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CASE REPORT

Myoclonus and hypercalcemia in a dog with poorly differentiated lymphoproliferative neoplasia

Cassia H. Z. Hare | Joy Archer | Emilie Cloup | Marie-Aude Genain | Katherine Hughes | Katie E. McCallum | Lisa Alves

Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom

Correspondence
Cassia H. Z. Hare, Queen's Veterinary School Hospital, Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, United Kingdom.
Email: chzh2@cam.ac.uk

A 1-year, 8-month-old Rhodesian Ridgeback was presented with obtundation, ambulatory tetraparesis, and myoclonus. Initial clinical findings included ionized hypercalcemia with an apparent marked increase in parathyroid hormone, thrombocytopenia, and nonregenerative anemia. Low numbers of circulating atypical cells were noted on blood film evaluation. Brain magnetic resonance imaging identified an extra-axial contrast enhancing subtentorial lesion, and cerebrospinal fluid (CSF) analysis documented a marked atypical lymphocytic pleocytosis. Flow cytometry performed on the CSF demonstrated expression of only CD45, CD90, and MHC class II, with Pax5 positivity on subsequent immunohistochemistry. The final diagnosis was of B-cell lymphoblastic lymphoma or acute leukemia, given the distribution of disease and the presence of significant bone marrow infiltration alongside an aggressive clinical course. The unusual immunophenotype of the neoplastic cells and hypercalcemia presented antemortem diagnostic challenges, highlighting the need for a multidisciplinary approach and caution in the interpretation of clinical abnormalities in cases with multiple comorbidities.

KEYWORDS

flow cytometry, immunohistochemistry, immunophenotyping, lymphoma, magnetic resonance imaging, seizures

1 | CASE REPORT

A 1-year, 8-month-old female neutered Rhodesian Ridgeback was presented to the Queen's Veterinary School Hospital for investigation of a week-long history of lethargy, inappetence, and abnormal behavior, consisting of vacant episodes with twitching of the head and forelimbs. The owner reported weight loss of 3 kg over the preceding month. Pertinent medical history included an episode of weight loss and vomiting 7 months previously during which ionized hypercalcemia, hyperphosphatemia, and increased symmetric dimethylarginine were documented by the primary care veterinarian. There was concurrent mild thrombocytopenia, and "atypical" lymphocytes were reported (blood film unavailable for review). These changes were attributed to presumptive ingestion of a toxic substance and treated symptomatically. No further investigations were performed.

At presentation, the dog was lethargic and mildly dehydrated, and on neurological examination, it was obtunded with ambulatory tetraparesis and proprioceptive ataxia. Postural reactions were reduced in all limbs. Spontaneous irregular myoclonic jerks were observed affecting the head and forelimbs both at rest and with movement, and there was irregular muscle fasciculation in the hind limbs. Patella reflexes were clonic bilaterally. There was a reproducible pain response on palpation of the cervical and lumbar spine. No cranial nerve abnormalities were detected.

Initial hematology documented marked thrombocytopenia ($30 \times 10^3/\mu L$); but despite repeated sampling, persistent platelet

Abbreviations: ALL, acute lymphoid leukemia; CNS, central nervous system; CSF, cerebrospinal fluid; FLAIR, fluid attenuated inversion recovery; LBL, lymphoblastic lymphoma; PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; T2WI, T2-weighted image.

