Acute myelomonocytic leukemia with multifocal manifestation and spinal cord infiltration in a dog

E. Dappiano[1], G. Cagnotti[1], C. Corona[2], F. Riondato[1], B. Iulini[2], M. D. Pintore[2], A. D'Angelo[1]

[1]Department of Veterinary Science, University of Turin, Grugliasco, Italy; [2]Istituto Zooprofilattico del Piemonte Liguria e Valle d'Aosta, Turin, Italy

https://doi.org/10.17236/sat00353

Eingereicht: 28.12.2020
Angenommen: 11.02.2022

Akute myelomonozytäre Leukämie mit multifokaler Manifestation und Rückenmarksinfiltration bei einem Hund

In der Literatur werden nur wenige Fälle myelomonozytärer Leukämien bei Hunden beschrieben, die mit neurologischen Symptomen assoziiert sind. Keine dieser Fälle stehen in Zusammenhang mit einer intraparenchymatösen Rückenmarksinfiltration durch neoplastische Zellen. Das Ziel dieser Kurzmitteilung ist die Beschreibung eines Falles einer akuten myelomonozytären Leukämie Subtyp M4 mit Rückenmarksinfiltration bei einem Hund.

Ein 3 Jahre alter, männlicher Golden Retriever wurde vorberichtlich mit Hyperthermie, Lymphadenomegalie, Leukozytose mit zirkulierenden Blasten, Anämie und Thrombozytopenie und akuter Paraplegie vorstellig. Anhand einer Immunphänotypisierung des peripheren Blutes mittels Durchflusszytometrie wurde eine akute myelomonozytäre Leukämie Subtyp M4 nachgewiesen. Der Hund wurde aufgrund klinischer Verschlechterung und ungünstiger Prognose euthanasiert. Die postmortale Untersuchung zeigte eine neoplastische Infiltration multipler Organe einschließlich des Rückenmarks.

Nach unserem Wissen ist dies der erste Fall einer akuten myelomonozytären Leukämie Subtyp M4 mit Rückenmarksinfiltration bei einem Hund. Bei Patienten mit akuter neurologischer Symptomatik mit Lokalisation im Rückenmark sollte eine Leukämie als eine Differenzialdiagnose mit einbezogen werden.

Schlüsselwörter: Hund, myeloproliferative Erkrankungen, Neurologie, Paraplegie, Rückenmarksneoplasie

Summary

Few cases of myelomonocytic leukemia associated with neurological signs have been described in dogs; none have been related to intraparenchymal spinal cord infiltration by neoplastic cells. This short communication describes a case of acute myelomonocytic leukemia subtype M4 in a dog with spinal cord infiltration.

A 3-year-old male Golden Retriever was presented with a history of hyperthermia, lymphadenomegaly, leukocytosis with circulating blast cells, anemia and thrombocytopenia, and acute onset paraplegia. Immunophenotyping of peripheral blood by flow cytometry was consistent with acute myelomonocytic leukemia subtype M4. The dog was euthanized because of clinical deterioration and unfavourable prognosis. Postmortem examination revealed multi-organ neoplastic infiltration, including the spinal cord.

To our knowledge, this is the first case of acute myelomonocytic leukemia subtype M4 in a dog with spinal cord infiltration. Our findings hold importance for including myelomonocytic leukemia in the differential diagnosis of patients with neurological signs due to spinal cord localisation.

Keywords: canine, myeloproliferative disorders, neurology, paraplegia, spinal cord neoplasia

Short communication

Myeloproliferative disorders are a group of neoplastic diseases caused by aberrant proliferation and/or decreased apoptosis of a clone of cells with defective maturation and function that infiltrate other tissues.[17] Since 1985 several revisions of the classification system and criteria for differentiation of these disorders have been made.[10,17] To date, the criteria are the type of blast cells identified, their percentage, and lineage specificity.

