T is for Tex

by

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Introduction

Lymphoproliferative disorder encompasses a vast spectrum of lymphoid neoplastic conditions that vary in presentation and progression. Lymphoma is one of the most common neoplasms in dogs and is typically high-grade.[1-3,6] In dogs, T-cell lymphoproliferative disorders tend to be more aggressive with shorter remission and survival times compared to B-cell neoplasms.[1-6,9,10] Roughly 20-40% of canine lymphoma is T-cell in origin and there is great variability in presentation and progression of disease.[1-6,9] It primarily affects dogs 6-12 years of age with the median being roughly 7 years of age, though any age can be affected.[1-4,6,10,11] Golden Retrievers, Labrador Retrievers, Mixed Breed Dogs and Boxers are overrepresented, with Boxers exhibiting a more aggressive disease.[1,2,4,10,11] Affected dogs may be subclinical, or can present for any number of clinical signs, which are often non-specific.[4,6,10] There are a number of treatment options published, and current literature suggests that T-cell lymphoma (TCL) may not be as responsive to CHOP protocols as B-cell lyphoma.[2,3] Variable median survival times (MST) for TCL has been reported, ranging from 136-507 days, with a number of factors impacting prognosis.[1-5,9,10] It is suggested that prognosis may be affected by specific immunophenotypes, which has become a topic of research over the past several years, and much work is still needed.[1,4]

Definitive diagnosis is typically made histologically. Cytologically, lymphoma can be challenging to differentiate from reactive hyperplasia, particularly if the sample is from lymphoid tissue as it commonly is. Immunohistochemistry may be used to detect CD3 and CD20 receptors, which are specific to T and B cells, respectively. However, it is not reliable for further immunophenotyping.[11] Flow cytometry can be useful in differentiating reactive hyperplasia from neoplasia, as with the latter will exhibit a monomorphic population rather than a mixed

population.[5] Flow cytometry has become the preferred test for immunophenotyping, as there are more encompassing marker panels available compared to immunohistochemistry.[1,5,6,10] PCR for antigen receptor rearrangement (PARR) can also be utilized but may be less sensitive than flow cytometry.[1,5,10,12] There are various immunophenotypes and involvement of any organ is possible. Possible negative prognostic indicators include low or absent MHC II expression, high mitotic index, presence of clinical signs, presence of concurrent leukemia, and cytopenias.[1,4,5,6,10] Presence of hypercalcemia and clinical stage have not been shown to have prognostic value.[1,5,6,10] Sex, age, and weight have not been shown to impact outcomes.[1-3] There is conflicting evidence whether absent expression of CD3, a T-cell marker, or neoplastic cell size are associated with prognostic value, with some studies suggesting loss of CD3 and larger cell size may be associated with more aggressive disease.[2-5,10,11] Small sample sizes and retrospective studies are current weaknesses in the literature overall.

Signalment and History

Tex is an approximately 5-year-old neutered male Australian Shepard who presented to MSU-CVM Emergency Service on April 24, 2021 for polyuria, polydipsia, and hypercalcemia. Tex's owner, a veterinarian, noticed behavioral changes and increased hiding over a few weeks. He then became less playful, reluctant to eat without someone watching, and had urinated inside, which is unusual for him. He also had a history of mild lameness. The first noted abnormal behavior on 4/16/21 and Tex was seen as his owner's clinic for further assessment. Physical exam revealed a tense abdomen and pain in his thoracic spine. Bloodwork at this time revealed an increased calcium, and leukopenia, and normal cPL. At that time, Tex had a USG of 1.018. Sedated cervical and thoracic radiographs were also taken at the time with no remarkable

findings. Tex was started on ampicillin and gabapentin, and blood was submitted to Michigan State for an ionized calcium, PTH, and PTHrp. Results of these tests reveal a persistent hypercalcemia, in addition to an elevated parathyroid hormone. On April 22nd, bloodwork was performed revealing a neutropenia, lymphopenia, eosinopenia and thrombocytopenia. ACTH stimulation was unremarkable. Repeat labwork revealed an elevated total calcium, normal cortisol, and unremarkable urinalysis. Bloodwork on April 23rd indicated hypernatremia and hyperchloremia. Rectal exam was unremarkable. On April 23rd Tex was given furosemide and intravenous fluids at twice maintenance. Several hours later, his calcium was 14.5, which was increased despite fluid therapy. His fluids were subsequently discontinued, and Tex was referred to MSU-CVM for further diagnostics and treatment. Tex had no previous medical concerns reported.

