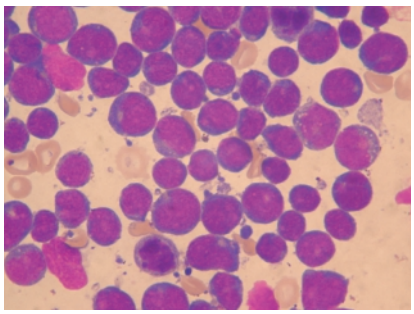

A series of laboratory-based investigations is required to reach a definitive diagnosis of leukaemia

Diagnosis and management of leukaemia in dogs and cats

JANE DOBSON, ELIZABETH VILLIERS AND JOANNA MORRIS

Jane Dobson graduated from the Royal Veterinary College, London, in 1982. She is a senior lecturer in veterinary clinical oncology at Cambridge.

Elizabeth Villiers graduated from Bristol in 1989. She is a lecturer in veterinary clinical pathology at Cambridge.

Joanna Morris graduated from Bristol in 1988. She is a senior lecturer in veterinary oncology at Glasgow.

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NEOPLASIA of the haematopoietic system is common in dogs and cats. In both species, lymphoma (eg, malignant lymphoma, lymphosarcoma) occurs far more frequently than leukaemia and multiple myeloma. Nevertheless, these conditions are important in the differential diagnosis of haematological and some metabolic problems. Animals with leukaemia do not present with an obvious tumour mass or lump, but rather with vague and non-specific clinical signs. Diagnosis, therefore, relies on a series of laboratory-based investigations – some more specialist than others. The aim of this article is to update practitioners on current diagnostic and treatment approaches for dogs and cats with leukaemia, using case examples as illustrations.

WHAT IS LEUKAEMIA?

Leukaemia is cancer of the bone marrow. It is defined as a progressive malignant disease of the blood-forming organs, marked by distorted proliferation and development of leucocytes and their precursors in the blood and bone marrow. In most instances, leukaemia is characterised by the presence of excessive numbers of abnormal neoplastic cells in both the peripheral blood and bone marrow. This is often accompanied by a reduction in cell numbers (cytopenia) as the normal bone marrow becomes overwhelmed by the neoplastic cells. Occasionally, the neoplastic process is contained within the bone marrow and is not accompanied by excessive numbers of abnormal cells in the circulation (so-called 'aleukaemic leukaemia').

CLASSIFICATION OF LEUKAEMIA

Leukaemia arises from neoplastic transformation of haematopoietic stem cells or their progeny in any of the lineages represented in the normal bone marrow. In the early stages of normal haematopoiesis, the stem and progenitor cells (colony-forming units and blast cells) remain relatively undifferentiated and retain the capacity for cell division and multiplication. As the cells become more differentiated and committed to a certain cell lineage, the capacity for replication is progressively diminished and ultimately lost in the mature cell lines seen in the peripheral blood.

Neoplastic transformation may occur at several stages of this proliferation–maturation process. Transformation

Definitions

Leukaemia
A progressive malignant disease of the blood-forming organs, marked by distorted proliferation and development of leucocytes and their precursors in the blood and bone marrow.

Myeloproliferative disease
A term used to describe all the non-lymphoid neoplastic and dysplastic conditions of haematopoietic cells.

Lymphoproliferative disease
A term used to describe all the neoplastic and dysplastic conditions arising from lymphoid cells. In addition to lymphoid leukaemia, lymphoproliferative disease includes lymphoma (lymphosarcoma) and plasma cell or multiple myeloma.

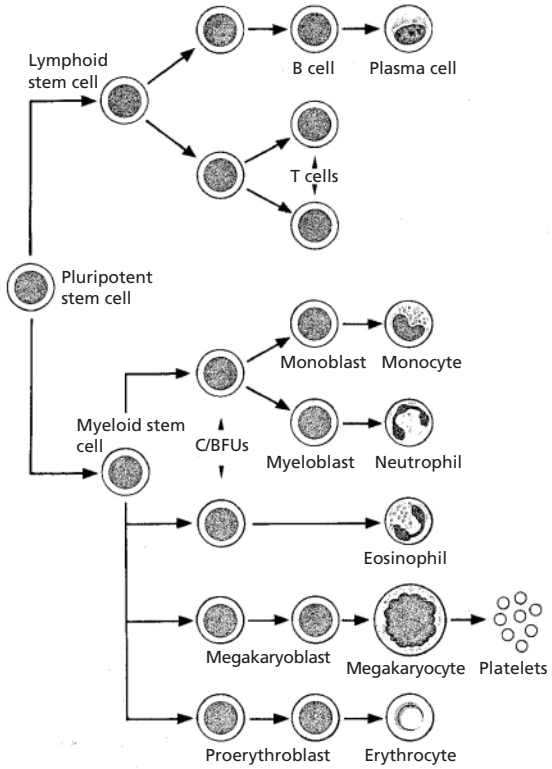
Acute leukaemia
An aggressive, rapidly progressing condition characterised by excessive numbers of abnormal, undifferentiated or 'blast' cells in both the bone marrow and peripheral blood, and accompanied by severe, non-regenerative cytopenia.

Chronic leukaemia
A less aggressive, slowly progressing condition, characterised by excessive numbers of mature, differentiated cells in the bone marrow and peripheral blood. This may or may not be accompanied by cytopenia (usually mild) in other cell lines.