aggregation made reliable platelet enumeration impossible. Thromboelastography was within normal limits, as were prothrombin time and activated partial thromboplastin time. There was borderline leukopenia ($5.42 \times 10^3/\mu L$) caused by mild lymphopenia with a normal neutrophil count. Occasional intermediate- to large-sized atypical cells with an irregularly round, paracentrally placed nucleus, ropy chromatin (rarely with 1-2 irregularly round prominent nucleoli), and a small amount of deeply basophilic cytoplasm were observed on the blood film (Supporting Information Figure S1). These accounted for 6% of a manual differential count performed on 200 cells ($0.32 \times 10^3/\mu L$). There was also moderate normocytic normochromic nonregenerative anemia (PCV, 28%). On biochemistry, there was total and ionized hypercalcemia (ionized calcium 7.2 mg/dL, reference interval 4.7-5.6 mg/dL). Parathyroid hormone (PTH) was markedly increased (>1000 pg/mL) with undetectable parathyroid hormone-related peptide (PTHrP). As hyperparathyroidism was considered clinically unlikely, further analysis was performed by the reference laboratory including serial sample dilution. This yielded a nonlinear result, suggestive of assay interference. There was concurrent mild hyperphosphatemia (6.1 mg/dL, reference interval 2.5-5.4 mg/dL) and increased C-reactive protein (37 mg/L, reference interval 0-8.2 mg/L) consistent with a systemic inflammatory response (full hematology and biochemistry results in Supporting Information Table S1). Infectious disease screening was negative (serology using an indirect fluorescent antibody test for *Toxoplasma gondii* and *Neospora caninum*; SNAP 4Dx Plus and Angio Detect; IDEXX Laboratories Inc, Westbrook, Maine). Urinalysis documented isosthenuria (specific gravity 1.010), attributed to the results of hypercalcemia and prior fluid treatment.

Abnormalities were not detected on radiographs of the thorax and lumbar spine; however, on abdominal ultrasound, the spleen was enlarged with heterogeneous echogenicity, the liver appeared heterogeneous, and there was a mild reduction in renal corticomedullary definition bilaterally. The parathyroid glands were prominent on ultrasound but within normal size limits (0.21 cm diameter). Electromyography under general anesthesia did not identify any abnormalities. Magnetic resonance imaging of the brain and cranial cervical spine (Esaote, VetMR 0.3Ts.p.a; Genoa, Italy) revealed a right-sided extra-axial triangular-shaped plaque-like area of contrast enhancement located just ventral to the tentorium cerebelli (Figure 1A). This lesion was not visible on any of the precontrast T1-weighted image (WI), T2WI, short tau inversion recovery, or fluid attenuated inversion recovery (FLAIR) sequences and extended the entire height of the tentorium cerebelli, with no mass effect on adjacent tissues. A meningeal tail was seen extending from the lesion dorsally and ventrally, and a fainter less-defined area of contrast uptake was seen along the ventral aspect of the cerebellum on the left side. Both retropharyngeal lymph nodes were prominent, with the left side slightly larger and more FLAIR hyperintense than the right side. Ventrally and caudally in the left retropharyngeal lymph node there was an ill-defined area of contrast uptake, corresponding to a T2WI hyperintensity on the precontrast sequences (Supporting Information Figure S2).

Cisternal cerebrospinal fluid (CSF) analysis documented a marked pleocytosis (1415 cells/μL) with a predominance of intermediate- to large-sized atypical round cells, considered of likely lymphoid origin and with an appearance similar to those observed in the blood

