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**Abstract:** A 2-year-old male African penguin (*Spheniscus demersus*) was presented to a veterinary teaching hospital for evaluation of a previously diagnosed subclinical, marked regenerative anemia. Physical examination at the zoological institution demonstrated biliverdinuria and pale oral mucous membranes. Diagnostic tests performed on the penguin since the diagnosis and prior to presentation to the veterinary teaching hospital included serial complete blood counts, plasma biochemistry panels, radiographic imaging, blood and plasma heavy metal testing, and infectious disease testing. The abnormal diagnostic test results were consistent with marked regenerative anemia and splenomegaly. At the veterinary teaching hospital, further diagnostic testing was ordered in an attempt to determine the cause of the biliverdinuria and pale oral mucous membranes. The diagnostic tests performed included a full-body contrast computed tomographic scan, upper gastrointestinal endoscopic procedure, bone marrow aspiration and evaluation, saline agglutination testing, blood *Plasmodium* species polymerase chain reaction screening, a vitamin profile panel, and repeat blood heavy metal testing. The complete blood count demonstrated a marked, regenerative anemia with the presence of dysplastic erythrocytes, and splenomegaly was found on the computed tomographic images without identifying a definitive cause. Primary disease differentials for the diagnosed regenerative anemia included a myelodysplastic syndrome and primary or secondary immune-mediated hemolytic anemia. The penguin was treated with oral prednisolone as an immunomodulatory agent; however, it did not result in a positive treatment response. The patient developed hyporexia, weight loss, and lethargy 2 months post presentation to the veterinary teaching hospital. Additional therapy with cyclophosphamide was initiated, and the penguin improved clinically, but then declined. The patient was euthanized due to a poor quality of life and prognosis 4 months after initial presentation and 1.5 years after the first complete blood count revealed the penguin to be anemic. Microscopic review of submitted postmortem tissue samples demonstrated a monomorphic population of neoplastic small lymphocytes infiltrating the spleen, consistent with splenic small cell lymphoma. The neoplastic cells did not label with the T-cell marker CD3 or B-cell markers CD20, CD79a, and Pax-5.