Unusual findings in a canine cerebrospinal fluid

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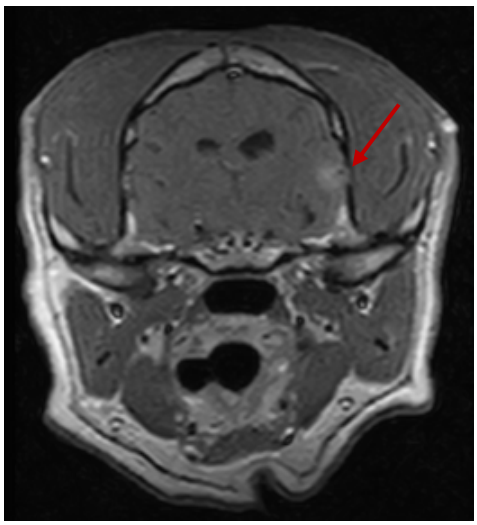
Signalment:
Dog, English Springer Spaniel, 8 years old, female neutered.

History:
The dog was presented as an emergency for investigation of progressive all four limb ataxia, staggering and behavioural change. Neurological symptoms started two weeks before referral with acute deterioration two days before. The dog had a solid mammary carcinoma surgically removed one year before, which recurred 6 months later and was also removed. Besides this, the patient had no other previous major medical or surgical problems.

Clinical findings:
On physical examination the dog was tachypneic and mucous membranes were hyperemic. The dog was obtunded, non-ambulatory tetraparetic with decreased postural reactions and increased spinal cord segmental reflexes in all four limbs. It also had obvious cervical hyperesthesia.

Diagnostic procedures:
Haematology, biochemistry, electrolytes and urinalysis were unremarkable. Thoracic radiographs revealed a diffuse mild broncho-interstitial lung pattern. Abdominal ultrasound revealed one small liver nodule which was sampled for cytology and was characterized by the presence of well differentiated hepatocytes with mild vacuolar cytoplasmic changes.
Magnetic resonance imaging revealed a mass lesion that was hyperintense on the T2-weighted and fluid FLAIR (fluid-attenuated inversion recovery) images with the perilesional oedema in the left temporal lobe. The mass was enhanced after contrast administration.



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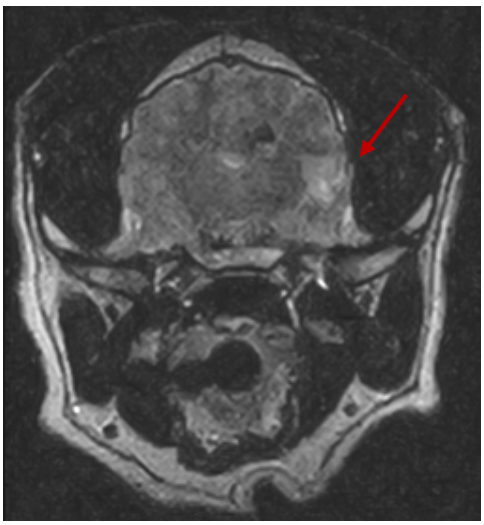

B

Fig.1A MRI, T1-weighted image after contrast administration, focal contrast enhancing lesion in the left temporal lobe (red arrow).
Fig. 1B MRI, FLAIR image, hyperintense lesion in the left temporal lobe (red arrow), mild perilesional hyperintensity suggesting perilesional oedema.

A CSF TAP was performed and the results are shown in the table and photomicrographs below.

CSF analysis:

	Values	**Reference intervals**
Appearance	Clear, colourless	
Nucleated cells (c/µL)	30	0-6
Red blood cells (c/µL)	90	0
Total protein (g/L)	0.25	0.14-0.30