Acute myelomonocytic leukemia (AML) results from the accumulation of immature myeloid blast cells in the bone marrow and peripheral blood. Different subtypes have been identified and defined as AML-M1, M2, M3, M4, M5a, M5b, M6, M6Er, and M7. Albeit a rare entity, AML is the most common form of acute leukemia in dogs.[10,17] AML-M4 is characterised by malignant proliferation of precursors of myeloid and monocytic cells and is one of the most common forms reported in dogs.[1,7,12,17] Few cases of AML associated with neurological signs have been described; none have been related to intraparenchymal spinal cord infiltration by neoplastic cells.[2–4,9]

The aim of this case report is to describe a case of AML-M4 in a dog with neurological signs due to spinal cord infiltration.

A three year-old intact male Golden Retriever was presented to the Veterinary Teaching Hospital, University of Turin because of an acute onset of paraplegia. On physical examination, the dog was lethargic and hyperthermic (rectal temperture 42 °C). The heart rate was 160 beats/min with hyperdynamic heart beat; the mucous membranes were congested; the capillary refill time was 1 sec. Submandibular, prescapular, and popliteal lymph nodes were markedly enlarged.

Neurologic examination disclosed a mildly obtunded mental state; gait analysis revealed flaccid paraplegia with absent nociception. The neurologic functions of the thoracic limbs were normal. No cranial nerve deficits were detected. Spinal reflexes were absent in both pelvic limbs; the anal sphincter tone was reduced and the perineal reflex was absent. Lower motor neuron urinary incontinence was reported. No palpable back pain was noticed upon examination. These findings were suggestive of a lumbosacral intumescence (L4 -S3 spinal cord segments) neuroantomical localization. The differential diagnosis included vascular disease, intervertebral disc disease or neoplasia.

Complete blood count (CBC) revealed mild nonregenerative anemia ($3{,}25 \times 10^{12}$ /L; reference interval [RI]: 5,4 – $8{,}6 \times 10^{12}$ /L), marked thrombocytopenia (72×10^9 /L; RI: 127,7 – 543×10^9 /L) with inadequate platelet estimate, marked leukocytosis ($88{,}16 \times 10^9$ /L; RI: 5,2 – $17{,}9 \times 10^9$ /L) characterized by neutrophilia ($34{,}38 \times 10^9$ /L; RI: 2,9 – $12{,}5 \times 10^9$ /L) with band neutrophils ($1{,}76 \times 10^9$ /L), monocytosis ($25{,}57 \times 10^9$ /L; RI: 0,2 – $1{,}2 \times 10^9$ /L), and atypical, medium to large-sized cells (22 %) with basophilic cytoplasm, roundish or convoluted/indented nuclei, finely stippled chromatin, and indistinct nucleoli (Figure 1A). The pre-referral biochemistry profile was within normal limits.

Based on clinical examination and blood work, fine needle aspiration of popliteal lymph nodes was performed. Cytological evaluation revealed infiltration with cells similar to those described in the peripheral blood (Figure1B). Numerous mitoses and a moderate increase in lymphocytes, granulocytes, and plasma cells were noted.

Immunophenotyping of peripheral blood and lymph nodes was performed by flow cytometry as previously described.[11] The antibody panel included CD45, CD5, CD21, CD14, CD11b, CD4, CD61, CD117, and CD34. Two particular populations of medium-to-large sized elements were recognized in the peripheral blood, together with mature granulocytes and monocytes and few small lymphocytes (Figure 2 A-C): CD45+ CD11b+dim CD14+dim cells (44 %; Figure 2B region P2) half of which expressing CD4, and CD45+ cells expressing no other markers except CD34 and CD117 (21 %; Figure 2B region P1 and Figure 3C region Q9 – LR). Lymph nodes were infiltrated by cells showing similar antigen exposition as peripheral blood (Figure 2 D–F).