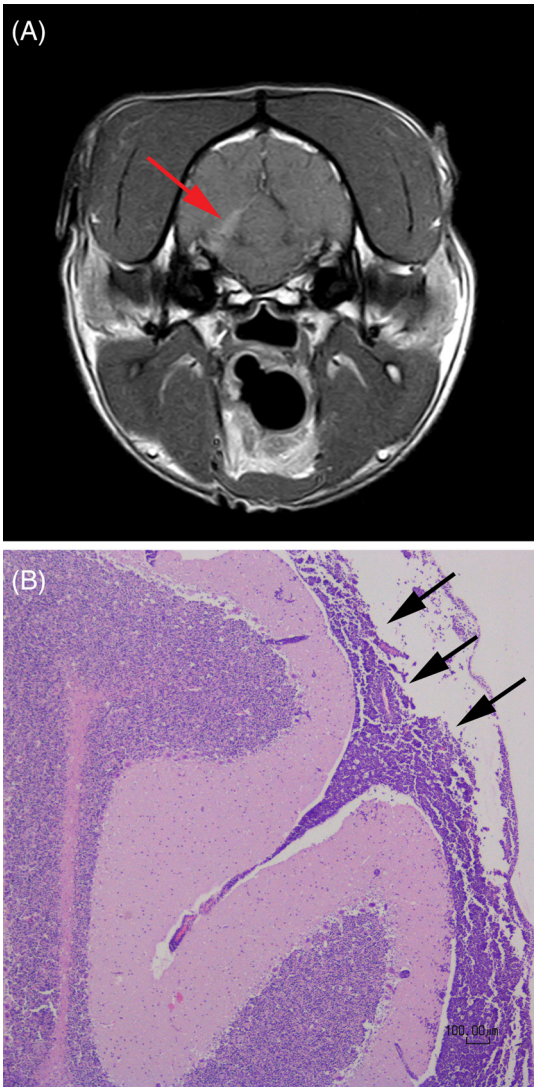

FIGURE 1 A, T1-weighted post-gadolinium transverse magnetic resonance imaging view of the brain at the level of the cerebellum demonstrating a wedge-shaped area of contrast uptake ventral to the right tentorium cerebelli (red arrow). B, Photomicrograph of the cerebellum. The cerebellar leptomeninges are markedly expanded by sheets of neoplastic round cells (black arrows). Hematoxylin and eosin. Scale bar = 100 μm

(Figure 2). Cerebrospinal fluid microprotein was also increased (0.9 g/L). Multicolor flow cytometry was performed using established laboratory protocols (BD Accuri C6 cytometer and software; BD Biosciences, San Jose, California, antibodies listed in Supporting Information Table S2), with data acquired on 10 000 gated events. A population of intermediate- to large-sized cells were gated according to their light scatter properties (Figure 3A). Positivity was determined by comparison to isotype controls, and a result was considered positive when the marker was expressed by more than 20% of gated cells, with the exception of CD34, where a cutoff value of 5% was used. The cells in the gated region displayed strong expression of the panleukocyte marker CD45, as well as CD90 and major histocompatability complex (MHC) class II. All other markers tested were negative, including the additional pan-leukocyte marker CD18, the T-cell markers CD3, CD4, CD5, CD8, and CD3-12, the B-cell markers CD21 and CD79a, and the myeloid