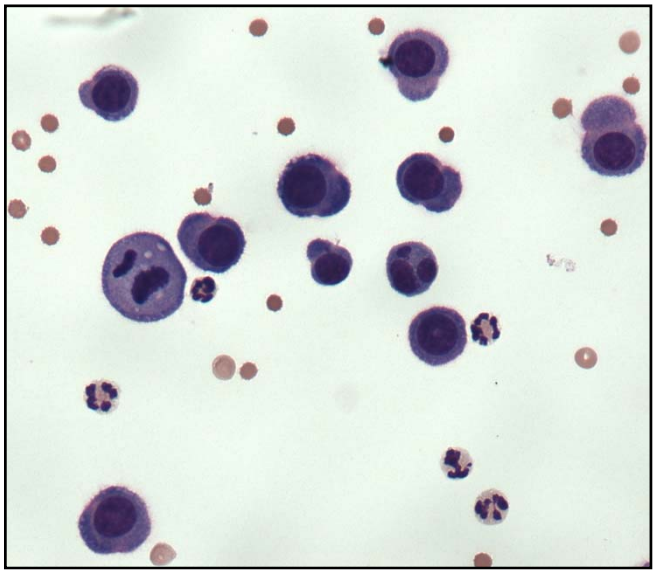

Fig.2 Cytospin preparation of CSF, May-Grunwals Giemsa stained, x50

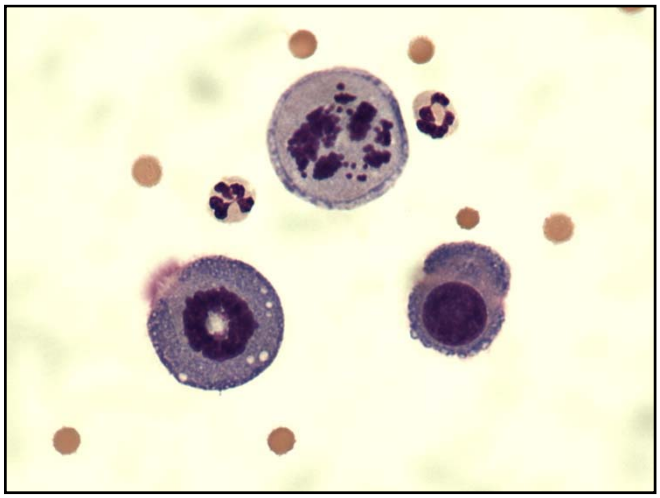

Fig. 3 Cytospin preparation of CSF, May-Grunwals Giemsa stained, x100

Questions:

1. What are your main differential diagnoses?
2. Which further tests would you suggest to confirm your diagnosis?

Further investigations and discussion:

Cytology:
CSF was collected aseptically from the atlanto-occipital cistern, revealing a total nucleated cell count of 30 c/µL and a red blood cell count of 90 c/µL. Protein concentration was 0.25 g/L. A cytospin preparation of CSF was prepared and stained with May-Grunwals Giemsa.
There was a clear background with small numbers of red blood cells. Nucleated cells included a prevalence of pleomorphic atypical discrete round cells (82%) and a lower proportion of neutrophils (13%) with rare small lymphocytes (5%).
The pleomorphic discrete cells were large with moderate amounts of lightly granular basophilic cytoplasm and well-defined borders. The nucleus was round, central to paracentral, large with a diameter of 20-25 microns, granular chromatin, with small dark round multiple prominent nucleoli. A few binucleated and trinucleated cells were also noted. Anisocytosis and anisokaryosis were marked. There were moderate numbers of atypical mitotic figures (1-2 per high power field 50x).
The cytologic interpretation was malignant neoplasia of unknown origin. The main differentials were primary tumours of the central nervous system, metastatic tumours, histiocytic neoplasia, and lymphoma.

Immunocytochemistry:

