The Journal of Veterinary Medical Science

NOTE

Pathology

Erythroblastic sarcoma in the thoracic cavity of a cow

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J. Vet. Med. Sci.
81(1): 134–137, 2019
doi: 10.1292/jvms.18-0413

Received: 17 July 2018
Accepted: 7 November 2018
Published online in J-STAGE: 19 November 2018

ABSTRACT. Erythroblastic sarcoma in a 10-year-old Japanese Black cow with anemia is described. Tumor masses or nodules were located mainly in the thoracic cavity, and some lymph nodes were slightly enlarged. Although neoplastic involvement of the bone marrow was detected, the cow was not leukemic. The diagnosis was made based on the localized distribution of neoplastic lesions, no increase of intravascular nucleated cell number, deeply eosinophilic cytoplasm in some tumor cells, and frequent immunoreactivity of the tumor cells for hemoglobin. The tumor cells were characterized by marked pleomorphism and atypia; such morphological deviation from their normal counterparts may be connected with functional deviation resulting in the sarcomatous growth of these erythroid cells.

KEY WORDS: cattle, cytological atypia, erythroblastic sarcoma, hemoglobin, pure erythroid leukemia

In humans, myeloid sarcoma is a rare manifestation that is characterized by the occurrence of one or more myeloid tumor masses occurring at an extramedullary site [5]. Erythroblastic sarcoma is a myeloid sarcoma, and was reported for the first time in 2011 in humans [23]. This and an additional case occurred in infants with pure erythroid leukemia (acute erythremic myelosis) [14]. In contrast, de novo or primary erythroblastic sarcoma was detected in two persons aged 67 and 79, who did not develop acute leukemia [6, 18].

Reports of myeloid sarcoma are extremely rare in farm animals. Neutrophilic granulocytic sarcoma has been described in an 8-year-old cow [19] and a 3-year-old sow [12], and eosinophilic granulocytic sarcoma in a 5-year-old female pig [3]. Erythroid leukemia is also rare but has been reported in a calf [24] and a foal [7]; in the former, the number of nucleated cells was 30,800/μl in the blood. Here, we report a case of erythroblastic sarcoma which presented as intrathoracic tumor masses in a 10-year-old cow.

A 10-year-old Japanese Black cow presented with a staggering gait and anorexia. On day 2, the cow was still anorexic and depressed, with a yellowish white color in the labial mucosa. On day 3, blood examination showed a red blood cell count of 2,410,000/μl, a hematocrit value of 14% and a white blood cell count of 5,400/μl with 73% lymphocytes, 3.5% proerythroblastic tumor cells, 8% segmented neutrophils, 12% band neutrophils, 1% eosinophils and 2.5% monocytes. Serum lactate dehydrogenase activity (LDH) was elevated significantly (3,870 IU/l). An enzyme-linked immunosorbent assay for detecting bovine leukemia virus (BLV) antibodies tested positive (JNC, Tokyo, Japan). On day 4, euthanasia was considered because of poor prognosis, but the animal died during transportation for necropsy examination.

Necropsy revealed large amounts of bloody pleural effusion. Several subpleural neoplastic masses or nodules, 2–7 cm in diameter, which were solid and homogeneous in consistency and dark red to grayish white in color, were detected between the thoracic aortae and the first to 13th thoracic vertebrae (Fig. 1A). These were adherent to the left side of the vertebral bodies. Similar masses or nodules were on the diaphragmatic pleura (two masses, 7 × 3 × 3 cm in each), in the left intercostal muscle (5 × 3 × 3 cm), on the sternum (smaller than in the intercostal muscle), and on the left costal periosteum (1 cm in diameter). Three nodules, 1–2 cm in diameter, were located on the greater omentum, urinary bladder mucosa and left kidney. The vertebral and sternal bone marrow was white and could not be distinguished from fatty marrow. Some lymph nodes, such as the left superficial cervical, sternal, bronchial, left ruminal, renal and medial iliac were slightly enlarged. The spleen and liver appeared normal in size.

Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin

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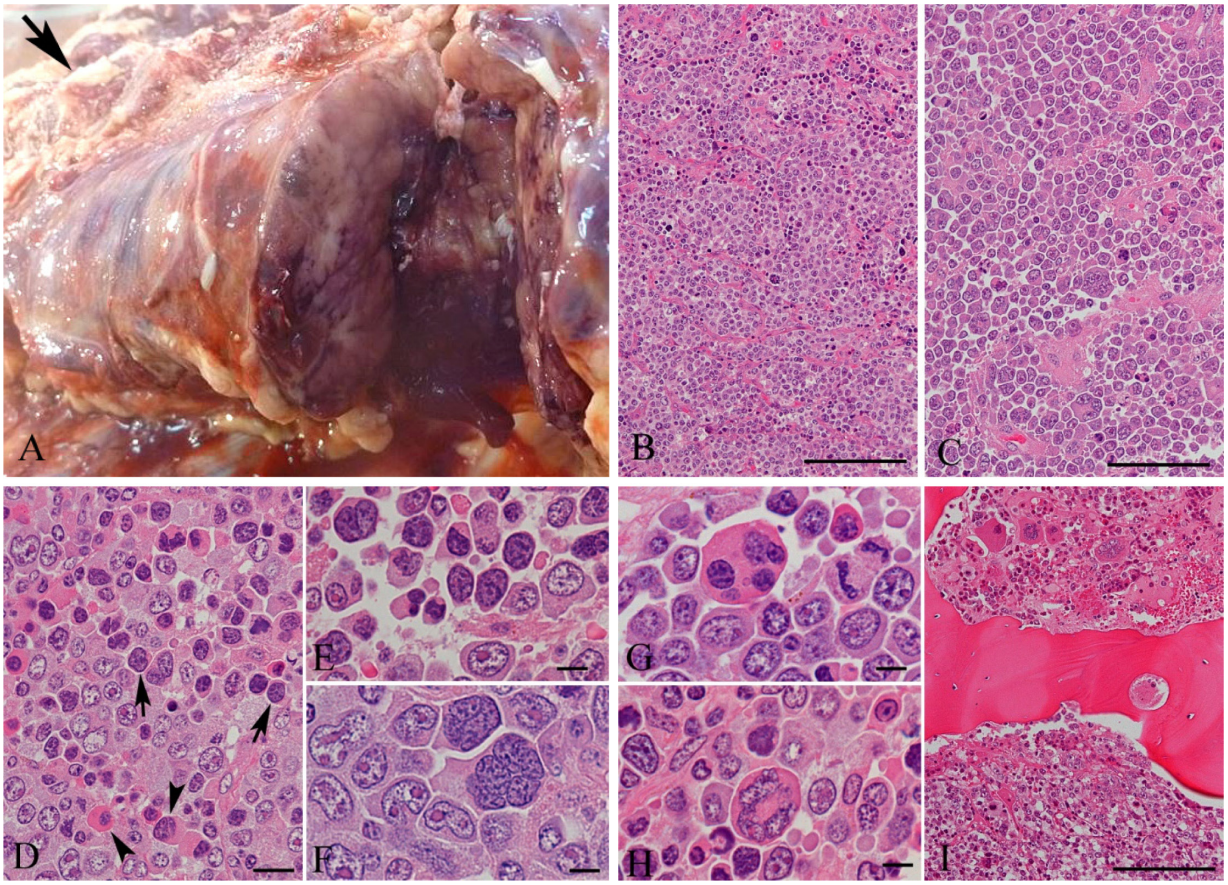

Fig. 1. Gross anatomy and histology. (A) Intrathoracic tumor masses. A bulging cut surface is visible by partial resection of tumor masses situated along halved thoracic vertebrae (arrow). (B) The same tumor as in A. This area is composed predominantly (top) or exclusively (bottom) of proerythroblastic tumor cells. HE. Bar=100 μm. (C) Left ruminal lymph node. The tissue consists of large or giant tumor cells with basophilic cytoplasm and frequently with two or multiple nuclei. HE. Bar=50 μm. (D) Left superficial cervical node. Erythroblastic tumor cells with similar chromatin condensation have scanty (arrows) or abundant eosinophilic (arrowheads) cytoplasm. HE. Bar=10 μm. (E) The same node as in D. Some tumor cells contain dark nuclei and basophilic or amphophilic cytoplasm. HE. Bar=5 μm. (F) The same node as in D. The nuclei of multinucleated tumor cells appear darker than those of surrounding proerythroblastic tumor cells, but the cytoplasm stain similarly. HE. Bar=5 μm. (G) The same node as in D. A giant tumor cell with eosinophilic cytoplasm has both dark and less dark nuclei. HE. Bar=5 μm. (H) The same node as in D. Immature nuclei with irregular contours are observed in a giant tumor cell, but the cytoplasm is maturing. HE. Bar=5 μm. (I) Sternal bone marrow. This specimen shows areas composed mainly of tumor cells (bottom) or normal hematopoietic cells (top). HE. Bar=100 μm.