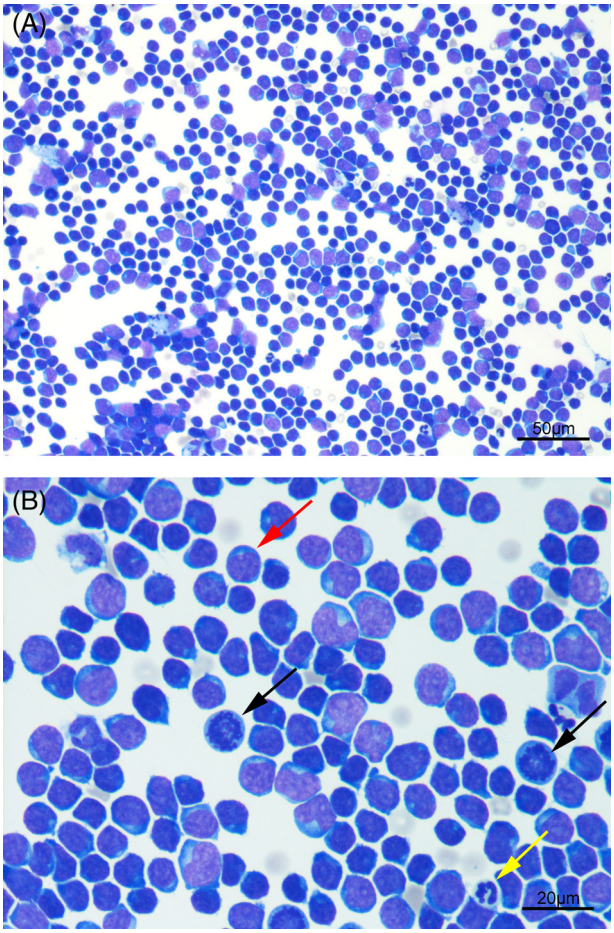

FIGURE 2 A and B, Cytospin preparation of cerebrospinal fluid stained with Wright's Giemsa. A, The preparation is highly cellular, and the predominant cells are a population of intermediate- to large-sized atypical round cells (×200 magnification, scale bar = 50 μm). B, The atypical cells (example indicated by red arrow) are on average 1-1.5 times the neutrophil diameter (nondegenerate neutrophil indicated by the yellow arrow for size comparison), with an irregularly round to indented nucleus and moderate amount of basophilic cytoplasm. Chromatin is ropy with 1-3 prominent nucleoli. Frequent mitotic figures are present (black arrows) (×500 magnification, scale bar = 20 μm)

markers CD14 and myeloperoxidase. CD34, the marker most frequently used to identify blasts in acute leukemia (ALL), was also negative. A preliminary diagnosis of poorly differentiated hematopoietic neoplasia with a likely lymphoid origin was made. Flow cytometric evaluation of the peripheral blood suggested the presence of a small population of cells with similar scatter properties and immunophenotype to those in the CSF (approximately 4.6% of events when cellular debris was excluded, as shown in Figure 3B,C), believed likely to correspond to those seen on the blood film.

Initial symptomatic treatment included levetiracetam (20 mg/kg q8h) and prednisolone (approximately 1 mg/kg q24h). The dog remained stable for 2 days; however, because of the poor prognosis associated with central nervous system (CNS) lymphoproliferative neoplasia, the owner elected for euthanasia, and a full postmortem examination was requested. Gross findings supported the imaging findings and included a moderate loss of the folia pattern in the cerebellum and the presence of multifocal areas of soft, homogenous tan to pale pink

