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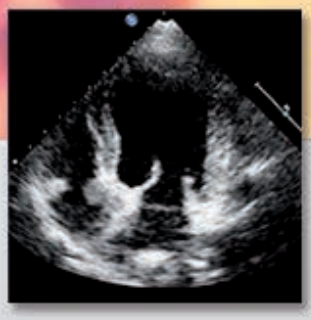
Volume 24(1), Spring 2014

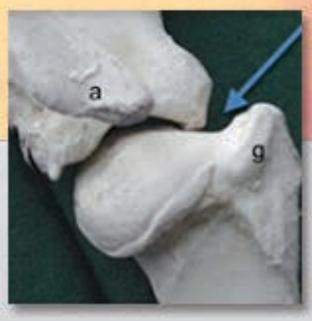
Advances in the therapy of congestive heart failure

Faulty Feathers

Anaesthetic arthrography of the shoulder joint in dogs

Mast Cell Tumour Advances





Treating epilepsy in dogs, Mammary lumps: the impact of spaying, Brain pains, Spindle toxins as chemotherapeutic agents in veterinary medicine, and more ...

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These refer to the species (in green) of animal or the veterinary discipline (in blue) relevant for the article.


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FECAVA LECTURE

Advances in the therapy of congestive heart failure in dogs and cats

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SUMMARY

Myxomatous mitral valve disease (MMVD) and dilated cardiomyopathy (DCM) in dogs, and different forms of cardiomyopathy in cats, may lead to signs of congestive heart failure (CHF). Traditionally, signs of CHF have been treated with a diuretic together with other adjunct therapy, such as digoxin, but very little data from clinical trials was available to support treatment strategies. During the last 20 years, an important paradigm shift has occurred in the demand for data to support our clinical decisions in dogs and cats with heart disease. Today, several drugs have in clinical trials been shown to improve outcome in small animal CHF patients, as indicated by improved quality of life and increased survival time. Not only have the growing number of drug trials provided information on drug efficacy, but these trials have also provided important information on prognostic value of clinical tests and a deeper understanding of disease progression. This article reviews current treatment alternatives from asymptomatic to progressed stages, associated with severe clinical signs of CHF, in dogs with MMVD or DCM and in cats with cardiomyopathy.

Introduction

Dogs and cats with heart disease may progress into congestive heart failure (CHF) at different rates. Some patients slowly exacerbate clinical signs over days or even weeks, with progressive, but slow, increase in respiratory rates. Other patients may rapidly progress from asymptomatic heart disease into severe acute congestive heart failure (CHF). However, dogs and cats previously stabilized on CHF therapy may also exacerbate clinical signs into severe life-threatening CHF, leading to the pet being admitted into the emergency ward. The American College of Veterinary Internal Medicine (ACVIM) proposed a new scheme of classification of heart failure, with classes ranging from A to D, where A represents patients free of heart disease, but belonging to a risk group for developing it, and D represents patients with severe refractory heart failure (Fig. 1) (Atkins, Bonagura et al. 2009).

Although this scheme was intended for dogs with myxomatous mitral valve disease (MMVD), it may, to some extent, be applicable to dogs with dilated cardiomyopathy (DCM) or cats with cardiomyopathy. For instance, some dogs with DCM, particularly Doberman Pinschers, may present with arrhythmias causing clinical signs but with comparably normal radiographs and echocardiograms, and the ACVIM classification scheme may not be useful in classifying these dogs.

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STAGE A	STAGE B1	STAGE B2	STAGE C	STAGE D
Risk of heart disease (HD); no structural HD documented; e.g.: genetics, concurrent systemic disease with cardiovascular implications	HD documented; no signs of cardiomegaly or CHF present	HD documented; signs of cardiomegaly present but no CHF present	**Past or current clinical signs of objectively documented CHF** Acute CHF +/- low output signs requiring hospitalizaion Clinical signs mild enough to allow home therapy or dogs previously stabilized during hospitalization for acute CHF	Refractory CHF not responding to maximal/optimum medical therapy (ancillary methods needed to keep animal alive)

Figure 1. ACVIM Consensus statement classification of canine congestive heart failure (Atkins, Bonagura et al. 2009).

Furthermore, we have grouped the different forms of feline cardiomyopathies, (Ferasin 2009) i.e. hypertrophic, dilated, restrictive, or unclassified into one group, partly for simplicity, but also because it is incompletely known if the different forms of cardiomyopathy benefit from different types of CHF therapy (Ferasin 2009).

Risk populations (Class A)

This group denotes individuals without heart disease, but belonging to a risk population for developing it. No therapy is indicated at this stage, but screening for heart disease is typically conducted in this category of dogs and cats.

Asymptomatic heart disease (Class B)

Class B identifies patients with structural heart disease, but that have never developed clinical signs caused by CHF. Because of important clinical implications for prognosis and treatment in dogs with MMVD, the ACVIM panel further subdivided Stage B into Class B1 and B2. Class B1 refers to asymptomatic patients that have no radiographic or echocardiographic evidence of cardiac remodelling in response to MMVD. Class B2 refers to asymptomatic patients that have haemodynamically significant heart disease, as evidenced by radiographic or echocardiographic findings of left-sided heart enlargement.

Myxomatous mitral valve disease

Several drug trials have been conducted in Stage B MMVD dogs, but none of these studies could show a prophylactic effect in delaying the onset of CHF.

The ACE-inhibitors are by far the most intensively studied pharmacological modality in Class B MMVD dogs. The currently available database for this type of therapy in Class B MMVD dogs includes a number of clinical trials, which in veterinary medicine is to be regarded as substantial documentation. The rationale for using this type of therapy is that suppression of the renin-angiotensin-aldosterone system (RAAS) has the potential to lead to a more favourable haemodynamic situation by vasodilatation of systemic arteries, by counteracting fluid retention and by counteracting the progressive left ventricular and left atrial remodelling process occurring in response to mitral regurgitation (MR).

Two prospective placebo controlled clinical trials in dogs (the SVEP and the VetProof studies) (Kvart, Häggström et al. 2002, Atkins, Keene et al. 2007) investigated the effects of enalapril compared to placebo in delaying the onset of CHF in dogs with MMVD. The two trials showed a comparably similar outcome with a non-significant difference in the primary outcome variable (time from initiation of therapy until diagnosed CHF) between the two treatment groups (Fig. 2). These are interesting findings because one trial only included 229 Cavalier King Charles Spaniels (the SVEP trial) in Class B1 (n=122) and B2 (n=107) (Kvart, Häggström et al. 2002), whereas the other included 124 dogs of multiple breeds (the VetProof study) in Class B 2 only (Atkins, Keene et al. 2007), and results indicate a similar response to ACE-inhibitor therapy and rate of progression in the two populations. Currently, there are other large clinical trials on-going where the prophylactic effect of substances other than ACE-inhibitors are tested versus placebo in dogs in stage B MMVD, but results are not expected to be available in the near future.

Dilated cardiomyopathy

For dogs with DCM, a retrospective study including 91 dogs

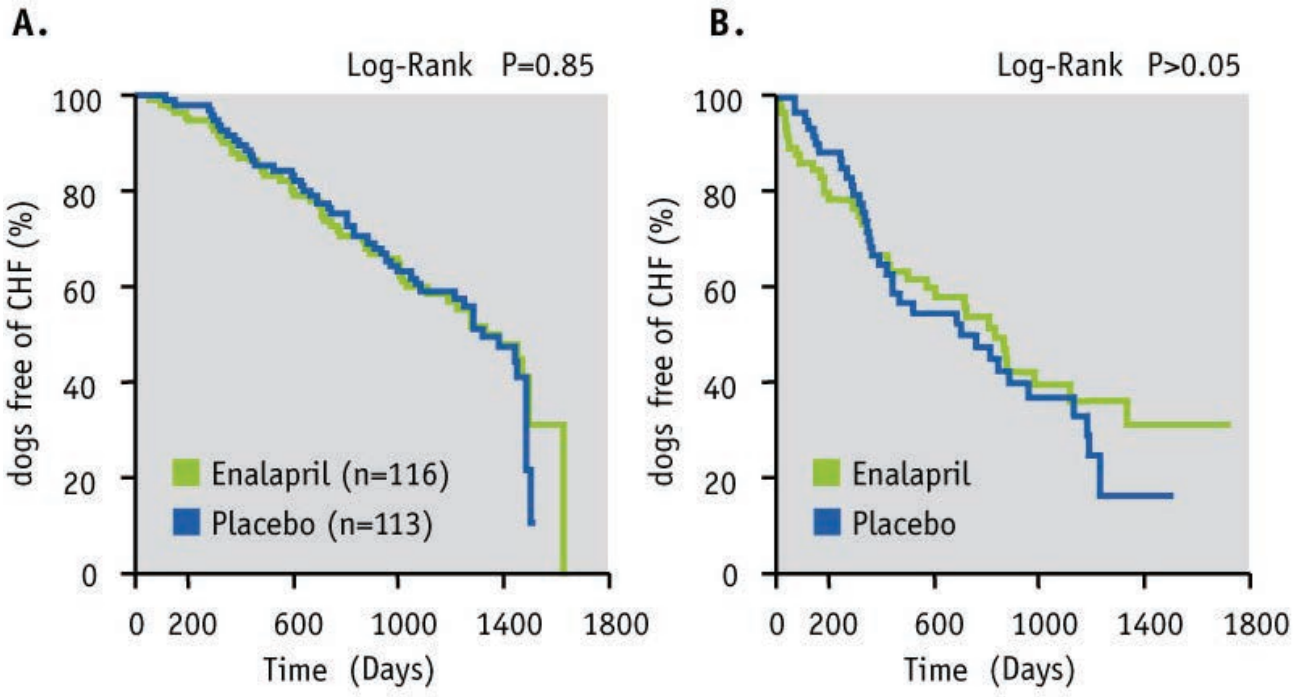

Figure 2. (A) Kaplan Meier curves from the SVEP-trial, which included 229 Cavalier King Charles Spaniels with MMVD in Class B1 and B2 randomly allocated to either monotherapy with enalapril or placebo (Kvart, Häggström et al. 2002). The endpoint was when CHF was diagnosed. The median CHF-free time was 1150 days for dogs in the enalapril group and 1130 days in the placebo group, and this difference was not significant (Log-rank test P=0.85). (B) Kaplan Meier curves from the VetProof-trial, which included 139 MMVD dogs in Class B2 randomly allocated to either monotherapy with enalapril or placebo. (Atkins, Keene et al. 2007) The endpoint was when CHF was diagnosed. The median CHF-free times for treatment and placebo groups were 851 and 778 days, respectively (P > 0.05). Reproduced with permission from publisher.

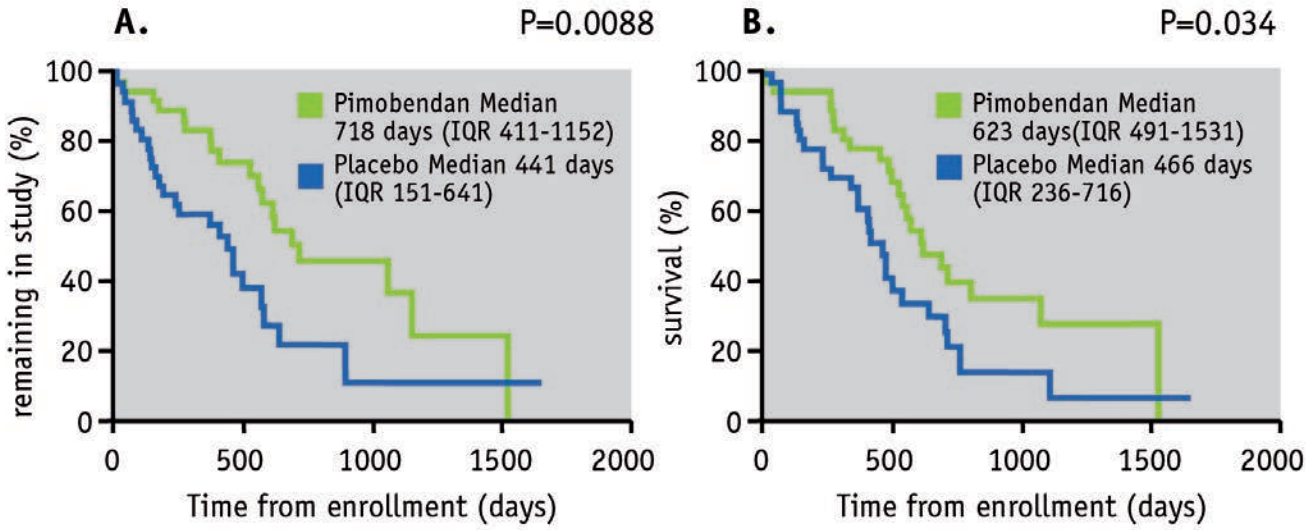

Figure 3. Kaplan Meier survival curves from the PROTECT study, which included 76 Doberman Pinschers with asymptomatic (Class B) DCM randomly allocated to pimobendan monotherapy or placebo (Summerfield, Boswood et al. 2012). (A) shows the estimated percentage of dogs in each group that have not yet met the primary endpoint, which was a composite endpoint of CHF or sudden death, against time. (B) shows survival curves for the all-cause mortality analysis, plotting the estimated percentage of surviving dogs in each group, against time. MST, median survival time; IQR: interquartile range. Reproduced with permission from publisher.

(57 received benazepril and 34 received no ACE-inhibitor) suggested that the ACE-inhibitor benazepril delayed the onset of CHF in Doberman Pinschers with asymptomatic (Class B) DCM (O'Grady, O'Sullivan et al. 2009).

More recently, the results from a clinical placebo-controlled, parallel group multicentre study, the PROTECT Study (Summerfield, Boswood et al. 2012) was published. This study comprised Doberman Pinschers recruited in the UK and North America (Fig. 3). The composite primary endpoint was defined as either onset of CHF or sudden death, and time to death from all causes was a secondary endpoint. Pimobendan administered to Doberman Pinschers with DCM in Class B prolonged the time to the onset of CHF or sudden

death (Median 718 days, versus the placebo group 441 days, P = 0.0088), and increased survival time (Median 623 days versus the placebo group 466 days, P = 0.034). Furthermore, pimobendan conferred a reduction in cardiac size, a reduction that appeared to be a primary effector for the prolonged asymptomatic period and survival. Although the PROTECT study was restricted to Doberman Pinschers, the results speak in favour of treating dogs of other breeds with DCM in Class B with pimobendan.

Finally, there is evidence that some Cocker spaniels develop a form of DCM responsive to taurine and carnitine supplementation (Kittleson, Keene et al. 1997). Taurine supplementation is typically given at a dose of 500 mg/dog PO q12h.

Feline Cardiomyopathy

As in dogs with MMVD, there are no clinical trials showing that cats with asymptomatic (Class B) cardiomyopathy benefit from medical therapy, the exception being feline DCM induced by taurine deficiency (see below, Pion, Kittleson et al. 1987).

Several drugs have been suggested to be beneficial in this setting and they include beta-receptor antagonists, such as atenolol 6.25-12.5 mg/cat PO q12h, ACE-inhibitors (type and dose varies) and diltiazem at 7.5 mg/cat PO q8h. Cats diagnosed with DCM should be evaluated for presence of taurine deficiency, and receive taurine supplementation if needed.

Many specialists initiate antithrombotic therapy in cats with cardiomyopathy in Class B and left atrial enlargement, because this finding has been associated with an increased risk of a thromboembolic event (Smith, Tobias et al. 2003, Payne, Luis Fuentes et al. 2010, Trehiou-Sechi, Tissier et al. 2012). Typical drugs used in this scenario include aspirin given at a dose of 75 mg/cat or 5 mg/cat PO q72h, but clopidogrel at 18.75 mg/cat PO q24h has become much more popular since the results of the FATCAT study was presented as an abstract (Hogan, Fox et al. 2013). This study was designed as a blinded prospective study where the rate of re-thrombosis in cats previously diagnosed with aortic thromboembolism were compared in cats receiving aspirin and in cats receiving clopidogrel. The outcome of the trial was that cats receiving clopidogrel had a more favourable outcome. (Hogan, Fox et al. 2013)

Symptomatic heart disease (Class C)

Class C of the ACVIM classification denotes dogs and cats that have developed signs of CHF and/or low output signs (e.g. exercise intolerance, weakness, syncope). Congestive signs predominate and are indicative of pulmonary congestion and oedema that have developed when disease progression and neurohumoral activation are such that compensatory mechanisms can no longer maintain pulmonary venous pressures at a level to prevent pulmonary oedema. Veterinary patients with severe signs of CHF require hospitalization for stabilization with the goal of chronic management at home.

Very few studies are available on efficacy of medical treatments for acute CHF and treatment modalities are often based on clinical experience. On the other hand, the vast majority of clinical trials in veterinary cardiovascular medicine concern long-term oral therapy of Class C MMVD (dogs with mild to moderate signs of CHF or those having been stabilized during hospitalization). The data in this category of veterinary patients is relatively bountiful, although the strength of evidence for beneficial actions of drugs used in small animal cardiovascular medicine in terms of size of study populations in the clinical trials and meta-analysis, when possible, is generally weaker than in human medicine.

Myxomatous mitral valve disease

ACVIM Class C includes dogs with clinical signs of acute CHF, secondary to severe MMVD, requiring hospitalization. Class C patients in acute CHF may exhibit signs of CHF for the first time on the day of presentation or their signs may be recurrent. The crisis may represent exacerbation due to disease progression; a complication (such as rupture of major chordae tendinae, ruptured left atrium [these dogs present with signs of low-output failure] or onset of arrhythmia such as ventricular tachycardia or atrial fibrillation); or be the result of excessive exercise, or fluid administration. In general, therapy for these dogs is aimed at alleviating clinical signs by regulating the patient's hemodynamic status, optimizing preload, afterload, heart rate and myocardial contractility, to relieve clinical signs associated with increased venous pressures and low forward cardiac output. Some animals may experience severe anxiety due to the presence of CHF and sedation may be indicated.

The ACVIM Consensus Panel reached consensus for the following measures (Atkins, Bonagura et al. 2009):

- **Furosemide** administered IV, IM, or SC either as bolus doses in the range of 1-4 mg/kg q 2-6h, or as constant rate infusion (CRI) (in dogs with severe signs of CHF and poor response to bolus administration) at 1-2 mg/kg/h. Treatment success is monitored by assessment of respiratory rate and severity of dyspnoea. Intensity of furosemide therapy is adjusted in accordance with change in the patient's condition. Furosemide is used to decrease capillary and venous pressures by contracting the extracellular fluid compartment and reducing cardiac filling pressures (preload), thereby reducing the tendency for pulmonary oedema and cavitary effusions. However, although loop-diuretics were included in the vast majority of veterinary clinical trials, there are no controlled clinical studies comparing either furosemide or torasemide with either placebo or another diuretic agent in ACVIM Class C dogs.
- **Pimobendan** PO at a dosage of 0.25-0.3 mg/kg q12h. Pimobendan is an "inodilator", which means that the drug acts as a vasodilator and a positive inotrope, and these effects are mediated through suppression of phosphodiesterase III and by sensitizing the myocardial contractile proteins to calcium ions (Pagel, Hettrick et al. 1996, Pagel, Hettrick et al. 1996). The clinical documentation of pimobendan's efficacy in acute CHF dogs is limited, but its use here is supported by one clinical study (Fig. 4) (Häggström, Lord et al. 2013) its haemodynamic actions, (Pagel, Hettrick et al. 1996, Pagel, Hettrick et al. 1996) experimental studies (pacing model) in dogs, (Ohte, Cheng et al. 1997) and clinical experience. An injectable form of pimobendan is currently being launched in many countries and this formulation is likely to be used in dogs with acute CHF. Although myocardial dysfunction does develop in long-standing MMVD and MR, it is less easily documented clinically than in diseases with profound myocardial failure, such as DCM. Inotropic agents may, therefore,