Diagnostic Examination

Prior to presentation, Tex had thoracic and cervical radiographs performed, which were unremarkable, bloodwork revealed hypercalcemia, and a PTH panel sent to Michigan State Diagnostic Laboratory. The report dated April 21st, 2021 showed elevated parathyroid hormone with no detectable PTHrp. On presentation, April 24th, 2021, physical exam was unremarkable. A venous blood gas confirmed persistent hypercalcemia (iCa 1.61 mmol/L). He was started on fluid diuresis in attempt to bring his calcium back into the normal range. On April 25th, a venous blood gas was repeated and the hypercalcemia was still present (1.62 mmol/L). An abdominal ultrasound was performed, revealing gallbladder sludge and left nephrolithiasis, but was otherwise non-diagnostic. Fine needle aspirates were obtained from the liver and spleen, cytology of which was unremarkable aside from mild splenic extramedullary hematopoiesis.

Ultrasound of the cervical soft tissues revealed and irregularly shaped, smoothly marginated nodule on the caudal aspect of the right thyroid gland as well as thickening of the right thyroid gland (5.6 mm) compared to the left (3 mm). The right parathyroid gland was normal in appearance but measured slightly larger than the left (1.7 mm on the right, 1.1 mm on the left). On April 26th, a CBC revealed a mild anemia (RBC 5.49 $x10^6$/ul), thrombocytopenia (96 $x10^3$/ul), mild leukopenia (4.22 $x10^3$/ul) characterized by lymphopenia (759.6 /ul) and monocytopenia (84.4 /ul), mild hypoproteinemia (5.8 g/dl), and, incidentally, Pelger-Heut anomaly. Serum chemistry revealed mild hypokalemia (3.35 mmol/L), mildly elevated creatinine (1.83 mg/dl), moderate hypercalcemia (13.0 mg/dl), and mild hypomagnesemia (1.4 mg/dl). His ionized calcium was rechecked on which continued to be elevated at 1.85 mmol/L despite fluid diuresis. On April 27th, a Renal Profile was performed, revealing persistently elevated creatinine (1.65 mg/dl) and calcium (13.6 mg/dl). On April 27th, Tex had a right parathyroidectomy performed and the tissues were submitted for histopathology, which was reported to contain normal thyroid tissue, a small lymph node, and two atrophied parathyroid glands with no evidence of neoplasia. On April 28th, it was discovered that Tex's malignancy profile had been erroneously mixed up with that of a horse, and that Tex's submitted sample was normal, and the working diagnosis of primary hyperparathyroidism was nullified.

Having ruled out primary hyperparathyroidism, additional diagnostics were required to further investigate. A venous blood gas revealed persistence of hypercalcemia (iCa 1.81 mmol/L) despite fluid therapy and parathyroidectomy. On April 29th, a CBC and serum chemistry were repeated, revealing mild anemia (Hct 31.5%), thrombocytopenia (112 $x10^3$/ul), mild hypoproteinemia (5.5 g/dl), elevated creatinine (1.83 mg/dl), mild hypoalbuminemia (2.3 g/dl), increasingly elevated calcium (14.3 mg/dl), and mild hypomagnesemia (1.2 mg/dl). A venous

blood gas confirmed an elevated ionized calcium (1.76 mmol/L). On April 29th, a Histoplasmosis antigen test and *Heterobilharzia americana* PCR were sent out to MiraVista and Texas A&M, respectively, both of which came back below detectable limits. A bone marrow biopsy was performed, which reported a population of small to medium sized cells of which 60-70% stained positive for CD3, a T-cell marker. About 50% of these cells also stained positive for CD20, a B-cell marker. These results were most consistent with a T-cell lymphoid neoplasm with dual expression. His prescapular lymph nodes, which were palpable but not overtly enlarged, were aspirated on 5/4/21. Cytology revealed increased numbers of intermediate to large lymphocytes and lymphoblasts with variably distinct nucleoli. Flow cytometry was sent out, reporting high expression of CD4, CD3, and CD5 in the T-cell population. A renal profile revealed azotemia (32 mg/dl, creatinine 2.82 mg/dl) and mild hyperphosphatemia (5.3 mg/dl).