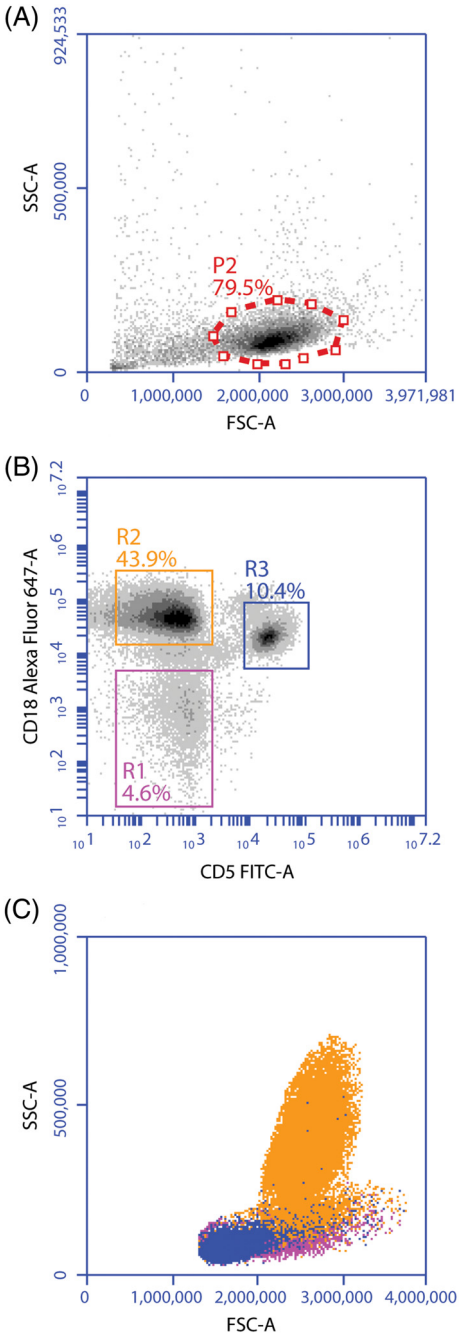

FIGURE 3 A-C, Plots displaying the results of flow cytometric analysis of the cerebrospinal fluid (CSF) and the blood. A, Representative plot displaying scatter properties of the cells in the CSF, with forward scatter corresponding to size and side scatter to complexity. The red dotted line surrounds the events gated for further analysis, believed to correspond to the cells seen on cytology. B, Cell populations in the blood illustrated using color back-gating by expression of CD18 (panleukocyte marker) and CD5 (T-cell marker). The events (cells) in the pink box are double negative for CD18 and CD5, corresponding to the circulating atypical cells (4.6%). The events in the blue box are double positive for CD18 and CD5, corresponding to a residual population of circulating T-cells (10.4%). The events in the orange box are positive for CD18 but negative for CD5, corresponding predominantly to the normal circulating neutrophils and monocytes. C, Representative plot displaying the scatter properties of the events (cells) in the blood defined in (B). Events with a low forward scatter (debris) have been excluded. Note that the cells shown in pink (CD18/CD5 double negative atypical cells) have similar scatter properties to those in the CSF shown in (A)

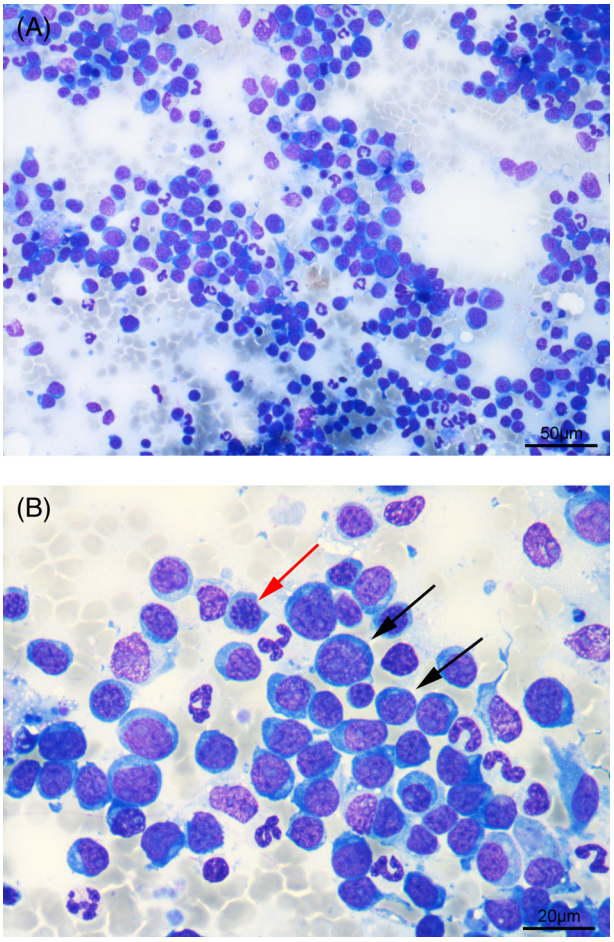

FIGURE 4 A and B, Cytology of the hypercellular bone marrow, demonstrating a population of cells similar to those observed in the cerebrospinal fluid. These accounted for 56% of a 500-cell differential count. May-Grünwald Giemsa. A, Magnification ×200, scale bar = 50 µm. B, Magnification ×500, scale bar = 20 µm. The black arrows indicate examples of the neoplastic cells, whereas the red arrow indicates a rare erythroid lineage cell. There are also low numbers of band and mature neutrophils and occasional small lymphocytes

tissue overlying the spinal cord. Cytology of the bone marrow was hypercellular with significant infiltration (approximately 56%) by atypical cells with a similar morphology to those in the CSF and blood (Figure 4). Special staining with alkaline phosphatase was negative. There was concurrent erythroid hypoplasia, although megakaryocyte numbers appeared adequate with an increased proportion of immature megakaryocytes. These findings were confirmed on core biopsy.