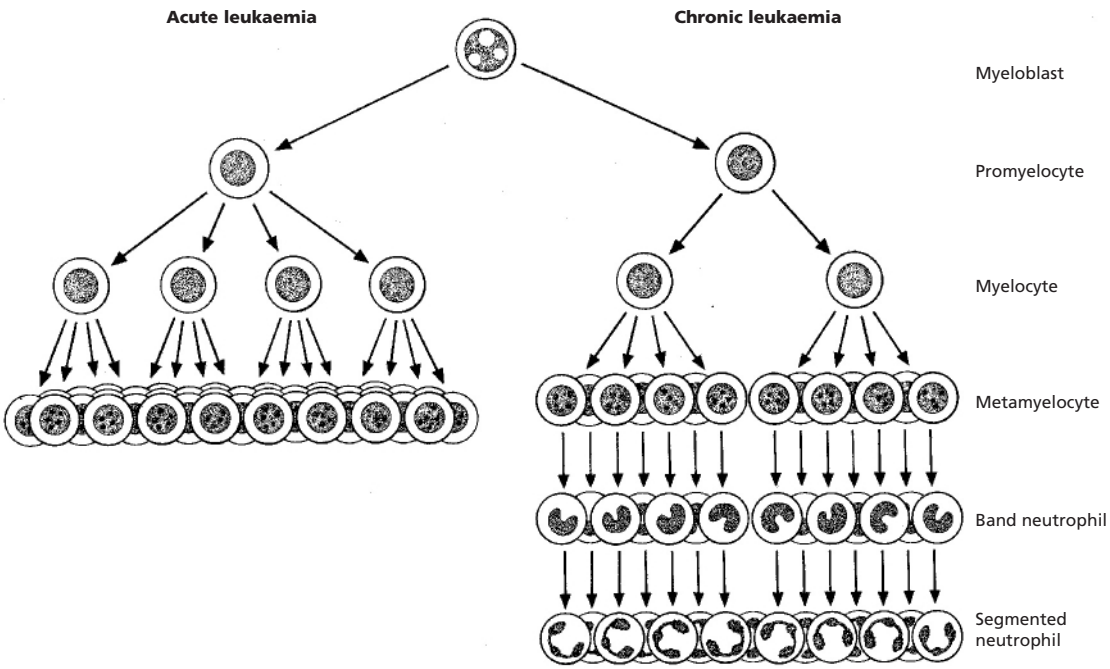
of stem cells or early precursors results in a massive proliferation of undifferentiated cells that are incapable of maturation (ie, acute leukaemia). Transformation of late precursor cells results in an overproduction of mature, differentiated cells (ie, chronic leukaemia). The division between acute and chronic leukaemia is not absolute and, sometimes, cases that share features of each may be encountered. Furthermore, there are instances of chronic-type leukaemia progressing to a more acute disease, termed 'blast cell crisis'.

Leukaemia may thus be classified according to the cell lineage involved and whether it is acute or chronic (see table below). Leukaemia of eosinophils, basophils and mast cells can also occur in acute and chronic forms, but these are all extremely rare in animals. In the lymphoid series, neoplastic transformation can result in a lymphoid leukaemia (as above), but may also take the more familiar form of lymphoma where the disease affects principally the tissues or organs of the lymphoid system (eg, lymph nodes, thymus) and tissues through which lymphocytes normally travel (eg, gut, skin).

Laboratory diagnosis and classification of leukaemia has traditionally relied on morphological examination of cells; however, this is very subjective and neoplastic blast cells in blood or bone marrow may be difficult to classify because of their aberrant morphology. Cytochemical stains (eg, chloroacetate esterase, myeloperoxi-



Normal bone marrow and haematopoiesis. The different cell lineages recognised in peripheral blood are the progeny of pluripotent stem cells. C/BFUs Colony blast-forming units. From Morris and Dobson (2000), reproduced with permission from BSAVA



Differences between acute and chronic leukaemia in the stage of neoplastic transformation in the bone marrow and outcome. From Morris and Dobson (2000), reproduced with permission from BSAVA

CLASSIFICATION OF LEUKAEMIA

Lineage	*Acute*	*Chronic*
Myeloid		
Granulocytic	Acute myeloid leukaemia (AML)	Chronic myeloid/granulocytic leukaemia (CML/CGL)
Monocytic	Acute myelomonocytic leukaemia (AMML) Acute monocytic and monoblastic leukaemia (AMoL)	Chronic myelomonocytic leukaemia
Erythroid	Erythaemic myelosis and erythroleukaemia*	Primary polycythaemia
Megakaryocytic	Acute megakaryoblastic leukaemia*	Essential thrombocytosis
Lymphoid	Acute lymphoblastic leukaemia (ALL)	Chronic lymphocytic leukaemia (CLL) Multiple myeloma

*Very rare in animals

CLASSIFICATION OF LEUKAEMIA USING FLOW CYTOMETRY

Class of leukaemia	*Staining characteristics* *Positive expression of cell surface markers*
T cell acute lymphoid leukaemia	CD3-ε, CD5 and surface CD3, CD34 most cases, Thy 1, ± CD4, CD8
B cell acute lymphoid leukaemia	CD79a, ± CD21
Acute myeloid leukaemia AML-M1	MPO, MAC-387, CD4 and neutrophil-specific antibody, CD11a-c
Acute monoblastic leukaemia AML-M5	CD14, MAC-387, and usually CD11a-c
Acute myelomonocytic leukaemia AML-M4	Neutrophil-specific antibody, CD14, MPO and MAC-387, CD11a-c

NB CD34 is positive in most cases of acute leukaemia (both myeloid and lymphoid) and is negative in cases of chronic leukaemia and stage V lymphoma

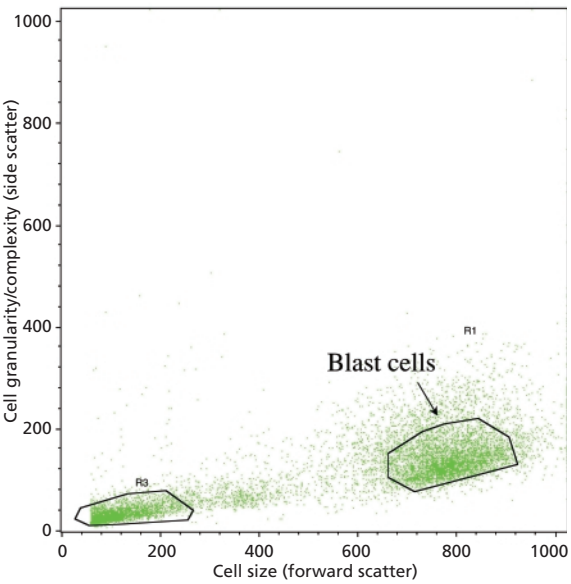
dase and lipase) have been used to aid classification, but have limited application. More recently, antibodies to cell surface markers have become available to identify different classes of leucocyte. Immunophenotyping by flow cytometry is now used routinely in the diagnosis of human leukaemia and is becoming more widely available in veterinary medicine. This involves staining aliquots of cells with a wide panel of antibodies (see table above and graphs below). Antibodies are available for identifying T and B lymphoid, monocytic and granulocytic cells and their precursors, as well as platelet precursors. In addition, an antibody recognising CD34 is useful for distinguishing between acute and chronic leukaemia, as CD34 is only expressed by stem cells and early blast precursors, and not by mature cells. These techniques are still being developed for veterinary use, but it is hoped that, in time, the prognostic value of such results will be determined. In the UK, immunophenotyping of canine leukaemia can be carried out at the University of Cambridge.

