

Acute Myeloid Leukemia in an Irish Setter

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Abstract

An eight year old male castrated Irish Setter presented to the Emergency/Critical Care Service at Cornell University's Hospital for Animals in April 2007 after an acute onset of emesis and melena that was preceded by a two week history of lethargy, anorexia, and weight loss. Significant physical exam findings included tachycardia with 'snappy' pulses, pale and tacky mucous membranes, and popliteal lymph node asymmetry. Baseline bloodwork revealed moderate anemia, leukocytosis, neutropenia with a regenerative left shift, lymphocytosis, thrombocytopenia, hypoalbuminemia, and elevated liver enzyme activity. Blood cytology identified two distinct populations of circulating immature blast cells, suggestive of either lymphoma or acute leukemia. Bone marrow cytology and flow cytometry were performed after initial stabilization of the patient and led to a diagnosis of acute myeloid leukemia. This case presentation serves as a model to discuss a clinically relevant approach to diagnosing acute myeloid leukemia.

Signalment and Case History

Hunter Graziano, an eight year old male castrated Irish Setter, initially presented to his referring veterinarian for evaluation of a two week history of lethargy, anorexia, and weight loss. A complete blood count performed at that time revealed anemia [27.5% (36-60%)], lymphocytosis [35,956/ul (690-4500/ul)], monocytosis [2,828/ul (0-840/ul)], and neutropenia [1616/ul (2060-10,600/ul)]. Hunter was treated with doxycycline and cyproheptadine, to which he was only minimally responsive. His clinical signs progressed to include emesis and melena, and on April 13, 2007, he presented to the Emergency Service at Cornell University's Hospital for Animals.

Clinical Findings and Initial Diagnostic Examination

On presentation to Cornell, Hunter was quiet, alert, and responsive. He was tachycardic with strong, 'snappy' pulses and pale, tacky mucous membranes. His popliteal lymph nodes were asymmetric and he had multiple, non-fixed, soft subcutaneous masses along his left and right lateral thorax. The remainder of his physical examination was unremarkable.

Initial diagnostics prior to initiation of therapy included baseline bloodwork. A complete blood count (CBC) revealed macrocytic, normochromic, nonregenerative anemia [26% (42-57%)], neutropenia [2.4 thou/uL (3.4-9.7 thou/uL)], thrombocytopenia [72 thou/uL (179-483 thou/uL)], lymphocytosis [6.3 thou/uL (1.2-4.7 thou/uL)], monocytosis [12.4 thou/uL (0.1-1 thou/uL)], and increased levels of circulating blasts. His chemistry panel demonstrated hypoalbuminemia [2.8g/dL (3.1-4.1 g/dL)] and non-specific elevations in liver and cholestatic enzyme activities, [ALT 276 U/L, (25-106 U/L); AST 85 U/L, (16-50 U/L); ALK PHOS 2447 U/L (12-122 U/L); GGT 40 U/L (GGT: 0-10 U/L)].

Cytologic evaluation of Hunter's blood revealed two distinct populations of circulating blasts. One population consisted of large round cells with round nuclei, finely stippled chromatin, and a scant amount of dark blue cytoplasm. The second population consisted of large round cells with slightly indented nuclei and light blue cytoplasm with faint pink granulation (Figure 1).

Problem List

From these initial diagnostic evaluations, Hunter's problem list included anemia, thrombocytopenia, leukocytosis, lymphocytosis, monocytosis, neutropenia with a regenerative left shift, elevations in circulating blasts, hypoalbuminemia, non-specific elevations in liver and cholestatic enzyme activities, and focal lymphadenopathy.

Differential Diagnoses

For each abnormality that was identified on Hunter's baseline bloodwork, there were several differential diagnoses to consider. Anemia can result from blood loss, hemolysis, or decreased production of erythrocytes. Hunter had a history of melena, however the amount of blood lost in his stool was not quantified at the time of initial presentation, nor did blood loss in his feces persist during his stay at Cornell. Therefore, melena was unlikely to be the sole cause for Hunter's anemia. Similarly, no spherocytes or microagglutination were reported on the CBC, which argued against hemolysis as the source of anemia. Finally, the lack of a regenerative reticulocyte response to anemia supported aberrant or reduced erythrocyte production.