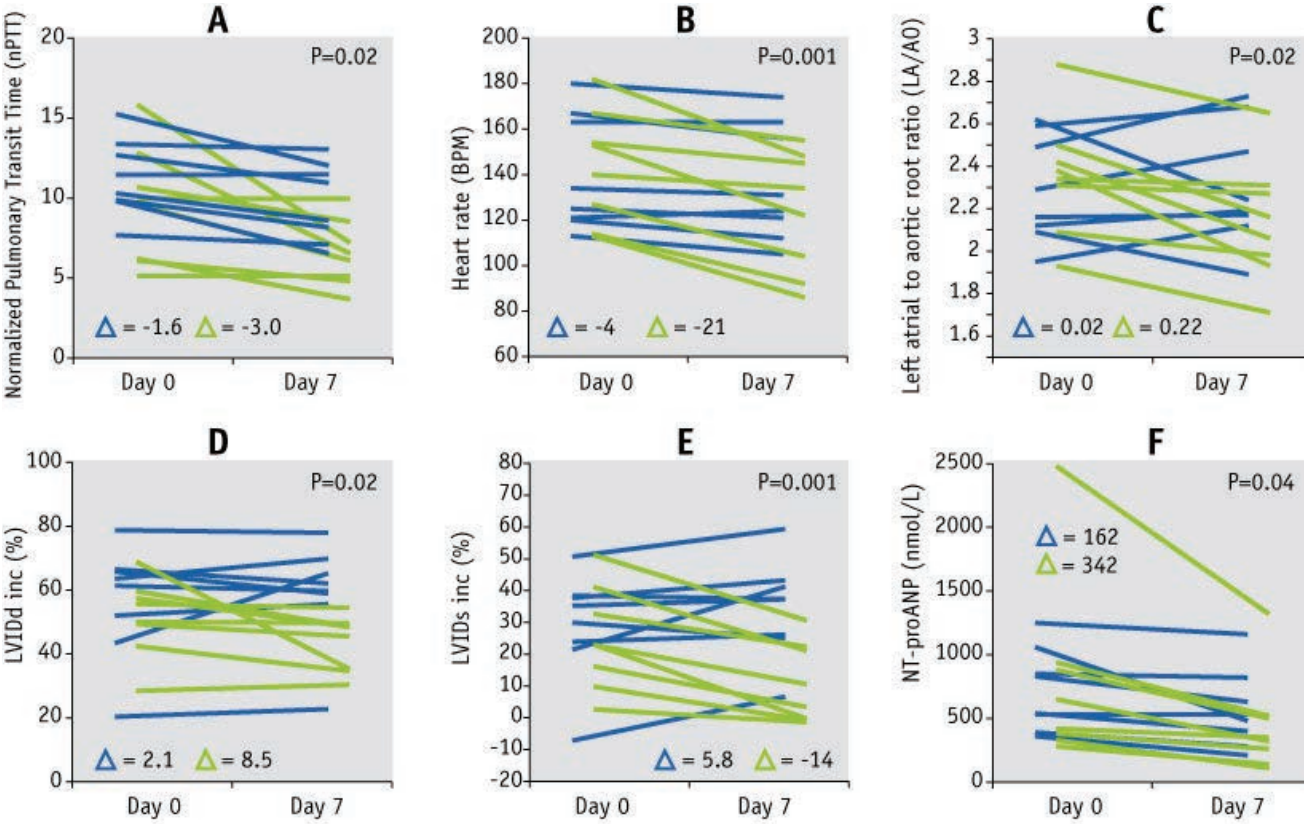

Figure 4. Changes in (A) heart rate normalized pulmonary transit time (nPTT), (B) heart rate, (C) Left atrial to aortic root ratio (LA/Ao), (D) percentage increase from expected value in left ventricular internal diameter in diastole (LVIDd inc.), (E) percentage increase from expected value in left ventricular internal diameter in systole (LVIDs inc.) and (F) plasma concentrations of N-terminal pro-atrial natriuretic peptide (NT-proANP) in 16 dogs with CHF due to MMVD. Green lines indicate dogs receiving pimobendan and blue lines indicate dogs receiving benazepril. negative values indicate group mean change over the 7-day period, where negative values indicate a decrease for that particular variable. Pimobendan, in comparison to benazepril, conferred a reduction in all 6 variables over the 7-day period. From Häggström et al. 2013 (Häggström, Lord et al. 2013) reproduced with permission from publisher.

seem less logical in this setting. Their supposed efficacy is likely due to several factors. First, there is evidence for systolic dysfunction in moderate to severe MMVD (Kittleson, Eyster et al. 1984, Borgarelli, Tarducci et al. 2007). Secondly, inotropic agents are not limited to improving contractility, as they provide beneficial effects on diastolic function and reduction of afterload (Pagel, Hettrick et al. 1996, Pagel, Hettrick et al. 1996). Lastly, inotropic agents reduce mitral valve orifice size, thereby reducing MR (Keren, Laniado et al. 1989).

- **Oxygen supplementation**, if needed, can be administered via a humidity and temperature-controlled oxygen cage or incubator or via a nasal oxygen cannula.
- Mechanical treatments (e.g. abdominal **paracentesis** and **thoracocentesis**) are recommended to remove effusions judged sufficient to impair ventilation or cause respiratory distress.
- Provide **optimal nursing care**, including maintenance of an appropriate environmental temperature and humidity, elevation of the head on pillows, and placement of sedated patients in sternal posture.

However, no consensus was reached for the following pharmacological measures:

- ACE-inhibitors. Although the ACE-inhibitors have been extensively studied in chronic management of MMVD in dogs, their efficacy and safety in acute CHF is less clear. Pharmacokinetic studies demonstrate an onset of action within 1-3 hours for orally administered benazepril as well as enalapril (Hamlin and Nakayama 1998). One study reports of a reduction in capillary wedge pressure when an ACE-inhibitor was added to furosemide, compared to furosemide alone (IMPROVE 1995).
- Nitro-glycerine ointment. This drug is used for its venodilatory (off-loading) effects, causing blood to pool in splanchnic vessels, thereby allowing for dissipation of pulmonary oedema fluid as preload falls. The clinical documentation of this type of therapy is limited to one report of increased splenic capacity in response to nitro-glycerine ointment in normal dogs. (Parameswaran, Hamlin et al. 1999)
- Dobutamine. This agent, a synthetic sympathomimetic, produces improvement in cardiac performance (myocardial contractility) by combining primarily with myocardial beta-1 receptors (Vatner, McRitchie et al. 1974, Kittleson 1980). Disadvantages of this drug include the short half-life, requiring that it be used as a CRI, and the fact that down-regulation of Beta-1 receptors occurs within 48-72 hours after initiation of therapy (Vatner, McRitchie et al. 1974, Kittleson 1980), rendering the drug ineffective after this time. There are no clinical trials involving dobutamine in naturally occurring canine heart disease.
- Hydralazine. This drug is an arteriolar dilator (Kittleson, Eyster et al. 1983). By reducing left ventricular afterload, MR as well as left atrial and left ventricular volumes and pressures may be reduced, with improvement in forward cardiac output. In canine patients with MR and refractory CHF, oral hydralazine reduced systolic blood pressure, systemic vascular resistance, pulmonary capillary wedge pressure, and radiographic evidence of pulmonary oedema (Kittleson, Eyster et al. 1983, Nakayama, Nishijima et al. 2007). These circulatory changes are associated with increased RAAS activity (Häggström, Hansson et al. 1996) and dogs receiving this type of therapy should be monitored carefully for hypotension (Atkins, Bonagura et al. 2009).

Dilated Cardiomyopathy

Treatment of dogs with DCM in acute CHF is comparably similar to strategies outlined above for MMVD dogs, with a few exceptions. Dogs with DCM in acute CHF are more likely to also present with severe signs of low-output heart failure as a consequence of low forward cardiac output. Therefore, these dogs are likely to benefit more from inotropic support. Because dogs with DCM may present with low-output heart failure, DCM dogs with acute CHF are usually not treated with a vasodilating agent, such as hydralazine. Furthermore, these dogs are more likely to present with a haemodynamically significant arrhythmia, such as atrial fibrillation and/or ventricular tachycardia. These arrhythmias need management if the CHF therapy is to be successful.

Frequently used drugs for managing atrial fibrillation are the combination of digoxin at 0.22mg/m2 PO q12h and diltiazem 0.5-1.5 mg/kg PO q8h, and for ventricular arrhythmias, lidocaine IV bolus at 1-2 mg/kg (maximum 8 mg/kg) or infusion at 40-80 mcg/kg/min, or mexilitine PO 4-8 mg/kg q8-12h, or sotalol PO 0.5-2 mg/kg q12h or less frequently amiodarone PO at 10 mg/kg q12h loading dose then 5-8 mg/kg q24h. Some of these anti-arrhythmic drugs have a negative inotropic effect, which needs consideration in the management of DCM dogs in acute CHF.

Feline cardiomyopathy

Cats with cardiomyopathy in acute CHF need special consideration. Some of these cats have very severe signs of dyspnoea, causing the cat to panic. Therefore, some clinical tests, such as thoracic radiographs may have to wait. Ideally, an IV cannula should be placed, but, again, this may have to wait until the cat is stabilized. Sedation may be indicated. Furosemide can also be administered IM or SC and the cat placed in an oxygen tent. Like dogs, treatment success is monitored through measuring respiratory rate and severity of dyspnoea. The following treatments may be considered in cardiomyopathic cats with acute CHF:

- Furosemide (IV, IM, SC, 1-4 mg/kg/2h, then reduce to 1-2 mg/kg or CRI 4-6 mg/kg over 24 h in case of poor response). Cats are more likely to develop signs of dehydration and electrolyte disturbances when intensively treated with furosemide. Cats should therefore be monitored for development of such adverse side reactions and treated accordingly.
- Oxygen supplementation, if needed, can be administered via a humidity and temperature-controlled oxygen cage or cage/tent oxygenator
- Mechanical treatments (e.g. thoracocentesis and abdominal paracentesis) are recommended to remove effusions judged sufficient to impair ventilation or cause respiratory distress
- Pimobendan (0.625 to 1.25 mg/cat q12 h PO). Pimobendan is currently not approved for use in cats in the EU or USA. Some specialists only use pimobendan in cats with CHF but without evidence of left ventricular outflow obstruction, whereas others use it in all cats with cardiomyopathy in CHF. The use of pimobendan in cats with cardiomyopathy is currently not supported by clinical trials, and only the pharmacokinetic characteristics (Hanzlicek, Gehring et al. 2012), and case series have been published (Macgregor, Rush et al. 2011, Gordon, Saunders et al. 2012, Hambrook and Bennett 2012). These series suggest that pimobendan is well tolerated in cats. The effect of pimobendan in cats is currently studied in an on-going clinical trial.
- Nitro-glycerine ointment (2%) (for venodilation, 6 mm cutaneous (earflap) q12h, wear gloves)
- As needed, antiplatelet/anticoagulants, such as aspirin or clopidogrel (for dosages, see under Class B cardiomyopathic cats) or unfractioned heparin SC at 150/250 mcg/kg q6-8h or CRI 20-50 mcg/kg/h at a low fluid rate, or low-molecular heparin, such as dalteparin SC at 100-180 IU/kg q12-24h, or enoxaparin SC at 1.25 mg/kg q12h (Smith, Rozanski et al. 2004, Alwood, Downend et al. 2007).

Cats in acute CHF may present in cardiogenic shock, which is defined as hypothermia (<37°C), often bradycardia and systolic arterial pressure (SAP) <70 mmHg. These cats need special treatments and the following measures can be considered:

- Passive warming (lamp warming, blankets, etc.)
- Dobutamine infusion (start 2.5 µg/kg/min increase to 5-10 µg/kg/min)
- Pimobendan (0.625 to 1.25 mg/cat q12 h PO)
- Furosemide/thoracocentesis (used as appropriate)

Home therapy

Dogs and cats with CHF may present with clinical signs mild enough to allow home therapy, or they may have been previously stabilized during hospitalization for acute CHF. These patients can typically be managed with multiple oral medications. Most clinical studies in veterinary cardiovascular medicine are aimed at investigating pharmacological effects and outcomes in this disease category. Drugs used in this setting include the diuretics furosemide, torasemide, spironolactone and hydrochlorothiazide; positive inotropes/calcium sensitizers, such as pimobendan; anti-RAAS therapy with ACE-inhibitor and/or spironolactone; positive inotropes with negative chronotropic properties such as digoxin; vasodilating drugs, such as amlodipine and hydralazine; and beta-adrenergic antagonists.

Myxomatous mitral valve disease

The ACVIM panelists recommended the following agents for home therapy (so-called “triple therapy”) for Class C dogs by consensus:

- **Furosemide** (wide dosage range 1–2 mg/kg PO q12h to 4–6 mg/kg PO q8h)
- **Pimobendan** (0.25–0.3 mg/kg q12h)
- **ACE-inhibitors** (e.g. enalapril 0.5 mg/kg PO q12h)

No consensus was reached for the following drugs:

- Spironolactone (0.25–2.0 mg/kg PO q12–24h) (Consensus statement published before the spironolactone clinical trial (Bernay, Bland et al. 2010))
- Digoxin (0.22 mg/m2 q12h PO) both for treatment of heart failure and rate control in atrial fibrillation
- Beta-adrenergic receptor antagonists (a minority of the panel advocated using these drugs after the CHF

was controlled; the presence of atrial fibrillation strengthens the opinion of these panellists, as beta-adrenergic receptor blockers will help to slow the ventricular response to atrial fibrillation as well as potentially slowing disease progression).

- Some panellists would initiate diltiazem in patients with atrial fibrillation for ventricular rate control; it is now known that the combination of digoxin and diltiazem provides better rate control in atrial fibrillation than either agent alone (Gelzer, Kraus et al. 2009).
- Cough suppressants, such as hydrocodone PO at 0.22 mg/kg q8-24h
- Bronchodilators, such as aminophylline PO at 10mg/kg q8h, sustained release theophylline PO at 20 mg/kg q24h PO, or terbutaline PO at 0.2 mg/kg q12h.

Clinical trials in MMVD Class C dogs (long-term therapy)

Pimobendan

Veterinary clinical studies concerning pimobendan in dogs with CHF attributable to MMVD have shown improved quality of life (QoL) variables and a reduced risk for adverse events (death, recurrent CHF or additional veterinary visits etc.) when compared to a positive control (ramipril) (Smith, French et al. 2005, Lombard, Jöns et al. 2006). To study the effect of pimobendan on survival in dogs with CHF secondary to MMVD, the QUEST (Quality of life and Extension of Survival Time) study was undertaken (Häggström, Boswood et al. 2008). The aim of the study was to compare the time taken to reach the primary endpoint for dogs receiving either pimobendan or benazepril in conjunction with other therapy. The primary endpoint was a composite of spontaneous cardiac death, euthanasia for cardiac reasons or withdrawal from the study due to treatment failure. Two-hundred and sixty dogs were recruited at 28 centres in Europe, Canada, and Australia, making it the largest prospective, blinded survival study so far undertaken in canine cardiology. Dogs were of small and medium-sized breeds, and were only included in the study if they had, at some point, demonstrated convincing radiographic evidence of left-sided CHF. After enrolment in the study the dogs were randomised to receive either pimobendan, plus standard therapy or benazepril plus standard therapy. They were then re-evaluated at regular intervals and followed until they reached the study endpoint, were censored from the study for other reasons, or the study was concluded (whichever occurred first). Dogs receiving pimobendan plus standard therapy had a longer survival time compared to those receiving benazepril plus standard therapy (pimobendan 267 days versus benazepril 140 days, P=0.0099) (Fig. 5). The benefit of pimobendan persisted after adjusting for all baseline variables (fig. 6). Several other baseline variables were shown to influence survival time. Most of these were direct or indirect indicators of disease severity.

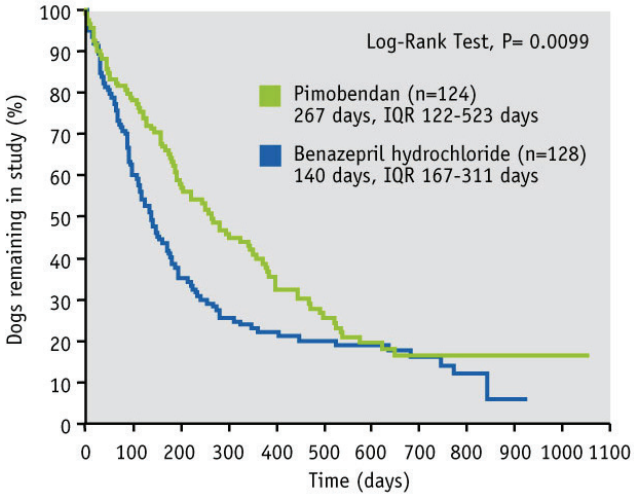

Figure 5. Kaplan-Meier plot of percentage dogs in the QUEST trial (Häggström, Boswood et al. 2008) as a function of time in 124 dogs treated with pimobendan and in 128 dogs treated with benazepril. The trial included only MMVD dogs in ACVIM Class C and the endpoint was a composite of cardiac related death, euthanasia and defined treatment failure. The pimobendan dogs had a significantly longer time period to reach the primary endpoint compared to the benazepril treated (pimobendan 267 days, IQR 122-523 days vs. benazepril 140 days, IQR 67-311 days; P=0.0099). Reproduced with permission from publisher.

Analysis of longitudinal QUEST trial data showed that pimobendan results in a similar quality of life for the duration of the period of treatment (Häggström, Boswood et al. 2013). However, results showed that pimobendan therapy reduced heart size (Vertebral heart scale (VHS), Left ventricular diameter in systole and diastole corrected for body weight) (Fig. 7) and resulted in a higher body temperature, sodium concentration, total protein and packed cell volume by comparison with benazepril. The differences between the treatment groups in the latter 3 variables indicate less retention of free water in the pimobendan-treated dogs. Furthermore, the necessity for intensification of concurrent therapy in patients in the QUEST study was evaluated by looking at the time to intensification of therapy and the frequency of use of commonly used concurrent medications. The reason an investigator decided to increase the dose of one or more of the baseline therapies or add another agent was

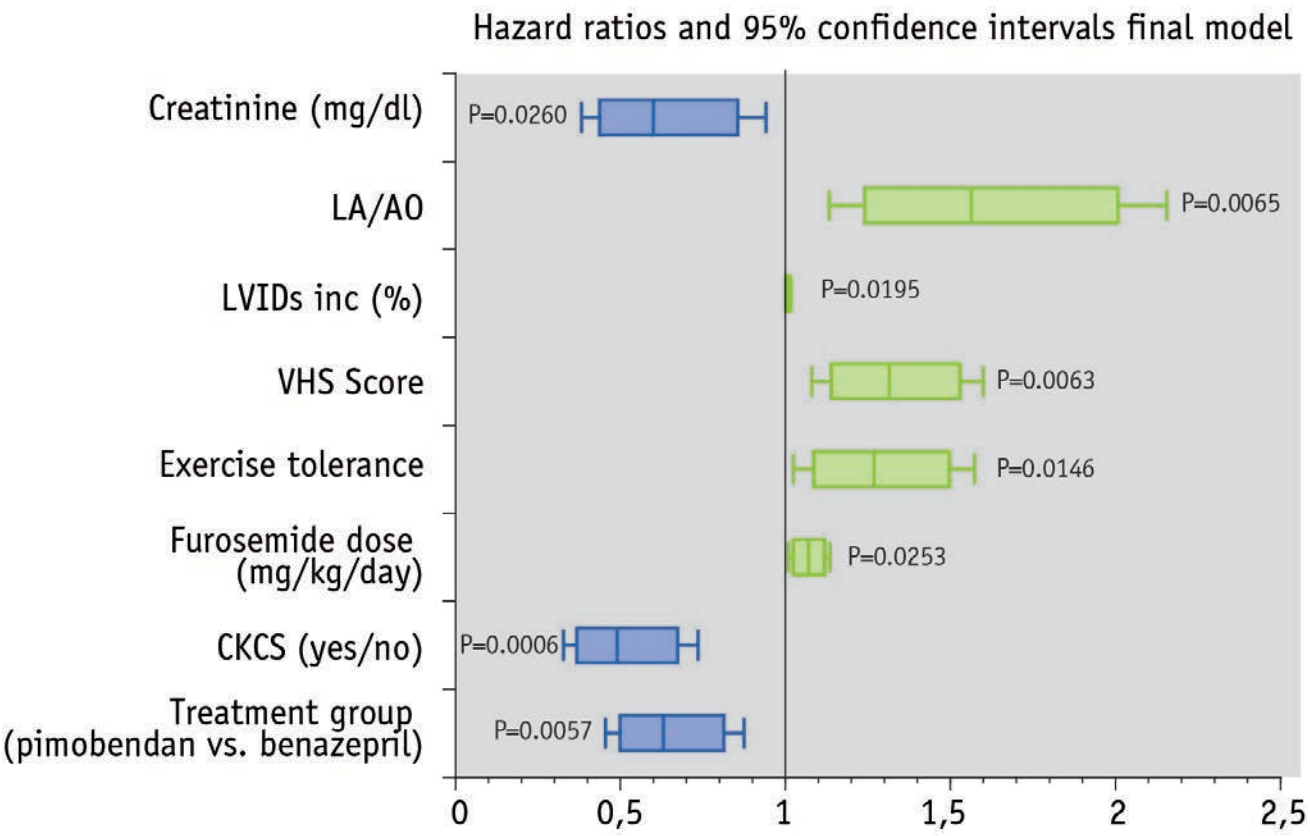

Figure 6. Hazard ratios and 95% confidence limits obtained from the final model of the backwards multivariate Cox Proportional Hazard analysis including possible confounding baseline variables in 252 dogs with MMVD in ACVIM Class C included in the QUEST trial (Häggström, Boswood et al. 2008). Factors associated with a reduction in hazard ratio included pimobendan treatment, the breed Cavalier King Charles Spaniel (CKCS), and increased serum creatinine concentrations. Variables associated with an increased hazard ratio included higher increased daily furosemide dose, worse exercise tolerance score, higher increased vertebral heart scale (VHS) score, percentage increase from expected value in left ventricular internal diameter in systole (LVIDs inc.) and left atrial to aortic root ratio (LA/Ao). Reproduced with permission from publisher.

