Proceedings of the 33rd World Small Animal Veterinary Congress

Dublin, Ireland - 2008

Next WSAVA Congress :

Canine leukaemia: are we any further forward?

Laura Blackwood BVMS, MVM, PhD, CertVR, DipECVIM-CA(Onc), MRCVS
Small Animal Teaching Hospital, University Of Liverpool, Leahurst Campus, Chester High Road, Neston, Merseyside CH64 7TE, UK

Introduction

Leukaemia is characterised by a neoplastic proliferation of haemopoietic precursors in the bone marrow, usually accompanied by a leukocytosis caused by high numbers of circulating neoplastic cells in the blood. Leukaemic cells may also infiltrate other organs (especially liver, spleen and peripheral lymph nodes). Occasionally, leukaemia may arise in the spleen rather than the bone marrow. Leukaemia may be acute or chronic, and may arise form either lymphoid or myeloid cells.

Acute leukaemia

Acute leukaemia occurs when neoplastic transformation occurs at the stem cell/committed blast stage. The neoplastic cells proliferate rapidly and in an uncontrolled manner with arrested or defective maturation because they have little differentiation potential. Acute leukaemia can occur in any age or breed of dog, with a young to middle aged predisposition, but accounts for less than 10% of all haematopoietic tumours in dogs. The clinical course is rapid, and signs are severe. Marrow infiltration due to uncontrolled proliferation of tumour cells results in crowding of normal marrow elements, competition for nutrients, failure of marrow to elaborate stimulatory factors and the build up of inhibitory factors released by the neoplastic cells. As a consequence of this, normal blood cell production is reduced. The first manifestation of failure of normal haematopoiesis is often neutropenia, and the main clinical consequence is sepsis and pyrexia. Concurrent thrombocytopenia is common, and some patients develop thrombocytopenia first, resulting in petechial and ecchymotic haemorrhages, epistaxis or melaena. Red blood cells have a longer circulating lifespan than neutrophils or platelets, so anaemia develops much later as pre-existing cells maintain levels for longer, and severe anaemia tends to occur in dogs only if there is immune mediated destruction or ongoing severe blood loss. Peripheral leukocytosis may cause hyperviscosity, resulting in bleeding diatheses, ocular changes, neurological signs, renal impairment and thromboembolic disease. Organ infiltration by leukaemic cells results in mild lymphadenopathy, hepatosplenomegaly, and organ specific signs such as seizuring. Paraneoplastic syndromes can also contribute to clinical signs.

Chronic leukaemia

Chronic leukaemias occur when the neoplastic transformation occurs in either a stem cell or later cell but progeny retain a strong tendency to differentiate. They are even less common than acute leukaemias, and tend to affect middle aged to older animals. Although proliferation is uncontrolled the cells are morphologically well differentiated (though often functionally abnormal). Chronic leukaemias generally have an insidious onset of less severe clinical signs, and less profound -cytopenias, but may still present acutely.

Overall, signs are severe in acute leukaemias, and generally mild in chronic.

Are we any further forward? Diagnostic tests

For many years, diagnosis of acute leukaemia in veterinary patients has been based on a combination of morphological evaluation of blood and/or bone marrow smears, and haemogram analysis. A diagnosis of acute leukaemia is based on the finding of >30% blasts in the peripheral blood or bone marrow, with concurrent -cytopenias (neutropenia, thrombocytopenia and/or anaemia). Differentiation between acute myeloid (AML) and lymphoid (ALL) leukaemia was most often based on morphological characteristics alone, but this is known to be unreliable, and in many cases the lineage of origin was unknown or wrongly determined. Cytochemical tests have rarely been used in canine patients due to the lack of specific stains for lymphoid cells, and the fact that negative staining with myeloid stains does not imply lymphoid origin i.e. only some myeloid leukaemias can be identified.

