Acute B-lymphoid leukemia in a mare: a case report

B. Bezdekova[1], M. Faldyna[2], O. Zapletal[3], P. Jahn[1]

[1]Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic
[3]University Hospital, Brno, Czech Republic

ABSTRACT: An 8-year-old Friesen mare was admitted to the Equine Clinic, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic with ventral edema and weight loss which had proceeded over the course of a few weeks. Clinical examination revealed poor body condition, depression, weakness, pale mucous membranes, enlarged mandibular lymph nodes and ventral edema. Thoracic percussion revealed fluid level reaching the shoulder on both sides. CBC revealed a severe disproportion of red and white cells. The horse was anemic and there was leucocytosis with the majority being blast cells (leucocyte concentration was 450.4×10^9/l) which included mitotic figures. The serum chemistry profile revealed hypoalbuminemia and increased alkaline phosphatase bone isoenzyme (BALP was 784.3 μkat/l). Based on the number of precursors in the peripheral blood acute leukemia was diagnosed. The peripheral blood was examined by flow cytometry and cytochemistry. The final diagnosis was determined as acute B cell lymphoid leukemia. The mare was treated with corticosteroids. On the second day of treatment the white blood cell count and alkaline phosphatase level decreased. The improvement continued until the fifth day, when the mare's status deteriorated and the horse was euthanized. Gross examination was carried out but no neoplastic mass was discovered. Final differentiation between primary and secondary lymphoid leukemia was not possible in this case.

Keywords: horse; leukemia; flow cytometry; cytochemistry; lymphoma

Leukemias are rare in horses in comparison to other species (McClure, 2000). Leukemias are defined as the presence of neoplastic haemopoietic cells in the circulation and they are classified as leukemic, subleukemic, or aleukemic based on the number of neoplastic cells in the blood, and acute or chronic based on the maturity of the neoplastic cells. Acute leukemia has a blast-like morphology whereas chronic indicates that the leukemic cells have a mature morphology (McClure, 2000). Regarding their cellular origin leukemias are categorized as lymphoid or myeloid and they can be classified as myelogenous or lymphoid based on their origin. B cell origin of lymphoid leukemias seems to be less frequent than T cell lymphoid leukemia in horses (Dascanio et al., 1992; McClure et al., 2001; Rendle et al., 2007).

Lymphoproliferative diseases in horses include lymphoma, lymphocytic (lymphoid) leukemia, and plasma cell myeloma. Lymphoid leukemia (LL) can be primary or secondary on the base of primary bone marrow neoplasia or secondary metastasis of lymphoma into bone marrow (McClure, 2000). There is scant published data regarding the treatment of leukoproliferative disease in horses (Byrne et al., 1991; Gerber et al., 2002; Saulez et al., 2004; Rendle et al., 2007).

Clinical case

An 8-year-old Friesen mare was admitted to the Equine Clinic, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

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with ventral edema and weight loss which had lasted for several weeks. The owner bought the horse one month previously just after the weaning of her current foal. The mare was treated with broad spectrum antibiotics and nonsteroidal anti-inflammatory drugs in the field for several days.

The body weight of the mare was 560 kg. Her appetite was normal. Clinical examination revealed poor body condition, depression, weakness, pale mucous membranes, enlarged submandibular lymph nodes and ventral edema which extended to the cranial thorax. The hind limbs were also edematous. Heart rate was 86 beats per minute, respiratory rate 80 per minute, rectal temperature 37.6°C. Heart sounds flared across the thoracic cavity. Thoracic percussion revealed fluid level to the shoulder on both sides.

