Treatment of Chronic Lymphocytic Leukemia in a Ring-Tailed Lemur (*Lemur catta*)

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ABSTRACT

A 19-year-old male ring-tailed lemur (*Lemur catta*) presented with a decreased appetite, lethargy, and splenomegaly. The hemogram revealed a severe lymphocytic leukocytosis and a tentative diagnosis of chronic lymphocytic leukemia was made. Treatment was initiated with oral prednisone and chlorambucil. The lemur improved clinically and the lymphocyte count decreased progressively. The lemur remaining clinically stable for 6 months until its death due to complications presumed unrelated to its primary condition.

Key words: chemotherapy, chlorambucil, glucocorticosteroid, lemur, leukemia, neoplasia.

CASE REPORT

A 19 year old, male ring-tailed lemur (*Lemur catta*) weighing 2.8kg was examined due to lethargy and weakness. The lemur was part of a group of lemurs at the Safari, Zoological Centres, Tel Aviv, Ramat Gan. As clinical signs persisted for 48 hours, it was anesthetized using isoflurane in oxygen (Forane, Abbot, Berkshire, UK) by facemask. A complete physical examination, blood collection, radiographs, and abdominal ultrasound was carried out. Physical examination and abdominal ultrasound revealed splenomegaly. Thoracic radiographs were within normal limits.

The hemogram revealed a severe leukocytosis (white blood cells [WBC] 142.9 x 10^3/μL) (reference interval 8.642±3.751 x10^3/μL (1)) and severe lymphocytosis (94.3 x10^3/μL) (reference interval 8.642±3.751 x10^3/μL(1)), with no evidence of anemia, neutropenia or thrombocytopenia. Blood smear examination showed a severe lymphocytosis, mostly consisting of large atypical lymphocytes, with a relatively low nuclear to cytoplasm (N:C) ratio, light blue cytoplasm, and mildly heterochromatic nuclei without apparent nucleoli (Figure 1). Roughly 2% of the lymphocytes were small, normal looking lymphocytes, with a high N:C ratio. Neutrophils were mature with no cytoplasmic toxicity. Platelet numbers were estimated to be normal. No morphologic changes were observed in the erythrocytes. Serum biochemistry was unremarkable. The findings supported a diagnosis of chronic lymphocytic leukemia, as the cells comprised a monomorphic population of large lymphocytes with high nuclear to cytoplasmic ratio and reticular nuclear chromatin pattern with no apparent nucleoli.

Treatment was initiated using chlorambucil (Leukeran, Excella GmbH, Feucht, Germany, 2 mg (one tablet) PO q24h(0.56mg/kg)) and prednisone (Prednisine, Rekah Pharmaceutical Industries, Holon, Israel, 5 mg PO q24h (1.8 mg/kg). Following six days of treatment, the lemur had improved clinically and spleen size was decreased. Three weeks after initiation of treatment the (hemogram showed a marked decrease in the leukocyte and lymphocyte counts (64.01 x10^3/μL and 59.53 x10^3/μL, respectively) but the blood smear examination revealed similar findings as initially found. Small, normal looking lymphocytes were estimated at 5% of all lymphocytes. Neutrophils were hypersegmented, with no cytoplasmic toxicity, although occasional large gran-

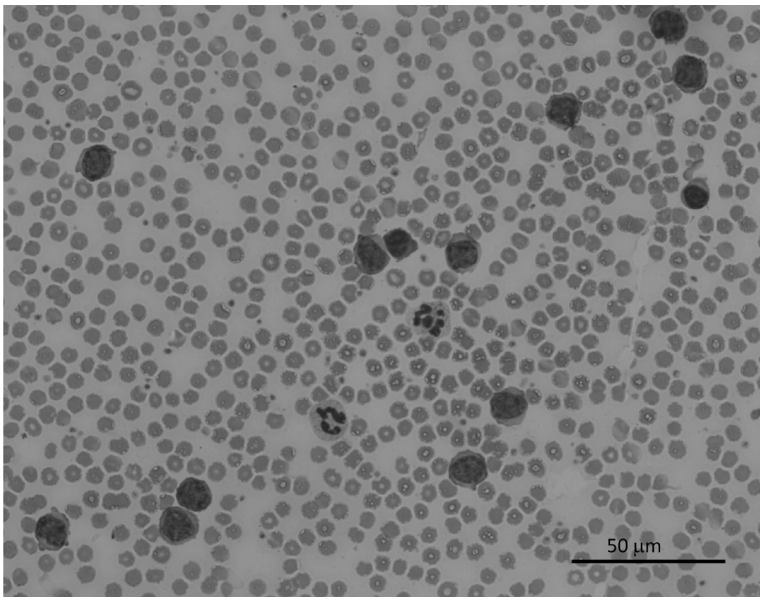

Figure 1: Peripheral blood smear from a ring-tailed lemur with chronic lymphocytic leukemia. Note the many atypical, medium sized lymphocytes, roughly the same size of the neutrophil. The nucleus to cytoplasm ratio of these lymphocytes is lower than normal. Nucleoli cannot be seen (Modified Wright's stain, original magnification X200).