Major histopathological findings included the presence of multifocal to coalescing infiltrative sheets and clusters of intermediate-sized neoplastic round cells in the cerebellum, spinal cord, and nerve roots (Figures 1B and 5). On immunohistochemistry, the cells exhibited strongly positive nuclear staining for Pax5, indicating a B-cell origin. Other lymphoid markers including CD3, CD79a, and CD20 were negative. The normal architecture of the retropharyngeal lymph nodes was effaced by sheets of similar neoplastic cells, which exhibited a mitotic rate of 87 per 10 high-power fields (per 2.37 mm^2). Additional morphological diagnoses included moderate chronic interstitial nephritis and mild-to-moderate multifocal infarction of the kidneys. The parathyroid glands were microscopically within normal limits. The final histopathological diagnosis was of intermediate-sized B-cell lymphoproliferative neoplasia affecting the spinal cord, retropharyngeal lymph nodes, and bone marrow. In combination with the antemortem findings, the overall picture was considered suggestive of B-cell lymphoblastic lymphoma (LBL) or ALL.

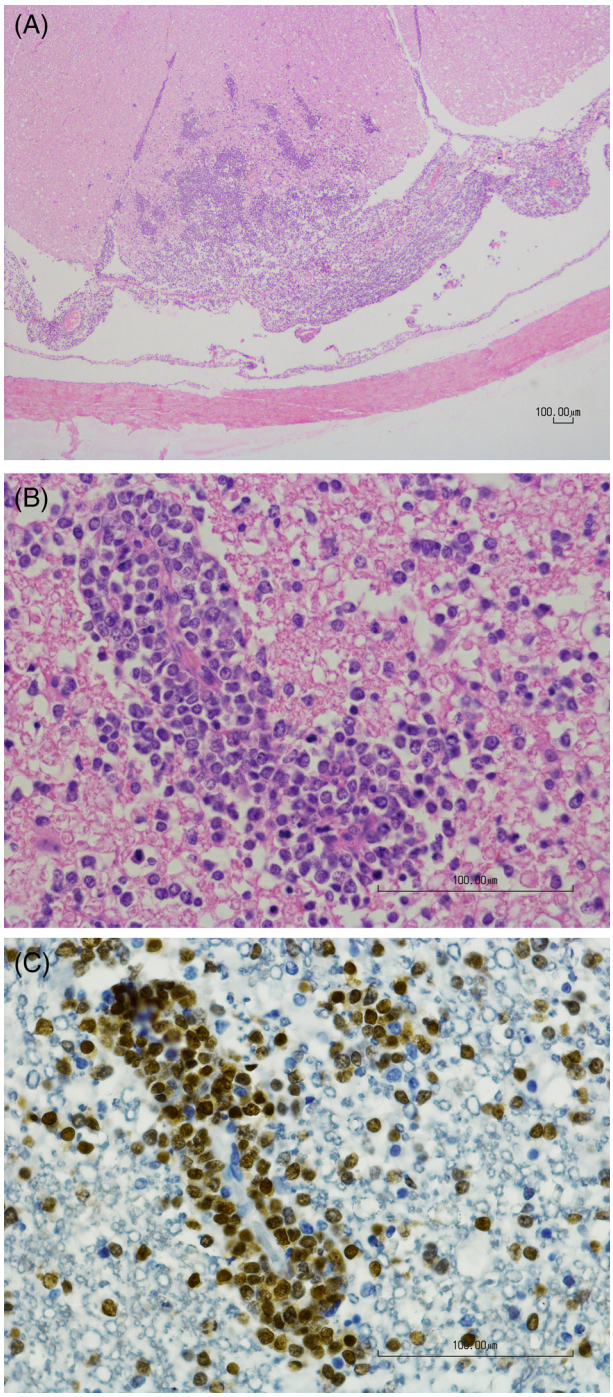

FIGURE 5 A-C, Photomicrographs of histopathological sections of the spinal cord. A, Multifocal to coalescing infiltrates of neoplastic round cells expand the leptomeninges and underlying white matter. Hematoxylin and eosin. Scale bar = 100 µm. B, Higher magnification of the spinal white matter showing a perivascular infiltrate of neoplastic cells. Scale bar = 100 µm. C, The majority of the neoplastic cells exhibit intense nuclear staining for Pax5 with hematoxylin counterstain. Scale bar = 100 µm