CBC revealed a severe disproportion of red and white cells [PCV 0.34 l/l (reference range 0.30–0.44 l/l); of which the red blood cell portion was 17% and the white blood cell portion was also 17%]. The horse was anemic [erythrocytes level 3.44×10^{12}/l (6.4–9.8 $\times 10^{12}$/l); hemoglobin 95 g/l (111–169 g/l)]. There was leucocytosis with the majority of blast cells [leucocytes level 450.4×10^{9}/l (4.1–10.1 $\times 10^{9}$/l); segmented neutrophiles 7% (31.528×10^{9}/l)]; band neutrophiles 0%; immature cells 90% (405.36×10^{9}/l); lymphocytes 3% (13.512×10^{9}/l); eosinophiles 0%; basophiles 0% and thrombocytopenia [platelets 74×10^{9}/l (117–256 $\times 10^{9}$/l)]. Mitotic figures were seen in some blast cells. The serum chemistry profile revealed hypoalbuminemia [1.7 g/dl (reference range 3.4–4.3 g/dl)] and increased alkaline phosphatase (ALP) [813.54 μkat/l (reference range 0.42–1.33 μkat/l)]. Alkaline phosphatase bone isoenzyme (BALP) was 784.3 μkat/l (96.4% from total ALP). The other findings were increased blood urea nitrogen (BUN) [12.2 mmol/l (reference range 3.5–8.0 mmol/l)], aspartate aminotransferase [10.8 μkat/l (2.5–6.8 μkat/l)], gamma glutamyl transferase [1.04 μkat/l (0–0.53μkat/l)], lactate dehydrogenase [47.98 μkat/l (1.27–6.82 μkat/l)] and bile acids [57.7 μmol/l (0–20 μmol/l)]. Other blood chemistry parameters, including globulins, bilirubin, creatinin, creatin kinase, calcium, magnesium, phosporus, sodium, potassium and chloride were within reference ranges. Electrophoresis of blood serum was performed on a cellulose acetate membrane. Results as shown in Figure 1 confirmed hypoalbuminemia. Moreover, an increase in the α1 fraction was detected (18.6% of total protein; for comparison, in three healthy horses used as controls, the fraction comprised 3.1%, 4.4% and 6.2% of total protein as in Figure 1).

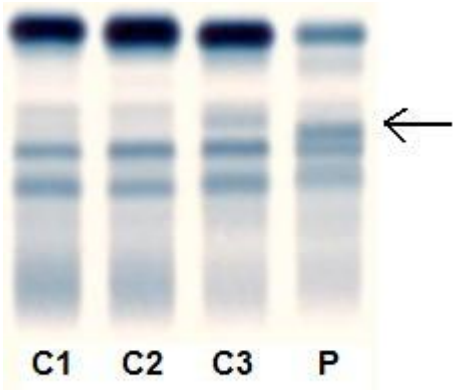

Figure 1. Results of electrophoretic examination of sera from three healthy controls (C1–C3) and the patient (P). Arrow shows elevated α1 fraction in patients' sera

Further examination included endoscopy of the upper airways, x-ray examination of the thorax, abdominal and thoracic ultrasonography. Diagnostic imaging procedures revealed an increased amount of fluid in the thoracic cavity and a slightly increased amount of peritoneal fluid. Thoracocentesis was performed and pleural fluid was examined. Total protein was 9.2 g/l, red blood cells 0.04×10^{12}/l and white blood cells 3.0×10^{9}/l. Multiple small lymphocytes with cytoplasmatic vacuoles and a small number of large lymphocytes with nucleoli were present. Urinalysis excluded proteinuria.

On the basis of the number of precursor cells in the peripheral blood a diagnosis of acute leukemia was made. The blood was examined using flow cytometry and cytochemistry staining. Cytochemistry included reaction to peroxidase, PAS and acid phosphatase reaction in this case. Negative results confirmed the lymphoid origin of the blast cells. For flow cytometry, cells were stained by a whole blood-lysing indirect technique as described previously (Jelinek et al., 2006). The antibodies used were as follows: mouse anti-equine CD4 (HB61A, VMRD, Inc., Pullman, U.S.A.), anti-equine CD8 (73/6.9.1, VMRD), anti-equine IgM (1.9/3.2, VMRD), cross reacting antibody directed against CD44 (BAG40A, VMRD), against CD172a (DH59B, VMRD), against CD14 (Tük4, DakoCytomation, Denmark), against CD79α (HM57, PE-conjugated, DakoCytomation) and FITC-conjugated F(ab') fragment of affinity purified polyclonal rabbit anti-human lactoferrin antibodies (DakoCytomation). Non-conjugated antibodies were visualized using goat anti-mouse