Tex was ultimately diagnosed with T-cell lymphoproliferative disorder on April 30, 2021 based on bone marrow results, persistent hypercalcemia, and clinical signs. It is possible his malignancy originated in the bone marrow, which blurs the line between lymphoma and leukemia. Though cytologically a blastic population was described, CD34 marker was negative, suggesting the malignancy did not involve a progenitor population. Further, lymphoblastic lymphoma was not entirely ruled out due to lack of testing for TCRαβ. As such, his official diagnosis was listed as T-cell lymphoproliferative disorder. However, stage Vb multicentric T-cell lymphoma is most likely in terms of prevalence, histological appearance, and expression of differentiated T-cell markers. Unfortunately, Tex's azotemia never resolved, suggesting renal disease secondary to chronic hypercalcemia. His intermittent left forelimb lameness, historic right hindlimb lameness and historic thoracic spinal pain were attributed to skeletal calcium leeching secondary to chronically elevated PTHrp. For treatment planning purposes, an MDR1

panel was sent out to Washington State University VPCL, as Australian Shepherds are known to be predisposed and MDR1 mutations are known to increase susceptibility for various drug toxicoses. Tex was classified as heterozygous MDR1.

Pathophysiology

Lymphoproliferative disorders encompass a spectrum of malignant lymphoid neoplasms, largely separated into lymphoma and leukemias. The exact etiology is not fully understood, but there are likely genetic, environmental, and infectious factors that contribute. Neoplasia is induced by initiation, which is alteration of DNA by exposure to a carcinogen, and promotion, where the cells switch to excessive proliferation.[8] These two processes may or may not occur simultaneously.[8] Mutations in a specific proto-oncogene, ROS1, have been described in three Boxers with TCL.[11] Increased activation of phosphatidylinositol 3-kinase and related kinases, which are signaling molecules involved in pathways regulating cell cycle and survival, has been demonstrated in neoplastic CD4+ T lymphocytes.[11] Concurrent inhibition of a tumor suppression gene, PTEN, was demonstrated in this same population.[11] These regulatory changes are thought to be primary mechanisms in CD4+ TCL, though the exact order and cause-and-effect relationships remain unclear.[11] Additionally, neoplastic CD4+ T lymphocytes have been shown to have absent or decreased expression of CD30, a target protein for tumor necrosis factor receptors, facilitating escape from host immunity.[11] Various other changes in gene expression have been identified for CD4+ TCL, altering regulation of signaling pathways and expression of membrane proteins ultimately leading to increased proliferation and decreased destruction.[11]

Various anatomic classifications, types of T-cells, and immunophenotypes exist, each of which affects clinical manifestation, progression of disease, and response to treatment.[1,5,6,9] Multicentric TCL is most commonly seen in dogs, characterized by generalized lymphadenopathy, and can progress to involvement of the liver, spleen, and/or other organs.[1,6] The most common T-cells found in lymph nodes are T-helper cells, which are normally positive for CD4, CD3, CD5, and MHC II expression.[4,5,11] Thus, for multicentric lymphoma, the most common immunophenotype is CD3+, CD4+, CD45+ with low or absent MHC II expression.[1,5,6,10] Loss of MHC II expression has been associated with more aggressive disease as it plays a significant role in decreased detection and destruction by host immunity.[1,2,6,10,11] Less common in lymphoid tissues, T-cytotoxic cells are characterized by normal expression of CD8, CD3, CD5, and MHC II.[5] Mediastinal lymphoma originates from thymic lymphocytes that typically exhibit dual expression of CD4 and CD8 and is associated with a poor prognosis.[1,5,6] Increased numbers of small cell CD4+/CD8+ T lymphocytes exceeding 10% of the overall population is diagnostic of mediastinal TCL.[5] Cells exhibiting CD11d or TCRγδ are uncommon, but typically originate from the spleen or alimentary tract, with associated neoplasms being more aggressive than multicentric TCL.[1,4-6] In contrast, an indolent T-zone lymphoma (TZL) has been described which is clinically mild with relatively long survival times.[5,10] TZL originates from lymph nodes and is often CD4+/CD45-.[5,10] Neoplastic cells can alter surface antigen expression not only at the induction of malignancy, but throughout any point in the disease process.[1] Absence of CD3, CD5, CD45, or MHC II expression, absence or dual expression of CD4 and CD8, or expression of B-cell targets are the most common changes seen with neoplastic transformation and can be supportive of a diagnosis.[5] Due to the numerous possibilities, defining specific immunophenotypes and cutoff values for TCL is challenging.[5] There are abundant other

anatomic, morphologic, and phenotypic subtypes of T-cell lymphoma described that are beyond the scope of this paper. It should be noted that many of these different diseases can overlap, while they may originate from one specific place they will often progress to involvement of many of the same tissues, though the cell of origin may have unique neoplastic patterns that could affect aggressiveness and response to treatment.