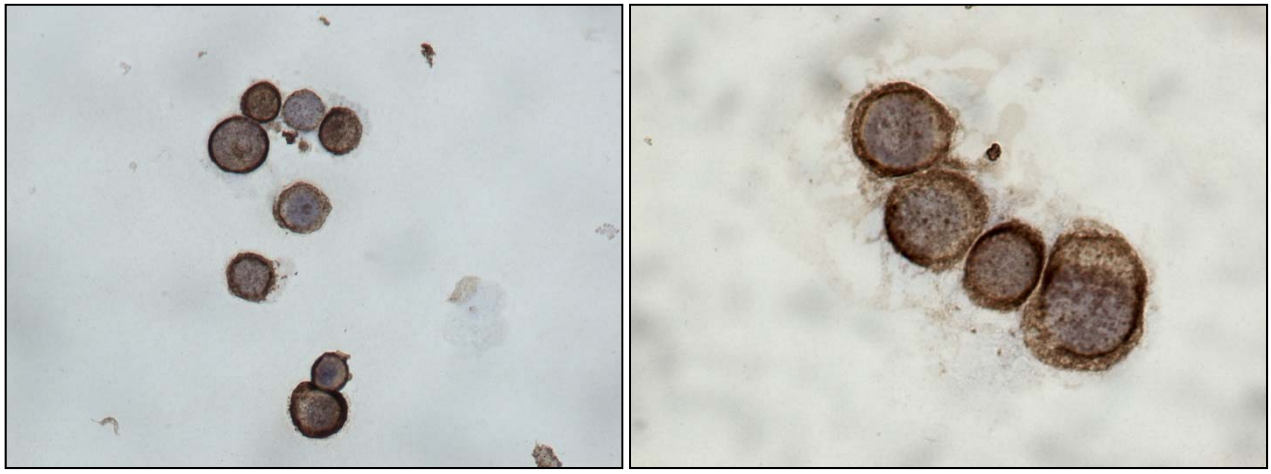

Fig. 4 Cytospin preparation from CSF, Cytokeratin, x 50, x100

Immunocytochemistry was performed on cytospin preparations. Cells stained strongly positive for cytokeratin showing an intense membrane-localized expression. They were negative for vimentin, CD3, CD79a and CD18. A diagnosis of malignant epithelial neoplasia (carcinoma) was made.

A primary malignant epithelial tumour of the central nervous system was considered unlikely, based on the localisation of the lesion in the brain, the MRI findings and the results of cytology and immunocytochemistry. With the prior history of mammary carcinoma, this was assumed to be a metastatic mammary carcinoma.

Treatment:
Treatment with high dose of glucocorticosteroids (dexamethasone, 0.2 mg/kg iv once a day), per-oral chemotherapy (lomustine, 60 mg/m2 per os) and a single intrathecal administration of cytarabine (100 mg/m2) and methotrexate (2.5 mg/m2) was initiated. The dog did not improve and was euthanized after 6 days according to the owner's wishes. Post-mortem examination was not permitted and histology was not available.

Discussion:

Neoplastic cells rarely exfoliate into cerebrospinal fluid in dogs with primary or metastatic neoplasia localized in the central nervous system. This is likely to be related to their location, often arising in a site remote from the ventricles, and the poorly exfoliative nature of CNS tumours. In this case, cytology was helpful in making a diagnosis of malignant neoplasia although the cytological features were not indicative of a specific type of tumour and immunocytochemistry was required. Cells were strongly positive for cytokeratin and failed to express CD18, CD3, CD79a and vimentin supporting the epithelial origin of the cells and eliminating histiocytic sarcoma, lymphoma, plasma cell tumour and mesenchymal tumours as possible differential diagnoses. Metastatic epithelial tumours, especially mammary carcinoma, expressing cytokeratin have been described in dogs. Meningioma is the only primary CNS tumour which could not be completely excluded by cytology and which was compatible with the localization of the lesion and the MRI findings. However, these tumours are uniformly and strongly positive for vimentin expression and only occasionally may have a variable expression of cytokeratin. The lack of expression of vimentin in this case excluded meningioma. These findings led to a diagnosis of a metastatic carcinoma, presumably a mammary carcinoma given the history of a previously excised mammary carcinoma. Metastatic mammary carcinoma are characterized mainly in humans by extensive multiple meningeal involvement as a consequence of haematogenous dissemination although focal involvement of the leptomeninges has also been described. In our case a single lesion in the left temporal lobe was found. Epithelial tumours usually exfoliate in cohesive clumps or sheets because cells adhere to each other by tight junctions (desmosomes). However, metastatic carcinoma in CSF with a discrete round cell appearance has been described in humans and also in the dog. To the author's knowledge, the reason of this is unknown.

The results of this study show the importance of cytology for the identification of neoplasia in the CSF and the value of immunocytochemistry as a non-invasive technique for a correct morphological interpretation of malignant pleocytosis. Histology remains the gold standard for the definitive diagnosis of these tumours.

References:

Behling-Kelly E et al, Neoplastic pleocytosis in a dog with metastatic mammary carcinoma and meningeal carcinomatosis, Vet Clin Pathol, 39/2 (2010), 247-252

Meuten DJ, Tumours in domestic animals (fourth edition), Blackwell (2002), 697-738

Pumarola M, Balash M, Meningeal carcinomatosis in a dog, Vet Rec, 25 (1996), 523-524

Raskin RE, Meyer DJ, Canine and Feline cytology (second edition), Elsevier (2010), 333-349

Spriggs AI, Malignant cells in cerebrospinal fluids, J Clin Path, 7, (1954), 122-130

Stampley AR et al, Meningeal carcinomatosis secondary to a colonic signet-ring cell carcinoma in a dog, J Am Anim Hosp Assoc, 23 (1986), 655-658