Based on these findings, a diagnosis of acute myeloid leukemia with lymph node infiltration was made and AML-M4 was suggested due to the myelomonocytic differentiation. Soon after hospitalization, the patient's general clinical condition deteriorated, as the dog became

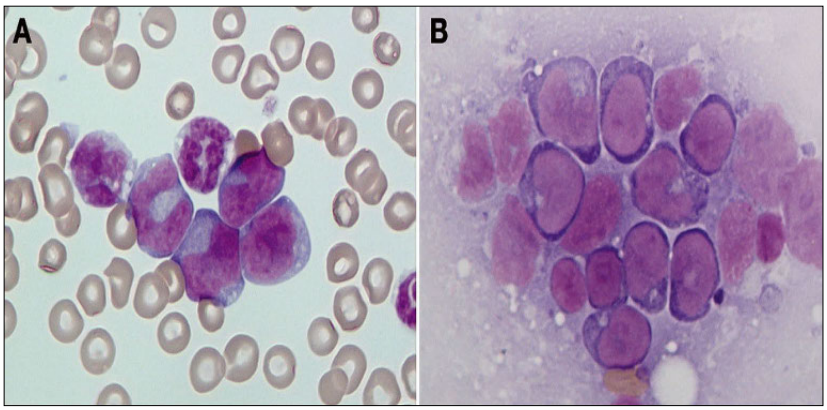

Figure 1: Atypical cells in peripheral blood (A) and lymph node (B) of Golden Retriever with acute myelomonocytic leukemia. Medium to large sized cells with basophilic cytoplasm, roundish or convoluted/indented nuclei, finely stippled chromatin, and indistinct nucleoli. Original magnification 1000 x.

more obtunded, dispnoic and with persistent hyperthermia Given the poor prognosis, the owner declined bone marrow aspiration for diagnosis confirmation and elected humane euthanasia. A postmortem exam was performed.

Gross pathology findings included splenomegaly, hepatomegaly, urinary stasis, and endocardiosis, other than enlarged peripheral and central lymph nodes. Macroscopically, dark red and greenish multifocal areas were observed in the cervical, thoracic, and lumbar tract of the spinal cord (Figure 3). No brain lesions were detected. Tissues (brain, spinal cord, stomach, intestine, lungs, liver, spleen, kidneys, peripheral and mesenteric lymph nodes, bronchial lymph nodes, myocardium, pericardium, gastrocnemius muscle) were processed by conventional techniques, embedded in paraffin, sectioned in slices about 4 ± 2 µm thick, and stained with hematoxylin and eosin (H&E) stain. Concerning central nervous system, brain sections examined