Lymphoma, or lymphosarcoma, most commonly originates from lymph nodes, but can arise from liver, spleen, or other tissues.[9] Lymphoid neoplasm can occasionally be localized, but almost always becomes systemic. It can progress to involvement of the bone marrow, referred to as leukemic transformation. There is an established WHO staging system I-V, with substage "a" assigned to patients who lack clinical signs, and substage "b" classifying patients who are clinical for their disease. Most dogs are stage III or higher at the time of diagnosis.[4,6]

Affected dogs commonly present with generalized lymphadenopathy. They may present for nonspecific signs such as weight loss, lethargy, hyporexia, coughing, regurgitation, vomiting, diarrhea, etc. [1,4,6,9] Signs may be a direct result of the location or infiltration of lesions including neurological signs, hepatosplenomegaly, lymphedema, cutaneous lesions, ocular lesions, pallor, petechiae and/or ecchymoses, and many others.[1,4,6] There are numerous paraneoplastic syndromes. The most common bloodwork findings in dogs with T-cell lymphoproliferative disorders include hypercalcemia, which is uncommon in dogs with B-cell lymphoma, and thrombocytopenia.[6,9] Overt lymphadenopathy is absent in up to 40% of patients with hypercalcemia.[6,9] Other common paraneoplastic syndromes include cytopenias, gammopathies, hypoglycemia, and polyneuropathies.[1,6,9] Polyuria and polydipsia may be seen with hypercalcemia. Anemia, thrombocytopenia, and neutropenia can be seen with infiltration of the bone marrow or immune-mediated destruction.[1,6]

Lymphoid leukemia is a rare malignancy of immature lymphoid precursors in the bone marrow, with T-cell being more common than B-cell in origin.[7,9,11] This is separated into acute lymphoid leukemia (ALL) and chronic lymphoid leukemia (CLL).[7,9,11] Dogs with CLL are older (>10 years), are often subclinical, and have slower disease progression with survival times of 2-3 years.[7,9] Diagnosis of CLL is often incidental.[9] CLLs can undergo blastic transformation, in which they suddenly become more aggressive and respond poorly to treatment.[7] Dogs with ALL are middle aged to older, present with lethargy, anorexia, PU/PD, shifting leg lameness, and neurological signs.[7,9] While dogs with lymphoma can often be asymptomatic, dogs with leukemia more consistently demonstrate clinical signs of disease and cytopenias, though lymphadenopathy is generally mild and hypercalcemia is not typically present.[7,9,11] Increased blasts in circulation is diagnostic.[7,9] If neoplastic cells are not found in peripheral circulation, termed aleukemic or subleukemic, diagnosis can be made based off bone marrow biopsy or flow cytometry.[7,9] ALL resembles CD4+ multicentric TCL in terms of an aggressive disease course and poor prognosis.[5,9,10] ALL can sometimes be differentiated from TCL based on histopathological differences, as ALLs can be poorly differentiated and may exhibit different staining characteristics, but can also look very similar.[7,10,11] They tend to be positive for CD45, and may lack expression of CD3, CD4, and/or CD5.[7] Lymphoblastic lymphoma is a rare, aggressive malignancy arising from precursor cells in the thymus or lymph nodes rather than the bone marrow as with ALL, and typically expresses TCRαβ.[1,5,9-11] Positive expression of CD34, a stem cell marker, by flow cytometry is diagnostic of precursor origin.[11] Leukemias and lymphoblastic lymphoma generally do not respond favorably to treatment and develop resistance very rapidly.[7,9,11]

Treatment

Surgery and radiotherapy may be beneficial in select situations, but are generally not practical.[6,9] Combination chemotherapy is therefore the treatment of choice, with a number of protocols described.[1-4,6,9] It should be intuitive that chemotherapy is associated with longer survival times than no treatment, and combination therapy has been shown to be more effective than single-agent therapy in terms of PFI and MST.[1] CHOP protocols tend to be a common first choice for canine lymphoma in general, though it seems to be more successful in B-cell lymphoma than T-cell.[2,3] Lomustine has become a more favorable choice for canine TCL due to concerns for chemotherapeutic resistance.[2,3] Increased expression of p-glycoprotein, both innate and acquired, has become a known factor in chemotherapeutic resistance for TCL, conveying resistance to antimicrotubular drugs, anthracyclines such as doxorubicin, and prednisolone.[2,4,9,13] Alkylating agents such as lomustine and procarbazine are not common substrates for efflux, and may yield better results compared to other agents.[4,9] Additionally, neoplastic T-lymphocytes contain low numbers of AGT and MGMT proteins that play a key role in DNA repair, supporting the use of alkylating agents that result in DNA damage.[3] Another well-described factor leading to chemotherapeutic resistance is the administration of glucocorticoids prior to induction protocols.[4,6,9] A study comparing lomustine-based protocols with other protocols reported an overall median survival time of 136 days and response rate of 80%, while lomustine-based protocols achieved a median survival time of and a response rate of 86%, although this was not statistically significant.[4] This same study also suggested that higher response rates were achieved with inclusion of procarbazine in the protocol.[4] One study reported a progression-free interval of 146 days and a median survival of 179 days in a population of dogs with TCL that were primarily treated with CHOP, while another study investigating a LOPP protocol in 35