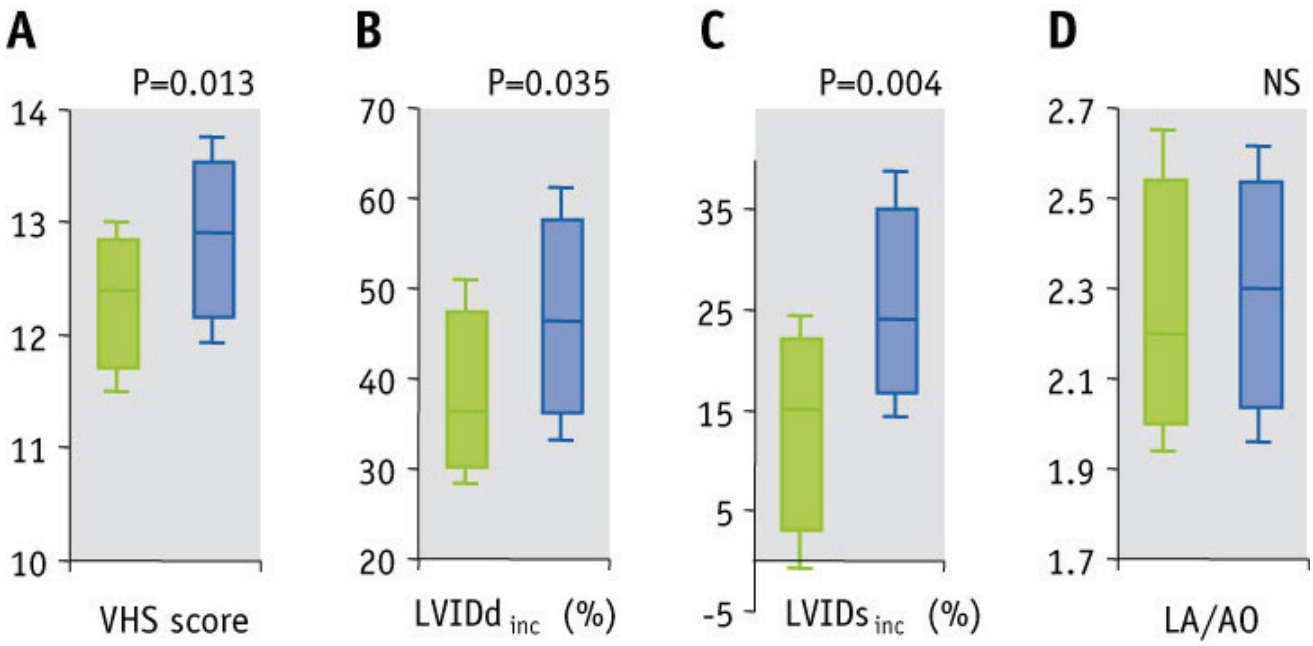

*Figure 7. Median area under curve (AUC) adjusted for days and interquartile range (IQR) for dogs with MMVD in ACVIM Class C included in the QUEST trial illustrated by box and whisker plots, where the pimobendan group is coloured blue and benazepril group is coloured green (Häggström, Boswood et al. 2013). Dogs treated with pimobendan significantly smaller heart sizes during the trial in comparison with benazepril treated dogs, as indicated by (**A**) vertebral heart scale scores (VHS), (**B**) percentage increase from expected value in left ventricular internal diameter in systole (LVIDs inc) and (**C**) diastole (LVIDd inc). (**D**) Left atrial to aortic root ratio (LA/Ao) was similar in the two treatment groups.*

because he/she believed the dog would benefit from the change due to a deterioration in the condition of the dog or the owner being concerned that their pet was failing to achieve an adequate QoL on their current treatment regimen. Thus although the average values for QoL variables were similar in the two treatment groups over the duration of the study, dogs in the benazepril group required intensification of treatment earlier to maintain their QoL (Fig 8). The similarity of QoL in the two groups is therefore probably a consequence of investigators being at liberty to modify concurrent medication to maintain an acceptable QoL for their patients. Thus, the results showed that the benazepril group required alteration of their therapy sooner and more frequent administration of other medications (spironolactone and digoxin) to maintain the same quality of life as dogs receiving pimobendan (Häggström, Boswood et al. 2013).

ACE inhibitors

By far the most thoroughly studied class of drugs in the veterinary cardiovascular armamentarium is the ACE-inhibitor, particularly in CHF caused by MMVD. In fact, the introduction of the ACE-inhibitors to the veterinary market in the early 1990s and the clinical trials, upon which market approval was based, represented a landmark in the advancement of evidence based veterinary medicine.
At present, the results from 4 prospective placebo-controlled or comparative clinical trials (COVE 1995, IMPROVE 1995, Ettinger, Benitz et al. 1998, BENCH 1999, Lombard, Jöns et al. 2006, Häggström, Boswood et al. 2008) and 5 other open label trials (Häggström, Hansson et al. 1996, Sent, Haarer-Kindler et al. 2000, Sent, Haarer-Kindler et al. 2000, Amberger, Chetboul et al. 2004, Moesgaard, Pedersen et al. 2005) and a plethora of experimental studies have been published. Although these studies include different ACE-inhibitors (e.g. enalapril, benazepril, ramipril, imidipril), each agent is a so-called second generation ACE-inhibitor, which is converted in the liver to an active metabolite. These agents have plasma half-lives of approximately 8-16h (Hamlin and Nakayama 1998). Some (benazepril and enalapril) do differ slightly with regards to tissue penetration and route of excretion (Lefebvre, Laroute et al. 1999).

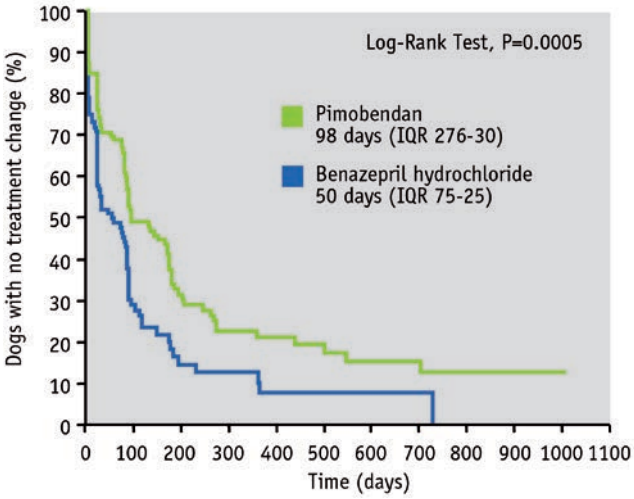

Figure 8. Kaplan-Meier plot of percentage dogs in the QUEST trial (Häggström, Boswood et al. 2008) with no intensification of heart failure therapy as a function of time in 124 dogs treated with pimobendan and in 128 dogs treated with benazepril. Dogs receiving pimobendan had a significantly longer time period (median 98 days, IQR 30–276 days) before experiencing any intensification their treatment compared to those receiving benazepril (59 days, IQR 11–121 days)(p = 0.0005). Reproduced with permission from publisher.

Clinical studies show that ACE-inhibitor are indicated in advanced MMVD with CHF, as adjunct therapy to diuretics, because dogs receiving an ACE-inhibitor benefitted in terms of clinical signs, exercise tolerance (COVE 1995, IMPROVE 1995, Hamlin, Benitz et al. 1997) and increased time until death, euthanasia or treatment failure, when compared to dogs not receiving an ACE-inhibitor (Fig. 9) (Ettinger, Benitz et al. 1998, BENCH 1999). Adverse side reactions are rare in the dose range that is recommended for dogs (DeLellis and Kittleson 1992, COVE 1995, Ettinger, Benitz et al. 1998, BENCH 1999, Kvart, Häggström et al. 2002, Atkins, Keene et al. 2007). One reason for a low rate of adverse side reactions is that the ACE-inhibitors are comparatively weak in comparison to other vasodilators, such as amlodipine or hydralazine.

ACE-inhibitors are particularly well suited in Class C because they theoretically blunt vasodilator- and loop diuretic-induced RAAS activation (Atkins, Rausch et al. 2007) although recent work has shown that this might be less than anticipated with furosemide-induced RAAS activation.(Lantis, Atkins et al. 2010).

Spironolactone

Spironolactone, a relatively old drug, experienced a revival after being shown in the RALES study to improve survival in people with severe CHF (Pitt, Zannad et al. 1999).
The rationale for using spironolactone in dogs (and people) with CHF includes both its (weak) diuretic effect and possibly an anti-fibrotic effect. Moreover, mineral corticoid receptor blockers have been suggested useful in blocking cardiac remodelling, electrical remodelling, reducing vascular and renal fibrosis, as well

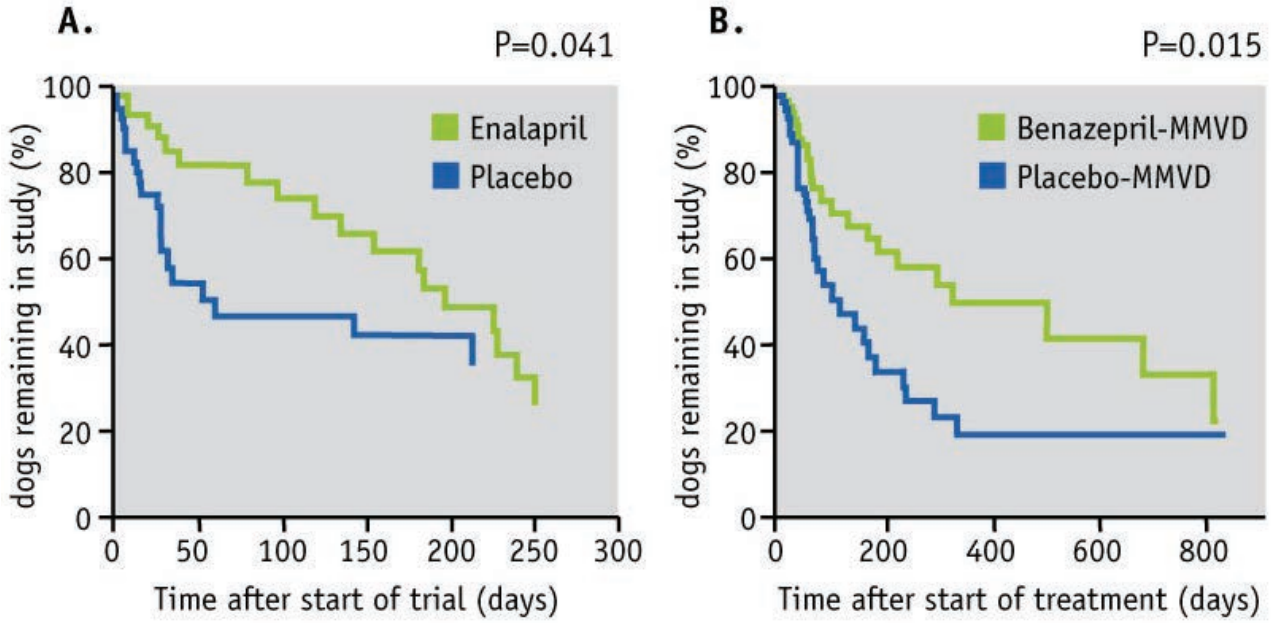

*Figure 9. Kaplan-Meier plots of percentage of dogs in (**A**) the LIVE study (enalapril) (Ettinger, Benitz et al. 1998) and (**B**) the BENCH trial (benazepril) (BENCH 1999) as a function of time. Both trials included dogs with MMVD in ACVIM Class C, and in both trials, the primary endpoint was death, euthanasia, or treatment failure leading to withdrawal of the dog from the study. The LIVE study comprised 67 dogs with myxomatous mitral valve disease (33 dogs received enalapril and 34 placebo) and the BENCH trial included 125 dogs (70 dogs received benazepril and 55 placebo). The difference in number of days between placebo- and ACE-inhibitor-treated groups was significant in both studies (LIVE study P = 0.041 and BENCH trial P= 0.015). From Ettinger et al. (Ettinger, Benitz et al. 1998) and BENCH Study Group (BENCH 1999) with permission from the publishers.*

as contributing to vascular health both in anatomical compliance and physiological vasodilatory competence (Bomback and Klemmer 2007). Spironolactone's diuretic effect is dependent on the degree of RAAS activation (Tan, Schlosshan et al. 2004). Its typical use has been as an adjunct to furosemide therapy. Depending on the furosemide dosage, plasma aldosterone concentrations recrudesce in some dogs receiving ACE-inhibitor and furosemide, a finding which has been attributed to the fact that ACE-inhibitors do not completely/permanently block ACE-activity (Häggström, Hansson et al. 1996, Lantis, Atkins et al. 2010). Furthermore, while an antifibrotic effect of spironolactone has been shown in experimental hypertension in rats and in canine infarction studies (Weber and Brilla 1991, Suzuki, Morita et al. 2002), it has not been demonstrated in dogs with naturally-occurring heart disease. Regardless, spironolactone was recently approved for use within the European Union in dogs with CHF, secondary to MMVD, as adjunctive therapy at a dosage of 2 mg/kg q 24h. This approval was based on improved survival in double-blind, placebo-controlled trials in dogs receiving spironolactone and standard therapy as compared to the placebo group (Fig. 10) (Bernay, Bland et al. 2010). This study had not been published at the time that the Consensus Panel made its recommendations. Spironolactone is now available as a combination tablet with benazepril.

Beta-adrenergic antagonists

The ACVIM Consensus Panel members were unanimous in advising against the use of beta-adrenergic antagonists (beta blockers) in dogs with active CHF, because these agents may exacerbate the signs of CHF (Kittleson 2000, Atkins, Bonagura et al. 2009, Olsen, Häggström et al. 2010). The expert recommendation of the ACVIM panel is that, if beta-adrenergic antagonists therapy is to be instituted in Class C MMVD, its use should be limited to dogs that have been stabilized and the dosage should be gradually increased with careful monitoring (Atkins, Bonagura et al. 2009).

Dilated cardiomyopathy

Chronic home therapy of dogs with DCM is similar to that of dogs with MMVD. In comparison to placebo, pimobendan has been shown to prolong survival times in Doberman Pinschers with DCM (Fuentes, Corcoran et al. 2002, O'Grady, Minors et al. 2008, Summerfield, Boswood et al. 2012) and ACE-inhibitors have been shown to improve quality of life variables in two large clinical studies (COVE 1995, IMPROVE 1995). Because dogs with DCM are more likely to present with an arrhythmia, antiarrhythmic drugs may be indicated in these dogs. Atrial fibrillation and ventricular arrhythmias are the most common forms and drugs and these arrhythmias may be treated as outlined for Class B DCM dogs above.

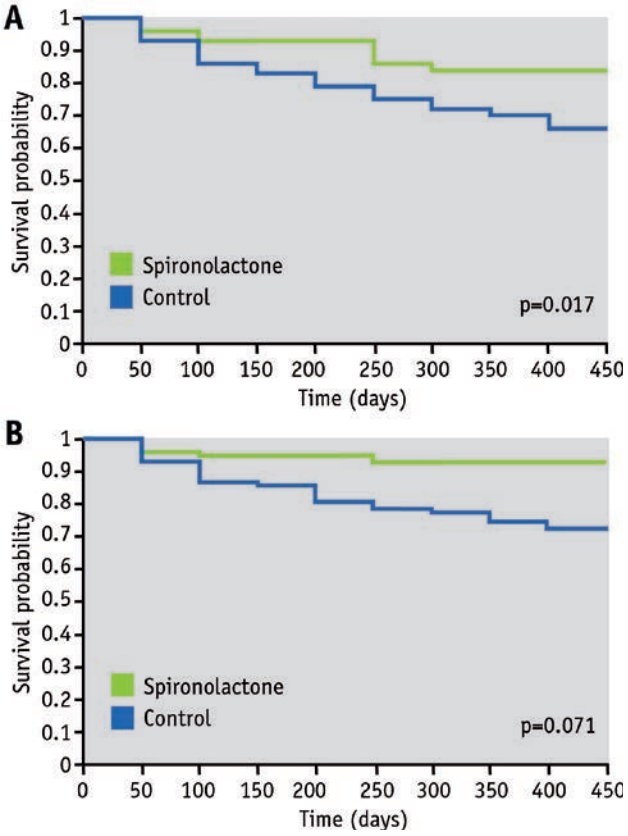

*Figure 10. Kaplan-Meier plot of percentage dogs in the study as a function of time (Intention to treat population, n=212) in a placebo controlled trial investigating the effect of spironolactone in comparison to placebo in dogs with MMVD and CHF (Bernay, Bland et al. 2010). **A**. Dogs with MMVD treated with spironolactone had a significant longer time period in the study to reach the primary endpoint, which was a composite of cardiac death, euthanasia because of CHF, and worsening of CHF. **B**. Dogs with MMVD treated with spironolactone had a significant longer time period in the study to suffer cardiac related death (sudden death or cardiac related euthanasia). Reproduced with permission from publisher.*

Feline cardiomyopathy

There are few published clinical trials involving chronic heart failure therapy in cats. It is furthermore not known if different forms of feline cardiomyopathy benefit from different types of CHF therapy (Ferasin 2009). Medical management of this category of cats is therefore restricted to other studies, such as case series, or anecdotal evidence and opinions. Cats receiving chronic CHF therapy at home have traditionally received:

- Furosemide PO 1-3 mg/kg q12 -24h or lower

Plus one or more of the following drugs:

- ACE-inhibitor PO, enalapril or benazepril 0.25 to 0.5 mg/kg q12-24h
- Diltiazem PO 1.5-2.5 mg/kg q8h (not in cats with recent onset of CHF)
- Beta-blocker, e.g. atenolol 6.25-12.5 mg/cat q12-24 h (not in cats with recent onset of CHF)

And

- As needed, antiplatelet/anticoagulants, such as aspirin or clopidogrel (for dosages see under class B cardiomyopathic cats) or unfractioned heparin, or low-molecular heparin (for dosages see under acute therapy of Class C cardiomyopathic cats)

The following drugs have gained recent popularity:

- Pimobendan PO 0.625 to 1.25 mg/cat q12 h (Macgregor, Rush et al. 2011, Gordon, Saunders et al. 2012, Hambrook and Bennett 2012),
- Spironolactone PO 1-2 mg/kg q12h. Adverse side reactions have been described in cats receiving spironolactone (MacDonald, Kittleson et al. 2008).

Clinical trials: feline cardiomyopathy (chronic therapy)

One clinical trial including cats with symptomatic cardiomyopathy was presented at a congress, but has hitherto not been published (Fox 2003). This trial was designed as a prospective blinded trial with 4 treatment groups on top of furosemide therapy: placebo, diltiazem, enalapril and atenolol groups. The reported results were that cats receiving atenolol had the shortest survival time, whereas the enalapril treated cats had the longest. This trial has influenced how many specialists treat symptomatic cardiomyopathic cats as evidenced by a published survey (Rishniw and Pion 2011). According to this survey, most cats are today treated with an ACE-inhibitor and furosemide, beta-adrenergic antagonists are used primarily to treat cats in atrial fibrillation and/or severe dynamic left ventricular outflow tract obstruction, and diltiazem is less frequently used.

Refractory heart failure (Class D)

Class D denotes dogs and cats which have developed signs of CHF and/or low output signs (e.g. exercise intolerance, weakness, syncope) and which have been treated and relapsed or failed to respond to standard CHF therapy consisting of recommended drugs and dosages listed under Class C. Dogs and cats in class D are more likely in need of body cavity centesis than in other classes.

Very limited information of drug effects in the form of clinical studies is available for dogs and cats in refractory heart failure. This stage represent advanced stages of

the disease, which means that prognosis is poor, and measures instituted may only serve to buy limited extra time. Accordingly, owners should be educated about the prognosis.

Myxomatous mitral valve disease

In addition to drugs and measures listed under Class C, the ACVIM Consensus Panel reached consensus for the following additional pharmacological measures:

- Intensification of furosemide therapy. Acute therapy: furosemide administered IV either as a bolus in the range of ≥2 mg/kg q2-6h, or as constant rate infusions at 1 mg/kg/h until respiratory function is normal. Long-term therapy: diuresis can be increased by increasing the dosage and/or dosing frequency from 1–2 mg/kg PO q12h to 4–6 mg/kg PO q6h, if owners are able to accomplish this, or by substituting one dosage per day with subcutaneous furosemide.
- Addition of diuretics other than furosemide which work on different sites in the nephron, such as hydrochlorothiazide PO at 1-2 mg/kg q12-48h to BID and/or spironolactone PO at 2 mg/kg q12h (Paul 2002) and/or by replacing one or more doses furosemide per day with torasemide at 0.1 times the furosemide dosage (Oyama, Peddle et al. 2011).
- Adding an extra dose of pimobendan per day to PO 0.25–0.3 mg/kg q8h (Atkins, DeFranscesco et al. 2013).
- More aggressive afterload reduction: with blood pressure monitoring, constant rate infusion of sodium nitroprusside at 0.1-0.5 µg/kg/min or oral drugs such as hydralazine PO at 0.5-2 mg/kg q12h or amlodipine PO at 0.05-0.1 mg/kg q24h in dogs with severe life-threatening unresponsive or poorly responsive pulmonary oedema. This therapy should be instituted with caution because it may produce an excessive fall in systemic blood pressure (recommended systolic blood pressure above 85mmHg and mean arterial pressure above 60mmHg). It is advisable to combine this therapy with a positive inotrope, such as pimobendan or dobutamine infusion.