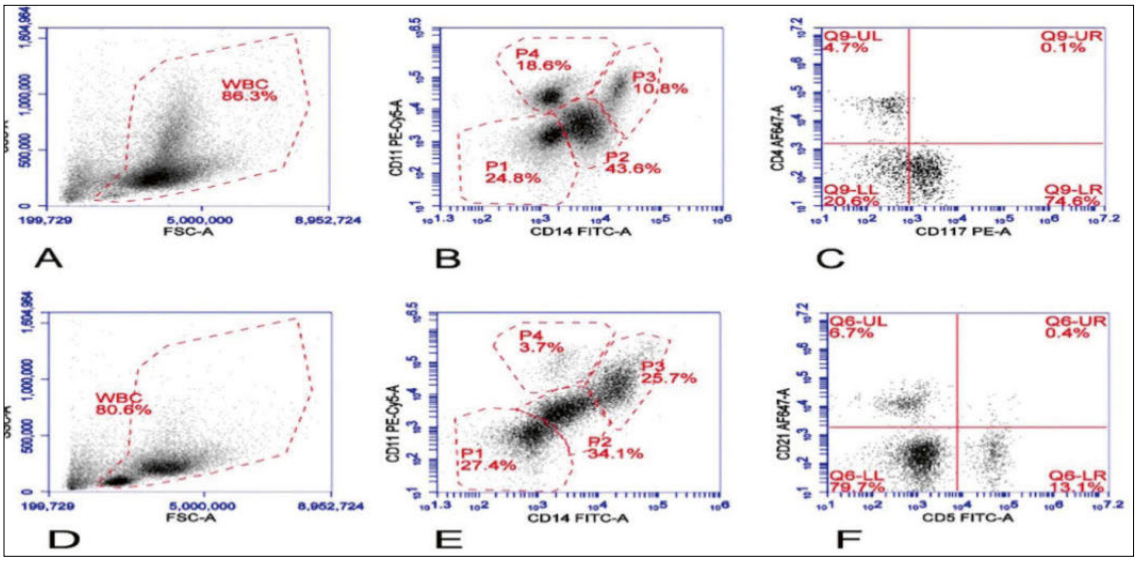

Figure 2: Flow cytometric analysis of peripheral blood and lymph node aspirate in a Golden Retriever with acute myelomonocytic leukemia..

A–C. Flow cytometric analysis of peripheral blood: **A.** Forward scatter (FSC) vs. side scatter (SSC) plot depicting a morphological white blood cell (WBC) gate after doublet exclusion (not shown). **B.** CD14 vs. CD11b plot of WBC-gated events. Three CD11b+ populations (myeloid lineage) with different levels of CD11b and/or CD14 (P2, P3, and P4) and an undifferentiated population (P1) are recognized. Lymphoid population in P1 is < 2 % (not shown). **C.** CD117 vs CD4 plot of P1-gated events. The population is made mainly of CD117 + events (Q9-LR); CD4+ events are small lymphocytes.

D–F. Flow cytometric analysis of lymph node aspirate: D and E. Nodal population presenting with scatter properties (D) and antigen expression (E) similar to those described in peripheral blood (Fig.1A and 1B). F. CD5 vs CD21 plot of WBC-gated events. Percentage of CD5 + or CD21 + cells showing that most of P1 events are small residual lymphocytes

Figure 3: Macroscopic pictures of thoracic spinal cord lesions in a Golden Retriever with acute myelomonocytic leukemia.

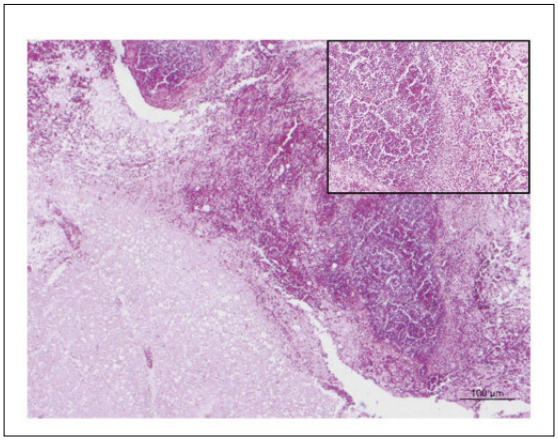

Figure 4: Thoracic spinal cord infiltration of neoplastic cells and hemorrhagic areas (2,5×) – (insert 10×) in a Golden Retriever with acute myelomonocytic leukemia.

included obex, pons, cerebellum, thalamus, hippocampus, basal nuclei and parietal, occipital and frontal cortices. Spinal cord evaluation included sections of the cervical segments, cervico-thoracic intumescence, thoraco-lumbar segments, and lumbo-sacral intumescence.

Microscopic examination revealed diffuse proliferation of neoplastic cells in the spinal cord meninges, epidural adipose tissue, nerve roots, and gray matter of the spinal cord at different degree of severity (Figure 4). The neoplastic cells were irregularly round, with a high nucleus-cytoplasm ratio, round nuclei, and distinct large nucleoli. The mitotic count was nine mitotic figures per ten high-power field (HE 40 x). Hemorrhagic areas and neutrophils were detected (Figure 5). Diffuse neoplastic infiltration was also observed in the lymphonodal, myocardial, pericardial, hepatic, renal, intestinal, muscle, and lung tissues. No neuropathological lesions were found in brain sections examined.

