Use of chemotherapy for treatment of a mixed-cell thoracic lymphoma in a horse

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A 4-year-old Oldenburg mare (weight, 610 kg [1,340 lb]) was evaluated because of a 4-day history of anorexia, coughing, and pyrexia of unknown origin. Prior to admission, the referring veterinarian had administered phenylbutazone (2 g, PO, q 12 h) and cefitiofur sodium (1 g, IM, q 12 h). The owner reported that the horse coughed during exercise and displayed minimal interest in its immediate surroundings.

On physical examination (day 1), the mare was obtunded, pyrexic (rectal temperature, 40°C [104°F]), and coughed occasionally. On thoracic auscultation, wheezes were detected bilaterally in the dorsocaudal lung fields; serous, nonodorous nasal discharge was evident bilaterally. Thoracic ultrasonography revealed a small amount of pleural fluid in both lungs; radiography revealed a focal, poorly defined, 8-cm-diameter opacity between the carina and diaphragm. To evaluate the respiratory tract further, a tracheal aspirate specimen was collected aseptically and submitted for aerobic and anaerobic bacteriologic culture. On that day, mature neutrophilia (11,952 cells/µL), band neutrophilia (714 bands/µL), hyperfibrinogenemia (800 mg/dL), and hypoproteinemia (5.7 g/dL) were evident.

A thoracic ultrasonography revealed a focal, poorly defined, 8-cm-diameter opacity between the carina and diaphragm. To evaluate the respiratory tract further, a tracheal aspirate specimen was collected aseptically and submitted for aerobic and anaerobic bacteriologic culture. On that day, mature neutrophilia (11,952 cells/µL), band neutrophilia (714 bands/µL), hyperfibrinogenemia (800 mg/dL), and hypoproteinemia (5.7 g/dL) were evident.

Immunophenotyping is a useful adjunct to routine cytologic evaluation of pleural fluid; results should be compared with results of cytologic and histologic examination of fine-needle aspirates and biopsies when possible.

Chemotherapeutic-induced remission of thoracic lymphoma in horses may be achieved.

Lymphoma should be suspected in horses with recurrent pleural effusion.

Chemotherapeutic-induced remission of thoracic lymphoma in horses may be achieved.

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Biochemical analyses included mature neutrophilia (7,424 cells/µL; reference range, 3,000 to 6,000 cells/µL), band neutrophilia (232 bands/µL; reference range, <100 bands/µL), hyperfibrinogenemia (800 mg of fibrinogen/dL; reference range, 100 to 400 mg/dL), hypoalbuminemia (2.8 g of albumin/dL; reference range, 2.9 to 3.8 g/dL), and hyperbilirubinemia (6.7 mg of bilirubin/dL; reference range, 0.8 to 2.6 mg/dL). Cytologic evaluation of the tracheal aspirate specimen revealed a predominance of neutrophils, RBCs, few macrophages with occasional erythrophagocytosis, mucus, and moderate amounts of acellular debris. These findings were consistent with suppurative inflammation. No signs of pain in the feet were detected with hoof testers, but because digital pulses were distinctly palpitable on day 2, treatment with pentoxifylline (8.5 mg/kg [3.9 mg/lb], PO, q 12 h) and 2% nitroglycerin ointment (15 mg applied topically on digital arteries q 24 h) was begun. On day 3, the mare suddenly became tachypneic (66 breaths/min). Thoracic ultrasonography revealed atelectasis and anechoic pleural fluid in the right hemithorax, whereas multifocal pulmonary consolidation with poor respiratory excursion was detected in the left hemithorax. Drainage of the right hemithorax by use of a sterile teat canula yielded 2 L of thick, red fluid (protein concentration, 4.1 g/dL; cytologic examination of this pleural fluid revealed evidence of suppurative inflammation and hemorrhage (Table 1). A sample of the pleural fluid was submitted for aerobic and anaerobic bacteriologic culture. On that day, results of hematologic and serum biochemical analyses indicated mature neutrophilia (7,735 cells/µL), band neutrophilia (714 cells/µL), hyperfibrinogenemia (800 mg/dL), and hypoproteinemia (5.7 g of protein/dL; reference range, 3.9 to 7.6 g/dL) as a result of low albumin concentration (2.6 g/dL).