Differential diagnoses for thrombocytopenia include increased destruction, increased utilization, decreased production, or sequestration of platelets. Hunter's left shift was non-specific for infection or inflammation, and his hypoalbuminemia could be explained by either decreased production secondary to hepatic dysfunction, loss (as from a protein-losing nephropathy or enteropathy), or decreased intake of protein.

There are two main differential diagnoses for elevations in circulating blasts in the peripheral bloodstream: lymphoma and leukemia. The former is characterized by a proliferation of neoplastic lymphoid cells arising outside of the bone marrow, whereas the latter is neoplastic proliferation of cells arising within the bone marrow itself.

Initial Stabilization

Hunter was moderately dehydrated on initial presentation. This was managed with intravenous fluid therapy. However, following diuresis his packed cell volume decreased from 26% to 16%. Hunter was blood-typed as DEA 1.1 positive and transfused with one unit of packed red blood cells. Hunter's temperature peaked at 103.2 degrees Fahrenheit within the first hour of the transfusion, however, this resolved without additional medical management and there

were no further complications. By two hours post-transfusion, Hunter's packed cell volume had risen to 22%. At this point, Hunter was stable enough for additional diagnostics.

Further Diagnostic Evaluation and Diagnosis

Thoracic radiographs were unremarkable, as was abdominal ultrasonography, with the exception of mild splenomegaly with normal echogenicity. Fine needle aspirates of the subcutaneous masses along Hunter's lateral thorax revealed benign lipomas.

Cytology of bone marrow collected from Hunter's left humerus revealed increased cellularity (Figure 2). Few normal hematopoietic cells were identified, and those present were largely outnumbered by blasts, which heavily infiltrated the marrow. Two distinct populations of blasts were noted, which bore striking resemblance to those found in the peripheral bloodstream (Figure 3). The presence of blasts in such high numbers in both the peripheral bloodstream and bone marrow was suggestive of acute leukemia.

The next step in Hunter's diagnostic work-up was to determine the cell lineage of these blasts in order to definitively diagnose Hunter with either an acute myeloid or acute lymphoid leukemia. Flow cytometry was utilized in Hunter's case to determine cell lineage. In flow cytometry, the specimen containing the cells of interest is fed through a column, wherein a detector measures the amount of scatter produced when directed light is refracted from each cell. There are two forms of scatter: forward scatter and side scatter. Forward scatter is determined by cell size: the larger the cell, the more forward scatter. By contrast, side scatter is contingent upon cell complexity: the more granular the cytoplasm of the cell in question, the greater the side scatter. Therefore smaller, less granular cells such as lymphocytes have less scatter, whereas larger, more granular cells like neutrophils produce relatively more scatter.

The utility of flow cytometry as it pertains to Hunter's case is that fluorescent antibodies can be added to a liquid suspension containing the cells of interest. These fluorescent antibodies can then bind to epitopes on the blasts in question, and the intensity of fluorescence is reported by the detector. Because certain epitopes are associated with specific cell lineages, the presence or absence of this binding can confirm or refute cell lineage.

To determine whether Hunter's blast cell populations were of lymphoid origin, the blasts were screened for the presence of cell surface markers CD3, CD5, and CD21. CD3 and CD5 are epitopes found predominantly on T cells. The majority of Hunter's blasts were negative for both T cell markers (Figure 4). Similarly, Hunter's blast cell populations were negative for the presence of CD21, an epitope found predominantly on B cells (Figure 5). These findings supported the conclusion that the neoplastic cells were not of lymphoid origin.

Hunter's blast cell populations were then evaluated for the presence or absence of markers (CD11b, CD11c, and CD14) that would confirm or refute myeloid lineage. Hunter's blasts were strongly positive for all three cell surface markers (Figure 6). Specifically, 94% of the blast cell population tested positive for CD11b; 92% for CD11c; 86% for CD14.