Few cases of myelomonocytic leukemia associated with neurological signs have been described; none have been related to intraparenchymal spinal cord infiltration by neoplastic cells.[2–4,9]

Central nervous system involvement in myelomonocytic leukemia was first reported in 1974. Infiltration of leukocytes in the brain and other organs was found in a 2,5-year-old Border Collie presented because of neurological signs. However no details of the neurological examination or neuroanatomical localisation were given. The spinal cord wasn't examined histologically.[2]

A more consistent presentation of intracranial and peripheral nervous system involvement was described in the case of a three year-old male Labrador displaying dropped jaw, bilateral atrophy of the cranial skeletal muscles and mildly enlarged lymph nodes. Neurological examination revealed bilateral motor and sensory deficits of the trigeminal nerve, bilaterally reduced pupillary reflex , and reduced swallowing reflex, suggesting lesions of the trigeminal, the optic, and the hypoglossal nerves, respectively. Further investigations showed blast cells of monocytic origin in peripheral blood, lymph node and bone marrow aspirate smears, leading to the diagnosis of myelomonocytic leukemia with central nervous system involvement. Necropsy confirmed involvement of the trigeminal, hypoglossal, and optic nerves, with multifocal aggregates of neoplastic cells also found in the leptomeninges of the brain and spinal cord, choroid plexus, and spinal nerves.[4] In another case, a five-year-old male Doberman Pinscher displayed dropped jaw and masticatory muscle atrophy in association with Horner's syndrome.[3]

More recently, a case of myelomonocytic leukemia associated with spinal neuroanatomical localisation was described in a two year-old intact male mixed-breed presented because of lethargy, anorexia, and cervical pain rapidly deteriorating to tetraparesis. Findings from the neurological examination suggested a lesion affecting C1 – C5 spinal cord segments. Further investigations revealed that the spinal neurological signs were due to extradural compression at the level of C3 – C4 as confirmed at necropsy. Microscopic examination of the extradural mass revealed diffuse proliferation of neoplastic cells that were found to have monocytic origin on cytochemical staining. Also in this case, no direct spinal cord infiltration was reported.[9]

In a more recent retrospective study of 35 dogs diagnosed with AML, six were reported to display neurolog-

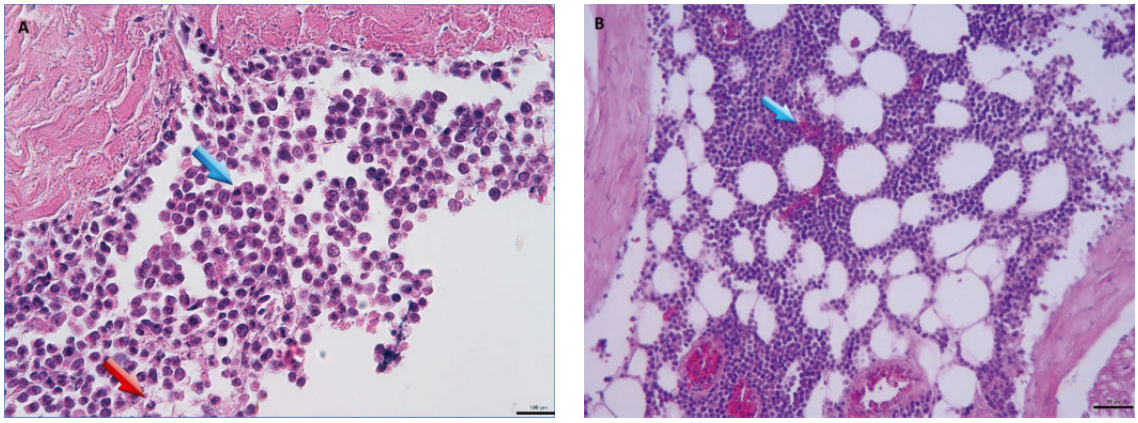

Figure 5: Photomicrographs of spinal cord meninges **(A)** and epidural adipose tissue **(B)** from a Golden Retriever with acute myelomonocytic leukemia. Diffuse proliferation of irregularly round neoplastic cells characterized by a high nucleus-cytoplasm ratio, round nuclei, and distinct large nucleoli (blue arrow – figure 5.A). Neutrophils (red arrow – figure 5.a) and hemorrhagic areas (blue arrow – figure 5.B) are also seen. H&E.