No consensus was reached for the same drugs listed under Class C, with the addition of:

- Sildenafil PO at 0.5-1 mg/kg q12h up to 2-3 mg/kg q12h in case of pulmonary hypertension (Bach, Rozanski et al. 2006, Kellum and Stepien 2007).

Dilated cardiomyopathy

Similar to Class C, treatment of dogs with DCM in refractory CHF is comparably similar to strategies outlined above for MMVD dogs, with a few exceptions. Dogs with DCM in acute CHF are more likely to also present with severe signs of low-output heart failure as a consequence of low forward cardiac output, and are therefore likely to benefit more from inotropic support but less from vasodilating agents. Furthermore, these dogs are more likely to present with a haemodynamically significant arrhythmia that needs management if the CHF therapy is to be successful.

Feline cardiomyopathy

Very little is known concerning optimal management of refractory CHF in cats. The same measures may be attempted as in MMVD class D dogs outlined above, with the exception of aggressive afterload reduction, which is usually not practiced in cats. For home therapy, it is important to recognize that cats are less likely to accept polypharmacy and they are more likely to suffer from adverse side reactions from intensive diuretic therapy than dogs. Cats are accordingly often treated with lower furosemide dosages than outlined above for MMVD dogs in Class D. Finally, cats in this class are more likely in need of antiplatelet/anticoagulant treatment owing to presence of clots or a hypercoagulable state.

Concluding Comments

In conclusion, while there are many unanswered questions in the management of CHF in dogs and cats, clearly progress has been made in our understanding as to which pharmacological agents may and may not be useful; how we might use them; and in which particular circumstances they will be most valuable. Perhaps even more importantly, we have observed a shift in the demand for data to support our clinical decisions, by both academic and private practitioners and by the public. There are, however, areas that need further studies, particularly optimal management of acute CHF and therapy of cats with cardiomyopathy.

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FECAVA LECTURE

Mast Cell Tumour Advances

Laura Blackwood[1]

SUMMARY

Background

Mast cell tumours (MCTs) are neoplastic proliferations of mast cells. Cutaneous MCTs arise from tissue mast cells in the dermis and subcutaneous tissue. MCTs account for 7-21% of canine skin tumours [1]. Although tumours can affect dogs of any age or breed, Boxers, Boston terriers, Pugs and Retrievers are predisposed. Shar-Peis are predisposed to developing aggressive tumours at a relatively young age. In Boxers and Pugs, less aggressive tumours are more frequently seen, but these breeds still develop high grade tumours [2-4]. The aetiology is unknown.

Although most mast cell tumours can be cured by surgical resection, some are very challenging to manage. This is because they show very variable biological behaviour in terms of invasiveness of the primary tumour, metastatic rate and paraneoplastic effects. Identifying the more aggressive tumours allows better management of these cases.

Key words: mast cell tumour, grade, prognostic indicators, treatment

This paper is based on the FECAVA Lecture delivered at the 19th FECAVA EuroCongress in Dublin, Ireland in October 2013.*

Clinical Presentation

Most mast cell tumours in dogs present as a cutaneous (or subcutaneous) mass which may fluctuate in size, or be associated with local oedema, swelling or erythema (figure 1). This is caused by local effects of factors released by mast cell degranulation (histamine, heparin, proteases). These factors can also cause local coagulation abnormalities (figure 2) and possibly delayed wound healing (figure 3). Some patients will show systemic effects of the inflammatory mediators contained in mast cell granules (predominantly gastrointestinal signs as a result of histamine release) [2 3 5]. Rarely, massive histamine release can cause anaphylaxis or collapse.

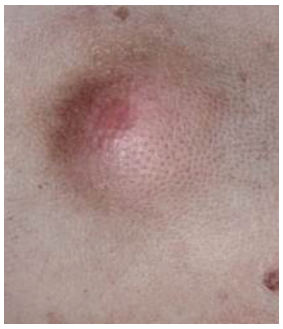

Figure 1: Mast cell tumour arising on the thoracic wall of a middle-aged female Staffordshire bull terrier. There is a raised mass, with erythema of the overlying skin and local oedema. The mass had been clinically mistaken for a lipoma previously.

Clinical examination can identify tumours likely to show aggressive behaviour (figure 4), and this is suggested by [5,6]:

- rapid growth
- large size
- local irritation, inflammation or oedema
- local infiltration or poor demarcation from adjacent tissues
- ulceration
- satellite nodules

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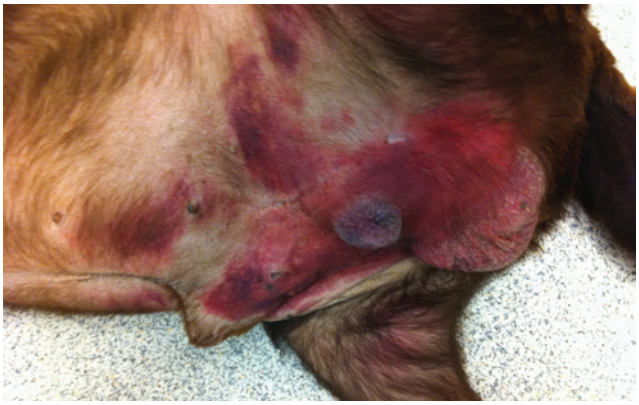

Figure 2: Ventrum of an 11-year-old male Labrador with a mast cell tumour arising adjacent to the scrotum. Note the extensive ecchymotic haemorrhage due to local effects of mast cell degranulation. The dog also has inguinal nodal metastases.

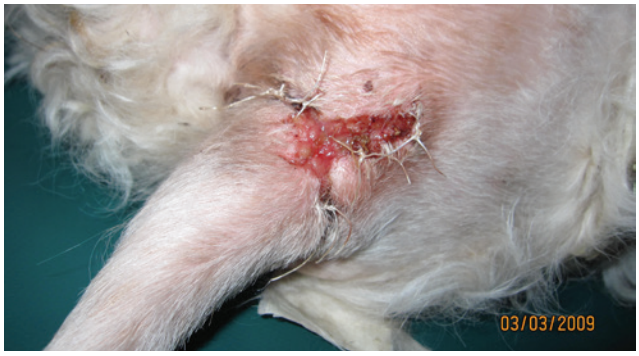

Figure 3: Wound dehiscence after removal of a mast cell tumour from the dorsum of the tail of a 6-year-old female neutered cross breed dog.

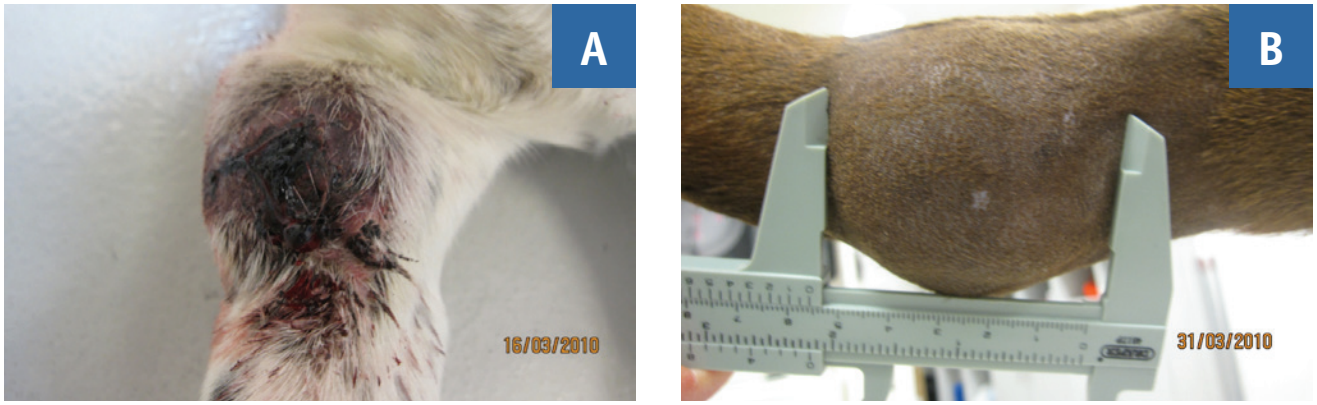

Figure 4: Examples of clinical features associated with a poorer prognosis. A. cutaneous mass on the proximal forelimb of a 10-year-old female neutered crossbreed dog. The mass had grown rapidly and was ulcerated and haemorrhagic. It caused local irritation and dog had to wear an Elizabethan collar to prevent self-trauma. This is a Grade III mast cell tumour. B. A very poorly demarcated, oedematous mast cell tumour on the lateral aspect of the hock of an 8-year-old male Boxer cross.

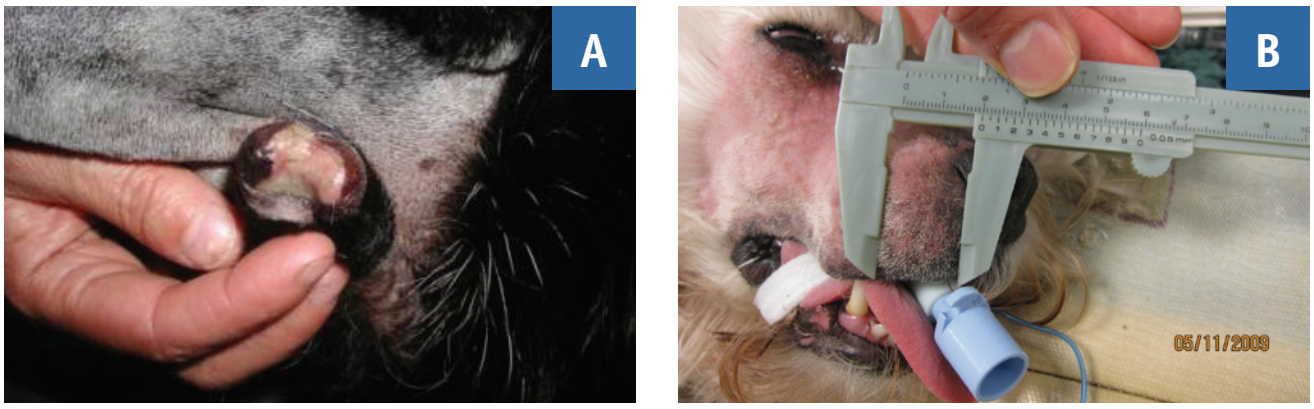

Figure 5: Examples of MCTs in difficult sites. A. A 10-year-old male neutered Collie cross who presented with an eight week history of intermittent vomiting, and had iron deficiency anaemia on haematology. This was due to a preputial mast cell tumour. Mast cell degranulation had resulted in gastrointestinal ulceration, vomiting and iron deficiency anaemia. B. A middle-aged male West Highland White Terrier with a mast cell tumour affecting the muzzle. This tumour was managed using neo-adjunctive chemotherapy, surgery and radiation therapy.

It is controversial whether tumours arising in the nail bed, oral mucosa, muzzle, prepuce, perineum and at mucocutaneous junctions have a poorer prognosis than other sites [1,7-9] (figure 5). Contributing factors may be a higher frequency of histopathologically more aggressive (higher grade) mast cell tumours, and difficulty in achieving complete excision in these sites. Rarely, tumours involve the viscera, intestine or bone marrow and these tumours carry a poor prognosis [10-12].

Up to about 40% of dogs that develop one mast cell tumour will develop more mast cell tumours, and these can occur sequentially or concurrently [4,13,14]. Golden Retrievers, Labrador Retrievers, Boxers and Weimaraners are predisposed. Each tumour should be treated as a *de novo* tumour and most work suggests survival times are the same as for those with solitary tumours of the same grade and stage [6,13], though development of multiple synchronous tumours has been associated with a poorer prognosis [15]. These tumours must be differentiated from tumour regrowth, satellite lesions associated with an aggressive primary tumour or metastatic disease, which are all associated with a poorer prognosis.

Approach

The key aspects of management of MCTs are diagnosis to allow appropriate management, and identification of tumours which are likely to recur or metastasise, which may not be cured by local/wide excision.
Diagnosis of MCTs is generally easy, as more than 90% of tumours can be confirmed by cytological evaluation of a fine needle aspirate (FNA) [16] (figure 6, 7 and 8). Cytology cannot accurately grade mast cell tumours: this is based on histopathological criteria. Histopathology is also required for evaluation of tumour margins. Ideally, submit samples to a laboratory you know evaluates shaved margins.

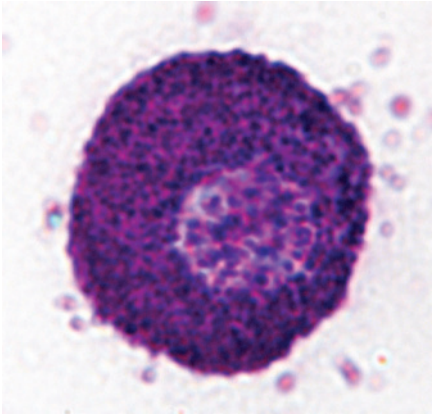

Figure 6: A well granulated canine mast cell.

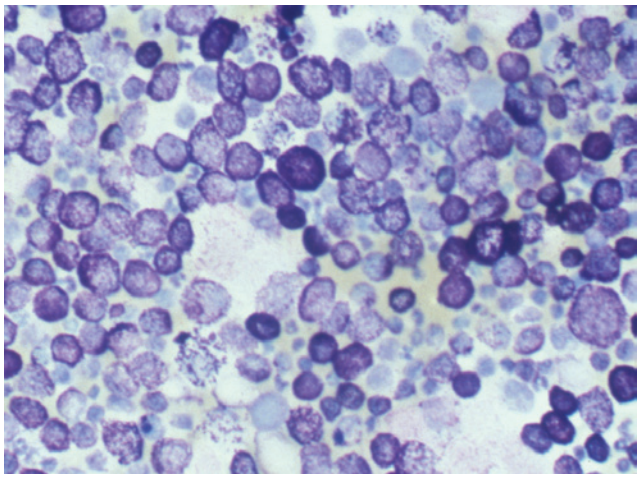

Figure 7: fine needle aspirate from a cutaneous mass on the neck of a 9-year-old male Boxer. Large numbers of round cells with variably granulated cytoplasm, often obscuring the nucleus, are seen. This is diagnostic of a mast cell tumour.

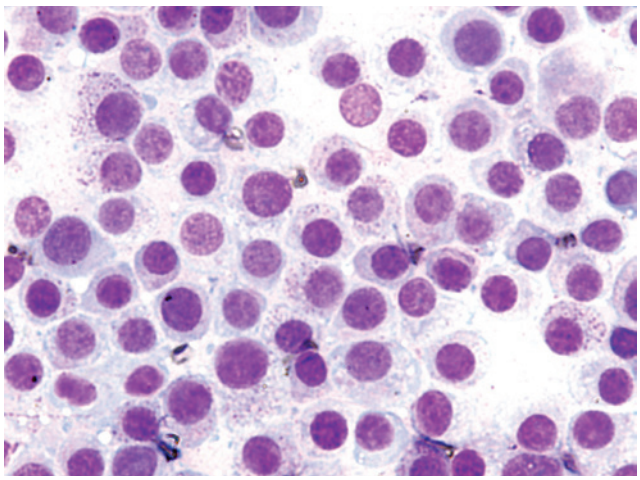

Figure 8: fine needle aspirate from a cutaneous mass on the dorsum of a 12-year-old female crossbreed dog. There is moderately pleomorphic population of round cells, many of which show sparse cytoplasmic granulation. This is diagnostic of a mast cell tumour, and the pleomorphism and sparse granulation along with other cytological features suggest this is not a low grade tumour, though histopathology is required for accurate grading.

Prognostic Indicators in Canine MCTs

Tumour Stage

There is no simple universally accepted staging system for MCTs. The approach to staging the MCT patient is summarised in figure 9. The first site of metastasis is usually the local lymph node/nodes [17] (figures 10 and 11), and liver and spleen are the main sites for distant metastasis[2,4]. Tumour stage is important in mast cell tumour patients, and nodal metastasis is a recognised poor prognostic indicator[13]. However, studies have shown that patients

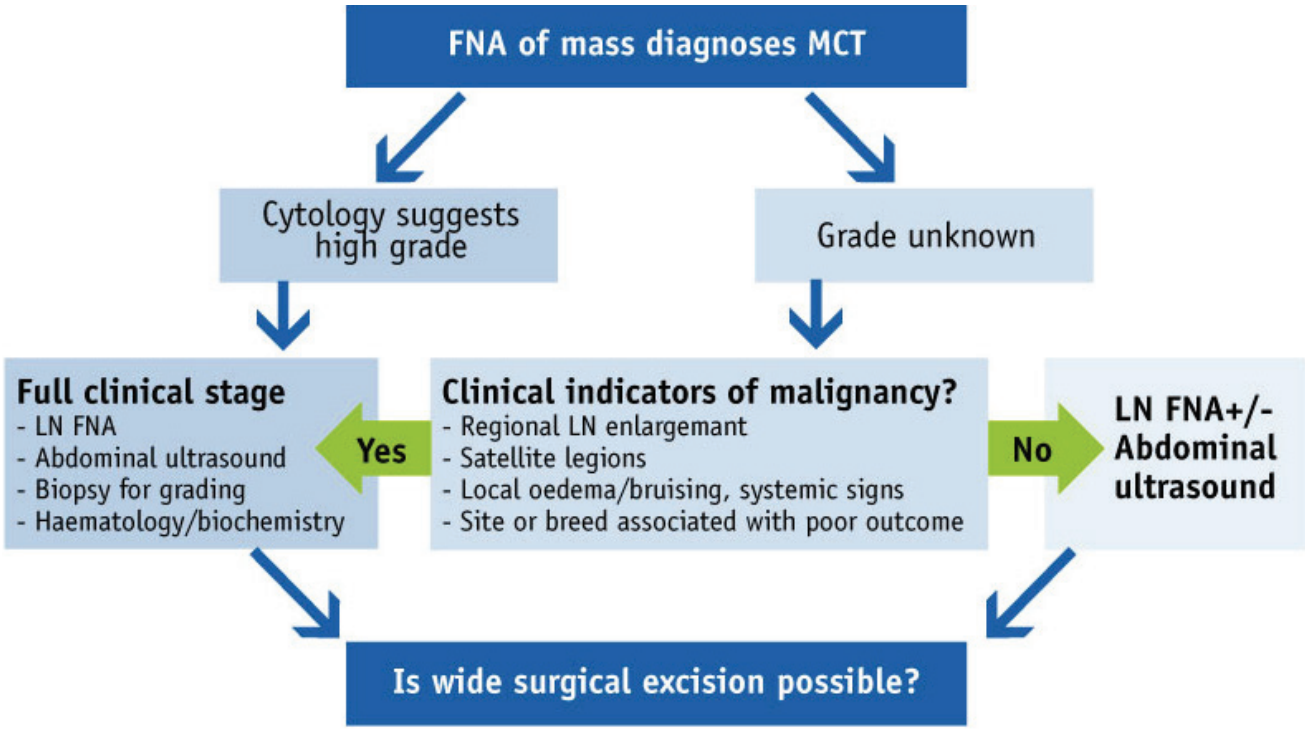

Figure 9: Approach to cutaneous mast cell tumours diagnosed on cytology (in dogs)

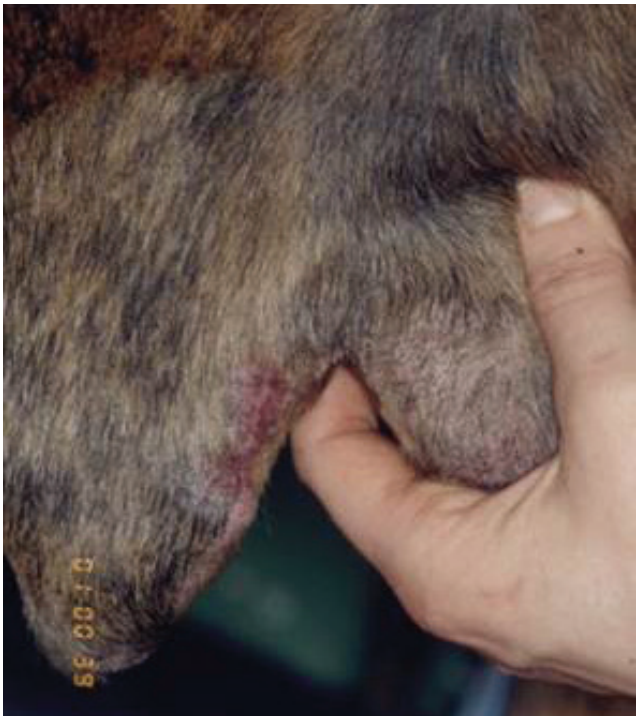

Figure 10: Left axilla of an 8-year-old neutered female Boxer. The irregular scar on the elbow is from recent resection of a cutaneous mass, diagnosed as a Grade II MCT. The markedly enlarged left axillary lymph node, affected by metastatic disease, is being palpated. Generally, tumours of the distal forelimb metastasise to the prescapular node, while tumours of the proximal forelimb metastasise to the axillary node.