To summarize, the lack of fluorescent antibody binding to T and B cell markers by the majority of Hunter's blast cells confirmed that the majority of blasts were not of lymphoid origin. By contrast, the significant binding of fluorescent antibodies to myeloid markers CD11b, CD11c, and CD14 on the majority of blasts in the peripheral circulation confirmed that the blasts were of myeloid origin. Consequently, Hunter was definitively diagnosed with acute myeloid leukemia.

Discussion

Leukemia is a hematopoietic neoplasm that most commonly arises within the bone marrow. In general, leukemias can be classified according to cell lineage and chronicity.

To understand how leukemias are classified according to cell line, it is important to review the process of hematopoiesis. Normal hematopoiesis begins in the bone marrow with a reserve of pluripotent stem cells. Each stem cell proliferates, differentiates, and matures into one of two cell lines: myeloid and lymphoid. The lymphoid cell line ultimately gives rise to T cells, B cells, and natural killer cells, whereas the myeloid cell line gives rise to platelets, erythrocytes, monocytes, and granulocytes.

Following this distinction, lymphoid leukemias involve cell populations that are of lymphoid origin – that is, the abnormal cell populations arise from T, B, or natural killer cells. By contrast, myeloid leukemias involve cell populations of granulocytic, monocytic, megakaryocytic, or erythrocytic origin. Of these cell lines, monocytes are the most common myeloid line to be affected in the canine patient (Ogilvie).

Leukemias may also be described by morphology. They may be either acute or chronic, depending upon the degree of cellular differentiation (Withrow). Acute leukemias result from uncontrolled proliferation of cells that fail to properly mature. By contrast, chronic leukemias are typically comprised of neoplastic cells that morphologically resemble their well-differentiated counterpart (Withrow).

Another definition of acute versus chronic leukemias is determined by clinical onset. Acute leukemias are characterized by sudden onset of clinical signs and rapid decline. In contrast, chronic leukemias tend to develop over an extended period of time, with slowly progressing clinical signs (Withrow).

The patient described in this case report was diagnosed with acute myeloid leukemia. Myeloid leukemias are rare in the canine patient and occur with ten times less frequency than lymphoproliferative disorders (Withrow). It is thought that myeloid leukemias are more common in large, female dogs, however there does not seem to be any specific age or breed predisposition (Ogilvie).

Acute myeloid leukemia typically presents with sudden onset of non-specific signs. Lethargy, anorexia, and sudden weight loss are most commonly observed by caregivers (Ogilvie), and were initially reported by Hunter's owners. Occasionally, the patient may also present with a shifting-limb lameness, which is attributed to pain caused by severe infiltration of the bone marrow by malignant cells; this was not appreciated in Hunter (Ogilvie). Mucous membranes are often pale due to underlying anemia, and approximately one-third of all dogs with acute myeloid leukemia develop ocular changes, including retinal detachment, hyphema, glaucoma, chorioretinitis, chemosis, and conjunctivitis (Ogilvie).

As neoplastic cells continue to accumulate in the bone marrow, they may spill into the peripheral bloodstream and eventually invade other organs, especially lymph nodes, spleen, liver, lungs, and kidneys (Hamlin). Infiltration of the bone marrow by blasts may become so overwhelming that the neoplastic cells displace the normal populations of hematopoietic cells in a process known as myelophthisis.

As the normal hematopoietic stem cell populations decline, patients become peripherally pancytopenic. Hemorrhage and infection are common sequelae, and may be more deleterious to the patient than the underlying disease itself.

A diagnostic work-up of acute myeloid leukemia begins with taking a thorough history and performing a complete physical examination, baseline bloodwork and imaging. Finding

elevated numbers of blast cells in the bloodstream is suggestive of either lymphoma or leukemia. Bone marrow cytology plays a key role in attempting to identify the origin of the neoplastic process. An evaluation of marrow in which the blast cells comprise $\geq 30\%$ of the total cell population is consistent with acute leukemia.