ical abnormalities. But because no further details were given, spinal cord involvement in these cases remains unknown.[7]

In the present case clinical signs of flaccid paraplegia, pelvic limb areflexia, and bladder and anal dysfunction were explained by the AML-M4 infiltration of the lumbosacral spinal cord, as confirmed by necropsy. Postmortem examination also revealed AML-M4 infiltration of the thoracic and, to a minor extent, the cervical spinal cord, suggesting a rapidly progressive and relentless process. In these areas the infiltration was less pronounced which may account for the absence of clinical signs on thoracic limbs. Cranial nerves were not sampled and histopathologically analysed because of lack of clinical signs.

A long list of differential diagnosis can be included in dogs with lumbosacral intumescence (L4-S3 spinal cord segments) neuroanatomical localization: vascular, inflammatory, traumatic, anomalous, neoplastic, and degenerative diseases.[5,8] Our findings hold importance for including AML and leukemia in general, in the differential diagnosis of patients with neurological signs due to spinal cord localisation with concurrent other clinical and laboratory signs (e.g., hyperthermia, lymphadenopathy, hepatomegaly, marked leukocytosis, anemia, thrombocytopenia). In leukemic patients hyperthermia can be caused by secondary infections or by the malignant cells themselves, producing chemical signals that cause the body to elevate the core temperature. From a clinical and diagnostic point of view no signs of infections were noted in this case, and this finding was confirmed by post-mortem examination.[6] Flow cytometry should be used in patients that present with leukemic profiles that also display neurologic signs.

Lymphadenopathy was once reported as an uncommon finding in AML. Some studies even excluded patients with this clinical sign from analysis in an attempt to differentiate dogs with lymphoma from those with acute leukemia. More recent studies, however, report that up to 74 % of patients diagnosed with AML display peripheral lymphadenopathy on physical examination.[7]

Immunophenotyping by flow cytometry of peripheral blood allowed for rapid diagnosis in the present case. CD34 is an hallmark of hempoietic precursor cells;[16] CD11b and CD14 are expressed by myeloid and monocytic elements, respectively, and CD4 identifies T-helper cells but it is exposed also in mature neutrophils in the dog.[15] The presence of a consistent population of undifferentiated precursor cells (CD34+CD117+) together with the increase of elements of myelomonocytic lineage (CD11b+ and CD14+), both mature and immature according to CD4 expression, lead to the diagnosis of acute leukemia with myelomonocytic differentiation.[15] Although a bone marrow sample was not available to confirm the diagnosis, the bi-cytopenia (anemia and thrombocytopenia) detected in the peripheral blood was indicative of bone marrow involvement. While anemia and thrombocytopenia are very frequent, neutrophil count is more variable and a neutrophilia is not a rare presentation.[7] Primary neoplastic extramedullary proliferation of myeloid precurors (myeloid or granulocytic sarcoma) can not be rouled out. However, it is very rare,[11] it is mostly associated to acute leukemias[14] and with the current available flow cytometric panels and the very limited knowledge in the veterinary species, a differential diagnosis would still not be possible. Indeed, this fast and relatively inexpensive test should be part of the diagnostic workup in dogs presenting with these clinical signs, in particular when associated with peripheral blood alterations and the presence of circulating ablnormal cells.