ular lymphocytes, plasmatoid lymphocytes and large platelets were present. All subsequent collections were performed under manual restraint. Over the following week, the lemur was stable, and hemogram performed three weeks after treatment initiation revealed a further decrease in the leukocyte and lymphocyte counts (32.54 x10^3/µL and 31.24 x10^3/µL respectively), however, the neutrophil count was mildly decreased (1.3 x 10^3/µL (reference interval 4.267±2.938 x10^3/µL (1))). Lymphoid cells morphology was similar to that observed in previous smears. Rare polychromasia was noted, and neutrophils were mature with no cytoplasmic toxicity.

Due to the decreased leukocyte and lymphocyte counts, the chlorambucil dose was decreased (2 mg PO q48h, (0.56mg/kg)). Prednisone was planned to be unchanged, however, due to a misunderstanding, it was also reduced (5 mg PO q48h (1.8 mg/kg)). Over the following period, the lemur remained clinically stable. A hemogram performed 6 weeks from treatment initiation demonstrated a further decline in leukocyte and lymphocyte counts (13.44 x 10^3/µL and 10.35 x 10^3/µL, respectively) and an improved neutrophil count (2.82 x 10^3/µL). The morphologic characteristics upon blood smear examination were similar to previous findings. Rare polychromasia and monocytes were observed.

Ten weeks from initiation of treatment the hemogram revealed a stable number of lymphocytes (8.84 x 10^3/µL), however, neutropenia was again was observed (1.49 x 10^3/µL) and as a result the chlorambucil dose was further decreased (2 mg PO (one tablet, 0.56mg/kg), with a cycle of one tablet on day one, a second tablet on day 3 and a third tablet on day 6). Blood smear examination revealed the same morphologic characteristics in the lymphoid cells. Neutrophils were hypersegmented with no cytoplasmic toxicity. Three months from treatment initiation, although the leukocyte (8.90 x 10^3/µl) and lymphocyte (8.01x 10^3/µl) counts were stable, the neutropenia had deteriorated (0.80 x 10^3/µL). Neutrophils were mature with no cytoplasmic toxicity. As the lemur was clinically normal, no changes were made in the treatment and the next hemogram was performed one and a half months later (4.5 months from treatment initiation) when leukocyte (11.09 x 10^3/µL) and lymphocyte (9.43 x 10^3/µL) counts were stable and neutrophils had improved slightly (1.55 x 10^3/µL). Lymphoid cells had similar morphologic characteristics as observed previously. Neutrophil morphology was normal. From initiation of treatment, and during the next 5 months, the lemur was clinically normal, ate and drank normally and no abnormal behavioral abnormalities were detected.

After six months of treatment, the lemur presented with both fore- and hindlimb skin lacerations and lameness which were presumably due to a bite wounds. At this time, no treatment was thought necessary, and the physical examination under manual restraint detected growth of a previously documented cutaneous tail mass and a mobile subcutaneous abdominal mass. Hemograms performed at this time and 2 weeks later (6.5 months from initiation of treatment) showed a decrease in the total leukocyte, lymphocyte and neutrophil counts and a mild anemia. The results of the examination of blood smears were unchanged from previous checkups. Neutrophils were hypersegmented with no cytoplasmic toxicity. In the second blood smear, a single nucleated red blood cell was observed but no polychromasia was detected. Serum chemistry revealed a decrease in albumin concentration (3.2 g/dL, reference interval 5.7±0.9(1)) and increase in globulin concentration (3.4 g/dL, reference interval 1.6±0.9(1)).

On physical examination six and a half months after the

initiation of treatment, a skin laceration was found, and suspected to be a bite wound. Therefore, antibiotic treatment cefixime (Supran, Teva Pharmaceutical Industries, Petah-Tikva, Israel, 5 mg/kg PO q24h) and topical mupirocin (Mupirocin, Teva Pharmaceutical Industries, Petah-Tikva, Israel, once daily) was initiated. The chemistry panel from this blood collection demonstrated an increase in total bilirubin (1.6 mg/dL reference interval 0.6±0.4(1)). Two days later following initiation of treatment, the lemur showed reduced attitude, coughing, sneezing and marked nasal discharge. However, over the following four days, the lemur improved clinically, and its appetite returned to normal, but it was found dead on the fifth day of antibiotic treatment. A necropsy was not performed due to autolysis.