The next step is to determine whether the acute leukemia is myeloid or lymphoid in origin. This distinction cannot be made solely based upon the cytologic morphology of the blasts (Withrow). Instead, cell lineage can only be determined by immunophenotyping, cytochemistry, flow cytometry, or polymerase chain reaction. Immunostaining is often helpful at differentiating acute myeloid leukemia from acute lymphoid leukemia, as the former is characterized by populations of abnormal cells that stain positive for CD1c, CD11, CD4, CD14, or DM-5, while the latter cells stain with CD3, CD4, CD8 (T cell), or CD79a (B cell, amongst others) (Withrow). In Hunter's case, flow cytometry was of great utility. The lack of binding of fluorescently labeled antibodies directed against B and T cell epitopes on the majority of Hunter's blasts confirmed that the neoplastic cells were not of lymphoid origin. However, the binding of antibodies to myeloid markers CD14, CD11b, and CD11c confirmed the diagnosis of acute myeloid leukemia.

Acute myeloid leukemia carries a very poor prognosis, both with and without treatment. Patients that forego treatment often survive fewer than two weeks following initial diagnosis, while those that undergo treatment have an expected survival of weeks to months (Withrow). Poor survival time is a consequence of the paucity of knowledge about the disease due to its relative rarity in veterinary patients, the lack of standardized chemotherapy protocols, nondurable response to currently employed treatments, and the severity of anemia and opportunistic infections that accompany myelophthisis (Withrow). Nevertheless, chemotherapy

may be attempted, with the goal being to induce and maintain remission, thereby prolonging life. The most commonly reported chemotherapeutic protocol outlined in the current literature involves alternating cycles of doxorubicin (Adriamycin[®]) and cytosine arabinoside (Cytosar[®]) therapy. Prednisone and 6-thioguanine may also be used (Ogilvie). It was once thought that the use of colony-stimulating factors may be of some benefit in supporting normal bone marrow populations and promoting differentiation of the immature neoplastic cells, however, the use of such factors remains controversial because they may instead support the growth of malignant populations (Withrow). Additional ancillary therapies may include red blood cell transfusions and systemic antibiotics to support against consequences of peripheral pancytopenia, and gastroprotectant medications for chemotherapy-induced gastrointestinal toxicity.

Regardless of which therapy is administered, prognosis for patients with acute myeloid leukemia remains poor. Clinical responses to chemotherapy are invariably short-lived, and all patients will succumb to either progression of the disease itself or, more likely, to secondary complications.

Outcome

Hunter's owners elected to initiate chemotherapy. He was initially treated with L-asparaginase pending flow cytometry results. His packed cell volume remained low at 22%, but his attitude and appetite were improved. Once acute myeloid leukemia was definitively diagnosed, doxorubicin was administered, with plans to cycle between doxorubicin and Cytosar chemotherapy.

Hunter was discharged to the care of his owners on April 20, 2007 on Clavamox, prednisone, and famotidine. His packed cell volume was improved at 26% on April 26, 2007, and his platelet count had risen to 136 thousand/uL.

By May 10, 2007, Hunter's hematocrit was 33% and repeat bone marrow cytology revealed marked improvement in the number of neoplastic myeloid cells, which represented at this time just 7% of the myeloid population, compared to greater than 50% at initial presentation; there was also a more appropriate mixture of hematopoietic cells of myeloid and erythroid lineage (Figure 7). Blasts were still present in the peripheral blood, but also in fewer numbers than before. Considering his positive response to treatment thus far, Hunter's next dose of chemotherapy was administered.

Complications began to arise soon after the second course of doxorubicin. On May 18, 2007, Hunter was tachycardic with an elevated temperature of 103.6 degrees Fahrenheit. A complete blood count showed increased numbers of blasts. Hunter was started on Unasyn for broad spectrum antibiotic coverage. An electrocardiogram revealed sinus tachycardia with rare atrial premature contractions, but no evidence of cumulative cardiac toxicity from doxorubicin therapy. Hunter's fever persisted, however, due to concern that his cancer was progressing, his owners elected to continue chemotherapy. Cytosar was administered and Hunter was discharged.