2 | DISCUSSION

This case of myoclonus in a dog was associated with poorly differentiated lymphoproliferative neoplasia with presumed CNS invasion. The findings on neurological examination correlated well with the multifocal distribution of lesions confirmed at postmortem; however, the unusual immunophenotype of the neoplastic cells identified using flow cytometry made definitive antemortem diagnosis challenging. The dog's history and hypercalcemia presented additional diagnostic and interpretive challenges, as did the presence of a presumed interfering substance in the PTH assay.

Myoclonus is the presence of sudden, shock-like involuntary movements and is an infrequent presentation in dogs with neurological disease. It can be classified according to phenotype, etiology, pathophysiology, neuroanatomical localization, pharmacological response, and moment of occurrence.[1] In veterinary medicine, classification as epileptic and nonepileptic myoclonus has been proposed based on the presence or absence of generalized tonic-clonic seizures. In this case, the myoclonic movements were noninducible sporadic muscular contractions (positive myoclonus) of the head and cervical region. The dog had concurrent obtundation and episodic vacancies that could suggest the presence of seizure activity. However, in the absence of generalized tonic-clonic seizures and electroencephalographic evidence of abnormal cerebrocortical activity, epileptic myoclonus is difficult to diagnose. Instead, an etiological classification of nonepileptic or symptomatic myoclonus might be preferable in this case.

The most frequent reports of symptomatic myoclonus in dogs involve canine distemper virus infection or more rarely lead toxicosis or other inflammatory CNS disease.[2] The pathogenesis of myoclonus in these conditions is incompletely understood, although it is believed to be the result of focal lesions causing pathological changes to the function of lower motor neurons of the spinal cord and cranial nerves. There are several reports of humans with both intracranial and extracranial neoplasms presenting with myoclonus either as part of paraneoplastic syndromes, as occurs in opsoclonus myoclonus syndrome,[3] or because of a direct insult on the nervous tissue. In most cases, these conditions are believed to have an immune-mediated basis involving onconeuronal antibodies, even in cases where the location of lesions within the CNS could explain the signs.[4] Histopathology in this case demonstrated infiltrative neoplastic lymphocytes distributed multifocally in the CNS, and thus, a direct disturbance of the cortical or spinal neuronal function can be hypothesized as the cause of myoclonus. However, as suggested in the literature, an indirect immune-mediated cause might be more plausible, either involving onconeuronal antibodies (which can be measured in human CSF) or immune dysregulation as can occur in neoplasms of the immune system.[5–7]

Lymphoid neoplasia with CNS involvement has been widely reported in dogs, most often as part of a multicentric process and rarely as a primary CNS neoplasm. In dogs, neurological signs occur most often during relapse of previously diagnosed lymphoma,[8] although in humans, CNS involvement is a recognized risk factor in many lymphoproliferative diseases, especially LBL/ALL. A single case report has described B-ALL with meningeal metastasis in a dog.[9] The findings in our case are most consistent with CNS metastasis (secondary involvement), and the distribution of lesions on histopathology was similar to those described in a recent paper documenting a single case of B-LBL with a perivascular and meningeal pattern.[10]