Treatment of AML has been unrewarding to date in veterinary medicine. There is too little information about the response of specific subtypes of leukemia to uniform chemotherapeutic protocols because of the rarity of the disease and because of the high rate of euthanasia prior to treatment initiation due to poor long-term prognosis.[7,17] Also in the present case, the owner elected humane euthanasia before the initiation of treatment due to the severity of clinical and neurological signs.

In conclusion, this case report describes the first case of AML-M4 in a dog with spinal cord infiltration. Our findings hold importance for including AML in the differential diagnosis of patients with neurological signs.

Leucémie aiguë myélomonocytaire avec manifestation multifocale et infiltration de la moelle épinière chez un chien

Peu de cas de leucémie myélomonocytaire associés à des signes neurologiques ont été décrits chez le chien ; aucun n'était lié à une infiltration intraparenchymateuse de la moelle épinière par des cellules néoplasiques. Cette courte communication décrit un cas de leucémie aiguë myélomonocytaire de sous-type M4 chez un chien avec infiltration de la moelle épinière.

Un Golden Retriever mâle de 3 ans a été présenté avec une anamnèse d'hyperthermie, de lymphadénomégalie, de leucocytose avec des cellules blastiques circulantes, d'anémie et de thrombocytopénie et de paraplégie d'apparition aiguë. L'immunophénotypage du sang périphérique par cytométrie de flux était compatible avec une leucémie myélomonocytaire aiguë de sous-type M4. Le chien a été euthanasié en raison de la détérioration de son état clinique et du pronostic défavorable. L'examen post-mortem a révélé une infiltration néoplasique multi-organique, y compris la moelle épinière.

À notre connaissance, il s'agit du premier cas de leucémie aiguë myélomonocytaire de sous-type M4 chez un chien avec infiltration de la moelle épinière. Nos résultats sont importants pour inclure la leucémie myélomonocytaire dans le diagnostic différentiel chez les patients présentant des signes neurologiques dus à une localisation médullaire.

Mots clés: chien, troubles myéloprolifératifs, neurologie, paraplégie, néoplasie de la moelle épinière

Leucemia mielomonocitica acuta con manifestazione multifocale con infiltrazione del midollo spinale in un cane

La letteratura riporta solo pochi casi di leucemia mielomonocitica nei cani associata a segni neurologici e nessuno di questi casi è associato a un'infiltrazione intraparenchimatosa del midollo spinale da parte di cellule neoplastiche. Lo scopo di questa breve relazione è di descrivere un caso di leucemia mielomonocitica acuta di sottotipo M4 con infiltrazione del midollo spinale in un cane.

Un golden retriever maschio di 3 anni è stato presentato con ipertermia, linfoadenomegalia, leucocitosi con blasti circolanti, anemia, trombocitopenia e paraplegia acuta. L'immunofenotipizzazione del sangue periferico mediante citometria a flusso ha rivelato una leucemia mielomonocitica acuta di sottotipo M4. Il cane è stato sottoposto ad eutanasia a causa del deterioramento clinico e della prognosi sfavorevole. L'esame post-mortem ha rivelato l'infiltrazione neoplastica di più organi, compreso il midollo spinale.

A nostra conoscenza, questo è il primo caso di leucemia mielomonocitica acuta di sottotipo M4 in un cane con infiltrazione del midollo spinale. I nostri risultati sono importanti per includere la leucemia mielomonocitica nella diagnosi differenziale dei pazienti con segni neurologici con localizzazione del midollo spinale.

Parole chiave: cane, disturbi mieloproliferativi, neurologia, paraplegia, neoplasia del midollo spinale

Literaturnachweis

[1] Adam F, Villiers E, Watson S, Coyne K, Blackwood L: Clinical pathological and epidemiological assessment of morphologically and immunologically confirmed canine leukaemia. Vet Comp Oncol. 2009: 7(3): 181–195.

[2] Barthel CH: Acute Myelomonocytic Leukemia in a Dog. Vet Pathol 1974: 11: 79–86.