By May 22, 2007, Hunter was vomiting at home. Two days later, his owner found him recumbent, with reportedly white oral mucous membranes. He was humanely euthanized later that same day by his referring veterinarian, approximately four weeks after his initial diagnosis of acute myeloid leukemia.

References

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Appendix

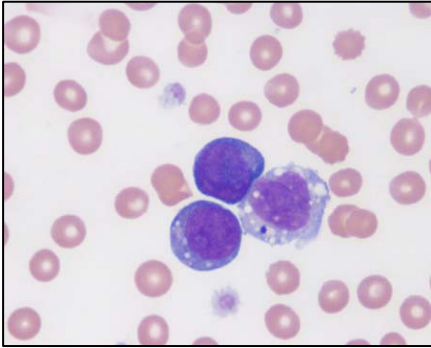


Figure 1: Two distinct populations of blast cells in the peripheral blood at initial presentation

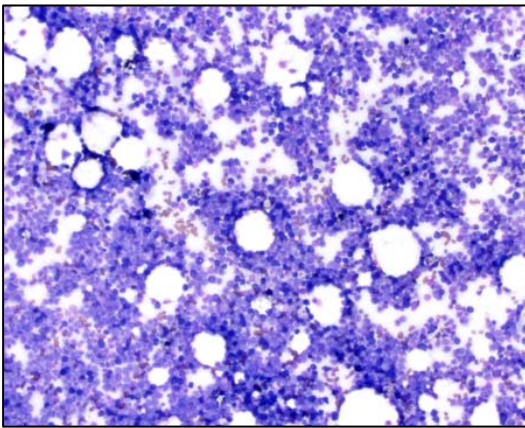


Figure 2: Increased cellularity within the bone marrow at initial presentation

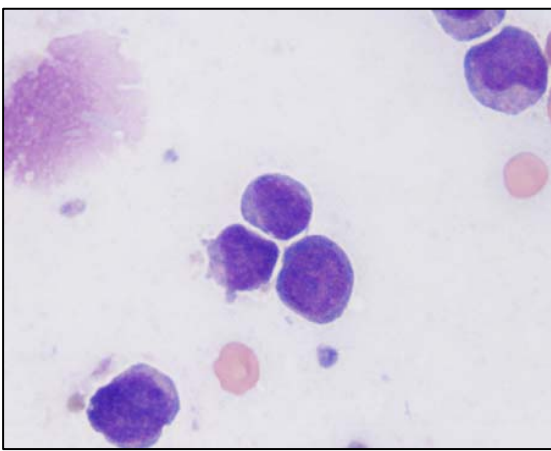


Figure 3: Close-up examination of the blast cells within the bone marrow at initial presentation

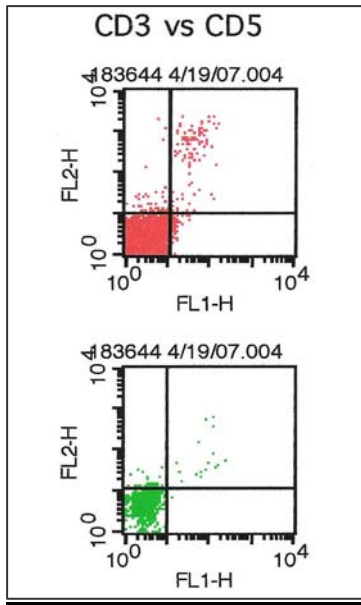


Figure 4: Flow cytometry data evaluating Hunter's peripheral blood for T cell markers CD3 and CD5. The red and green markers represent the two gated blast cell populations identified cytologically. The majority of Hunter's blasts fall within the lower left hand quadrant of the dot plot, indicating that the majority are negative for T cell markers.