Table 1. Primary antibodies utilized for immunohistochemistry

Antibody	Clone	Type	Dilution	Manufacturer	Antigen retrieval
Hemoglobin		pAb	Pd	Lipshaw, Pittsburgh, PA, U.S.A.	None
Factor XIII-related antigen		pAb	Pd	BioGenex, San Ramon, CA, U.S.A.	0.05% pepsin
CD3		pAb	1:50	Dako A/S, Glostrup, Denmark	0.05% pepsin
CD79a	HM57	mAb	1:25	Dako A/S, Glostrup, Denmark	Mh (pH6)
Myeloid/histiocyte antigen	MAC387	mAb	1:25	Dako A/S, Glostrup, Denmark	0.05% pepsin
CD68	EBM11	mAb	1:50	Dako A/S, Glostrup, Denmark	0.05% pepsin
Macrophage	HAM56	mAb	1:25	Dako Corp., Carpinteria, CA, U.S.A.	Mh (pH6)
Tryptase	AA-1	mAb	1:200	Lab Vision, Fremont, CA, U.S.A.	Mh (pH6)

pAb, rabbit polyclonal antibody; mAb, mouse monoclonal antibody; Pd, prediluted; Mh, microwave heating.

(HE), Giemsa, Berlin blue and naphthol AS-D chloroacetate esterase (CAE). Some sections were selected, dewaxed, and labeled by the streptavidin-biotin-peroxidase complex (SAB) method. The primary antibodies employed are presented in Table 1. Subsequent procedures were carried out by means of an immunoperoxidase labeling system (Nichirei, Tokyo, Japan).

Histologically, tumor masses or nodules detected at necropsy were composed of neoplastic tissue, in which proerythroblastic or erythroblastic tumor cells grew diffusely, in sheets or in clusters (Fig. 1B). Similar histological findings were obtained in the other

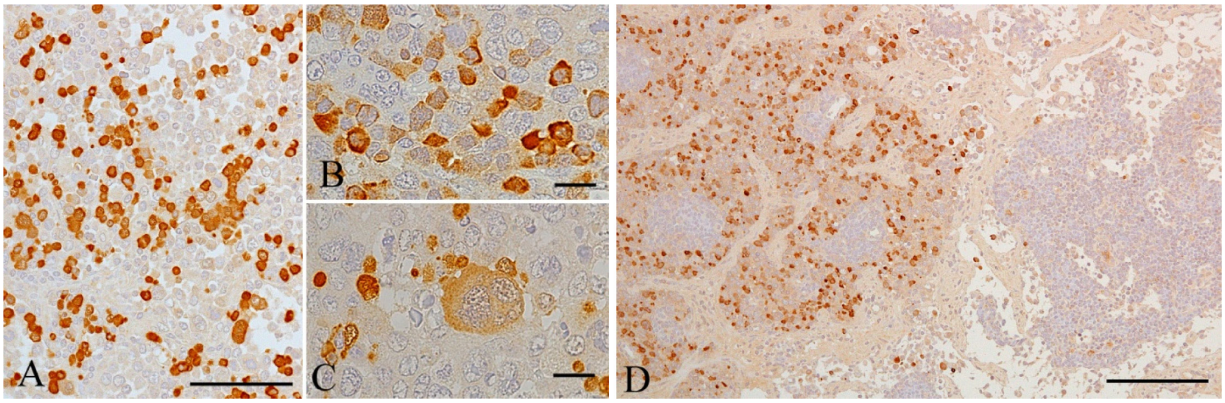

Fig. 2. Immunohistochemistry. (A) Sternal lymph node. Hemoglobin-positive tumor cells show a variation in cell size. SAB. Bar=50 μm. (B) Left superficial cervical node. Relatively small erythroblastic tumor cells are hemoglobin positive with various staining intensity. SAB. Bar=10 μm. (C) Sternal node. A giant tumor cell with large blastic nuclei stains weakly for hemoglobin. SAB. Bar=10 μm. (D) The same node as in B. Because this is the transitional area, lymphatic tissue remains, despite massive intrasinus infiltration of tumor cells frequently expressing hemoglobin (left half). In the right half, in contrast, positive cells are exceedingly rare. SAB. Bar=100 μm.

neoplastic lesions. Cytologically, the tumor cells could be divided into immature and mature forms, with the former predominating. In the former, the cells were large or very large in size, with round, oval or irregularly contoured nuclei, moderately condensed chromatin and small to large nucleoli. Multiple nuclei were not infrequent (Fig. 1C). The cytoplasm was variable in amount, and was basophilic. In the mature form, most of the cells were characterized by hyperchromatic nuclei, deeply eosinophilic cytoplasm and small cell size (Fig. 1D), although small or large cells with hyperchromatic nuclei and basophilic cytoplasm were detected (Fig. 1E and 1F). In contrast, deeply eosinophilic cytoplasm was rarely seen in larger or giant cells with single or multiple nuclei and varying chromatin condensation (Fig. 1G and 1H). Mitotic figures were plentiful.