On day 4, thoracic ultrasonography revealed a minimal amount of pleural fluid in the right hemithorax that was considered to be a result of the pleural drainage performed the previous day; however, pulmonary consolidation was still evident in the left hemithorax from the seventh through tenth intercostal spaces. By day 5, acro-bic and anaerobic bacteriologic cultures of pleural fluid, the tracheal aspirate specimen, and blood had yielded no growth of bacteria. Results of hematologic and serum biochemical analyses indicated leukocytosis (16,600 WBCs/µL; reference range, 6,000 to 12,000 WBCs/µL) as a result of mature neutrophilia (11,952 cells/µL) and hyperfibrinogenemia (700 mg/dL); total serum protein concentration (6.8 g/dL) was within reference limits with a low albumin-to-globulin ratio (0.55; reference value, 1.0) as a result of hypoalbuminemia (2.4 g/dL) and hyperglobulinemia (4.4 g of globulin/dL; reference range, 3.0 to 3.8 g/dL). Intravenous fluid administration was
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The horse was discharged on day 11.

Three days after discharge from the hospital (day 15 with regard to the initial examination), the horse was readmitted for further evaluation after an acute episode of colic that morning; the referring veterinarian had administered flunixin meglumine (1.1 mg/kg, IV). On examination, the mare was tachycardic (60 beats/min) and tachypneic (44 breaths/min), with bilateral wheezes audible via auscultation of the thorax. Findings of rectal palpation and cytologic examination of peritoneal fluid obtained via abdominocentesis were within reference limits. Edema in the pectoral region obtained via abdominocentesis was within reference limits. On day 7 because digital pulses were within reference limits. On day 8, results of hematologic analyses indicated less severe leukocytosis (14,400 WBCs/µL), mature neutrophilia (11,376 cells/µL), and hyperfibrinogenemia (500 mg/dL). Administration of gentamicin was discontinued on day 8; treatment with penicillin G potassium and metronidazole was discontinued on day 9. Because of incomplete resolution of the chronic active inflammation, administration of a potentiated sulfonamide (25 mg/kg [11.4 mg/lb], PO, q 12 h) was begun on day 9. The horse was discharged on day 11.

Drainage of the left and right hemithoraces yielded 1 and 7 L of cloudy pleural fluid, respectively. The pH of the pleural fluid from the left hemithorax was 7.3 (reference limit, 7.3 ± 0.1), the glucose concentration was 138 mg/dL (reference limit, 97.2 ± 33.5 mg/dL), and the lactate concentration was 2.23 mmol/L (reference limit, 1.63 ± 1.2 mg/dL).1 Biochemical analysis was not performed on the pleural fluid from the right hemithorax.

The fluids obtained from both pleural spaces were cytologically similar (Table 1). The pleural fluid from the left and right hemithoraces was red and yellow, respectively, and contained 35,000 and 27,000 nucleated cells/µL (reference range, < 10,000 cells/µL), respectively; the total protein concentrations in both samples were characteristic of an exudate (4.2 g of protein/dL of pleural fluid). Lymphocytes predominated in both samples of pleural fluid (composing 50% of cells in the fluid in the left hemithorax and 65% of cells in the fluid in the right hemithorax); large numbers of prolymphocytes and lymphoblasts were observed, although small lymphocytes predominated (Fig 1). In some of the large lymphocytes, nuclear blebbing, cleaved nuclei, and occasional cloverleaf-shaped nuclei were noted.

Table 1—Results of cytologic evaluation and bacteriologic culture of specimens of pleural fluid obtained from the right and left hemithoraces of a horse with thoracic lymphoma

<table>
<thead>
<tr>
<th>Characteristics of fluid</th>
<th>Right hemithorax</th>
<th>Left hemithorax</th>
<th>Right hemithorax</th>
<th>Left hemithorax</th>
<th>Left hemithorax</th>
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</thead>
<tbody>
<tr>
<td>Nucleated cells (No. of cells/µL)</td>
<td>45,000</td>
<td>35,000</td>
<td>27,000</td>
<td>375,000</td>
<td>32,800</td>
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<td>Neutrophils (%)</td>
<td>67</td>
<td>25</td>
<td>13</td>
<td>95</td>
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<tr>
<td>Mononuclear cells (%)</td>
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<td>25</td>
<td>22</td>
<td>4</td>
<td>10</td>
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<td>Lymphocytes (%)</td>
<td>3</td>
<td>50</td>
<td>65</td>
<td>1</td>
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<tr>
<td>Results of aerobic and anaerobic bacteriologic culture</td>
<td>Normal</td>
<td>Abnormal</td>
<td>Abnormal</td>
<td>Normal</td>
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<td>Morphologic features of lymphocytes</td>
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NA = Not assessed.