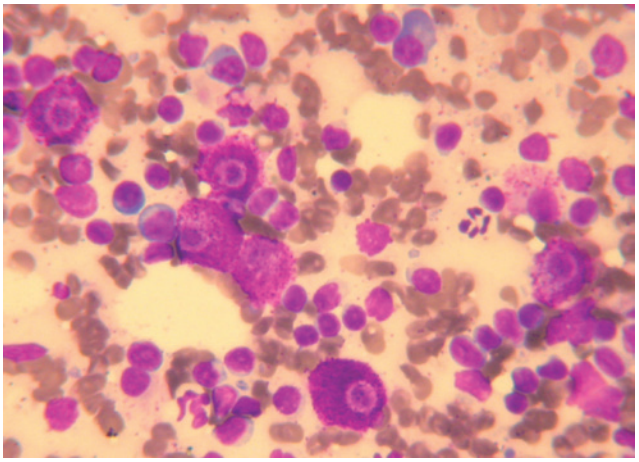

Figure 11: Fine needle aspirate showing metastases of a mast cell tumour on the right hind of a 10-year-old MN Retriever to the right popliteal lymph node. Note the background red blood cells due to haemodilution, which may be a result of release of heparin from the mast cells.

with nodal metastases (without distant metastases) can do well when they are treated with multimodality therapy, particularly surgery and radiation therapy [18].

Tumour Grade

Histopathological grade is the single most important factor in determining mast cell tumour prognosis. For many years, the Patnaik grading system has been used [19]. This classifies tumours based on their extent, cellularity, cell morphology and mitotic index, and grades them from

Grade I (well differentiated, low grade) through Grade II (intermediate grade) to Grade III (poorly differentiated, high grade) (figure 12). This system has been widely used for the last 30 years, and has been of huge benefit in the management of MCTs. However, over time some limitations have become apparent. It is a relatively complex and subjective system, and agreement between pathologists is poor, with approximately 60% agreement for Grade I and Grade II tumours, and 75% for Grade III tumours [20 21]. In addition, while the prognosis for Grade I tumours is good, and for Grade III tumours is bad, the behaviour of intermediate Grade tumours remains somewhat unpredictable, with up to 22% metastasising. In an effort to address these problems, and to identify the tumours in the intermediate grade group that may behave aggressively, an alternative system has been developed. The Kiupel grading system [21] is a simplified grading system which divides tumours into high or low grade tumours. The diagnosis of a high grade tumour is based on the presence of one or more clear characteristics (figure 13). Recent work has suggested that this system will identify tumours with a poor prognosis. In addition, the grading system is less subjective and higher agreement occurs between pathologists [22, 23].

Grade	Histological criteria
I Well differentiated	Monomorphic round cells with distinct cytoplasm, medium sized intracytoplasmic granules, no mitotic figures noted. Compact groups or rows of neoplastic cells confined to dermis
II Intermediately differentiated	Some pleomorphic cells-round to ovoid in shape. Some cells having less distinct cytoplasm with large and hyperchromatic intracytoplasmic granules but others have distinct cytoplasm with fine granules. Areas of oedema or necrosis are noted. Mitotic figures are 0-2 per high power field. Tumour infiltrating lower dermis/ subcutaneous tissue.
III Poorly differentiated	Dense sheets of pleomorphic cells with indistinct cytoplasm with fine or not obvious intracytoplasmic granules. Mitotic figures 3-6 per high power field. Oedema, haemorrhage, necrosis and ulceration common. Tumour infiltrating lower dermis/ subcutaneous tissue.

Figure 12: Patnaik [19] histological grading of canine mast cell tumours

Patnaik Grade I tumours and Kiupel low grade tumours will generally have a good prognosis, and, in most cases, adequate surgery should be curative. All Patnaik Grade III tumours, tumours where the pathologist is "hedging" between GII and GIII, and all Kiupel high grade tumours should be considered to have a significant risk of recurrence/metastasis.

Diagnosis of high-grade MCTs based on the presence of any one of the following criteria

- At least 7 mitotic figures in 10 high-power fields (hpf)
- At least 3 multinucleated (3 or more nuclei) cells in 10 hpf
- At least 3 bizarre nuclei in 10 hpf
- Karyomegaly (i.e. nuclear diameters of at least 10% of neoplastic cells vary by at least two-fold).

All other tumours are considered low grade

Figure 13: Kiupel[21] histopathological grading of canine mast cell tumours

Proliferation Indices

Several proliferation indices have been investigated in MCTs. In the 1980s, Bostock [24] reported that tumours with a high mitotic index had a poorer prognosis than those with a low mitotic index. This work has been developed recently. Other proliferation markers include Ki67, MCM7, PCNA, and AgNORs. Of these, Ki67 is currently the most widely used.

Mitotic Index

The mitotic index (MI) is determined by identifying the region with the highest overall mitotic activity, and counting the mitotic figures in 10 high power (x400) fields (figure 14). MI requires no additional stains or techniques, and should be part of every pathology report. MI is prognostic for survival, independent of histological grade. The cut-off values most commonly used are 5 or 7 [25-27]. Using a cut-off of 7, MI has also been shown to be predictive of recurrence [21]. Generally, tumours with high MI are aggressive. However, not all tumours with low MI are indolent.

Ki67

Ki67 is a proliferation marker detected by immunohisto-chemistry. Most laboratories simply express the result as a percentage of cells which stain positively. High Ki67 is associated with an increased risk of mortality, recurrence and metastasis, and is predictive of survival, independent of histopathological grade [27-30]. The usual cut-off is 1.8%,

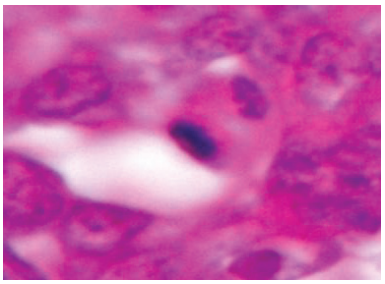

Figure 14: A cell in mitosis, in a canine mast cell tumour. The mitotic index (MI) is determined by identifying the region with the highest overall mitotic activity, and counting the mitotic figures in 10 high power (x400) fields

and tumours with a Ki67 of more than 1.8% are more likely to recur or metastasise.

Others

Minichromosome maintenance protein 7 (MCM 7) is another proliferation marker detected by immunohistochemistry, which predicts survival independent of grade [31]. Proliferating nuclear cell antigen (PCNA, detected by immunohistochemistry) and agryophilic nucleolar organiser regions AGNORs (detected by silver stain) are also prognostic indicators, but do not predict survival independent of histological grade [29,30,32].

KIT and Receptor Tyrosine Kinases

KIT is a receptor tyrosine kinase which is often activated in canine MCTs [22,33-35]. Receptor tyrosine kinases are transmembrane enzymes which provide a signalling connection between the extracellular environment and the cell interior/nucleus. The inactive RTK exists as a dormant monomer, but when the ligand binds, dimerisation occurs. This results in activation of the tyrosine kinase domain by phosphorylation, and activation of downstream pathways resulting in cell proliferation and survival (figure 15). Dysregulation of RTKs and their downstream pathways is common in cancer, and KIT is important in MCTs, with KIT dysregulation in 15-40% of canine MCT. KIT dysregulation is associated with a poor prognosis, increased risk of metastasis and local recurrence, independent of histological grade [22,33,36]. KIT dysregulation is also associated with a higher tumour proliferation index [35,37]. Most commonly, mutations in the KIT gene result in constitutive activation of the KIT protein, but other mechanisms are also involved. The result is ligand independent increased cell proliferation and alterations in migration, maturation and survival characteristics which favour tumour growth. In addition, other RTKs may be important in MCTs, particularly those involved in angiogenesis.

Surgical MCT Margins

For many years, the recommendation for MCT surgery was 3cm lateral margins and one deep fascial plane [2 38]. Before the 3cm rule, papers report recurrence rates of 30-50%, so adequate margins are important. Several recent studies have shown that for Patnaik grade I and II tumours less than 4cm in diameter, 2cm lateral margins and one fascial plane is

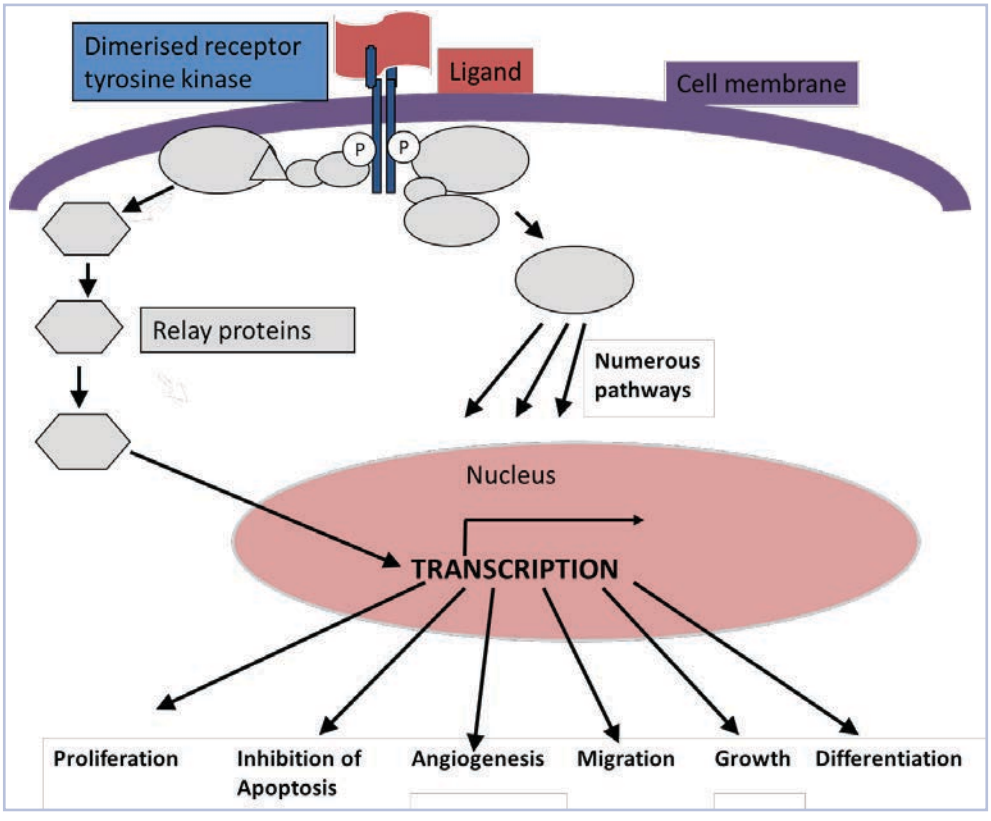

Figure 15: A diagrammatic representation of a receptor tyrosine kinase.

sufficient for most cases [39-41]. One study reports even smaller margins for grade I and II tumours up to 31mm[42]. This is encouraging, but caution needs to be applied, particularly in a practice situation where the margins aimed for may not be achieved (for example, if the surgeon "guesstimates" rather than measuring margins, or where the deep margin is inappropriate). For these margins to succeed, the deep margin should include the panniculus muscle (if present), the underlying fascia or, in its absence, the superficial layer of musculature [13,40,41]. These margins have not been evaluated for tumours over 4cm in diameter. High grade tumours are likely to recur irrespective of margins [5,43], but the recommendation is a lateral margin of at least 3cm plus the deep fascial plane [5].

Histological MCT Margins

Mast cell tumours release chemotactic factors which attract normal, inflammatory mast cells to the periphery of the tumour. This can make it very difficult for the histopathologist to determine margins, particularly in low grade tumours. Recent work has suggested that a histological tumour free margin (determined by microscopic examination of the excised tumour) of 3mm may be adequate for low grade tumours [43]. However, narrower margins have not been assessed. Most histopathologists define close/narrow margins as tumour cells within 1mm of the surgical margin [44].

Reported recurrence rates for dogs with Grade I and II or low grade tumours which have incomplete or narrow margins are generally low (less than 20% for Patnaik Grade II tumours)[39-41,45-48]. Options for these patients are en bloc resection of the scar, active monitoring or radiation therapy. After en bloc resection of apparently incompletely excised mast cell tumour scars, histopathology may reveal no mast cells in around a quarter of cases. Active monitoring is appropriate for some cases, but is not appropriate if there are gross signs of residual disease, there are pre-existing metastases, or for high grade tumours. It is also less desirable for tumours which have a high mitotic index or Ki67, or other markers of higher likelihood of recurrence.

Histologically tumour free margins are not a guarantee of surgical cure: up to 5-23 % of tumours with clean margins recur [16,39,47,48]. However, the recurrence rates with "dirty" margins is significantly increased compared to clean margins [49]. Recurrence is much more likely with high grade tumours.

Treatment Options

Traditional treatment options are surgery, radiation therapy and chemotherapy. Decision making is summarised in figures 16 and 17.

Surgery

Surgery remains the treatment of choice for most canine mast cell tumours [46 50]. The recommended margins are discussed above. Key to ensuring adequate surgery is a presurgical diagnosis of MCT.

Radiation therapy

Radiation therapy is most often used as a post operative adjunctive treatment after incomplete excision of tumours which are thought to have a significant risk of recurrence. Generally, irradiation of bulky disease is avoided due to the risks of mast cell tumour degranulation (resulting in systemic signs), and because larger tumour volume is associated with shorter disease-free intervals. Good recording of pre-surgical tumour site and dimensions (photographs, sketches, measurements), will reduce the risk of a "geographical miss" when animals are referred for post operative radiotherapy. Use of metal surgical clips, particularly on the body wall and proximal limbs, allow visualisation of the periphery of the surgical field which may not be predictable from the scar, as there may be substantial post surgical tissue migration. In addition, when tumours on the limb are irradiated, a strip of skin is usually spared to maintain lymphatic drainage: oblique and transverse incisions will make this more difficult to achieve without compromising margins. Where complete excision of tumours is impossible even with radical surgery, conservative or marginal excision followed by radiotherapy is often preferred over radical surgery [1,51-53] (figure 18). This is because complex surgery (including use of flaps) can delay radiotherapy or be associated with complications during and after radiotherapy [54].

Radiation therapy is associated with generally good outcomes. Adjuvant treatment for intermediate grade tumours results in 81-95% of patients achieving disease-free intervals of 1 to 2 years [51,53]. In addition, disease-free intervals of more than 3 years have been reported for dogs with regional lymph node metastases treated with surgery, radiotherapy, and chemotherapy [18,55,56] or prednisolone [56].

Chemotherapy

Chemotherapy is used in three ways. Firstly, it is used where systemic rather than locoregional treatment is

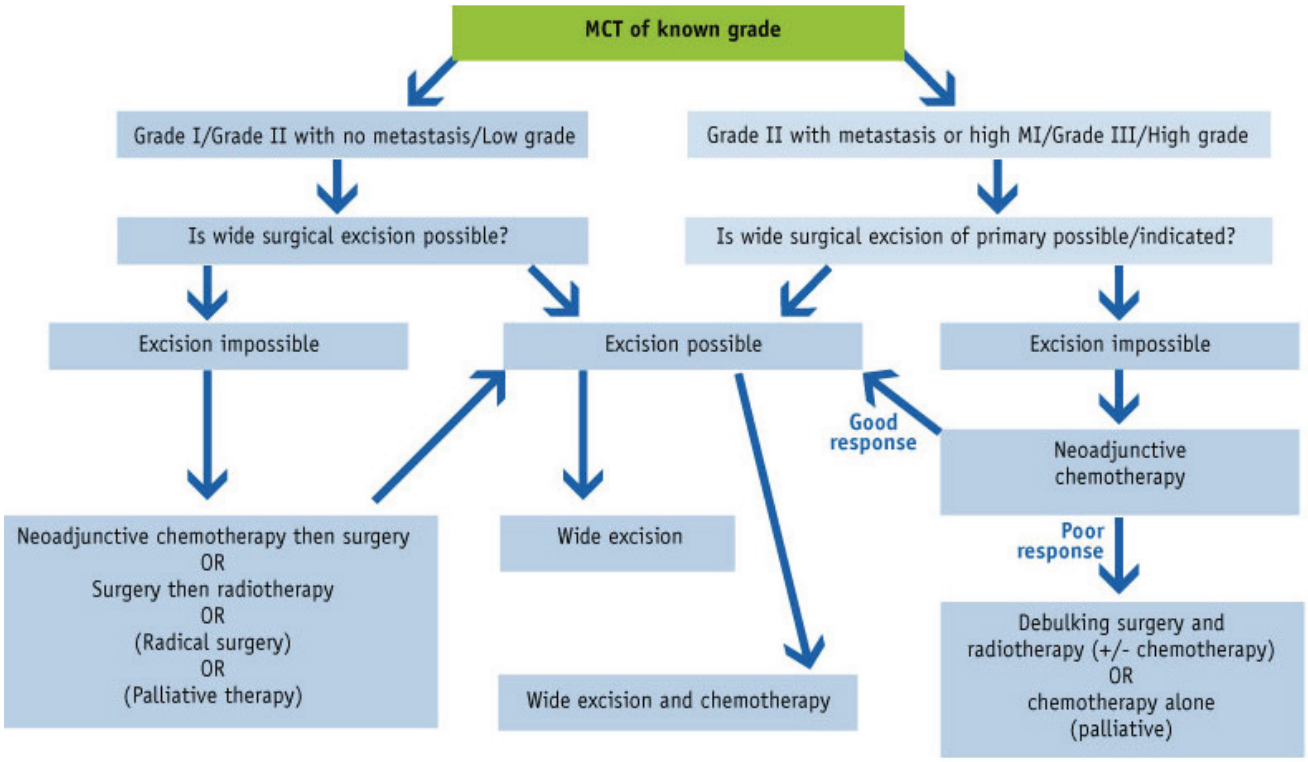

Figure 16: Summary of initial assessment of mast cell tumours for surgery and adjunctive treatment

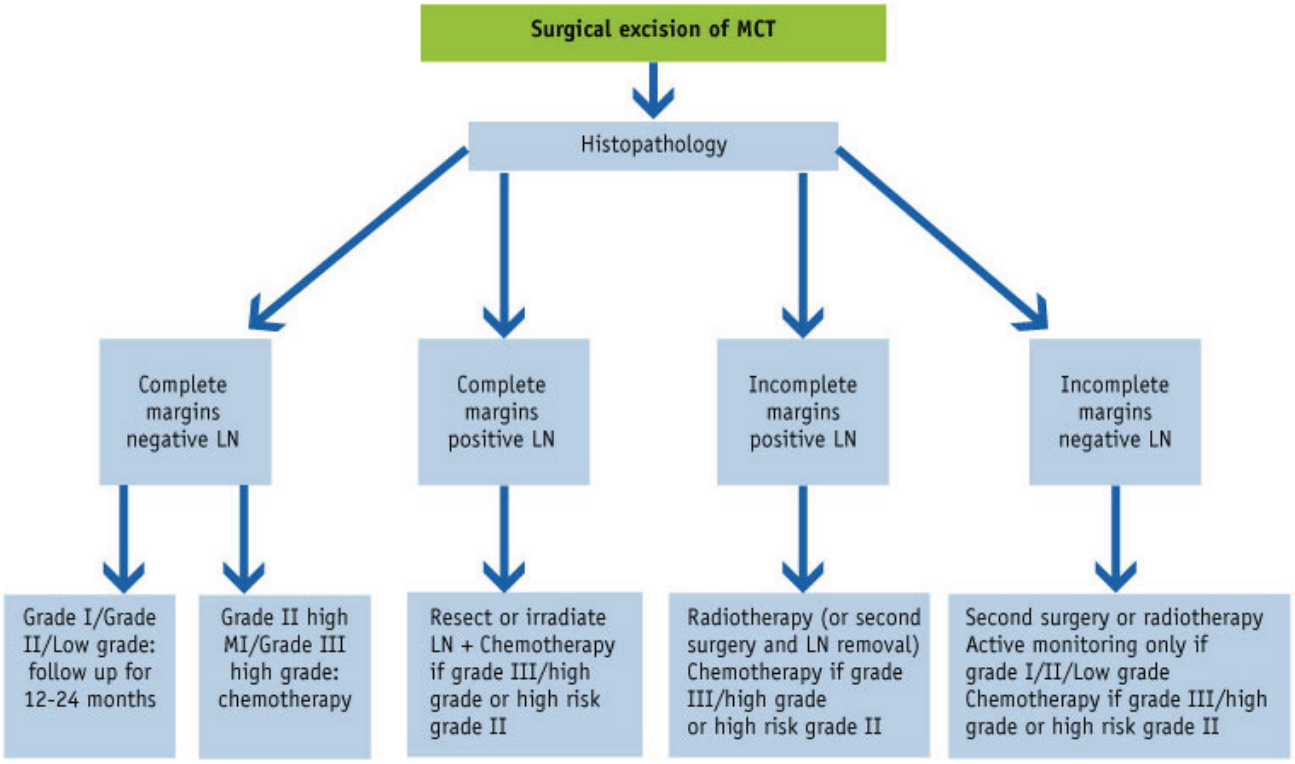

Figure 17: Summary of post-operative mast cell tumour options

required to treat, delay or prevent disseminated metastases in high grade tumours. Ideally, in these cases the primary tumour is managed by surgery (and/or radiotherapy) and chemotherapy is used adjunctively. Systemic therapy is clearly most appropriate for tumours with a high risk of metastases: grade III or Kiupel high grade tumours, tumours where the pathologist suggests Patnaik grade II/III and grade II tumours with risk factors for reduced survival and/or metastases such as high MI or Ki67. Some grade III/high grade tumours are treated with palliative chemotherapy only, where treatment of the primary is not appropriate (e.g. where there is high risk of wound breakdown or other surgical complications).