Although the thoracic spinal and sternal bone marrow was slightly to moderately infiltrated with tumor cells (Fig. 1I), areas of fatty tissue with or without normal hematopoietic cells were observed. The normal cells were largely neutrophilic promyelocytes, myelocytes or metamyelocytes, and smaller numbers of megakaryocytes or relatively mature erythroblasts were admixed with them. The architecture of most of the enlarged lymph nodes was nearly completely replaced by tumor tissues. In the left superficial cervical lymph node, one half of the tissue was heavily infiltrated by tumor cells, but the other half contained very few tumor cells. Tumor cells were extremely rare in certain other lymph nodes, such as salivary gland and gastrointestinal. Tumor cell necrosis was conspicuous in larger neoplastic lesions, and active erythrophagocytosis by tumor cells was observed in hemorrhagic areas. Intravascular tumor cells were few in number in the lungs, and there were small numbers of tumor cells within sinusoids of the liver and adrenal glands. Neoplastic infiltration was mild in the splenic red pulp, with very slight granulopoiesis and megakaryopoiesis. Many siderophages and marked iron deposits were demonstrated by Berlin blue staining.

Immunohistochemically, the tumor cells were frequently positive for hemoglobin (Fig. 2A) but negative for the other markers tested. Although smaller cells tended to stain positively for hemoglobin (Fig. 2B), some large tumor cells with single or multiple nuclei were also positive (Fig. 2C). Areas with and without tumor cells were highlighted by hemoglobin staining in the left superficial cervical lymph node (Fig. 2D).

Hemoglobin, an excellent marker for erythroid differentiation in cattle [23], was demonstrated to be present in maturing tumor cells in the current case. On the other hand, not only B cell, T cell and other hematopoietic cell markers but also intracytoplasmic granules were absent [1, 8, 10, 13, 19, 22, 25]. This tumor was therefore judged to be a neoplasm of the erythroid lineage. [24]. Although the thoracic bone marrow was affected by tumor, residual non-neoplastic hematopoietic and adipose tissues were extensive, and the tumor cells tended to form tumor masses or nodules and to be invasive into adjacent tissues rather than to proliferate in blood vessels. Only few tumor cells were hence observed in splenic red pulp, hepatic sinusoids and pulmonary capillaries, which are crowded with neoplastic cells in ordinary leukemias [16, 19]. On the basis of the clinical, macroscopic and histological results, a diagnosis of erythroblastic sarcoma was made [5].

In a previously reported case of bovine erythroid leukemia, the neoplastic cells were very similar to their normal counterpart [24]. In the present case, mononuclear or multinuclear giant cells were reminiscent of cells with megakaryocytic differentiation [10] or cells of BLV-associated pleomorphic B cell lymphoma [11]. As in the latter [9], the cytological pleomorphism and atypia indicate a marked deviation from the normal erythropoietic pathway, and may be linked to loss of function of the tumor cells to migrate to other sites. This loss was clearly demonstrated by the finding that only half of the lymphatic tissue was infiltrated by neoplastic cells in the left superficial cervical lymph node. Likewise, in a case of neutrophilic granulocytic sarcoma of thoracic cavity origin in an 8-year-old cow, agranular tumor cells often did not resemble normal myeloblasts [19]. These sarcomas were not thought to terminate as acute leukemias, because of the poor condition or subsequent death of the cows. Although primary myeloid sarcomas usually lead to myeloid leukemias in humans [15], patients with de novo erythroblastic sarcoma do not develop acute leukemia [6, 18].

Splenic hemosiderosis may occur in hemolytic anemia [20]. In the current case, marked iron deposits in the spleen may be due to necrosis of hemoglobin-positive tumor cells. The present animal was highly anemic, but severe extramedullary hematopoiesis suggestive of widespread neoplastic involvement of the bone marrow was not observed. Taking into account the few normal erythropoietic cells and greater numbers of hemoglobin-positive tumor cells, the anemia may be associated not only with neoplastic replacement of the bone marrow, but also high iron consumption by active neoplastic erythropoiesis. Alternatively, the animal may have been in an aplastic or cytopenic condition of the marrow leading to a hematopoietic malignancy [4].

In humans, erythroleukemia (erythroid/myeloid) is predominantly a disease of adults. Pure erythroid leukemia can occur at any age [2]. Erythroblastic sarcoma occurs in infants with pure erythroid leukemia [14, 23], whereas de novo or primary erythroblastic sarcoma occurs in older persons [6, 18]. Acute myeloid leukemias including a case of pure erythroid leukemia usually occur in calves or young cattle [10, 17, 19, 21, 24], but myeloid sarcomas have been observed in adult or aged cattle and swine [3, 12, 19]. In cattle, myeloid sarcomas, characterized by the age predilection as well as no elevation of white blood cell count, formation of large tumor masses and unusual cytology of neoplastic cells [19], are quite different from common myeloid neoplasms (leukemias). These features are presumably the reason why case reports of this disease are so rare.

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