Figure 1—Photomicrograph of a preparation of pleural fluid from a 4-year-old horse with thoracic lymphoma. Notice that approximately 40% of the cells are small lymphocytes with compact, densely stained chromatin, occasional shallow nuclear indentations; and scant amounts of cytoplasm. Large lymphocytes with finely distributed chromatin, inconspicuous nuclei, and abundant pale cytoplasm compose 25% of the cells in the sample. Other cells detected include well-preserved neutrophils, large monocytes, and occasional plasma cells. Wright-Giemsa stain; bar = 10 µm.
leaf-shaped nuclei were detected. Scattered mitotic figures (0 to 2/hpf) were observed, but few of these were considered abnormal cytologically (Fig 2). Additionally, numerous large mononuclear cells were present in both samples (composing 25% of cells in the fluid in the left hemithorax and 22% of cells in the fluid in the right hemithorax), and leukophagocytosis was common among the mononuclear cells in both fluid samples. The findings of the cytologic evaluation of pleural fluid samples obtained on day 15 were consistent with lymphoma with secondary chronic inflammation.

To further classify the lymphoma, samples of pleural fluid were cytocentrifuged to obtain cells for immunophenotyping. Immunophenotyping (based on a previously described technique2) was performed on 5 × 10⁴ cells with streptavidin-horseradish peroxidase. Briefly, cells were centrifuged onto glass slides, fixed in acetone for 2 minutes, and air-dried. The slides were rinsed with phosphate-buffered saline (PBS) solution. Endogenous peroxidase was blocked with 0.3% hydrogen peroxide in 0.1% sodium azide in PBS solution for 15 minutes and then blocked with 10% horse serum for 10 minutes at room temperature (20ºC). Primary antibodies (BLA36+ [mouse monoclonal], CD79α [mouse monoclonal], and CD3 [rabbit polyclonal]) were diluted for optimum reactivity as established by titration and applied for 2 hours at 37ºC. Slides were rinsed in PBS solution and incubated with prediluted biotinylated horse anti-mouse IgG for 20 minutes at room temperature (20ºC). After rinsing with PBS solution, slides were incubated with the streptavidin-horseradish peroxidase conjugate for 15 minutes at room temperature (20ºC). Slides were again rinsed with PBS solution, and the chromagen was applied. Color development was monitored by examination under the light microscope, and the reaction was stopped by placing the slides in distilled water. Counterstaining with Gill’s No. 2 hematoxylin was performed for 2 minutes, followed by a rinse in tap water; slides were placed into PBS solution and then placed into distilled water. Finally, the slide preparations were coverslipped by use of a mounting medium and examined via light microscopy. Negative controls were also prepared; slides were processed in the same manner except that the primary antibody was substituted with normal mouse ascites, normal rabbit serum, or rabbit isotype control immunoglobulin. In the negative control slides, no cells were stained when the primary antibody was substituted with normal mouse ascites, normal rabbit serum, or rabbit isotype control immunoglobulin.

On examination of the slides, > 95% of the small lymphocytes (Fig 3) were CD3+ T cells, whereas most of the large lymphocytes (Fig 4) were BLA36+ B cells. A small proportion (5% of the 2 cell types combined) of large lymphocytes and plasma cells were CD79+. Large lymphocytes had weak cytoplasmic staining when labeled with a pan-equine immunoglobulin reagent, and results for IgG, IgM, and IgA were negative.
tive. The large lymphocytes were strongly positive for both κ and λ light chains. Results of immunopheno-
typing indicated a mixed-cell (T-cell-rich, B-cell) lymph-
oma.

The horse continued to receive a potentiated sul-
fonamide (25 mg/kg, PO, q 12 h) and flunixin meglu-
mine (1.1 mg/kg, IV, q 12 h) because of ongoing chronic
active pulmonary inflammation. On day 17, the mare had an acute episode of tachycardia (66
beats/min) and tachypnea (72 breaths/min); wheezes
and crackles associated with respiration were auscul-
tated bilaterally. Results of hematologic analyses indi-
cated mature neutrophilia (3,700 cells/µL), band neu-
 trophi (1,000 cells/µL), hyperfibrinogenemia (600
dL), and continued normocytic, normochromic anemia
(PCV, 24%). Thoracic ultrasonography of the left
hemithorax revealed poor pulmonary excursion, fibrin,
and small amounts of cloudy pleural fluid, com-
pared with the right hemithorax. Via thoracocentesis of
the fluid, there was no growth by aerobic or blood-cul-
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is not conclusive evidence for the absence of a thoracic tumor. The apparent lack of neoplastic cells in these samples may be the result of intermittent shedding of tumor cells (perhaps because populations of neoplastic cells have become sequestered) or an inability to retrieve fluid from the caudal lung lobes via transendoscopic lavage. In the horse of this report, other diagnostic modalities that could have been used included percutaneous transthoracic fine-needle aspiration or biopsy of the mediastinal or pulmonary mass.18