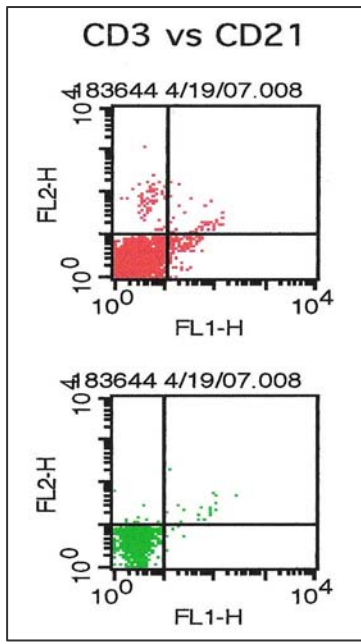
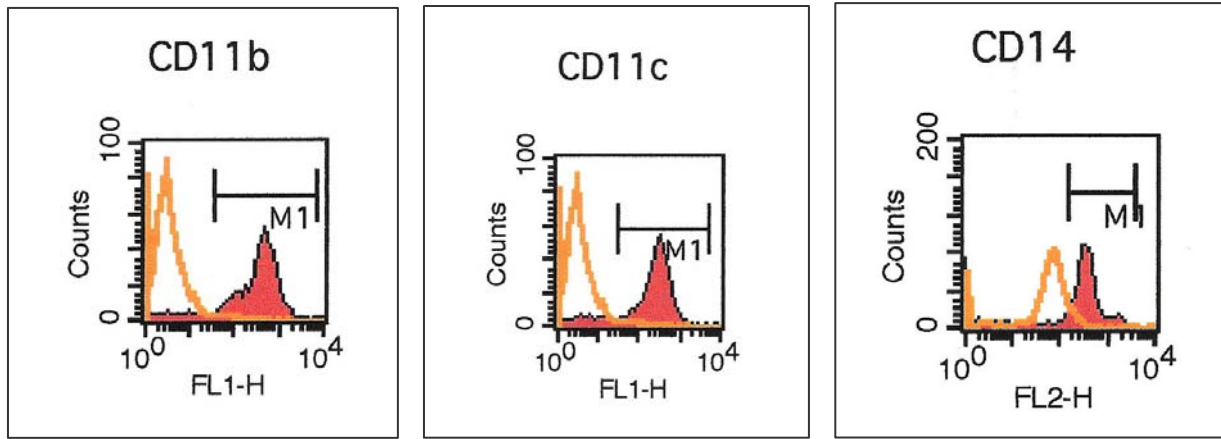


Figure 5: Flow cytometry data evaluating Hunter's peripheral blood for the B cell marker CD21. The majority of Hunter's blasts fall within the lower left hand quadrant of the dot plot, indicating that the majority are negative for B cell markers.



Figures 6a, b, and c (from left to right): Flow cytometry data evaluating Hunter’s peripheral blood blast cell populations for myeloid markers CD11b, CD11c, and CD14. The orange curve represents the negative control; cells falling within the line are said to be negative for the markers in question. Hunter’s blast cell populations, outlined in red, fall well outside of the negative control; therefore, they are said to be strongly positive for all three myeloid markers.

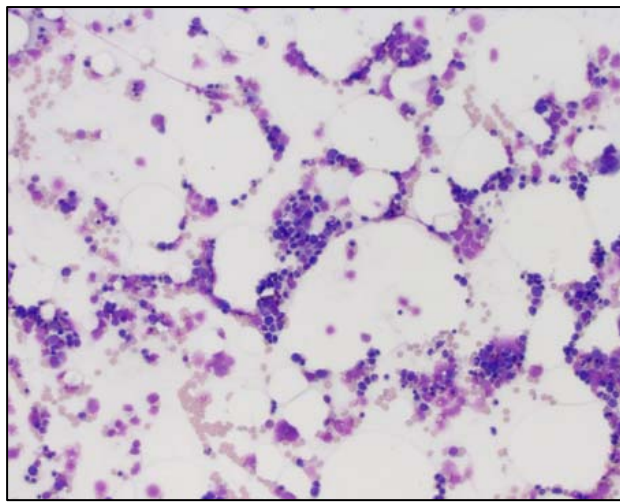


Figure 7: Bone marrow cytology following the first cycle of chemotherapy. Note the decreased cellularity when compared to the aspirate cytology at initial presentation.