DIAGNOSIS OF LEUKAEMIA

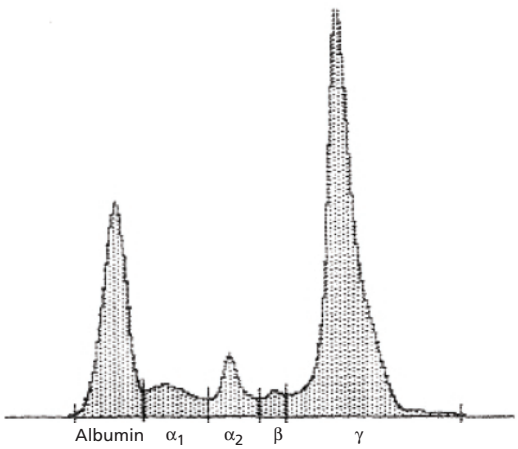
Most animals with leukaemia present with vague, non-specific clinical signs (eg, lethargy, weakness, inappetence and weight loss). These are attributable to the consequences of the disease process (eg, cytopenia, and metabolic or paraneoplastic complications) (see top table on page 25). Acute leukaemia tends to present with more severe clinical signs, both in terms of degree and speed of onset/progression of the disease. Chronic leukaemia may be associated with mild and/or recurrent clinical signs, and some cases are even asymptomatic. A diagnosis of leukaemia is rarely obvious or even possible on the basis of history or physical examination alone.

Invariably, a series of laboratory-based investigations is required to reach a definitive diagnosis of leukaemia and to assess the presence and severity of disease-related complications (see second table on page 25). Routine haematological assessment of a patient usually provides the first indication of leukaemia. Abnormal, 'leukaemic' cells may be detected in the blood and there may be gross leucocytosis accompanied by marked reductions in normal cells. Biochemistry may reflect paraneoplastic complications such as hypercalcaemia in cases of lymphoid leukaemias, hyperproteinaemia in animals with myeloma or chronic lymphocytic leukaemia (CLL), or organ failure due to neoplastic infiltration in patients with acute leukaemia.

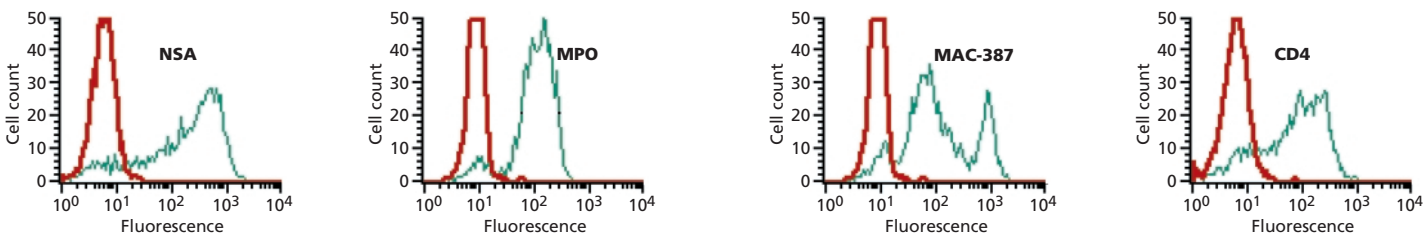
Radiography and/or ultrasonography may be indicated to assess possible neoplastic infiltration of internal



(right) Immunophenotyping using flow cytometry. This sample is from an eight-year-old crossbreed dog with leucocytosis consisting of large undifferentiated blast cells, neutropenia, anaemia and thrombocytopenia. Flow cytometry was performed on the peripheral blood. The 'dot plot' shows the presence of a population of blast cells



Serum electrophoretic trace from a dog with hypergammaglobulinaemia due to myeloma, showing the classic 'monoclonal' spike in the globulin range



Staining of the blast cells shown on the dot plot above with several antibodies. The green trace indicates the test antibody and the red trace shows the negative control. Positive staining is indicated by increased fluorescence relative to the negative control. These cells have been stained with neutrophil-specific antibody (NSA), myeloperoxidase (MPO), MAC-387 and CD4, demonstrating that they are neutrophil precursors. This dog was diagnosed as having acute myeloid leukaemia (AML-M1)

COMPLICATIONS AND CLINICAL SIGNS OF LEUKAEMIA

Complication	*Clinical signs*	*Notes*
Cytopenia(s) Leukaemic cells overwhelm normal marrow and suppress production of normal blood cells causing: – Non-regenerative anaemia – Neutropenia* – Thrombocytopenia	Lethargy Pyrexia due to sepsis or tumour-released pyrogens Petechial/ecchymotic haemorrhages, gingival bleeding	Secondary immune-mediated destruction of red blood cells and platelets can result from aberrant production of antibodies from neoplastic lymphoid cells, leading to immune-mediated haemolytic anaemia and/or thrombocytopenia
Disseminated intravascular coagulation (frequent terminal event, especially in cases of AML)	Bleeding diathesis	
Blood hyperviscosity Excessive numbers of circulating cells Hypergammaglobulinaemia (myeloma and CLL)	Neurological signs Ocular signs, including tortuosity of retinal blood vessels Tendency to bleed	Sluggish circulation and oxygen transport in critical capillary beds (eg, kidneys, brain). In cases with extremely high blood cell counts, aggregates or microthrombi of tumour cells may form
Hypercalcaemia Tumour production of humoral factors (eg, parathyroid hormone related protein) stimulates osteoclastic resorption of bone	Polydipsia/polyuria Lethargy and weakness Anorexia, vomiting Bradycardia	
Organ infiltration and failure Liver failure Renal failure	Signs associated with liver and renal failure	

*Most common, life-threatening complication. AML Acute myeloid leukaemia, CLL Chronic lymphocytic leukaemia

organs (especially the liver, spleen and lungs). Skeletal radiographs may be required if animals present with lameness or if myeloma is suspected.

Urine should be collected for analysis, particularly in cases with hypercalcaemia or hyperproteinaemia.

