23), and should therefore be done with caution. Nevertheless, this case demonstrated its potential usefulness, and it should therefore be considered when ocular manifestations are suspicious of neoplasia, primary local or metastatic.

In human patients presenting characteristic clinical signs, with and without known neoplasia, MC is diagnosed by imaging and CSF cytology, with diagnostic sensitivity of 45% for a single lumbar puncture, which increases to >77% following a second lumbar puncture (11). There are six reports of dogs suspected with MC in the veterinary literature (5-10), of which in three, CSF had been sampled (5, 8, 10), and in two of these, neoplastic cells were detected. Combined with the current report, this transforms the sensitivity of 75%. There is no information as to the diagnostic sensitivity of additional spinal punctures in dogs with MC, which may possibly be increased using this test, as reported in humans (11).

Presence of neoplastic cells in the CSF has been documented previously in both primary and secondary brain tumors. The most prevalent tumor diagnosed based on presence of neoplastic cells in the CSF is choroid plexus carcinoma, followed by histiocytic sarcoma, CNS lymphoma and mammary carcinoma (5, 8, 10, 13, 16-19, 24-26). Of the previous six MC cases (5-10), and combined with the present one, four resulted from spread of MGCA and two were diagnosed as choroid plexus carcinoma. Based on these results, it seems that MGCA is the most common tumor involved in MC in dogs, and the only extra-cranial tumors causing MC to lead to neoplastic cell shedding into the CSF.

The possibility that cells in the AH cytology could be round, small macrophages containing amorphous dark cytoplasmic material of retinal pigmented epithelium could not be entirely ignored cytologically. Nevertheless, the cytological findings in the CSF, together with the dog's history, made this interpretation unlikely. Chest survey radiographs were unremarkable in this case, although gross pathology revealed multiple small nodular masses in the lungs. CT is significantly more sensitive than survey thoracic radiography for detecting soft-tissue nodules in dogs, especially when nodules

are of small diameter, as in this case (27). Chest CT might have been warranted to demonstrate pulmonary metastases ante-mortem.

In conclusion, CSF cytology is a useful diagnostic tool in the diagnosis MC in dogs with MC and MGAC brain metastases. When ocular signs are present in such cases, aqueocentesis is also warranted.

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