Immunophenotyping

The introduction of immunophenotyping is the greatest advance in our approach to leukaemic patients in recent years. Immunophenotyping is based on the detection of cell surface markers usually assigned CD (clusters of differentiation) numbers, which are expressed by cells of myeloid or lymphoid type. CD markers are expressed at different stages of development and some markers are expressed only at restricted stages of cellular development, so in addition to differentiation between lymphoid and myeloid, immunophenotyping can give an indication of the stage of maturation arrest and clonal development. However, immunophenotyping is still a new technique in veterinary clinical pathology, and we don't know exactly at which stage of differentiation canine cells express each of the markers, so much is based on extrapolation from human data. In addition,

neoplastic cells can show aberrant expression profiles, so panels of antigens are much more likely to determine the cell of origin than single antibody tests, which are unreliable. Immunophenotyping based on flow cytometry on EDTA blood or marrow should be based on a comprehensive panel of antibodies: an example is given in Table 1. It is very important to remember that not all CD34 negative leukaemias are chronic (i.e. some acute leukaemias are not CD34 positive), and it is the author's experience that occasional animals presenting with signs compatible with acute leukaemia have a clinical pathological diagnosis of chronic lymphoid leukaemia based on immunophenotyping and morphology: these patients respond to therapy in similarly poor way to ALL patients.
Immunophenotyping can also help differentiate lymphoma with bone marrow involvement from leukaemia: clinical and clinicopathological differentiation of lymphoma and acute lymphoid leukaemia is summarised in Table 2 below.

In chronic leukaemias, the main diagnostic challenge is usually eliminating other causes of lymphocytosis, neutrophilia or erythrocytosis from the differential diagnosis list.
Differentiation of reactive and neoplastic lymphocytosis can be carried out by PCR evaluation of antigen receptor rearrangements (performed at Colorado State University). This test identifies a clonal population in neoplastic disease. In chronic lymphocytic leukaemia, immunophenotyping will determine T or B cell origin, and profiling is likely to be prognostically relevant.
Cytogenetic typing has historically been an important part of assessment of human leukaemias, though immunophenotyping and molecular genetic analysis are arguably now more important. There is little cytogenetic data for dogs, but a chromosome rearrangement homologous to the Philadelphia chromosome has recently been reported in canine chronic myeloid leukaemia.

Are we any further forward? Treatment
Treatment of acute leukaemias in canine patients is fraught with difficulty, and most patients do not achieve remission. The key difficulties are the advanced nature of the disease at presentation; the difficulties managing cytopenias while introducing myelosuppressive drugs; the difficulties adequately supporting patients, and the intrinsic resistance of many leukaemias, especially in the face of the relatively low doses of cytotoxic drugs used in veterinary medicine.
ALL is more common in the dog than AML, and might be expected to be more chemoresponsive, but still carries a very poor prognosis. This poor chemosensitivity is likely because the fundamental molecular genetic lesions involved in neoplastic transformation in ALL are different from those in lymphoma, and confer resistance to the actions of most cytotoxic drugs. If treatment of ALL is attempted, any of the protocols used to treat lymphoma may theoretically be used. However, in most cases, neutropenia and thrombocytopenia are limiting and initial treatment will consist of minimally myelosuppressive therapy (vincristine, L-asparaginase and prednisolone) with the best supportive therapy available. The introduction of more myelosuppressive agents is generally postponed until there has been some

Antigen/ clone	Cellular specificity
CD3, CD5	all T cells
CD4	T helper cells, neutrophils
CD8	cytotoxic T cells, natural killer (NK) cells
CD 11d	NK cells/cytotoxic T large granular lymphocytes
CD14	monocytes, macrophages
CD21	B cells
CD34	haemopoietic progenitor cells
CD45	all leukocytes
CD79a	B cells
CD90 (Thy1)	monocytes, macrophages, T cells, some B cells
MPO	neutrophils and their precursors
MAC387	monocytes, macrophages, neutrophils and their precursors

Table 1: Example of markers used in immunophenotyping of canine leukaemia

Lymphoma	Acute Lymphoid Leukaemia
Lower numbers of circulating neoplastic cells	Higher numbers of circulating neoplastic cells
Massive lymphadenopathy	Mild to moderate lymphadenopathy
May not be systemically ill	Usually systemically ill
Mild or no -cytopenias	Severe -cytopenias
Morphologically variable	Morphologically primitive blasts
CD34 negative	CD34 positive (usually)

Table 2: Clinical and clinicopathological differentiation of lymphoma and acute lymphoid leukaemia.

improvement in neutrophil and platelet counts. There are no series of treated canine ALL patients comparing protocols, and there is no favourable survival data, with best reported response rates of 30%. Treatment of AML is even less successful, and there are no well established veterinary protocols. Even with much more aggressive therapy, and much better supportive care, the cure rate in adult human ALL is still only 20-30%, and up to 50% in some subsets of AML. However, outcomes in human patients with acute leukaemia have improved over recent years.