Further, more specific evaluations may include:

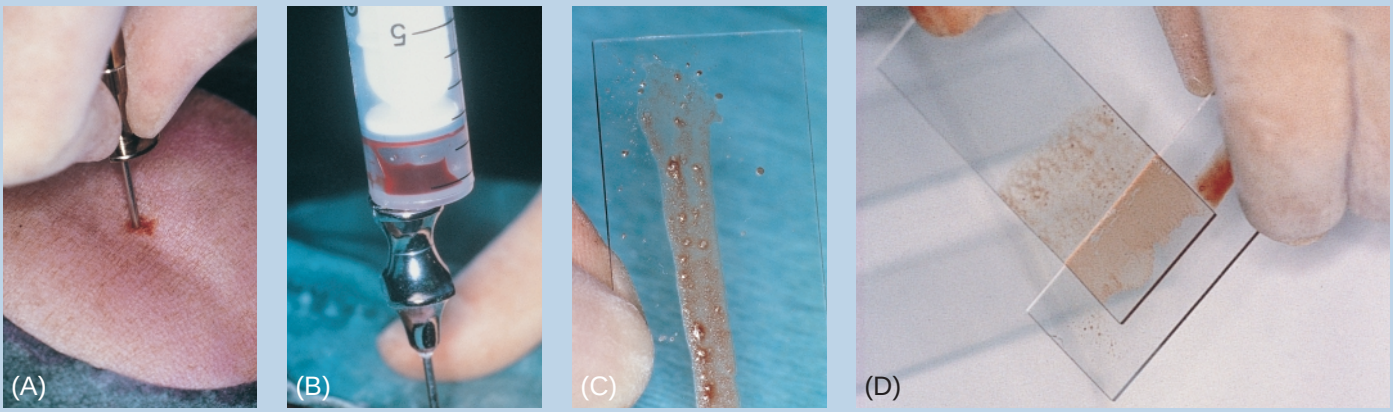
- SERUM PROTEIN ELECTROPHORESIS to determine the nature of hypergammaglobulinaemia. Myeloma and CLL are associated with a monoclonal spike in the globulin fraction, reflecting excessive production of one type of immunoglobulin (this may be IgG, IgA or IgM);
- A HAEMOSTATIC PROFILE, including whole blood clotting time and coagulation assays. Fibrin degradation products and D-dimers should be assessed if disseminated intravascular coagulation (DIC) is suspected.

INVESTIGATION OF LEUKAEMIA

Investigation	*Findings associated with leukaemia/clinical application*
Routine haematology	Anaemia, neutropenia, thrombocytopenia Leucocytosis Cells with abnormal morphology
Serum biochemistry	Hypercalcaemia Hypergammaglobulinaemia Renal failure Liver failure
Urinalysis	Low specific gravity Urinary tract infection
Clotting profile	Disseminated intravascular coagulation
Bone marrow aspirate and biopsy	Confirm diagnosis and classify leukaemia Assess normal erythroid and myeloid series
Diagnostic imaging	Assess organ involvement
Immunophenotyping	Classify leukaemia

Bone marrow evaluation

Evaluation of the bone marrow is important to confirm a diagnosis and also to provide a basis for determining the prognosis and treatment, both of which depend heavily on the degree of disruption of normal marrow elements. Bone marrow samples can be collected either by aspiration using a Klima-type needle with stylette, or by biopsy using a Jamshidi needle. Ideally, both an aspirate and biopsy should be collected as the information provided can be complementary.



Collecting a bone marrow sample by fine needle aspiration. (A) With the patient sedated and local anaesthetic infiltrated into the surrounding skin, muscle and periosteum, insert a Klima needle with a stylette in place into the dorsal wing of the iliac crest using a twisting action. (B) Once the needle is deeply seated within the bone, remove the stylette, connect a 10 ml syringe and apply suction to bring a sample of bone marrow bubbling into the syringe. (C) Withdraw the syringe and needle from the bone as one unit and place a few drops of bone marrow onto five to 10 clean glass slides tilted at an angle of about 45°. This allows the excess blood to run down the slide, thus reducing haemodilution of the sample. Globules of fat and flecks of marrow should be visible in a good sample of bone marrow. (D) Draw a second clean glass slide perpendicularly across the first to make a smear. Speed is important here to prevent the bone marrow from clotting before the smear is made. The sample is then air-dried and sent to a laboratory for fixation and staining. Note the same approach and positioning may be used with a Jamshidi needle to collect a core of bone marrow; this requires fixation in formalin and processing by standard histological methods

ACUTE LEUKAEMIA

Acute leukaemia (acute lymphoblastic leukaemia [ALL] and acute myeloid leukaemia [AML]) accounts for less than 10 per cent of all haematopoietic neoplasms. Most cases occur in young to middle-aged animals (mean, five to six years of age for ALL), but the disease can occur in older animals (range, one to 12 years of age). There appears to be a slight sex predisposition in dogs, with more males being affected at a ratio of 3:2. In cats, retroviruses are implicated in the aetiology of ALL, with up to two-thirds of affected cats being positive for feline leukaemia virus (FeLV).

CLINICAL FEATURES

Acute leukaemia is characterised by aggressive and rapid progression of disease. AML is more aggressive than ALL. In both forms, the early blast cells proliferate in the bone marrow at the expense of normal haematopoiesis, resulting in varying degrees of anaemia, thrombocytopenia and neutropenia. In the case of ALL, blast cells also spill over into the blood and infiltrate peripheral organs, especially the liver and spleen. Sepsis and uncontrolled haemorrhage due to DIC are common outcomes of AML.

DIAGNOSIS

The diagnosis of acute leukaemia is based on finding >30 per cent blast cells in the bone marrow, usually accompanied by similar cells in the peripheral blood. In humans, this lower limit has recently been reduced to 20 per cent and, in future, the accepted limit used in animals may be adjusted in line with this change. To differentiate between ALL and AML, evaluation of cell morphology may be helpful, but definitive diagnosis relies on the use of cytochemical stains such as myeloperoxidase and non-specific esterase, and/or immunophenotyping. In dogs, ALL may be differentiated from stage V lymphoma by milder lymphadenopathy and more severe

Case work-up

History

A six-year-old, entire male labrador retriever presented with a one- to two-week history of being 'off colour', slightly inappetent and lethargic.