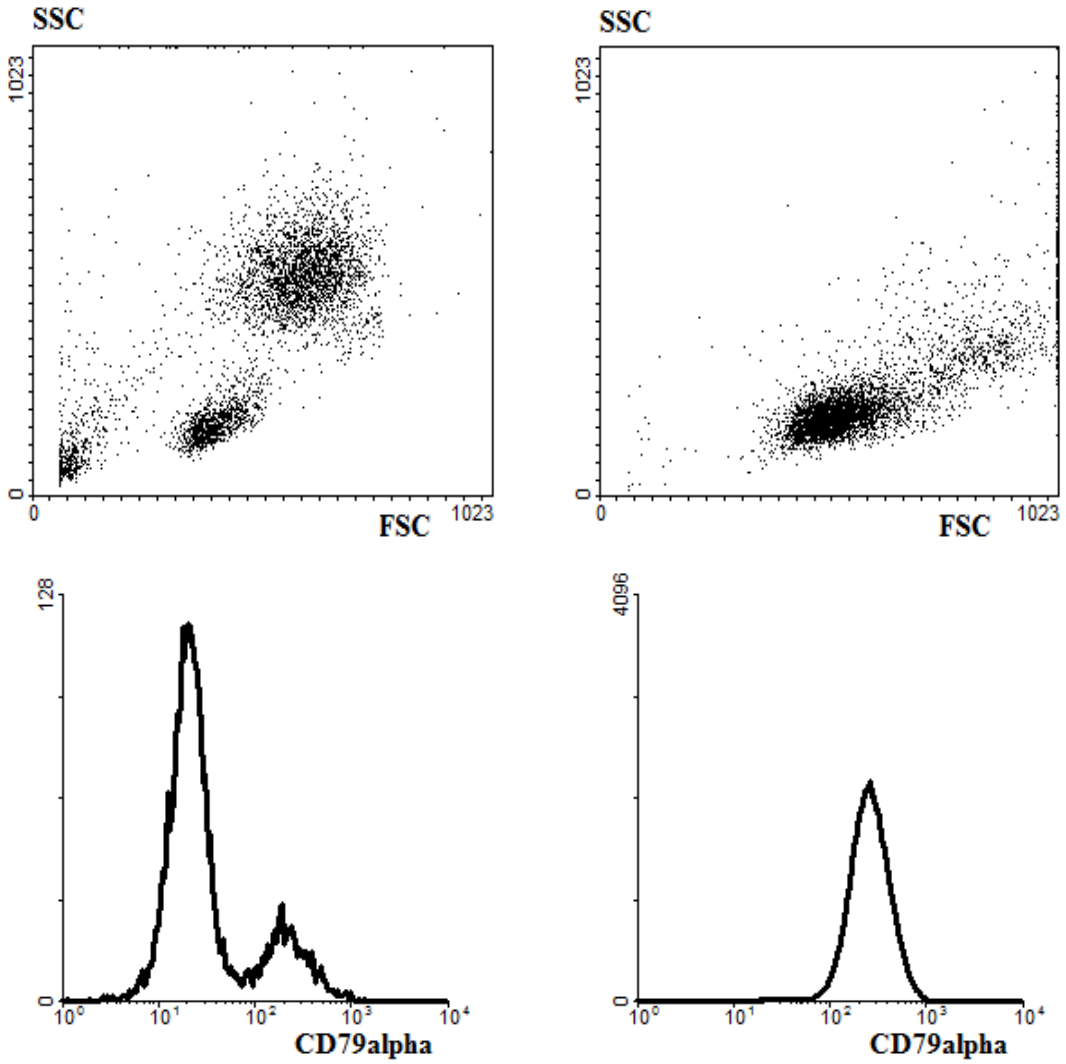

Figure 2. Results of flow cytometry analyses of peripheral blood leukocytes obtained from healthy control (left) and the patient (right). Light scatter properties of the cells are shown in the upper part of the picture. Expression of CD79 alpha – marker of B lymphocytes – are shown in the lower part