Recent advances in molecular biology involving immunohistochemical evaluation of formalin-fixed tissue have been applied to the investigation of lymphomas in horses; in 1 study,11 of 24 (46%) horses had tumors that were classified as T-cell-rich, B-cell lymphomas. To the authors’ knowledge, immunophenotyping has not been described as an aid to classification of thoracic lymphoma in horses. This technique should be considered when atypical lymphoblasts are detected in pleural fluid, thereby enabling classification of the type of lymphoma without requiring invasive techniques to obtain tissue samples. At present, immunophenotyping is a standard technique in human medicine, but the diagnosis of lymphoma subtypes depends on histologic assessments, and not all tumor subtypes are solely defined by cytochemical criteria.19

Detection of a mixture of small and large lymphocytes histologically or cytologically is indicative of 1 of 2 types of B-cell lymphoma. A diffuse mixed-cell type (also known as a centroblastic-centrocytic lymphoma according to the Kiel classification) can develop and consists entirely of B cells.20 Also, a T-cell-rich, B-cell lymphoma can develop with a mixture of large and small lymphocytes; this is composed mostly of small tumor infiltrating (reactive) T cells and lesser numbers of neoplastic B cells.20 These B cells, as observed in the horse of this report, are cytologically atypical and have large vesicular nuclei with prominent nucleoli and peripheral distribution of chromatin.

Few reports21 have described the use of a multiple-drug induction protocol in the chemotherapy treatment of lymphoma in horses. One such protocol consisted of cytarabine (200 to 300 mg/m², SC or IM, q 7 to 14 d), chlorambucil8 (20 mg/m², PO, q 14 d) or cyclophosphamide (200 mg/m², IV, q 14 to 21 d), and prednisone (1.1 to 2.2 mg/kg [0.5 to 1 mg/lb], PO, q 48 h). Administration of vincristine (0.5 mg/m², IV, q 7 d) can be added to the induction protocol if no clinical response is detected in the first 2 to 4 weeks of treatment with the chemotherapy agents.19 Should remission be achieved, the induction protocol can be used for an initial 2- to 3-month period; this is followed by a maintenance protocol during which the dose of prednisolone is gradually reduced and the administration interval for each chemotherapeutic drug is extended by 1 week for the next 2 to 3 months. Finally, the administration interval for each chemotherapeutic drug is extended by another week for the next 2 to 3 months until termination of chemotherapy in 6 to 8 months.21 Other reported protocols include cyclophosphamide (200 mg/m², IV, q 14 to 21 d) or 1-asparaginase (10,000 to 40,000 U/m², IM, q 14 to 21 d) and combinations of cytarabine or cyclophosphamide with prednisone.1 In humans, treatments of T-cell-rich, B-cell lymphoma is similar to that used for diffuse B-cell lymphoma and involves the administration of cyclophosphamide (750 mg/m², IV), doxorubicin9 (50 mg/m², IV), vincristine (1.4 mg/m², IV), rituximab10 (375 mg/m², IV), and prednisolone (50 mg/m², PO, q 24 h for 5 days).22

The most commonly encountered complications associated with administration of chemotherapeutic drugs in equids include anorexia, signs of depression, jugular thrombosis, laminitis, bone marrow damage, gastrointestinal and genitourinary tract toxicoses, and immunosuppression.23,24 Anemia and thrombocytopenia may develop as a result of bone marrow suppression, as detected in the horse of this report; depending on the severity of anemia and thrombocytopenia, blood transfusions may be warranted or further chemotherapeutic treatments postponed. Leukopenia and secondary infection are more commonly encountered; however, recommendations vary regarding the lower limit of WBC concentration at which chemotherapy should be suspended.23,24

Pharmacodynamics may influence selection of chemotherapeutic agents. Cytarabine administered IV is rapidly metabolized, compared with its metabolism after IM administration, whereas cyclophosphamide requires bioactivation to its active metabolite 4-hydroxy-cyclophosphamide by the liver microsomal oxidase system.21 In the treatment of horses with lymphoma, considerable success has been achieved with a chemotherapeutic protocol consisting of cytarabine, cyclophosphamide, and prednisolone.21 Cytarabine is cytotoxic to proliferating cells, and its action is specific for cells undergoing DNA synthesis.26 After hepatic bioactivation, cyclophosphamide interferes with the growth of proliferating malignant cells by causing cross-linking of tumor cell DNA.27 Corticosteroids are thought to initiate cell death by activation of endonucleases that destroy chromatin integrity in tumor cells.28

Primary lymphoma in horses is rare but may be treatable when diagnosed early. Immunophenotyping has been used to identify monoclonality in effusions as an indication of lymphomatous involvement in human medicine; however, clinicians should be aware that large numbers of reactive T cells may obscure detection of a population of neoplastic B cells.29 Immunophenotyping in conjunction with fine-needle aspiration and biopsy may be useful in the diagnosis, subtyping, and treatment of lymphoma.30

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