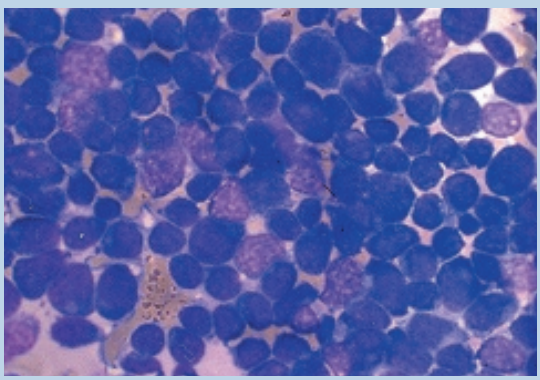
Physical examination

The dog was very quiet/depressed, pyrexic (40°C), in thin condition, and mucous membranes were pale. Abdominal palpation revealed possible splenomegaly, but no other abnormalities were detected. A blood sample was taken and submitted for haematology (see table, below right) as anaemia was suspected from the clinical examination.

Interpretation

- Normocytic, normochromic anaemia (non-regenerative)
- Severe thrombocytopenia
- Lymphocytosis with atypical cells present
- Severe neutropenia

Blood was submitted for flow cytometry to identify the type of abnormal cells. This revealed a single population of cells that stained strongly for CD79a and CD34. These findings indicate that this is a B cell leukaemia (CD79a is a B cell marker) and is acute (CD34 is expressed only in stem cells and early blast cells). Bone marrow evaluation was indicated and a representative field of a bone marrow aspirate is shown below left.



There is no recognisable erythroid or myeloid activity in this smear; this finding is consistent with the pancytopenia seen in the blood. The aspirate is dominated by lymphoid cells. The diagnosis was ALL and the prognosis was poor

Outcome

The dog was treated with L-asparaginase, vincristine and prednisolone and granulocyte-colony stimulating factor (G-CSF; Granocyte, Chugai Pharma UK). Within two weeks, the lymphocytosis had resolved and the neutrophil count had risen to 2·5 x 10^9/litre. This was maintained with vincristine and cyclophosphamide for six weeks, at which point the lymphocytosis recurred and the dog became progressively anaemic and neutropenic. It survived for just over two months following diagnosis.

HAEMATOLOGY RESULTS

	Patient	*Normal range*
Red blood cell count (x10^{12}/litre)	3·02	5·5 to 8·5
Packed cell volume (litre/litre)	0·215	0·37 to 0·55
Haemoglobin (g/dl)	7·2	12 to 18
Mean cell volume (fl)	71·3	60 to 77
Mean cell haemoglobin (pg)	23·9	19·5 to 24·5
Mean cell haemoglobin concentration (g/dl)	33·5	32 to 37
Plasma protein (g/litre)	75·0	60 to 80
Total white blood cell count (x10^9/litre)	66·8	6 to 17
Neutrophils (x10^9/litre)	0·016	3 to 11·5
Lymphocytes (x10^9/litre)	49·5	1 to 4·8
Monocytes (x10^9/litre)	17·3	0·2 to 1·5
Eosinophils (x10^9/litre)	0·00	0·1 to 1·3
Basophils (x10^9/litre)	0·053	0
Platelets (x10^9/litre)	6·01	175 to 500

DIFFERENTIATION OF ACUTE LYMPHOBLASTIC LEUKAEMIA FROM STAGE V CANINE LYMPHOMA

Clinical sign	*ALL*	*Stage V lymphoma*
Cytopenia	Severe	Mild
Lymphocytosis	++++	+/++
Lymphadenopathy	Mild	Gross
Hepatosplenomegaly	Yes	Yes
Patient status	Ill/depressed	Often bright and alert
CD34	Usually positive	Negative

Adapted from Morris and others (1993)

bone marrow infiltration, which results in more severe myelosuppression (see table above). CD34 is expressed by cells in ALL, but not in lymphoma.

MANAGEMENT

Supportive therapy

Supportive measures include fluid therapy for dehydration, nutritional support for anorexia, blood transfusion for severe loss of red blood cells or platelets, and broad-spectrum antibiotic therapy for secondary infections.

Specific treatment

The theoretical aim of therapy is to destroy leukaemic cells and allow the resumption of normal haematopoiesis. Ideally, treatment of acute leukaemia should be aggressive. Standard lymphoma protocols, especially those containing doxorubicin, are often advocated for animals with ALL. By contrast, cases of AML are frequently given cytosine arabinoside with anthracyclines because it may encourage differentiation of the blast cells; however, AML rarely responds to chemotherapy protocols and any response is short-lived.

In veterinary medicine, the use of chemotherapy in the treatment of acute leukaemia is severely restricted by the degree of myelosuppression caused by the disease. The inability to preserve sufficient levels of normal blood cells during treatment is a constant problem. Furthermore, the normal toxicity of cytotoxic agents may be enhanced by compromised hepatic and renal function. Most animal patients either succumb to overwhelming sepsis secondary to neutropenia, organ failure secondary to leukaemic infiltration, or DIC. Intensive medical care, bone marrow transplants and extracorporeal treatment of bone marrow are used for human patients, but are not routinely available for veterinary use. Human recombinant granulocyte-colony stimulating factors (eg, G-CSF and GM-CSF), which stimulate granulocyte precursors to increase neutrophil production and release from remaining normal marrow, may have a role in the management of canine leukaemia although this has yet to be fully evaluated in clinical cases.

PROGNOSIS

Any form of acute leukaemia carries a poor prognosis due to:

- Low rates of induction and maintenance of remission;
- Organ failure, which enhances the cytotoxic effects of the drugs;
- Septicaemia secondary to the disease or treatment.

The prognosis for ALL is slightly better than for AML. Twenty to 40 per cent of ALL cases go into remission, usually with short survival times of between one and three months, but occasionally longer. Survival times for AML rarely exceed three months.

CHRONIC LEUKAEMIA

Chronic leukaemia is less common than acute leukaemia. In dogs, CLL occurs more frequently than chronic myeloid leukaemia (CML). Affected animals are middle-aged to old (mean, 9·4 years of age for CLL). An increased incidence is reported in male dogs; in cases of CLL, the male to female ratio is 2:1. No breed predisposition has been reported. Chronic leukaemia is rare in cats.

PATHOGENESIS

Chronic leukaemia arises from the neoplastic transformation of late precursor cells in lymphoid and myeloid development, which leads to proliferation of fairly well differentiated cells. The disease is characterised by slow progression and relatively mild clinical signs. The neoplastic cells proliferate in the bone marrow at the expense of normal haematopoiesis, spilling over into the blood and infiltrating peripheral organs (eg, spleen, liver, lymph nodes). A change from mature cell to blast cell proliferation (a blast cell crisis) can occur as a terminal event with CML, but not CLL.