subisotype matched fluorochrome-conjugated polyclonal antibodies from Southern Biotechnology Assoc. (U.S.A.). For acquisition of the data, a flow cytometer FACSCalibur (Becton-Dickinson) operated with CellQuest software was used. WinMDI software was used for off-line data analyses. Based on light scatter properties, lymphocytes from the patient were slightly larger than lymphocytes from a control horse (Figure 2). Lymphocytes from the patient were negative for all markers except CD79α (Figure 2). Based on dye cytochemistry and flow cytometry analyses acute B cell lymphoid leukemia was determined as the final diagnosis.

The mare was treated with corticosteroids. An initial dexamethasone (Dexadreson a.u.v. inj., Intervet International B.V., Netherlands) dose (0.1 mg/kg *i.m.*) on the first day was followed by prednisolone (Prednisolon tbl., Dr. Kulich-Pharma, Czech Republic) therapy. On the second day 500 mg of prednisolone (total dose), on the third day 750 mg (total dose) and on the fourth day 1 000 mg (total dose) were applied. Corticosteroid therapy was supported by intravenous and oral rehydration plus electrolyte supplementation. The mare's clinical state was stable, submandibular lymph nodes decreased in size and pleural fluid partly disappeared. By the second day of treatment the white blood cell count had already decreased to 147 × 10^9/l and alkaline phosphatase had decreased to 523.24 μkat/l. The improvement continued until the fifth day, when the white blood cell count was 49.2 × 10^9/l and alkaline phosphatase was 221.62 μkat/l.

On the fifth day of treatment the mare's status deteriorated and the horse was euthanized at the owner's request.

Gross examination was carried out 18 hours after death. It was not possible to evaluate the majority of organs due to advanced postmortem changes (putrescence). Hemorrhagic fluid was found in the abdominal, thoracic and pericardial cavities. None of the examined lymph nodes were enlarged and no neoplastic mass was found. Histological examination of the deep cervical lymph node revealed dimorphic round cell neoplastic infiltrate. Similar changes were present in the liver and spleen. The other organs including the bone marrow were not suitable for examination.

DISCUSSION

Leukemic leukemia which is defined as an increase in white blood cell (WBC) count in the peripheral blood caused by a large number of neoplastic cells was presented in this case (McClure, 2000). Acute leukemia has a blast-like morphology whereas chronic leukemia indicates that the leukemic cells have a mature morphology (McClure, 2000). When there is neoplastic transformation of stem cells or early precursors and a subsequent release of poorly differentiated neoplastic cells into the circulation it is termed acute leukemia, whereas transformation of late precursors and release of mature differentiated cells is termed chronic leukemia. Blast cells exceeded 90% of blood white cells in this horse and acute leukemia was diagnosed.

Regarding their cellular origin leukemias are categorized as lymphoid or myeloid. The differentiation of leukemia into myelogenous or lymphoid can be problematic. Both originate from the same pluripotent stem cell and cell lines affected can vary depending on when in the maturation process the neoplasm occurs. Most reported lymphoid leukemias in horses have been diagnosed as acute lymphoid leukemia (ALL) (Roberts, 1977; Bernard et al., 1988; Kramer et al., 1993; Lester et al., 1993). With the availability of panels of monoclonal antibodies that recognize lymphocyte surface antigens it is possible to phenotype neoplastic lymphoid cells by immunohistochemistry and indirect immunofluorescent flow cytometry on circulating lymphocytes. Due to the large number of blast cells in the peripheral blood in this case, diagnosis could be made using cytochemical staining and flow cytometry. Cytochemical staining of blast cells in the peripheral blood is an accurate way of classifying cells from the peripheral blood of bone marrow aspirate (Rendle et al., 2007). Clear results were obtained from the negative reaction to peroxidase, PAS and acid phosphatase reaction in this case. Peroxidase stains the granules in myeloid cells black while undifferentiated blast cells remained unstained (pink). PAS stains glycogen in cells pink-violet. Blast cells remained unstained blue in our patient. Acid phosphatase (isoenzyme 5) staining was positive in some blast cells (red color, dot – like positivity). This confirmed the lymphoid origin of the blast cells in our patient. Flow cytometry confirmed the B cell origin of the lymphoid leukemia in our patient, which seems to be less frequent than T cell lymphoid leukemia. There exist reports on only 10 horses in total in which lymphoid leukemias with T or B cell origins have been studied. Two of these had LL of B cell origin, whereas in eight horses LL of T cell origin was confirmed (Dascanio et al., 1992; McClure et al., 2001; Rendle et al., 2007). Bone marrow aspirate was performed postmortem but unfortunately was not qualifiable.