[3] Carpenter JL, King NW, Abrams KL: Bilateral trigeminal nerve paralysis and Horner's syndrome associated with myelomonocytic neoplasia in a dog. J Am Vet Med Assoc. 1987: 191: 1594–1596.

[4] Christopher MM, Metz AL, Klausner J, Polzin D, Hayden DW: Acute myelomonocytic leukemia with neurologic manifestations in the dog. Vet Pathol. 1986: 23: 140–147.

[5] Coates JR: Paraparesis. In: Platt S, Olby N, ed. BSAVA manual of canine and feline neurology. 4th ed. Glouchester: BSAVA (British Small Animal Veterinary Association), 2013: 297–327.

[6] Cunha BA: Fever of unknown origin: clinical overview of classic and current concepts. Infect Dis Clin North Am. 2007: 21(4):867–915, vii

[7] Davis LL, Hume KR, Stokol T: A retrospective review of acute myeloid leukaemia in 35 dogs diagnosed by a combination of morphologic findings, flow cytometric immunophenotyping and cytochemical staining results (2007–2015). Vet Comp Oncol. 2018: 16: 268–275.

[8] De Lahunta A: Small animal spinal cord disease. In: de Lahunta A, et al., ed. Veterinary neuroanatomy and clinical neurology. 4th ed. St. Louis, Mo: Elsevier, 2015: 257–305.

[9] Hayashi A, Tanaka H, Kitamura M, Nishida H, Shimoda T, Ohashi F, Nakayama M: Acute myelomonocytic leukemia (AML-M4) in a dog with the extradural lesion. Vet Med Sci. 2011: 73:419–422.

[10] Jain NC, Blue JT, Grindem CB, Harvey JW, Kociba GJ, Krehbiel JD, Latimer KS, Raskin RE, Thrall MA, Zinkl JG: Proposed criteria for classification of acute myeloid leukemia in dogs and cats. Vet Clin Pathol. 1991: 20:63–82.

[11] Morosco DT, Cline CR, Owston MA, Kumar S, Dick EJJr. Spontaneous mediastinal myeloid sarcoma in a common marmoset (Callithrix jacchus) and review of the veterinary literature. Med Primatol. 2017 Apr;46(2):42–47.

[12] Novacco M, Comazzi S, Marconato L, Cozzi M, Stefanello D, Aresu L, Martini V: Prognostic factors in canine acute leukaemias: a retrospective study. Vet Comp Oncol. 2016: 14: 409–416.

[13] Riondato F, Miniscalco B, Poggi A, Arico A, Aresu L, Comazzi S, Martini V: Analytical and diagnostic validation of a flow cytometric strategy to quantify blood and marrow infiltration in dogs with large B-cell lymphoma. Cytometry B Clin Cytom. 2016: 90: 525–530.

[14] Shahin OA, Ravandi F: Myeloid sarcoma. Curr Opin Hematol. 2020 Mar;27(2):88–94

[15] Stokol T, Nickerson GA, Shuman M, Belcher N: Dogs with Acute Myeloid Leukemia Have Clonal Rearrangements in T and B Cell Receptors. Front Vet Sci. 2017; 31(4):76.

[16] Vernau W, Moore PF: An immunophenotypic study of canine leukaemias and preliminary assessment of clonality by polymerase chain reaction Vet Immunol Immunopathol 1999; 69: 145–164

[17] Young KM, Vail DM: Hematopoietic tumors – Canine Acute Myeloid Leukemia, Myeloproliferative Neoplasms, and Myelodysplasia. In: Elsevier, ed. Withrow & MacEwen's Small Animal Clinical Oncology, 5th ed. St. Louis, Mo., 2013: 653–665.

Korrespondenzadresse

Giulia Cagnotti
Department of Veterinary Science, University of Turin
Via Largo Braccini 2, 44 Grugliasco (TO)
IT-10095
Telefon: +39 011 670 90 73
E-Mail: giulia.cagnotti@unito.it