CLINICAL FEATURES

Fifty per cent of CLL cases may be asymptomatic and only detected on haematological examination. Other cases of CLL and all animals with CML show mild, progressive disease, with vague signs such as lethargy, anorexia, vomiting, pyrexia, polyuria, polydipsia and weight loss, all of which may wax and wane.

Clinical findings include:

- Myelosuppression. Anaemia, thrombocytopenia, lymphopenia and granulocytopenia are all milder than in cases of acute leukaemia;
- Secondary infections due to reduced humoral and cellular immunity;
- Monoclonal gammopathy in approximately 25 per cent of cases with CLL (although 10 per cent of cases may have reduced immunoglobulin levels);
- Pallor;
- Mild lymphadenopathy (more common in patients with CLL);
- Splenomegaly ± hepatomegaly;
- Skin infiltration;
- Hyperviscosity syndrome.

DIAGNOSIS

Chronic lymphocytic leukaemia

The diagnosis of CLL is based on the presence of >30 per cent small lymphocytes in the bone marrow, usually accompanied by similar cells in the peripheral blood. A marked circulating lymphocytosis is almost pathognomonic for CLL although, on occasion, lymphocyte numbers can exceed this level following a reactive/inflammatory process; in such cases, the lymphocytosis is usually transient and accompanied by obvious clinical signs associated with the primary disease process. Lymphocytosis (consisting of small lymphocytes) can also be seen as a result of overspill in small cell/lymphocytic lymphoma, but this is rare.

Chronic myeloid leukaemia

CML is characterised by marked neutrophilia, often with a severe left shift; sometimes the neutrophils exhibit atypical/dysplastic changes. There is often hepatosplenomegaly due to infiltration with granulocytic precursors. The bone

marrow is hypercellular and dominated by maturing granulocytic cells, with increased numbers of immature cells (myeloblasts not exceeding 20 per cent). CML can be very difficult to distinguish from extreme neutrophilia caused by an inflammatory/infectious process (so-called leukaemoid reaction) as the bone marrow and peripheral blood findings are similar. The diagnosis of CML is made by excluding all causes of inflammation, demonstrating granulopoiesis in the spleen or liver, and by the presence of dysplastic maturation (although not all animals show this). Toxic changes within neutrophils and increased inflammatory plasma proteins suggest a leukaemoid reaction. Cytochemical staining and flow cytometry are not helpful in distinguishing leukaemia from leukaemoid reactions.

CHEMOTHERAPY FOR CHRONIC LEUKAEMIA AND MYELOMA

	Drug	*Dose*
Chronic lymphocytic leukaemia	Chlorambucil	2 to 8 mg/m^2, orally, daily for seven to 14 days, then 2 mg/m^2 every 48 hours* *or* 20 mg/m^2, orally, as a single dose every two weeks
	Prednisolone	40 mg/m^2, orally, daily for seven days, then 20 mg/m^2 every 48 hours
Chronic myeloid leukaemia	Hydroxyurea (Hydroxycarbamide)	Dogs: 30 mg/kg, orally, daily for seven days, then 15 mg/kg daily for maintenance Cats: 10 to 12·5 mg/kg daily
	Busulfan	0·1 to 0·2 mg/kg, orally, daily
Multiple myeloma	Melphalan	2·0 mg/m^2, orally, daily for seven to 14 days, then every 48 hours†
	Prednisolone (plus bisphosphonate if skeletal lesions are present)	40 mg/m^2, orally, daily for seven to 14 days, reducing to 20 mg/m^2 every 48 hours, depending on response
Primary polycythaemia	Hydroxyurea (Hydroxycarbamide)	Dogs: 30 mg/kg, orally, daily for seven days, then 15 mg/kg daily for maintenance Cats: 10 to 12·5 mg/kg daily

Alternative dosing regimens reported in the literature for some of the drugs listed above:
*Chlorambucil: 0·2 mg/kg, orally, once daily for induction and 0·1 mg/kg, orally, once daily for maintenance
†Melphalan: initial starting dose 0·1 mg/kg, orally, once daily for 10 days, reducing to 0·05 mg/kg once daily or every other day for maintenance. Also, pulsed therapy at 7 mg/m^2, orally, for five days repeated every three weeks

MANAGEMENT

Supportive therapy

Paraneoplastic complications such as hypercalcaemia and hypergammaglobulinaemia may need to be addressed.

Specific treatment

- For asymptomatic cases of CLL, no treatment may be needed, although frequent monitoring and haematological screens are advisable.
- For symptomatic cases of CLL and animals with CML, chemotherapy is the treatment of choice. Drug selection depends on the type of disease and is summarised in the table, below left.

The aim of treatment is to restore the peripheral blood counts to within the normal range; the response to treatment is monitored by haematological findings. Once remission is achieved, maintenance therapy is continued at reduced doses and frequencies of the appropriate drugs in order to keep the white blood cell counts within the normal range.

PROGNOSIS

The prognosis for chronic leukaemia is more favourable than for acute leukaemia. Mean and median survival times for CLL may exceed one year, but are usually shorter for CML, which is associated with a greater risk of blast cell crisis.

MYELOMA

Myeloma (multiple myeloma, plasma cell myeloma) results from neoplastic proliferation of plasma cells (B lymphocytes) predominantly in the bone marrow. Animals with myelomas may present with a variety of vague and non-specific clinical signs such as weakness, lethargy and pyrexia. More specific signs depend on the secretory nature and bony involvement of the

Case work-up

History

A 12-year-old neutered female terrier cross (12·5 kg) was presented for evaluation of a recurrent soft tissue mass affecting the right carpus, previously diagnosed as a haemangiopericytoma. No other problems were reported.

Physical examination

The dog had a 2 to 3 cm, soft, reasonably circumscribed mass on the craniomedial aspect of the carpus. This did not appear to be painful and no lameness was observed. No other abnormalities were noted, except that the animal's spleen was enlarged and readily palpable. A blood sample was taken prior to surgical removal of the mass (see table).

Interpretation

- Massive leucocytosis due to lymphocytosis with mild neutrophilia
- Mild normocytic anaemia
- Mild hyperproteinaemia

Many of the lymphocyte nuclei contained nucleolar remnants; the nuclei had a condensed chromatin pattern and there was a thin rim of cytoplasm.