Most of the data on response to chemotherapy focuses on the response of primary tumours, rather than impact on

Figure 18: A 10-year-old female neutered crossbreed dog referred for radiation therapy after resection of a mast cell tumour with wound closure involving a pedicle flap. The irradiated area is large to include the whole flap (except the most proximal pedicle) and to avoid geographical miss as there were no accurate records of tumour location. The pegs seen cranially are used to help hold skin out of the field to spare a strip from radiation, to maintain lymphatic drainage. Good presurgical tumour recording, the use of metal surgical clips on body wall and proximal limbs and well planned surgery increase the likelihood of successful outcomes from radiotherapy.

survival/metastases. However, two studies involving dogs with grade III tumours or grade II tumours thought to be at risk of metastases or with nodal metastases have shown that chemotherapy (prednisolone and vinblastine, or prednisolone, vinblastine and lomustine) in combination with excision (and in some cases radiotherapy) results in disease-free intervals of more than 40 months [18,55]. Chemotherapy is thought to prolong survival in these cases. Certainly, the results compares favourably with the very poor survivals reported for Grade III tumours historically, where only 6 to 27% of patients were alive after one year [19,24].

Chemotherapy (or prednisolone alone) is also used in a neo-adjunctive setting to reduce tumour burden and improve the likelihood of achieving complete excision or to make it easier and safer to irradiate the mass [4,44].
Finally, chemotherapy is used to treat residual microscopic disease where further surgery is not possible and radiation therapy is not available. There is little data on outcomes in this setting. One study in 20 dogs reported 18 of the patients did not have local recurrence after one year [57]: however, there were many grade II tumours in the study, and the risk of recurrence for some patients was probably already low.

The most commonly used agents are vinblastine, prednisolone and lomustine. Some protocols are summarised in table 1. Familiarity with drug toxicities and adequate patient monitoring are required when these protocols are used [4].

There is no evidence to support the use of systemic chemotherapy in dogs with multiple (low grade) tumours.

Receptor Tyrosine Kinase Inhibitors

Receptor tyrosine kinase inhibitors (RTKIs or TKIs) are small molecule inhibitors, which usually act by blocking the ATP binding site and preventing phosphorylation of the tyrosine kinase, so there is no initiation of downstream signalling. Two TKIs are licensed for the treatment of dogs with MCTs, both for the treatment of recurrent and unresectable MCTs. These are masitinib mesylate (Masivet, AB Science) and toceranib phosphate (Palladia, Zoetis). Both were developed to target KIT [58,59]. Toceranib also targets VEGFR2, PDGFR and RTKs that play an important role in tumour angiogenesis and metastasis [2]. Both drugs have demonstrated efficacy as single agents in prospective clinical trials in dogs [60-62], where they delay time to disease progression. However, TKIs should not be considered as an alternative to surgical resection where this can be performed in practice or by a specialist surgeon. Currently, both drugs are most often used in non-resectable or recurrent mast cell disease where conventional therapy is not appropriate or available.
TKIs have a similar toxicity profile to chemotherapy drugs [60,61,63]. Side effects include neutropenia (usually mild but can be severe in some sensitive patients or where treatment is inappropriate). Gastrointestinal signs (mainly diarrhoea or reduced appetite) are relatively common. Other drug specific side effects are reported: for example muscle cramping is reported with toceranib and protein losing nephropathy with masitinib. Close clinical and clinicopathological monitoring is required (especially in the early stages of treatment). In most cases, side effects can be managed by "treatment holidays" and/or dose reductions.

Much work remains to be done investigating the most appropriate use of TKIs. It is likely that they may become part of a multimodality approach to MCTs, where both their anti KIT and anti-angiogenic effects contribute to response. They may also have a potential immunomodulatory effect and an effect on tumour stroma, which may be important in a range of tumours: this has been demonstrated for toceranib [64]. However, their role in multimodality therapy for MCTs remains largely investigational at the current time. There is little data on combination of TKIs with radiotherapy, or on their potential role in post-operative adjunctive therapy. Some work has

Table 1: Most commonly used chemotherapy protocols in the treatment of canine mast cell tumours (modified from Blackwood et al, 2012[4])

Drug	**Published total response rate** (sum of complete and partial responses for measurable disease)	Protocol	Comment / toxicity	References
Vinblastine and prednisolone	47%	Vinblastine 2mg/m² IV weekly for 4 weeks then fortnightly for 4 further treatments Prednisolone 2mg/kg PO once daily for one week, then 1mg/kg daily for 2 weeks, then 1mg/kg every other day	6 – 20 % toxicity: myelosuppression and GI toxicity. Can roll vinblastine out to 6 months. Dose escalation of vinblastine may be possible in some cases.	Thamm et al 1999 [66] Thamm et al 2006[55] Vickery et al 2008 [67]
Lomustine	44%	70mg/m² PO q 21d for 4 cycles	Lomustine is associated with myelosuppression, GI and hepatotoxicity. Toxicity of longer term monotherapy unknown if lomustine continued Lomustine often used as rescue therapy after vinblastine and prednisolone	Rassnick et al 1999[68]
Vinblastine/ lomustine (alternating vinblastine/ lomustine, one treatment q 14d)	57%	Vinblastine 2mg/m² IV week 1 then every 4th week Lomustine 60mg/m² PO week 3 then every 4th week	Planned protocol 4-6 cycles Toxicity in 54% of cases, mainly myelosuppression	Cooper et al 2009 [69]
Vinblastine / lomustine and prednisolone (alternating vinblastine/ lomustine, one treatment q 14d)	Not published (reported in opinionated review)	Vinblastine 2mg/m² IV week 1 then every 4th week Lomustine 70mg/m² PO week 3 then every 4th week Prednisolone 0.5mg/kg PO daily	Protocol continues for 6 months	Welle et al 2008[1]
Vinblastine / lomustine and prednisolone (alternating vinblastine/ lomustine, one treatment q 14d)	65%	Lomustine 70mg/m² PO week 1 then every 4th week Vinblastine 3.5mg/m² IV week 3 then every 4th week Prednisolone 2mg/kg PO daily for first two weeks then 1mg/kg daily until week 24 then tapered over 4 weeks.	Thirteen dogs required lomustine dose reduction and 17 dogs required vinblastine dose reduction (of 48 that received both) due to significant myelosuppression. 33% developed severe neutropenia, 28% hepatotoxicity. CARE advised in general practice. Twenty four week protocol.	Rassnick et al 2010[70]

been published reporting combined TKI and chemotherapy treatment. Because they have similar toxicity profiles to conventional chemotherapy drugs, combining these drugs with chemotherapy tends to result in reduction in the dose intensity of the conventional cytotoxic, to avoid toxicity, and the impact of this on outcome is unknown. However, data so far suggests the efficacy of combined treatment using toceranib and vinblastine may not be less than that of the cytotoxic alone [65].

As investigations continue into the best ways to utilise TKIs continue, it is likely new recommendations may be made.

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COMMISSIONED PAPER (NL)

Plumage disorders in psittacine birds - part 1: feather abnormalities

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SUMMARY

Plumage disorders in parrots represent one of the more common, but also one of the more challenging and frustrating problems that veterinarians dealing with parrots in their daily practice face on a day-to-day basis. Various types of plumage disorders may be identified, including stress marks, damaged or broken feathers, poor feather quality, feather discolouration, feather dystrophy, feather loss and feather damaging behaviour. This first article will deal with plumage disorders that result in feather abnormalities in psittacines, including the most common underlying causes, as well as their diagnostic work-up and treatment.

Keywords: Feathers; Feather disorders; Integument; Parrot; Plumage; Dermatology

This paper was commissioned by FECAVA

Introduction

Feathers are one of the most prominent features of a bird's anatomy that distinguishes them from all other classes of vertebrates. They serve many functions of which the most noticeable is enabling a bird to fly. In addition, feathers also serve an important role in the protection against the elements because of their insulating and waterproofing properties, whereas their colour and pattern may provide camouflage to protect them from predators and/or enable them to communicate with conspecifics (e.g. during courtship and mating). In general, seven types of feathers may be distinguished, each serving its own function (Fig. 1). These include 1) the long, stiff and asymmetrically shaped flight feathers of the wing (remiges) and tail (rectrices), which help to generate thrust and lift, thereby enabling flight; 2) the contour or covert feathers that line the bird's body and provide streamlining, waterproofing, camouflage and ability to communicate with conspecifics; 3) the small, fluffy down

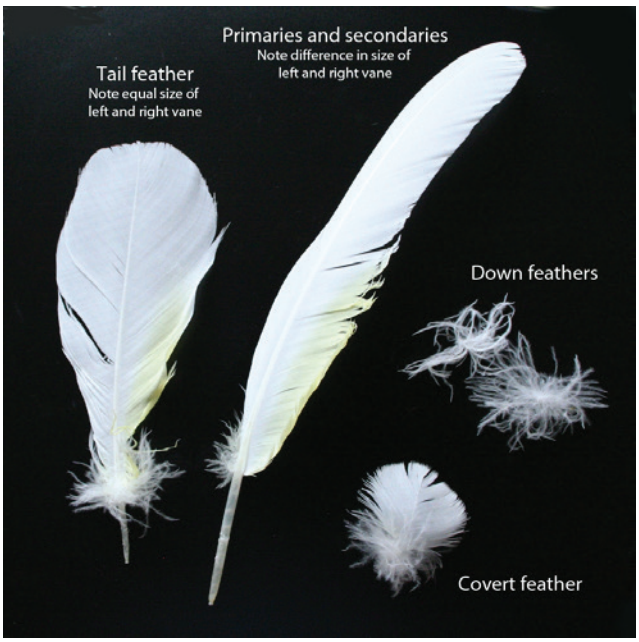

Figure 1. Feathers of a white or umbrella cockatoo (Cacatua alba). The four main types of feathers that can be distinguished in a parrot include: a) tail feathers or rectrices with a symmetrically shaped vane; b) the primary flight feathers or remiges with an asymmetrically shaped vane; c) covert feathers; and d) down feathers. The other feather types (i.e., semiplumes, powder down feathers, filoplumes and bristle feathers) are not depicted here.

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feathers that are located underneath the contour feathers and have an insulating function; 4) the semiplumes, that are slightly larger than down feathers and act as thermal insulation and help to increase buoyancy in aquatic birds; 5) the brittle powder down feathers that shed a fine waxy powder that forms a waterproof dressing for the contour feathers; 6) the long, fine, hairlike filoplumes that serve a proprioceptive function; and 7) the long, stiff bristle feathers that are located around the bird's mouth and eyes which may have a sensory function, similar to a cat's whiskers [1,2].

Each of these feathers is made up of keratin and can basically be divided into a) a calamus, which is the hollow portion of the shaft that inserts into the feather follicle; b) a rachis, which is the central, solid portion of the shaft to which the vane is attached; and c) a vane, which is the flattened part of the feather that is attached to either side of the rachis and comprises of barbs and barbules that overlap and interlock like zippers, giving the feather its strength and rigidity (Fig. 2). Throughout time,

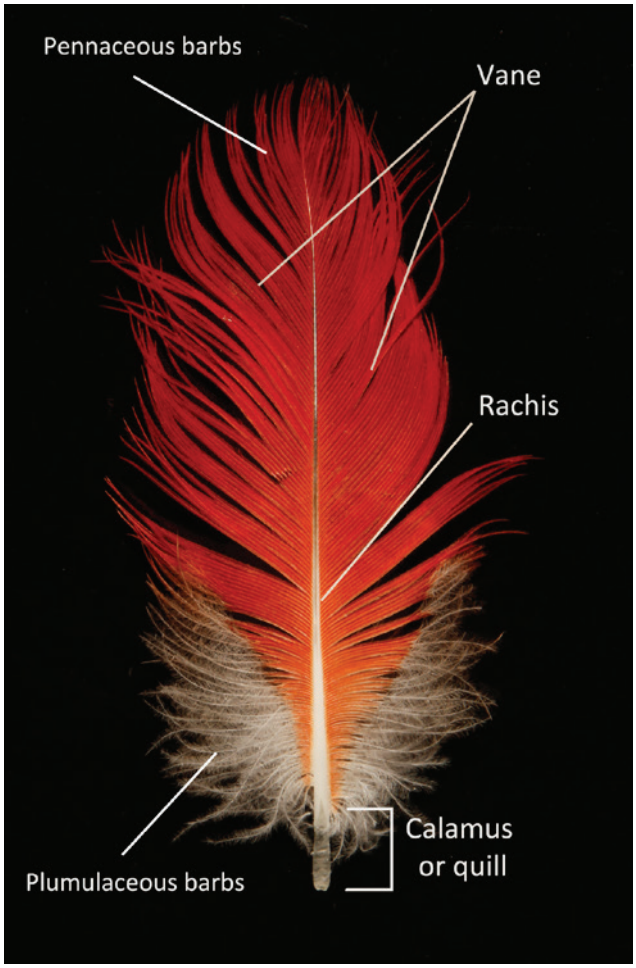

Figure 2. Feather of a Grey parrot (Psittacus erithacus erithacus). In general, each feather consists of a shaft (rachis) to which the vane of the feather is attached. The calamus (or quill) is the hollow, featherless portion of the shaft that is inserted into the feather follicle.

feathers suffer from wear and tear, thereby deteriorating their quality and reducing their ability to insulate and/or enabling the bird to fly. To prevent this deterioration from occurring, feathers are regularly shed and replaced by new ones in a process called moulting.
Various different diseases and disorders may affect the plumage of birds, resulting in poor feather quality, colour changes, malformations, loss of feathers and/or improper moulting. In addition, birds – in particular parrots – are presented commonly with signs of feather damaging behaviour (also referred to as feather plucking or feather picking), which can have a variety of medical and behavioural causes that result in the parrot pulling, biting and/or fraying its own feathers[3,4].

When confronted with a parrot with feather abnormalities, one should consider that, aside from a primary skin and feather disorder, a more serious, generalized or systemic disorder or a behavioural issue may be present. The list of potential aetiologies is long and includes a variety of infectious, toxic, nutritional, neoplastic, immune-mediated, metabolic, endocrine, behavioural, traumatic and management-related conditions[5-7]. In order to be able to effectively treat the bird and resolve the problem, it is thus of utmost importance to identify the underlying cause for the feather abnormalities. For this purpose, a full diagnostic work-up is warranted. As a first step, a thorough and comprehensive history needs to be obtained, which includes information on the presenting complaint (e.g. duration, initial appearance, progression, [responses to] previous treatments and presence of pruritus or self-inflicted trauma), husbandry, diet, living conditions, general condition and behaviour of the bird. Next, a full physical examination should be performed, in which attention should not only be paid to the overall condition of the skin, feathers and feather follicles, but also to identifying signs of a potential underlying generalized illness. If specific dermatologic lesions (e.g. nodules, papules, plaques, ulcers and/or exudate) and/or feather abnormalities are present, these should be examined closely and classified according to their type, localization and distribution. Dependent on the findings during the physical examination, diagnostic work-up may furthermore include a complete blood count and plasma biochemistry, faecal cytology including wet mount, diagnostic imaging (ultrasound, radiographs, CT imaging or MRI) and/or endoscopy to identify or exclude presence of a systemic illness or organ dysfunction (Table 1). In addition, specific tests may be performed to identify presence of a specific

Table 1. Diagnostic tests that may be performed in birds with feather abnormalities

Diagnostic test	Indications
CBC & Biochemistry	Hepatopathy, nephropathy, generalized infection or inflammatory process, diabetes mellitus, hypocalcemia
Toxicology	Suspected lead or zinc toxicosis. Collect heparinized whole blood (lead) or plasma/serum in non-rubber plastic or glass tubes
TSH stimulation test	Hypothyroidism
Faecal cytology (incl. wet mount and/or flotation)	Giardiasis (common in cockatiels), helminth infection, candidiasis, macrorhabdus ornithogaster infection (avian gastric yeast), bacterial gastroenteritis
Radiology	Heavy metal intoxication, reproductive disorder (e.g. egg binding), hepato-, spleno- or renomegaly, proventricular dilatation disease, pneumonia, airsacculitis, neoplastic conditions, musculoskeletal disease (e.g. osteoarthritis, osteomyelitis, fractures, osteosarcoma)
Ultrasound	Hepatomegaly, reproductive disorders (e.g. egg peritonitis, cystic ovary), neoplastic conditions, cardiac disease, ascites
Endoscopy	Air sacculitis, hepato- or nephropathy, splenomegaly, pancreatic disorders, reproductive disease
Skin scrapings	Ectoparasites, in particular mites (e.g. Knemidokoptes)
Impression smear, swab cytology or tape strip	Bacterial or fungal dermatitis, dermatophytosis, Malassezia, Candida, ectoparasites (e.g. feather mites, lice), pox virus
Fine needle aspirate	Skin neoplasia, xanthomatosis, feather follicle cyst, haematoma, bacterial dermatitis or abcess
Feather digest (using potassium hydroxide)	Ectoparasites (quill mites)
Feather pulp cytology	Bacterial or fungal folliculitis, PBFD or polyomavirus infection, quill mites
Culture	Bacterial or fungal dermatitis, folliculitis
Skin and/or feather follicle biopsy (histopathology)	Various infectious, inflammatory and/or neoplastic skin diseases, e.g. PBFD, polyomavirus, bacterial and fungal folliculitis, quill mite infestation, xanthomatosis, squamous cell carcinoma, feather follicle cysts
Intradermal skin testing	Hypersensitivity reactions, allergic skin disease. Thus far not found to be reliable due to the bird's diminished reaction to histamine
Tests for specific causative agents	• PCR testing on whole blood, feather pulp or tissue for Psittacine beak and feather disease virus (PBFD) • PCR testing on faecal swab or tissue for presence of Polyomavirus • PCR on cloacal swab/faeces and/or serologic testing for Avian Bornavirus (ABV) • PCR on conjunctival/choanal/cloacal swab and/or serologic testing for Chlamydia psittaci

pathogen, such as PCR on whole blood to screen for presence of circovirus, the virus causing Psittacine Beak and Feather Disease (PBFD). Other diagnostic procedures that may be used in the medical work-up of a patient with (localized) feather abnormalities and/or skin lesions include (superficial or deep) skin scrapings, fine needle aspirates, tape strip samples, impression smears, feather digest and/or feather pulp cytology, culture and sensitivity tests and/or histopathological examination of skin and/or feather biopsies (Table 1). When diagnostic work-up fails to identify an underlying medical condition for the feather abnormalities, self-inflicted trauma due to a psychogenic cause may be suspected, which subsequently requires the conduction of an extensive behavioural assessment to identify the triggering events.

The current review will focus on the various types of feather abnormalities that may be noted in parrots and discuss their most common aetiologies as well as the currently available techniques that may be employed in the diagnostic work-up, treatment and prevention of these disorders.

Stress Marks

Stress marks, also referred to as stress lines or stress bars, are translucent lines across the vane of a feather (Fig 3). They are generally oriented perpendicular to the shaft and represent segmental dysplasia that occurred in the developing barb and barbules due to a brief period of dysfunction in the epidermal collar from which the feather arises. Any type of disease or condition resulting in stress (e.g. due to transport, restraint, nutritional deficiencies, food deprivation or environmental stressors) that occurs at the time that a feather is growing will be able to induce these lesions[5,8,9]. Similarly, administration of exogenous corticosteroids during feather growth may induce such lesions[5]. Particularly in neonates, stress marks are a common finding as their feeding schedule is disrupted during the weaning process.
Stress marks may be readily identified upon spreading the wing and/or tail feathers and holding them against the light. Their clinical relevance is largely dependent on the extent to which they are present in a bird and the bird's general condition. A few stress marks in an otherwise healthy bird may merely have (temporary) aesthetic consequences and thus be of limited importance, whereas larger numbers of stress bars may be cause for concern and warrant further investigation to identify the underlying cause. The latter is often difficult as the marks are the result of a problem during feather development in the past and the inciting event does not need to be present anymore. However, if the underlying cause can be identified and eliminated or corrected, the abnormalities will generally resolve when the old, affected feathers are replaced by new ones during the next moult.