Lymphoproliferative diseases in horses include lymphomas, lymphocytic (lymphoid) leukemia, and plasma cell myeloma. Lymphoid leukemia (LL) can be primary or secondary. Primary lymphoid leukemia is defined as neoplastic lymhocytes originating within the bone marrow. Secondary lymphoid leukemia results from a lymphoma that has metastasized to the bone marrow (McClure, 2000). Differentiation between primary lymphoid leukemia and the leukemic phase of a lymphoma can be achieved with the finding of solid tumors in lymphoma cases. Most cases of lymphoid leukemia are secondary to a lymphoma and only a few cases of primary LL have been reported in horses (Roberts, 1977; Ringger et al., 1997; Clark et al., 1999). No solid tumor was found during clinical and gross examination in this case. However, a decrease in blast cells after corticosteroid therapy could also be accompanied with a reduction of any solid mass, which would therefore not be found during *post mortem* analysis. Final differentiation between primary and secondary lymphoid leukemia was not possible in this case.

There are no convincing reports regarding sex or age predisposition to lymphoproliferative diseases in horses. Acute lymphoid leukemias seem to be more common in younger horses, whereas chronic lymphoid leukemia seem to occur more frequently

in older horses. This mare was eight years old. ALL seems to be more common in geldings. Lymphoma has been diagnosed in horses of all ages (McClure, 2000). Clinical signs for leukoproliferative diseases are usually nonspecific. Horses affected with primary lymphocytic leukemia and myeloid leukemia usually have a history of acute onset of clinical signs over a period of a few weeks. Horses with lymphoma are frequently presented with a history of prolonged illness usually over a period of months (Burkhardt et al., 1984; Lester et al., 1993). Peripheral lymphadenopathy can accompany lymphomas as well as primary lymphocytic leukemia cases (McClure, 2000). It is possible that the ventral edema was caused by obstruction of drainage due to the increased numbers of lymphocytes circulating within the lymphatic and blood vessels or vascular damage by neoplastic lymphocytes in this case. Enlarged submandibular lymph nodes were also noted here, reflecting the increase in cell numbers. This case had a history of fever which could be due to secondary infection, pyrogens associated with neoplastic growth or tumor necrosis (Rebhun and Bertone, 1984).

Additional abnormalities in laboratory results were anemia, hypoalbuminemia and extremely increased levels of bone isoenzyme, alkaline phosphatase (BALP). Pancytopenia accompanying bone marrow neoplasias (caused by white cell neoplasia) is due to myelophtisis – a reduction in the bone marrow's normal cell forming function (Lester et al., 1993; Ringger et al., 1997; McClure, 2000). This horse had anemia, slight trompocytopenia and mature white blood cells were almost absent in the peripheral blood. Sporadic anisocytes and Howell-Jolly bodies were found. These changes could be mostly a result of bone marrow neoplasia, but without any bone marrow examination it is not possible to confirm this theory. Hypoalbuminemia has been reported in a few cases of lymphomas and LL (McClure, 2000; Rendle et al., 2007). Hypoalbuminemia in this case was not likely to be due to intestinal or renal disease because there were no clinical signs present which generally accompany disease of these organ systems. A high level of alkaline phosphatase was caused by increased bone isoenzyme (BALP). The most commonly increased fraction of ALP in horses is hepatic. Bone marrow neoplasia could cause increased BALP. ALP was not increased in five horses which suffered with lymphoid leukemia as described by Rendle et al. (2007), but the primary or secondary origin of LL was not assessed. The level of ALP in this case had decreased together with the number of blast cells in the peripheral blood due to therapy with corticosteroids. Intravascular leukostasis, aggregation of leukemic cells in blood vessels, is seen when the WBC concentration is greater than 200×10^9/l or when blast cells are greater than 40% of the WBC (McKee and Collins, 1974). It has been described as the cause of death in a horse with acute myelomonocytic leukemia (Buechner-Maxwell et al., 1994). Both intravital conditions were present in this case, but the presence of intravascular leukostasis was not found *post mortem* in this mare.