A bone marrow aspirate was collected, which showed a hypercellular marrow. Megakaryocyte numbers appeared normal. The granulocytic series was extremely active, maturation appeared orderly and was progressing to completion. The erythroid series was less active, but maturation was also progressing to completion. There was a marked increase in the number of small- and medium-sized lymphocytes (including some small cell clusters). Lymphoblasts were present at <10 per cent.

The cytology was more suggestive of a CLL than the acute lymphoblastic form. The lymphoid cells appeared reasonably well differentiated. There was clear evidence of a degree of normal haematopoietic activity. A diagnosis of CLL was reached and the prognosis was considered to be fair.

Outcome

The dog was treated with chlorambucil (Leukeran; GlaxoSmithKline) 2 mg once daily (5 mg/m^2) and prednisolone 20 mg once daily (40 mg/m^2) for seven days, and then at 10 mg every 48 hours. The resulting decrease in white blood cells is shown in the graph. At day 30, chlorambucil was reduced to 2 mg every 48 hours. The dog was maintained on prednisolone and chlorambucil for six months, at which point it developed recurrent lymphocytosis and splenomegaly.

tumour (see box below). The classical signs of myeloma are:

- Monoclonal hypergammaglobulinaemia;
- Multiple punched-out osteolytic skeletal lesions.

However, the presence of one or both of these signs is not sufficient for a definitive diagnosis, which depends on confirming the presence of neoplastic plasma cells in a bone marrow aspirate or biopsy sample.

THERAPY

Chemotherapy is the treatment of choice for myeloma and most protocols comprise a combination of an alkylating agent, either melphalan or cyclophosphamide, with prednisolone with or without vincristine (see table on page 28). Bisphosphonates (eg, clodronate, pamidronate) may be indicated in the treatment of animals with

Bence Jones proteinuria

Myeloma is reportedly characterised by the presence of light chain proteins (resulting from immunoglobulin molecules) in the urine. These proteins, being of smaller molecular size, may cross the glomerular barrier and appear in the urine; whereas, in theory, the larger complete immunoglobulin molecules are contained within the blood if the glomerular barrier is intact. The light chain proteins are termed 'Bence Jones' proteins after the laboratory test that determines their presence in urine. In humans, the finding of Bence Jones proteinuria is strongly indicative of myeloma. In dogs, Bence Jones proteinuria is only present in 30 to 40 per cent of cases with monoclonal gammopathy, making it a less useful test. In the authors' experience, analysis of urinary protein in cases with secretory myeloma usually yields an electrophoretic trace very similar to that of the plasma protein, indicating that the glomerular barrier is damaged by the time the disease is diagnosed.

Clinical features of myeloma

Cytopenia, pyrexia
Infiltration of the bone marrow with plasma cells can affect the production and maturation of other cell series. The resulting cytopenia, while not as dramatic as that associated with ALL, can give rise to some of the clinical signs of myeloma.

Hypergammaglobulinaemia
Neoplastic cells often retain their secretory function and produce large quantities of immunoglobulins. On plasma protein electrophoresis, this appears as a large, monoclonal 'spike' in the globulin fraction.

Hyperviscosity
Hyperviscosity results from overproduction of the multimeric immunoglobulins, IgA and IgM; IgG-secreting tumours do not cause this problem. Signs include a bleeding diathesis, neurological signs due to poor perfusion of the brain, thromboembolic disease and ocular changes, especially detached retinae.

Skeletal lesions
The tumour cells stimulate localised resorption of bone resulting in characteristic punched-out solitary or multiple osteolytic lesions in both the axial and appendicular skeleton. Skeletal pain may be the main presenting clinical sign. Spontaneous pathological fracture of a long bone or collapse of a vertebral body can occur and this would result in an acute presentation of the case.

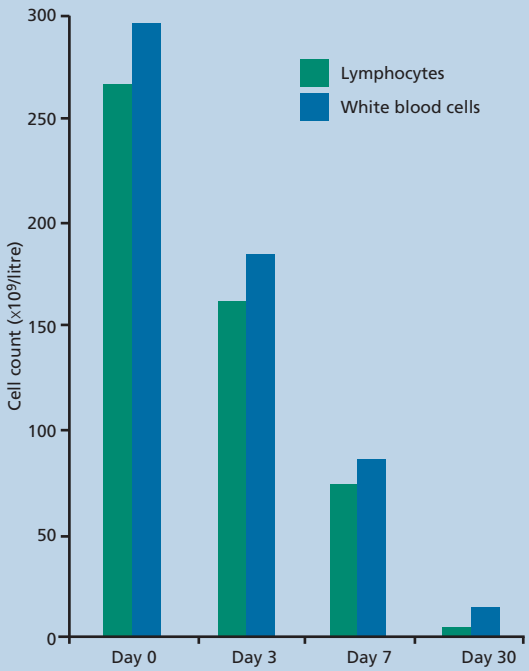
Hypercalcaemia
This occurs in 15 to 20 per cent of canine cases, and causes polydipsia and polyuria.

Renal disease
Light chain proteinuria (see box above) is present in association with monoclonal gammopathy in 30 to 40 per cent of cases and predisposes to the development of renal failure by causing renal tubular damage. Hypercalcaemia, amyloidosis and urinary tract infection may also contribute to renal disease.

Immunocompromise
Depressed normal globulin levels and myelosuppression predispose myeloma patients to secondary infections. This is known as 'immune cripples'.

HAEMATOLOGY RESULTS

	Patient	*Normal range*
Red blood cell count (x10^{12}/litre)	4·77	5·5 to 8·5
Packed cell volume (litre/litre)	0·302	0·37 to 0·55
Haemoglobin (g/dl)	12·5	12 to 18
Mean cell volume (fl)	63·4	60 to 77
Mean cell haemoglobin (pg)	26·3	19·5 to 24·5
Mean cell haemoglobin concentration (g/dl)	41·5	32 to 37
Plasma protein (g/litre)	86·0	60 to 80
Total white blood cell count (x10^{9}/litre)	215	6 to 17
Neutrophils (x10^{9}/litre)	23·7	3 to 11·5
Lymphocytes (x10^{9}/litre)	182	1 to 4·8
Monocytes (x10^{9}/litre)	5·15	0·2 to 1·5
Eosinophils (x10^{9}/litre)	0·102	0·1 to 1·3
Basophils (x10^{9}/litre)	3·52	0
Platelets (x10^{9}/litre)	313	175 to 500



skeletal lesions or hypercalcaemia, to help prevent further osteolysis.