Broken or abraded feathers

Broken or abraded feathers are a relatively common finding in caged birds (Fig. 4). Particularly the primary flight feathers, alula and carpal coverts and tail feathers are easily damaged[5]. Often, this damage is the result from errors in management, such as housing the bird in a small or overcrowded cage, or in a cage in which perches are too close together. Feather damage may furthermore be self-inflicted (see paragraph feather damaging behaviour) or occur due to trauma, e.g. during restraint, transport or crash landings, which are particularly common in parrots that are trimmed too aggressively. Other predisposing factors include malnutrition (particularly mineral [e.g. calcium, zinc, selenium, magnesium, manganese] and amino acid deficiencies) and/or stress which may result in weakening of the feathers, which are subsequently more prone to wear and tear[5,9]. To identify the potential underlying causes for the trauma, a thorough anamnesis is necessary, whereas a dermatologic examination may help to identify whether an improper wing trim or other feather abnormalities, resulting in a weakened feather (e.g. stress bars) are present. Elimination of the predisposing factors may help to prevent problems in future, but a moult is necessary to shed the damaged feathers. This usually does not pose a huge problem as the consequences are often merely aesthetic, but occasionally the damage may be so

Figure 3. Stress marks in the feather of a Blue and Gold macaw (Ara ararauna). These translucent lines in the vane of a feather are generally oriented perpendicular to the shaft and may develop in a growing feather during a transient period of stress or disease.

Figure 4. Abraded flight feathers in a Grey parrot (Psittacus erithacus erithacus). In this bird, the damage was self-inflicted and started after a poor wing trim. Note the ragged, split ends of the remaining shaft of the primary feathers.

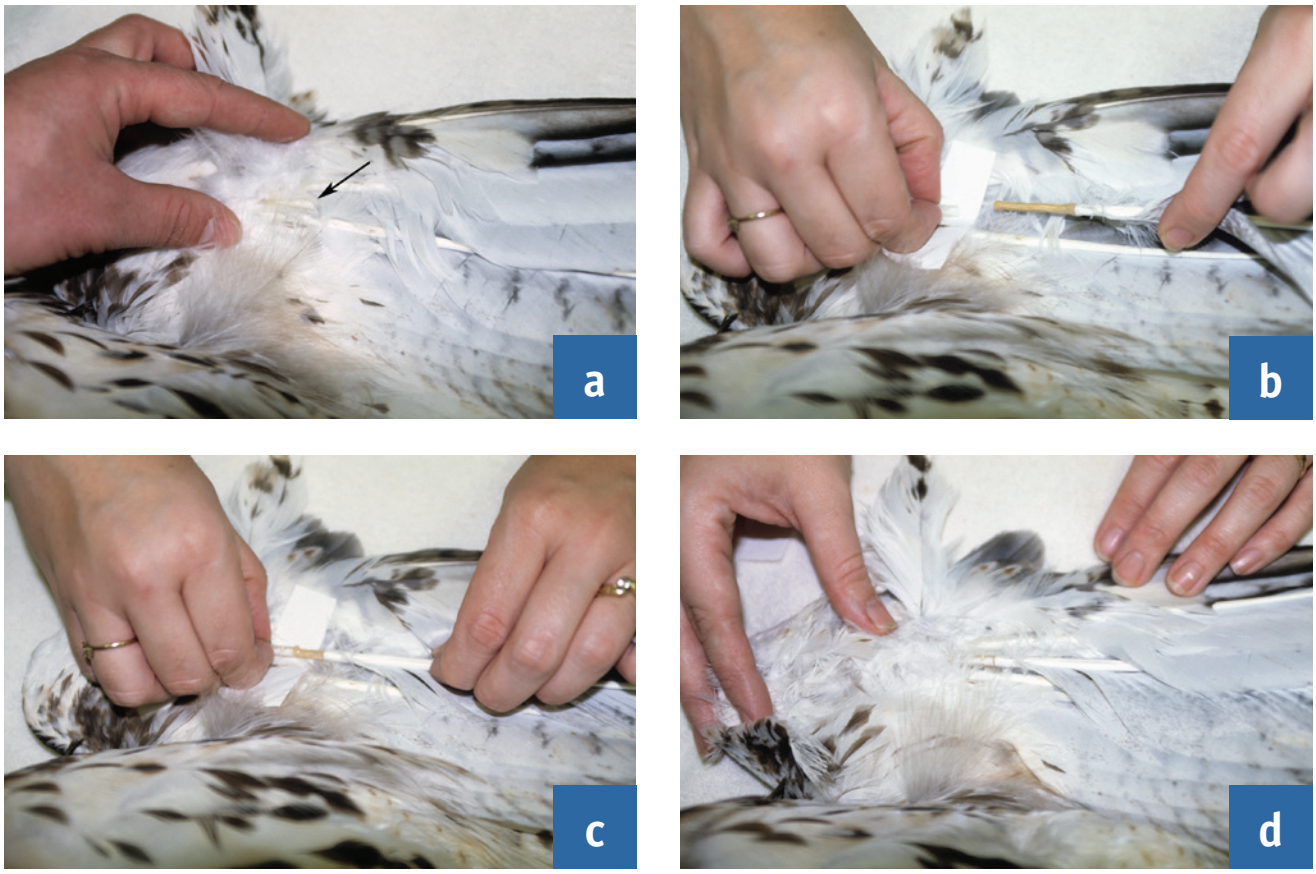

Figure 5. Imping technique to repair a primary wing feather in a bird of prey. First, the damaged or broken feather is clipped approximately 15-25 mm distal to the skin using a nail cutter or sharp scissors (a). Next, a donor feather is cut to the same length as the recipient feather, after which both shafts are cleaned out so that a thin bamboo stick fits tightly in the shaft (b). Using superglue, the two ends are subsequently glued together. Paper is placed underneath the imping site to prevent glue exposure to the adjacent feathers (c). Once connected and before the glue dries, the donor feather is rotated to achieve proper positioning (d).

severe that it results in difficulties with e.g. flight and balance. In such cases, the damaged flight feathers may be repaired using a technique called imping (Fig 5a-d). During the procedure, which is commonly used in falconry, donor feathers (preferably of the same bird or a healthy conspecific) are attached onto the hollow shaft of the damaged feather by the use of small splints[10]. Although the technique may be effective to restore the functionality of the feather in cases of iatrogenic damage, its use in birds with self-inflicted damage is more controversial and is advised primarily to remove the stimulus to chew at the cut ends of (poorly) clipped feathers[10].

Whereas damage to most feathers is primarily aesthetic, damage to newly emerging pin or blood feathers may be more problematic as this can result in severe haemorrhage (Fig. 6). Electrocautery or using caustic substances (e.g. silver nitrate) is not advised in such cases as this may result in damage to the feather follicle, resulting in cessation of feather growth or abnormally growing feathers[7]. To prevent this from occurring, manual pressure or application of a substance to stimulate coagulation (e.g. flour) may be used to control the bleeding. Alternatively, the damaged blood feather may be removed by slightly twisting and pulling the feather from its base in the direction of the feather growth, after which firm digital pressure is placed on the follicle until the bleeding has stopped[7].

Figure 6. Newly emerging pin or blood feather. (Black arrow)Trauma to such feathers may easily result in severe haemorrhage.

Figure 7. Wild Hyacinth macaw (Anodorhynchus hyacinthinus) with an unkempt plumage. The poor plumage quality in this bird may be explained by the fact that this bird (a female) was incubating her eggs. The relative small nesting site (a hollow tree) severely restricts her movements and ability to stretch the wings, thereby easily resulting in feather damage and an overall poor plumage quality. Also note the black discolouration of various covert feathers on the dorsal surface of the wing.

Poor feather quality

Birds with poor feather quality often present with a ragged, dull, unkempt plumage (Fig 7). This poor feather quality may arise from any condition that prevents a bird from preening, such as the use of Elizabethan collars, neck braces, beak malformations, obesity, arthritis, pathological fractures and other conditions that restrict the bird's movement[9,11]. Other factors that may negatively affect feather quality include housing conditions (e.g. low humidity, irregular or inappropriate photoperiod, exposure to aerosols or cigarette smoke), ingestion of toxins (e.g. trichothecene), malnutrition (e.g. vitamin A deficiency), feather or quill mites and chronic, systemic illness (e.g. infections, neoplasia, gastrointestinal, hepatic, pancreatic disease)[9,11-15]. Diagnostic work-up should therefore include a thorough history to identify management errors in housing, care and/or nutrition, combined with a full physical and dermatologic examination to identify presence of parasites or signs of underlying systemic illness. In the latter case, further diagnostic tests may be performed. Correcting the underlying cause usually will help to improve the feather quality, but one or more consecutive moults may be required to completely resolve the issue.

Malnutrition

Malnutrition is one of the most commonly encountered problems in captive psittacines. Many birds are still fed on all-seed diets which are deficient in a number of nutrients, including vitamins (e.g. vitamins A, D, K, B12, riboflavin, pantothenic acid, choline, niacin), minerals (e.g. calcium, phosphorus, sodium), trace elements (e.g. manganese, zinc, iron, iodine, selenium), and essential amino acids (particularly lysine and methionine) and fatty acids (omega-3 fatty acids) [14-17]. Many of these nutrients, such as essential amino and fatty acids, copper, zinc, vitamins A, B and E, are important for normal feather growth[18,19]. As a consequence, the normal moulting process may be delayed or interrupted in birds which are fed an imbalanced or deficient diets and may thereby give rise to development of depigmented or discoloured, poor quality feathers that break easily and have a dull, ragged-looking appearance (Fig. 8) [14,20]. Other signs of malnutrition include thickening and scaling of the skin, especially on the face, feet and legs[14,15]. Birds may furthermore be predisposed to develop skin infections and inflammation as a result of deficiencies, particularly hypovitaminosis A (Fig. 9) [14,15]. Although abnormalities are seldom specific, an underlying nutritional component should be suspected in any abnormally feathered bird that is primarily fed seeds. In such cases, treatment should at least be aimed at correction of the malnutrition and may include administration of multivitamin injections (5,000-20,000 IU/kg IM; based on vitamin A content), oral administration of supplements and conversion to a complete (pelleted) diet.

Feather or quill mites

Birds are host to a variety of different mites that may inhabit the skin and/or feathers[13]. Quill mites predominantly live and reproduce inside the feather quills where they feed on the available tissue and secretions[13]. Although many different species have been identified (e.g. *Syryngophilus*, *Dermoglyphus, Pterolichus, Analges* and *Harporhynchus spp.*), quill mites are a relatively uncommon cause for feather abnormalities in parrots[7,9]. When present in large numbers, they may cause damage

Figure 8. Amazon parrot (Amazona spp.) with a severely abnormal plumage resulting from chronic malnutrition. Note the feather discolouration and depigmentation as well as the overall dull- and ragged looking plumage of this bird. Surprisingly, the bird was not presented for these feather abnormalities, but rather for the presence of a tumour of the uropygial gland, which turned out to be a squamous cell carcinoma.

Figure 9. Grey parrot (Psittacus erithacus erithacus) with Klebsiella dermatitis. The bird was fed an all-seed diet, which probably predisposed the bird to the development of secondary infections. As a sequel to the dermatitis, the bird started to pluck its feathers, but fortunately the plucking ceased after treatment with antibiotics and conversion to a pelleted diet.

to the feathers by destroying the medulla from the quill to the rachis. Affected quills tend to break or bleed easily and may change in appearance from transparent to opaque. Although most quill mites primarily feed on the keratin and medulla of the feather quills, quill wall mites (e.g. *Syringophylus spp.*) primarily feed on feather follicle fluid and outer, unkeratinized layers of the feather germ of developing feathers, thereby triggering hyperkeratosis of the feather sheath.

Feather mites (e.g. *Protolichus, Dubininia spp.*) live between the barbs of the contour, wing and tail feathers and primarily feed on the feather fragments and skin debris[13]. Feather mites are generally considered apathogenic, but may cause problems when present in high numbers, non-host adapted species or birds with a compromised immune system[21]. The mites, which may be identified as tiny dark spots of grains of sand on the ventral surface of feathers, may cause irritation (leading to feather damaging behaviour) and induce feather lesions such as darkened areas, dots or stripes on bright feathers or bright dots and stripes on dark feathers.

Feather and quill mites are usually detected upon microscopic examination of the pulp and/or vane of a damaged or developing feather (Fig. 10). They can usually be treated effectively with ivermectin (0.2 mg/kg PO or IM for a minimum of two treatments given 10-14 days apart), selamectin (25 mg/kg topically q3-4 weeks), moxidectin (0.2-0.5 mg/kg orally) and/or topical acaricides (e.g. permethrin, pyrethrin, fipronil)[9,13,21].

Abnormal colouration of feathers

Feathers derive their colouration from pigments (melanins, porphyrins and carotenoids) and/or structural conditions

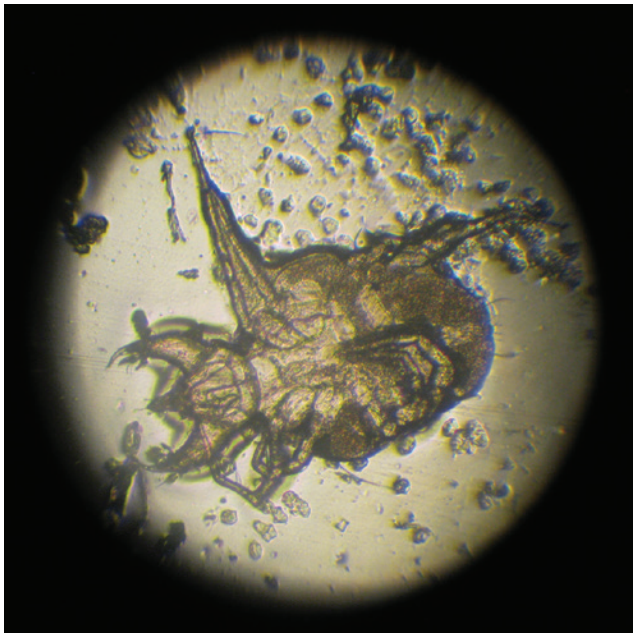

Figure 10. Feather mite, found in a Turquoise parrot (Neophema pulchella) with poor feather condition.

Figure 11. Black discolouration of the feathers due to chronic malnutrition in a conure

of the feather that modify or separate the components of white light[2]. As a consequence, any condition resulting in a change of the feather structure may also result in a change of colour. Changes in colouration of the feathers may be localized or generalized. The most common cause for a generalized change in feather colour is malnutrition (Fig. 11). A lack of dietary carotenoids, which give rise to the yellow, orange and red colours in the plumage, results in a muted feather colour[14]. Colour changes may also result due to low levels of non haem iron, which is needed for production of porphyrins that give rise to green and reddish colouration, or due to tyrosine and/or copper deficiencies which results in impaired melanin production and causes the feathers to become lighter[14]. Achromatosis (loss of colouration) of the primary feathers has furthermore been found to occur in cockatiels when fed choline or riboflavin deficient diets[15]. In such cases,

Figure 12. Reddish discolouration of the feathers on the chest in a peach-faced lovebird (Agapornis roseicollis).

correction of the malnutrition by conversion to a complete, pelleted diet may result in a change of colour back to normal after the subsequent moult.
Other causes of generalized feather discolouration include liver disease, chronic lead toxicosis, hypothyroidism, neoplasia (e.g. pituitary tumour), drug administration (e.g. thyroxine, fenbendazole), early circovirus infection (feather dystrophy) and genetic mutations (Fig. 12).
Localized colour changes involving a single or few feathers, in contrast, are most likely the result of a localized inflammatory process or trauma that affected the feather follicle during its development.

Feather cysts

Feather cysts usually present as oval or elongated lumps or masses in which a yellow-whitish material (keratin) accumulates (Fig. 13). Although these lesions can appear anywhere on the body, they are most commonly found on the wings. Particularly the soft-feathered canary breeds such as Gloucester and Norwich appear prone to developing feather cysts thereby raising suspicion of a

Figure 13. Norwich canary (Serinus canaria) with a feather follicle cyst. Particularly soft-feathered breeds are prone to developing such cysts, which are filled with white-yellowish keratinaceous material.

hereditary condition[22]. The condition may, however, also incidentally be encountered in parrots and parakeets. In these species, the cysts most often represent an acquired condition which may be the result of an infection, trauma or other condition that interferes with the normal feather growth[5,6]. Diagnosis is usually based on the clinical signs and appearance of the mass. Cytology of the mass may reveal erythrocytes, erythrophagocytosis, mixed-cell inflammation with a marked amount of background debris, multinucleated giant cells and/or presence of feather fragments. Surgery is often indicated to treat these cases, especially in those cases in which self-mutilation and/or (secondary) infections are present, and may involve curettage or surgical excision of the feather cyst, or of the whole feather tract, if multiple cysts are present[23].

Folliculitis

Folliculitis (inflammation of the feather follicles) is a condition that may develop due to (secondary) bacterial and/or fungal pathogens. Pathogens that have been implicated in folliculitis include *Staphylococcus* spp., *Aeromonas* spp., *Mycobacterium* spp., *Aspergillus* spp. and *Malassezia* spp. [7,24,25]. Grossly, the lesions are characterized by presence of perifollicular swelling and erythema, but hyperkeratosis, crust formation and discolouration of the feathers have also been noted when fungal organisms are involved (Fig. 14) [7]. Birds may furthermore show signs of pruritus and/or pain, which may be expressed by restlessness, shivering and/or feather damaging behaviour. To identify presence of a fungal or bacterial organism, an affected feather may be pulled and aseptically opened, after which the pulp may be cytologically examined using specific stains (e.g. Gram's stain) and/or cultured

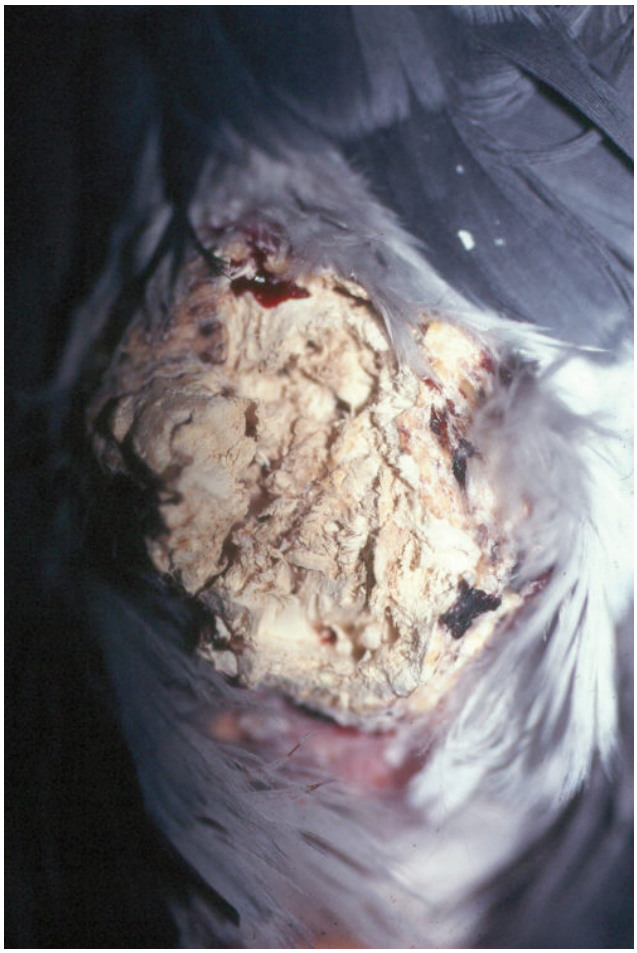

Figure 14. Fungal dermatitis due to Aspergillus spp. in a Grey parrot (Psittacus erithacus erithacus). Note the hyperkeratosis and crust formation of the skin.

to identify whether and which pathogenic organisms are present. Occasionally histologic examination of feather follicle biopsies may be needed to identify the cause of the folliculitis. In case of bacterial folliculitis, treatment is preferably based on the results of the sensitivity test; in case of fungal folliculitis, antimycotic drugs such as itraconazole, fluconazole, clotrimazole and terbinafine may be used. Prolonged treatment periods of >3 weeks are often needed to effectively eliminate the infection.