DISCUSSION

Although ring-tailed lemurs (*Lemur catta*) are one of the most common prosimian species in captivity, neoplasia has rarely been reported. A total of 14 cases of neoplasia in ring tailed lemurs have been reported, including single case reports of cholangiocarcinoma, hamartoma, renal tumor, mammary tumor, bone sarcoma, and a T cell rich-B-cell lymphoma(2-7). In a review of cases from the literature of captive prosimian population, the incidence rate of neoplasia was found to be 0.6-3.2% in lemurs (8). In this review, there was only one case of neoplasia in a ring tailed lemur reported (hepatic malignant fibrous histiocytoma) (8). While neoplasia was reported in all organ systems, the hematopoietic system was involved in 6-23% of the cases (captive versus wild populations) (8). These hematopoietic neoplasms included both lymphoma and lymphoma with leukemia (8).

Chronic lymphocytic leukemia (CLL) is characterized by an abnormal population of small, well differentiated lymphocytes, which mostly originate in the bone marrow, but may originate in the spleen (7). The neoplastic cells might or might not be circulating in the peripheral blood (7). Humans diagnosed with CLL generally are asymptomatic at presentation, and the diagnosis is often made when lymphocytosis is detected in a routine hemogram although clinical signs might include fatigue, anorexia, and weight loss (9). The physical examination might reveal mild lymphadenomegaly and splenomegaly (9). Due to the indolent nature of CLL, the question of whether treatment should be initiated is controversial (9). The indications to treat are based on the severity of clinical signs (weight loss, fatigue and fever), bone marrow failure (anemia, immune mediated hemolytic anemia, thrombocytopenia and/or immune mediated hemolytic thrombocytopenia), massive splenomegaly and lymhadenomegaly and progressive lymphocytosis (an increase in the absolute lymphocyte count of over 50% over a 2 month period or a predicted doubling time of less than six months (9). First-line treatment options in humans include single agent chemotherapy with alkylating agents, such as chlorambucil, or nucleoside analogues, such as fludarabine; other therapeutic measures include splenectomy and radiation (9).

Although the circulating lymphoid tumor cells were not the typical small lymphocytes, but rather intermediate, atypical cells, the diagnosis of CLL in the present case was based on the lack of lymphadenopathy or other masses at presentation; no peripheral cytopenias; and response to chemotherapy. Although cases of lymphoma and CLL have been previously reported in lemurs, this case was the first treatment documented for this taxon.

Treatment was initiated with chlorambucil and prednisone, based on past experience in humans as well as in other animal species, which within 10 days, led to a remarkable clinical improvement, evident upon physical examination, as well as by improvement in the hematologic picture as evident in subsequent hemograms. This treatment was continued for 6 months, with several dose adjustments. The lemur was monitored throughout that period, clinically and by hemograms and serum biochemistry. Chlorambucil-associated toxicity for humans is mostly due to myelosuppression, manifested by anemia, leukopenia and thrombocytopenia (10). No clinically-noticeable adverse effects were noted in this lemur. However, several chlorambucil dose reductions during this period were made due to identified neutropenia (1).

After six months, clinical deterioration was observed, manifested by lethargy and anorexia, coughing, sneezing and nasal discharge. However, it seemed likely that the general deterioration of the lemur was due a bacterial infection, resulting from the suspected bite wounds, and not due to CLL, because the lymphocyte count at that point was normal and stable. Evidence of a systemic inflammatory process, manifested by the increased globulin and decreased albumin concentration, was noted. If indeed the skin lacerations were infected due to bite wounds, possibly, chlorambucil and prednisone might have predisposed the lemur to be susceptible to spread of a local infection. Chlorambucil might have in-

duced neutropenia while prednisone might have decreased the immune response, both promoting sepsis and death. Unfortunately, although additional diagnostics were planned, the lemur died before these were completed.

This report has several limitations. Immunohistochemistry and full disease staging (e.g., aspirates of lymphoid tissues, spleen, liver and bone marrow) were not performed. A necropsy, that could have shed some light on the cause of death and the state of the neoplasia at the time of death, was not performed. Despite these limitations, the present case presented successful long term treatment of lymphoid neoplasia in a lemur. Treatment was well tolerated, with no apparent adverse effects, with exception of borderline neutropenia, and its administration by the zoo personnel was accomplished without difficulty. Most importantly, based on the zookeepers' assessments of the lemur, the treatment improved the lemur's quality of life.

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