dogs reported a PFI of 431 days and a MST of 507 days.[1,2] A separate study of 31 dogs reported a 97% response rate to their LOPP protocol with a disease-free interval of 176 days and a survival time of 323 days following initiation of treatment.[3] The difference in reported survival times among these two groups receiving LOPP protocols could be attributed to small sample sizes, differences in patient demographics, or could be due to differences with protocols, as the study with the longer survival time utilized doses closer to maximum tolerance. It may be worth noting that the study that reported longer survival times also reported a higher incidence of adverse effects at 86%, most of which were grade 1 or 2 out of 5, compared to 42%.[2,3] Common side effects include nausea, vomiting, diarrhea, neutropenia, and myelosuppression.[2,3] Chronic renal toxicity and hepatotoxicity have been reported effects of lomustine.[2,3]

In this case a modified LOPP protocol was chosen to include lomustine, vincristine, procarbazine, and prednisone, in combination with L-asparaginase. Lomustine is an alkylating agent that adds alkyl groups to DNA molecules by covalent bonding, resulting in reactive intermediates and double-strand breaks.[3,14] Procarbazine is another alkylating agent that acts by adding methyl groups to nucleic acids.[3,14] Vincristine is a vinca alkaloid that acts by inhibiting tubulin synthesis.[14] Prednisone is converted to active prednisolone, a glucocorticoid that binds to various cell receptors and decreases DNA replication.[14] L-asparaginase depletes asparagine, an essential amino acid required by various rapidly-dividing cells.[14] This protocol entailed one dose of L-asparaginase (400 IU/kg SC) on May 5th, 2021, and daily prednisone (1 mg/kg PO) was started while awaiting the MDR1 results, as decreased expression of functional p-glycoprotein has been associated with increased adverse effects of certain chemotherapeutic agents.[13] On May 17th, Tex received lomustine (55 mg/m^2 PO) and two days later started procarbazine (48 mg/m^2 PO) for 12 days while continuing to wait for MDR1 status. Once Tex was found to be

heterozygous, vincristine was included in his plan at the lower end of the normal range. The planned protocol included six four-week cycles starting June 1, 2021. Each cycle consisted of vincristine (0.5 mg/m2 IV) on Days 1 and 14, lomustine (55 mg/m^2 PO) on day 15, followed by procarbazine (48 mg/m^2 PO) started on Day 16 for twelve days. Denamarin was recommended daily and a CBC was recommended every 7 days.

Tex entered remission during his first cycle of chemo, evidenced by resolution of historic hypercalcemia, decreased size of peripheral lymph nodes, return to normal behavior, increased energy, and improved body condition. Tex was noted to have melena May 21st and was started on metronidazole, omeprazole, and sucralfate due to suspicion of a gastric ulcer secondary to prednisone administration. The prednisone was subsequently tapered and not restarted. During each cycle, particularly after the combination of vincristine and lomustine, Tex became increasingly neutropenic, receiving prophylactic Clavamox each time. After each dose of vincristine, Tex developed gastrointestinal signs to include nausea, inappetence, and diarrhea, that increased in severity with each cycle. Prior to starting his fifth cycle, his dose of lomustine was decreased (to 40 mg/m^2) in hopes of facilitating better tolerance. Lowering the dose of vincristine was discussed with the owner, who elected to maintain the current dose with administration of metaphylactic ondansetron and maropitant. On September 14th, a week after his fifth dose of vincristine, Tex had to be hospitalized for supportive care due to gastrotoxicity, lymphopenia, and profound, febrile neutropenia. He received intravenous Plasmalyte (72 ml/hr), enrofloxacin (10 mg/kg IV q24h), ampicillin sulbactam (30 mg/kg IV q8h), maropitant citrate (1 mg/kg IV q24h), pantoprazole (1 mg/kg IV q12h), and ondansetron (0.5 mg/kg IV q12h). He improved after about 36 hours of hospitalization and was subsequently discharged. The decision

was made to stop his chemotherapy after his fifth cycle, rather than completing the planned six cycles.

On September 30, 2021, Tex developed neurological signs with atypical seizure episodes, presumably a result of metastasis to the central nervous system and was started on levetiracetam XR. This marked the end of his progression-free interval, which lasted roughly twenty weeks. He was euthanized November 8, 2021, 192 days after established diagnosis, due to progression of disease.

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