The results of electrophoresis confirmed hypoalbuminemia. An increase in α1 fraction was detected. The α1 fraction includes α1-acid glycoprotein (orosomucoid, seromucoid), α-lipoprotein, α1-antitrypsin, α1-chymotrypsin. α1-acid glycoprotein belongs to a family of acute phase proteins that have also been described in horses (Taira et al., 1992). An elevated level of the glycoprotein has been described to be associated with inflammation or cancers (Falleti et al., 1993; Mackiewicz and Mackiewicz, 1995; Moore et al., 1997).

Data regarding leukoproliferative disease treatment in horses is limited. Prognosis is poor and not many owners are interested in investing in what is an almost desperate treatment. Few equine lymphoma treatment protocols have been described, but acute primary leukemias tend to last only a few weeks and these horses die or are euthanised within a couple of weeks of diagnosis. However the report of long term survival in three horses with LL could change this practice (Rendle et al., 2007). The use of radiation therapy, surgical resection, immunotherapy and chemotherapy has been described in leukoproliferative diseases in horses. A chemotherapeutical regimen of cyclophosphamid, vincristine sulfate and prednisolone was used to treat two horses. One pregnant mare survived for several months and successfully foaled (Byrne et al., 1991). Treatment with cytarabin, and cyclohosphamid followed by prednisolone was used with satisfactory success for a mixed cell thoracic lymphoma in a horse (Saulez et al., 2004). A combination of cytarabine, cyclophosphamid, vincristin and doxorubicin was used in a southern black rhinoceros with clinical remission achieved 19 days after the initiation of treatment. However the animal died because of congestive heart failure possibly secondary to treatment toxicity (Radcliffe et al., 2000). A single corticosteroid treatment, dexamethasone, was used for

intestinal T cell lymphoma in three horses, and two showed clinical improvement (Gerber et al., 2002). Others have used only corticosteroids in horses with little success although some horses seem to improve briefly as well as did the mare described here (Bernard et al., 1988; Ringger et al., 1997). Surprising results were obtained by Rendle et al. (2007); in their study three horses with LL were given corticosteroid treatment and survived from 11 months to 5 years. In this case a large decrease in blast cell number was noted, as well as a decrease in ALP activity. A massive decrease in blast cells after corticosteroid treatment was also reported in the Rendle et al. (2007) study. Clinical worsening on the fifth day in our case was most probably due to sepsis. Immunodeficiencies have been described in horses with leukoproliferative disorders. Blood cultivation was not done in this mare. The other possibility is tumor lysis syndrome (TLS), which was described in a horse with peritoneal mesothelioma (LaCarrubba et al., 2006). TLS is a well recognized oncologic emergency in humans characterized by severe metabolic derangements such as acute renal failure, hyperkalemia, hyperphosphatemia, hypokalcemia. The chemotherapeutic doses were lower at the beginning of the treatment of this horse to prevent TLS. The horse deteriorated rapidly but biochemical results on the day of euthanasia were not consistent with TLS occuring in this horse.

A case report of acute B cell lymphoid leukemia is described in this paper. The history, clinical picture and establishment of diagnosis were similar as in previously reported cases. However, we report an unusual biochemical result (BALP) and also rapid treatment response despite the short term survival in this case.

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Corresponding Author:

MVDr. Barbora Bezdekova, PhD., University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Equine Clinic, Palackeho 1–3, 612 42 Brno, Czech Republic
Tel. +420 603 858 524, Fax +420 541 562 395, E-mail: bbezdekova@vfu.cz