Immunophenotyping of lymphoproliferative neoplasms using flow cytometry is well established in human medicine and increasingly utilized in veterinary medicine. In this case, the flow cytometric findings were unusual in that the neoplastic cells lacked expression of all mature B- and T-cell markers. The expression of CD45 and MHC class II was considered nonspecific as both may be expressed by a wide variety of leukocytes. CD90 has traditionally been accepted as a marker of T-cells and monocytes in dogs,[11] and as such, a T-cell origin of the neoplastic cells was considered most likely antemortem. However, on immunohistochemistry, the expression of Pax5 (a highly specific B-cell marker in humans and dogs) was consistent with a B-cell neoplasm.[12,13] In human medicine, CD90 is considered more broadly as a marker of stem cells,[14] and some authors in the veterinary literature also interpret it as such[15] although specific evidence to demonstrate this is lacking. The expression of CD90 by a clinically aggressive B-cell neoplasm in this case lends support to this broader approach, and further investigations into the expression of CD90 in canine lymphoproliferative neoplasia could be beneficial. This may be of interest when expression of CD34 (stem cell marker associated with ALL) is absent, as can occur in some cases of clinically diagnosed ALL.[15]

The definitive classification of the neoplasm in this case is somewhat controversial given the difficulty in differentiating between poorly differentiated stage V lymphoma (eg, LBL) and ALL. In humans, the degree of bone marrow involvement is critical in making this distinction, with a cutoff of greater than 25% infiltration being consistent with ALL.[16] Similar definitions have been applied in the veterinary literature,[17] and according to these, the diagnosis in this case should be of B-ALL (given the 56% of neoplastic cells documented in the bone marrow). The clinical course and distribution of disease should also be considered, with lymphomas typically having more significant involvement of lymphoid tissues outside the bone marrow (not observed in this case). However, in the absence of confirmatory cytogenetic testing (routinely used in human medicine) and given the late stage at which dogs with lymphoma frequently present, it could be argued that such a cutoff is inappropriate and could result in overdiagnosis of ALL. Although the bone marrow involvement generally confers a worse prognosis in dogs with lymphoma,[18] the well-established poor prognosis of ALL in dogs might be more likely to result in euthanasia when such a diagnosis is made. In cases such as ours with CNS involvement, this is arguably less of a concern given the poor prognosis this confers alone. However, with further advances in veterinary diagnostics and treatment, this could become an important discussion point.

Platelet aggregation (hyperreactivity) is a common finding in dogs with lymphoma and is believed to play a role in tumor metastasis and confer an increased risk of disseminated intravascular coagulation.[19] Thrombocytopenia is a common finding in dogs with lymphoid neoplasia and is frequently multifactorial.[20] Given the presence of adequate megakaryocytes on bone marrow evaluation in this dog, the apparent thrombocytopenia was believed most likely primarily the result of increased platelet consumption or sequestration.

The cause of hypercalcemia in this case remains unexplained, with differentials including vitamin D secretion by neoplastic cells and osteolysis (although no specific evidence of this was identified).[21] Repeated samples were measured, making laboratory error unlikely. PTHrP was undetectable, excluding a classical hypercalcemia of

malignancy, and other differentials such as hypoadrenocorticism and renal disease were considered very unlikely in the absence of other suggestive findings. Ionized calcium (and phosphate) is expected to be above typical adult reference values in growing large breed dogs;[22] however, the degree of increase in PTH and age of this dog are likely incompatible. The marked increase in PTH documented during initial testing was considered inconsistent with the other clinical findings and signalment in this case. As such, further investigations were performed by the reference laboratory, including the addition of further (Ethylenediaminetetraacetic acid, EDTA) to the sample and the testing of serial dilutions. The lack of linearity in these dilutions suggested the presence of an assay interferent. A common cause of interference in human immunoassays is the presence of heterophilic antibodies,[23] and although the nature of the substance in this case remains unknown, this could provide an explanation given the known occurrence of antibody production and secondary immune-mediated disease in lymphoma. These findings highlight the need for caution in the interpretation of immunoassay results in dogs with significant underlying disease and inconsistent clinical signs.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflicts of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Consent was gained from the owner for all diagnostic procedures and use of case material for teaching and research purposes.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Cassia H. Z. Hare https://orcid.org/0000-0002-9306-4796
Katherine Hughes https://orcid.org/0000-0002-3331-1249
Katie E. McCallum https://orcid.org/0000-0001-6153-0687

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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