Radiotherapy can be of value in effecting a rapid remission of solitary or localised bony lesions provided that these are not too extensive. Pathological fractures can follow rapid remission of large skeletal lesions, but this may occur spontaneously in any case.

Animals that present with hyperviscosity syndromes may be treated via phlebotomy to reduce the viscosity of the blood prior to chemotherapy. This can be achieved by withdrawing 20 ml of blood per kg bodyweight and replacing it with an appropriate fluid.

In cases of myeloma, the tumour mass is not easily accessible or measurable, and other parameters must therefore be used to assess and monitor the response to treatment:

- In cases of hypergammaglobulinaemia, the plasma immunoglobulin concentration can be used as a guide to monitor the success of treatment and determine the frequency and dosage for maintenance therapy. The aim of treatment is to maintain the immunoglobulin concentration within the reference range;
- In cases of non-secretory myelomas, the response and maintenance therapy are dictated by remission of other clinical signs and haematological toxicity. In animals with skeletal involvement, the alleviation of signs of pain may be used for assessment. Radiological resolution of the skeletal lesions is not usually very helpful because, while some lesions may resolve with time, this is not always the case.

PROGNOSIS

The prognosis for myeloma is guarded, although most cases respond favourably to treatment and survival times of 12 to 18 months may be achieved.

MYELODYSPLASTIC SYNDROME

Myelodysplastic syndrome (MDS) is a group of acquired disorders arising from genetic mutations of haematopoietic stem cells. The aberrant stem cells give rise to cells that fail to mature properly and die within the bone marrow. This ineffective haematopoiesis results in peripheral cytopenia, most commonly anaemia and thrombocytopenia. Associated clinical signs include lethargy, anorexia, depression and pyrexia, hepatosplenomegaly, lymphadenopathy and weight loss. These signs may wax and wane. The bone marrow is normocellular or hypercellular, with abnormal maturation and dysplastic changes in one or more cell lines. Additional abnormalities in the peripheral blood may include macrocytic normochromic red cells (megaloblastic change), nucleated red cells without polychromasia, large bizarre platelets, and large, hypo- or hypersegmented neutrophils.

In cats, MDS is frequently associated with FeLV infection and usually carries a poor prognosis; survival times are a matter of weeks, although some cases have been reported to survive for more than a year following therapy with low dose cytarabine or aggressive combination chemotherapy. Affected FeLV-positive cats may also

Dysmyelopoiesis

Certain disease states and drugs can trigger secondary dysmyelopoiesis, resulting in similar haematological changes to MDS. Secondary dysmyelopoiesis differs from MDS in that it is not caused by genetic mutations and is reversible if the underlying cause is removed. Dysmyelopoiesis may occur secondarily to lymphoma, immune-mediated haemolytic anaemia, myeloma and, occasionally, infectious processes (eg, associated with pyometra); alternatively, it may be drug induced following therapy with cytotoxic agents, oestrogen, cephalosporins and phenobarbital. Differentiation of MDS and secondary dysmyelopoiesis may not be possible based on blood and bone marrow morphology alone and so, when a diagnosis of MDS is made, a search for an underlying cause should be carried out.

Future directions

Cytogenetic approaches

In human medicine, cytogenetic analysis is increasingly being used to classify leukaemia. Certain forms of leukaemia are identified by well-defined mutations (including translocations, inversions and deletions), which can correlate to outcome and also dictate the treatment offered. Such cytogenetic tests are not widely available for dogs and cats as yet. However, tests have been developed to identify mutations in lymphoid cells using polymerase chain reaction (PCR) technology. PCR analysis for antigen receptor rearrangements (PARR) uses primers that identify antigen receptor rearrangements on B and T cells. Primers are available for detecting the majority of T cell receptor gene rearrangements and Ig gene rearrangements in B cells, and, overall, can detect gene rearrangements in 91 per cent of lymphocytic neoplasms. PARR can be used to detect small numbers of circulating leukaemic cells in cases of lymphoid leukaemia and lymphoma, and for distinguishing between leukaemic and reactive lymphocytosis. PARR is carried out at Colorado State University in the USA, and samples can be sent there via commercial laboratories in the UK.

Molecular approaches

Advances in the molecular characterisation of myeloproliferative diseases in humans are leading to a much more targeted approach to therapy. Imatinib mesylate (Gleevec; Novartis) is a small molecule drug that inhibits the receptor tyrosine kinases of several genes (including Abl, Kit) and growth factor receptors (platelet-derived growth factor receptors A and B). The drug has activity against tumours in which there are activating mutations of these genes. For example, in most forms of CML, Abl is activated by fusion with the Bcr gene. Imatinib has shown impressive therapeutic activity in human patients with CML and other myeloproliferative disorders characterised by activating mutations of the PDGFR genes. Gleevec has been reported to be toxic in dogs, but another group of selective kinase inhibitors has been developed from small molecules with an indolinone structure. In a phase I clinical trial, one such agent (SU11654; Pfizer) showed activity against canine mast cell tumours with Kit mutations (London and others 2003). A better understanding and characterisation of feline and canine myeloproliferative diseases is required before such therapeutic approaches can be applied to veterinary patients.

develop lymphoma. In both cats and dogs, MDS may progress to AML.

MDS is subclassified according to the number of blast cells present and the ratio of myeloid:erythroid cells. Dogs with MDS with a blast cell count of <5 per cent seem to respond well to erythropoietin and supportive therapy, including antibiotics and blood transfusion. However, if the blast cell count is between 5 and 30 per cent (classified as MDS-excessive blasts), the response to treatment is poor and survival time is short.

MDS may be a primary idiopathic disorder, or may occur secondarily to an underlying disease, which in cats is most commonly FeLV infection. In dogs, MDS may arise as a late effect following treatment with cytotoxic drugs and radiation therapy.

SUMMARY

Although not as common as lymphoma, leukaemia encompasses an important group of neoplastic diseases affecting dogs and cats. Veterinary surgeons should be aware of these conditions and be able to interpret haematological results in the light of this knowledge. Evaluation of bone marrow is often key to the diagnosis and management of leukaemia. Bone marrow aspiration and biopsy techniques are simple and should be well within the capabilities of a small animal practice.

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