The main reasons for the improvement in survival times for human patients has been development of specialist tertiary care centres, where high dose intensity treatment with traditional chemotherapy drugs can be given with very intensive supportive care. It is unlikely that this level of supportive care will ever be available to canine patients because of the high costs involved, and many complex ethical issues. If we intensify our treatment regimes sufficiently in dogs, it is likely we will be able to induce remission by killing tumour cells, but we will not be able to adequately support patients to ensure acceptable mortality on induction, and an acceptable quality of life during and after induction. If any progress is to be made in the treatment of our patients we need effective therapeutic regimes which do not exacerbate the myelosuppression caused by the disease. Such new therapies can also be combined with the chemotherapy regimes established in human medicine and improve outcome in human patients.

New treatments for acute leukaemias in humans include new chemotherapy drugs and new formulations of old drugs (with greater efficacy and reduced toxicity), monoclonal antibodies to allow cytotoxic selectivity, and drugs directed to molecules in the cell signalling pathways that control cell cycle progression, gene transcription, cell motility, apoptosis and cell metabolism. In human medicine, in addition to clinical, morphological and immunological data, the molecular genetic lesions of a particular leukaemia are used to determine prognosis and also, in some cases, to direct therapy. Arguably the most common target molecules are receptor tyrosine kinases (RTKs), which are transmembrane enzymes activated by ligand binding to the receptor on the cell surface, resulting in relaying of signals into the cell, and activation of downstream effectors.

RTK inhibitors have been the focus of much research, and several compounds are established or under investigation in human clinics. The molecular genetics of the individual patient's cancer are important as these drugs have very specific targets. For example, inhibitors of the RTK *FLT3* are utilised in patients with activating mutations of this gene, which are common in acute myeloid leukaemia in humans. There is little work on the molecular genetics of canine leukaemia, but we have demonstrated *FLT3* mutations in only 3 of 61 dogs with leukaemia.

Another potential target is *ras*. The ras proteins are effectors for most RTKs, relaying signals to the nucleus when the ligand binds to the RTK. Activating mutations of the *ras* gene are found in up to 20% of human ALL and 45% of human AML patients. We have recently identified *ras* mutations in 14 of 36 (39%) dogs with immunophenotypically confirmed acute leukaemias. A number of *ras* inhibitors are in clinical trials, including the farnesyl transferase inhibitor tipiranib (Zarnestra) which is used in relapsed AML patients. Drugs of this type may eventually be available for evaluation in canine patients.

References

1. *Breen, M. & Modiano, J.F. (2008) Evolutionarily conserved cytogenetic changes in hematological malignancies of dogs and humans - Man and his best friend share more than companionship. Chromosome Research 16, 145-154.*
2. *Dobson J., Villiers E.J. & Morris J. (2006) Diagnosis and management of leukaemia in dogs and cats. In Practice 28, 22-31 (basic review)*
3. *Avery A.C. & Avery P.R. (2007) Determining the significance of persistent lymphocytosis. Veterinary Clinics of North America - Small Animal Practice 37, 267-282*
4. *Pui C.H. & Jeha S. (2007) New therapeutic strategies for the treatment of acute lymphoblastic leukaemia. Nature Reviews: Drug Discovery 6, 149-165.*
5. *Villiers, E., Baines, S., Law, A.M. & Mallows, V. (2006) Identification of acute myeloid leukemia in dogs using flow cytometry with myeloperoxidase, MAC387, and a canine neutrophil-specific antibody. Vet Clin Pathol 35, 55-71.*