Polyfolliculitis

Polyfolliculitis is a condition that is characterized by the appearance of multiple feathers from the same follicle (Fig. 15a and b). The condition is commonly seen in budgerigars, cockatiels and lovebirds and appears to primarily affect the feather tracts of the tail and dorsal region of the neck [5,7,26,27]. Newly emerging feathers which arise from the follicle often have short, stout quills with retained sheaths. The condition often results in pruritus, thereby provoking the birds to pluck their feathers and/or automutilate themselves. The exact aetiology remains

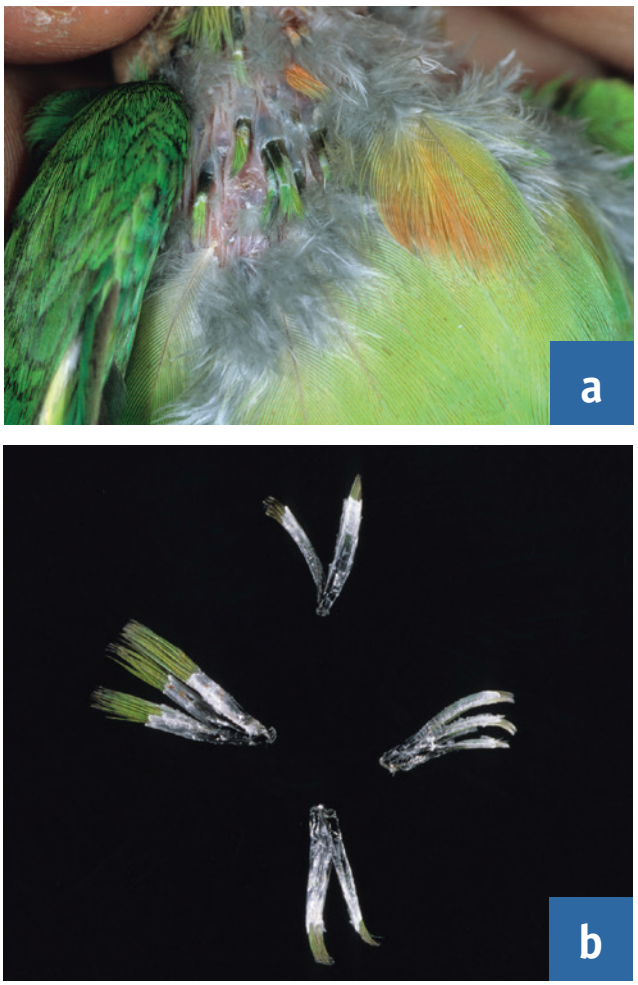

Figure 15. Polyfolliculitis in a lovebird (a). Note the abnormal appearance of the feathers, which often have short, stout quills with retained sheaths (b).

unknown, but viruses such as PBFD-virus, polyomavirus, poxvirus have long been suspected as the potential cause. Diagnosis is usually based on the clinical appearance of the feather follicles, and may be confirmed by histopathological examination of feather follicle biopsies which reveal the presence of multiple feather shafts arising from a single follicle, chronic inflammation beneath the pulp cap and thickening of the feather sheath. Occasionally, large, keratin-filled cysts (feather cysts) may also be noted. Because no definite aetiology has been established, treatment is usually palliative and consists of (manual or surgical) removal of the abnormal feathers, treatment of secondary infections and anti-inflammatory drugs [26].

Feather dystrophy

Feather dystrophy is characterized by the formation of abnormally shaped feathers and may result from direct or indirect damage to the follicular collar or developing feathers. The most common causes for feather dystrophy

Figure 16. Chrysanthemum or feather duster disease in a budgerigar (Melopsittacus undulatus).

in psittacine birds include infections with a circovirus that gives rise to the development of PBFD and polyomavirus, which are both discussed below.

In budgerigars, a hereditary condition has also been described [28]. The disease is characterized by a continuous growth of the contour, tail and flight feathers, giving the bird the appearance of a feather duster, hence the name 'feather duster disease' or 'chrysanthemum disease' (Fig. 16). Birds with this condition often appear less alert than nest mates and often cannot fly. There is no treatment for the condition; birds are often euthanized in the nest.

Psittacine beak and feather disease

Psittacine beak and feather disease (PBFD) is caused by a virus of the family Circoviridae and may affect both Old and New World parrots [29,30]. The disease, which is enzootic in Australia and transmitted via oral ingestion or inhalation of infected skin and feather dander, primarily affects juvenile and young adult birds up to 3 years of age. Two syndromes are recognized: 1) an acute form, which occurs predominantly in nestlings and juvenile Grey parrots (*Psittacus erithacus*) and is characterized by the rapid onset of leucopenia and liver necrosis that result in the development of secondary infections and general malaise; birds often die within a week after onset of the clinical signs, without obvious feather lesions present [31], but cases may also be noted in which generalized feather loss is present (Fig. 17) [29,30]; 2) a chronic form which usually becomes apparent when the bird goes through its first moult and the normal feathers are replaced by dystrophic feathers giving rise to progressive and often

Figure 17. Acute PBFD in young Grey parrots (Psittacus erithacus erithacus). Feather abnormalities may be generalized, but affected birds, especially young Grey parrots, may also die prior to developing the characteristic feather lesions, as a result of immunosuppression and secondary infections.

Figure 18. Sulphur-crested cockatoo (Cacatua galerita) with chronic feather abnormalities due to PBFD.

bilateral symmetrical feather, claw and beak deformities. The pattern of feather dystrophy may depend on various factors such as the stage of moult that the bird was in at the time of onset of the disease, and the type of parrot species involved [30,31]. Powder down feathers may be the first affected in cockatoos (*Cacatua* spp.), followed by other types of feathers when the disease progresses, eventually giving rise to a generalized pattern of feather abnormalities and feather loss (Fig. 18) [7,31]. In other species, feather

Figure 19. Reddish discolouration of the feathers in an adult Grey parrot (Psittacus erithacus erithacus) with PBFD.

Figure 20. Annular constriction and bending of the feathers in a bird with PBFD.

lesions may be mild and localized (e.g. in lovebirds [32]), primarily characterized by delayed moult or poor quality feathering (e.g. in Eclectus parrots [7]) or affect the colouration of feathers (e.g. in Grey parrots, Fig. 19 [33]).

Feather abnormalities commonly seen in PBFD-affected birds generally comprise of one or more of the following: thickened and/or retained feather sheath; haemorrhage within the shaft or pulp cavity; stunting or clubbing of feathers; stress lines across the vanes; annular constrictions of the calamus; curling of the feathers and feather necrosis of both contour and down feathers (Fig. 20). In the later stages, feather development is hampered until eventually all feather growth ceases and the bird becomes progressively bald. In addition to feather changes, beak and claw abnormalities (e.g. overgrowth, malformation, necrosis, fractures and fissures) may also develop [29,30]. The clinical course of the chronic form of the disease may run from months to years until the birds eventually succumb to secondary infections [29,30].
A presumptive diagnosis can usually be made based on the distinct clinical features of the disease, but further

diagnostic tests are needed to confirm the diagnosis. A polymerase chain reaction (PCR) on blood, fresh feather pulp or tissue samples is considered the most sensitive method for detecting latent or early infections, but may sometimes yield false-negative as well as false-positive results [7,29,30,34]. In those cases, histological examination of feather follicles (plucked feathers or a feather follicle biopsy) can be used to confirm the disease [7,30]. Other diagnostic tests to confirm presence of the virus include a haemagglutination assay (HA) on feather material, a haemagglutination inhibition test (HI) on blood. Serological testing with the use of HI, or enzyme-linked immunosorbent assays (ELISA) may in particular be useful to detect PBFD-infected flocks and/or demonstrate seroconversion in an individual bird, but not all of these aforementioned tests are available in every country [30]. There is no treatment for PBFD and affected birds may be euthanized once the disease progresses. Although experimental inactivated vaccines are available in Australia these do not prevent infection [35] and prevention is therefore aimed primarily at reducing spread of the disease by maintaining closed flocks, quarantine measures and (repeated) testing. Proper hygiene and disinfection protocols, including the use of glutaraldehyde as a disinfectant, are also recommended to reduce the risk of transmission [30].

Polyomavirus

Polyomavirus can infect all psittacine birds, but most commonly affects budgerigars (*Melopsittacus undulatus*), macaws (*Ara* spp.), conures (*Aratinga* and *Pyrrhura* spp.), caiques (*Pionites* spp.), Eclectus parrots (*Eclectus roratus*), Ringneck parrots (*Psittacula* spp.) and lovebirds (*Agapornis* spp.) [7]. Nestling and juvenile birds are most susceptible. In larger psittacine species the virus usually causes an acute, fatal disease in nestlings characterized by subcutaneous haemorrhage, hepatomegaly, anorexia, crop stasis, depression and sudden death without feather abnormalities present [30,36]. In budgerigars, feather dystrophy may develop in the nestlings that survive, similar to that seen in PBFD. This form of the disease, also termed 'French moult' follows a relatively typical pattern: budgerigars less than 2 weeks of age show a lack of powder down on the head and neck; those of 2 to 4 weeks of age show a lack of or incompletely developed flight feathers; and those older than 25 days of age will develop feathers, but the tail and/or some flight feathers may remain underdeveloped or absent [37]. The failure to develop rectrices and/or remiges generally renders the birds flightless. Often, these individuals are termed 'runners' or 'creepers' (Fig. 21) [7,30,36].

Figure 21. Polyomavirus infection in a budgerigar (Melopsittacus undulatus), resulting in a condition called 'French moult'. The primary flight feathers often do not develop in affected juveniles, essentially rendering them flightless. This is why such birds are often called 'runners' or 'creepers'.

A presumptive diagnosis of polyomavirus infection is usually based on the history, clinical findings and pathological features. Definite diagnosis requires serology or PCR testing of cloacal swabs, blood or tissues. PCR is currently the method of choice for many veterinarians. Due to the short viraemia and irregular shedding, however, many false-negative results may be obtained. Definite diagnosis may therefore sometimes require histopathologic examination of tissue samples to identify the presence of basophilic intranuclear inclusion bodies in various tissues (e.g. liver, spleen, kidney and feather follicles), which may be confirmed as polyomavirus using electron microscopy or in situ hybridization techniques [29,30]. Currently, there is no specific treatment for polyomavirus infection. A commercially available polyomavirus vaccine is available in the US to protect nestlings of large psittacines in aviaries in which the virus is endemic [38]. Other measures to control and prevent spread of polyomavirus in the population include hygiene and quarantine measures similar to those discussed for PBFD as well as PCR testing to identify viraemic and/or shedding birds. In addition, temporary cessation of breeding in flocks of affected budgerigars may help to reduce spread of the disease as the disease may be transmitted both vertically and horizontally [36].

Conclusions

A variety of different conditions may affect the plumage of parrots resulting in various feather abnormalities. For all of the disorders a thorough history and medical work-up are needed to identify any underlying causes that should be treated accordingly. Plumage disorders are, however, not restricted to development of abnormal feathers, but also include problems with feather growth, feather loss. Particularly self-inflicted pulling, plucking, biting, chewing or fraying of the feathers (so-called feather damaging behaviour) is commonly seen in parrots. The disorders involving problems with feather loss and feather growth, including feather damaging behaviour will be discussed in part 2 (EJCAP Summer 2014).

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Commissioned paper

Medical treatment of canine seizures – a review

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SUMMARY

Over the last decade, experience was gained with the use of newer human anticonvulsive medications for treating epilepsy in dogs. In addition, a new drug (imepitoin) was introduced on the European veterinary market. This paper provides an overview of the characteristics, advantages and disadvantages of the medications currently used in dogs suffering from epilepsy.

Introduction

An understanding of both the current concept of seizure classification and general principles of epilepsy treatment in dogs is necessary for appropriate decision making and successful treatment in dogs with epilepsy.

Classification

Epileptic seizures and epilepsy are not synonymous. An epileptic seizure is defined as paroxysmal hypersynchronous electrical brain activity with associated clinical signs, while the term epilepsy is used in cases of recurrent seizure activity only. Epileptic seizures may result from temporary metabolic or metabolic-toxic conditions. These patients rarely require permanent anticonvulsive therapy if the underlying metabolic disturbance can be identified and treated successfully. In those cases the term reactive epileptic seizures is used.

Epilepsy is commonly divided into idiopathic epilepsy and symptomatic epilepsy. In the latter, recurrent seizures occur as a consequence of a structural brain lesion or a metabolic disease initiating a self-perpetuating process of seizure activity. In contrast, in idiopathic epilepsy no underlying pathology can be identified despite extensive diagnostic tests. Therefore, the term epilepsy of unknown cause is sometimes applied. The classification is important as a dog presented for seizures may require further diagnostic tests if symptomatic epilepsy is suspected, since anticonvulsive therapy may be less effective if the underlying pathology is not addressed simultaneously. Dogs with idiopathic epilepsy usually experience their first seizure between 1 and 5 years of age, sometimes this time span is extended to 6 months and 7 years. The neurological examination performed between seizures is normal in those dogs. Dogs starting to seizure outside that timeframe or dogs with abnormal neurological examination between seizures, especially if those are lateralised, should be screened for any possible underlying pathology since symptomatic epilepsy is very likely. In one study, 79% of dogs experiencing their first seizure at an age of seven years or older an underlying pathology could be identified[1]. The term cryptogenic epilepsy is sometimes used in dogs experiencing their first seizure outside the typical age range for idiopathic epilepsy, but where extensive diagnostic tests have failed to identify the underlying cause.

In addition to the classification based on the underlying pathology, seizures can be classified as generalised or partial, according to the clinical signs.

Generalised seizures involve the entire forebrain and are characterised by loss of consciousness in combination with different types of abnormal motor activity (tonic-clonic, tonic, clonic, paralyzed) and autonomous nervous system dysfunction (urination, defecation, salivation).

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In contrast, dogs with partial seizures exhibit only some of the signs described above. They are subdivided into simple partial seizures and complex partial seizures. The first are characterised by motor activity affecting selective muscle groups only in dogs with normal mentation, whereas the term complex partial seizures requires some degree of abnormal mentation or behaviour alone or in combination with partial motor activity. It should be noted that partial seizures are not just "minor seizures" with less clinical relevance than generalised seizures!

General therapeutic principles

There is an on-going debate about the optimal time for initiating anticonvulsive therapy. Some prefer to start treatment rather early in the course of the disease in order to prevent structural brain changes setting up the brain for further seizures ("kindling"). Others tend to extend the therapy-free period as long as possible to limit side effects of chronic anticonvulsive therapy. There is obviously a fine line between effective seizure control and minimizing side effects of long-term medication. The following recommendations may serve as a guideline for initiating anticonvulsive therapy:

- on average more than one seizure every second month
- cluster of seizures (more than one seizure within 24 hours) independent of their frequency
- a single status epilepticus
- symptomatic epilepsy caused by a known structural brain lesion
- symptomatic epilepsy starting within 2 weeks following brain trauma

It is recommended to start with a single agent therapy. Combination therapy should only be considered if the upper end of the dose range does not result in desired seizure control. Sudden switching between different drugs or even between different brands of the same drug should be avoided as this may increase seizure frequency. Once anticonvulsive treatment has been started, subsequent life-long therapy is required in most cases. However, even following the principles of anticonvulsive therapy, treatment will result in seizure freedom in about 30-40% of dogs only. There are another 30% in which the seizure frequency can be reduced significantly, while 30-40% of dogs are so-called "none-responders", as the seizure frequency does not decrease by at least 50%.

Therapeutic options

Currently the following medications are available for veterinary use:

- phenobarbital
- imepitoin
- potassium bromide

In addition, the following human medications are used in dogs:

- levetiracetam
- gabapentin
- pregabalin
- topiramate
- zonisamide
- felbamate
- primidone

Veterinary products

Phenobarbital

Phenobarbital is currently still considered the medication of choice for initiating anticonvulsive therapy in dogs. It has been used in dogs for several decades now, providing data on its effectiveness, on when it can be used and when not, its potential side effects and risks, and how to react if complications occur. Therefore, phenobarbital is currently still recommended as first line treatment.

Phenobarbital is initiated at a dose of 2.5 mg/kg twice daily (BID) per os. There is a large individual variation in resulting blood levels. Serum blood levels should therefore be checked two weeks after initiating therapy and the individual dose should be adjusted accordingly. The owner should be informed that the medication might take up to two weeks to achieve full effectiveness. The desired serum level is 20-30 µg/ml, with levels < 15 µg/ml being not effective in many patients, whereas levels > 35 µg/ml lead to an increased risk of hepatotoxicity. The levels should be interpreted in the light of the achieved seizure control reported by the owner. For instance, there might be dogs that do not exhibit seizures anymore with a serum level between 10-15 µg/ml and therefore do not require an increase of the phenobarbital dose.

If a phenobarbital dose has to be adjusted, the new dose can simply be calculated due to a linear dosage-serum level relationship. Should the blood level be increased by 50%, the phenobarbital dose has to be increased by 50% as well.

Serum levels should be obtained directly before drug administration to obtain the lowest level of the day, bearing the highest risk of seizures. The circadian variation, however, is negligible in dose ranges below 10 mg/kg/day.

Once desired serum level and seizure control have been achieved, serum blood levels should be controlled again 3 months later. During the first 3 months of therapy the hepatocellular cytochrome-P-450 enzyme system is activated, resulting in increased phenobarbital metabolism. Therefore, a serum level well within the range of 20-30 µg/ml after 2 weeks of therapy may drop to subtherapeutic levels 3 months later; another dose adjustment might be necessary in those cases. Further serum level checks are recommended every 6 months. A decrease of the phenobarbital serum level of more than 20% without any dose change is indicative of poor owner compliance[2]. Visits for serum level checks should be used to control the liver parameters alanine aminotransferase, glucose and albumin. The liver enzyme alkaline phosphatase will be increased in most dogs on phenobarbital treatment due to enzyme induction and therefore, it is not suited for evaluating liver cell damage. If there is any suspicion of impaired liver function, a serum bile acid test should be performed. It should be noted that thyroid tests interfere with phenobarbital treatment and test results should be interpreted with caution in dogs receiving phenobarbital treatment.

Side effects of phenobarbital treatment can be grouped as follows:

- frequent side effects:
  - polyphagia
  - polydipsia/polyuria (do not restrict access to water!)
  - sedation (usually in the first 2-3 weeks after starting treatment only)
- less frequent side effects:
  - hepatotoxicity
  - anaemia, neutropenia, thrombocytopenia
  - ataxia
  - hyperexcitability (medication has to be discontinued)
  - pancreatitis

Hepatotoxicity can manifest itself as chronic liver cell damage or as fulminating hepatic failure in the hepatocutaneous syndrome (superficial necrolytic dermatitis). In those cases, phenobarbital has to be substituted by another medication.
The phenobarbital treatment protocol is summarised in Figure 1.

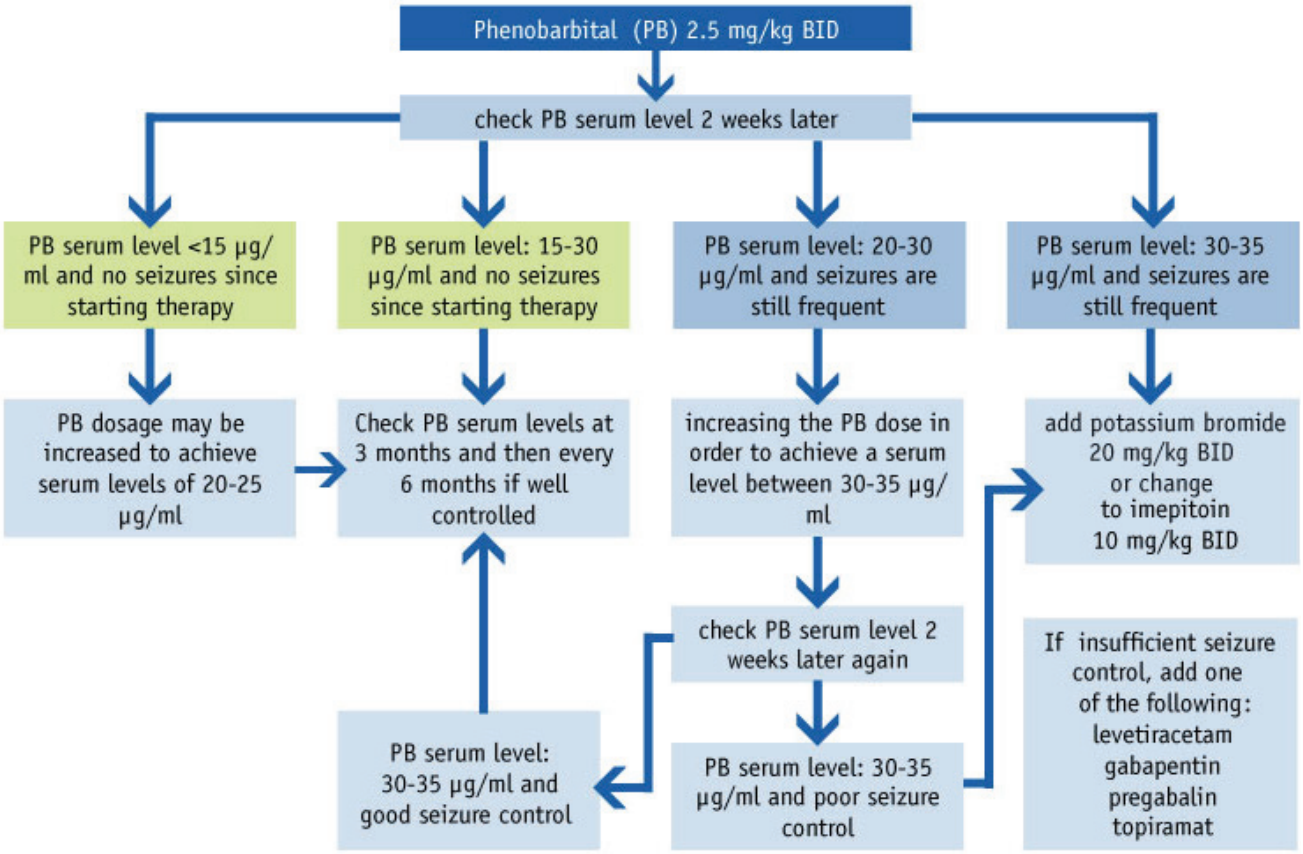

Figure 1: Phenobarbital treatment protocol for dogs with epilepsy

Imepitoin

Imepitoin (Pexion®, Boehringer Ingelheim) was introduced into the European market in early 2013 as the first anticonvulsive medication licensed for animals only. Imepitoin has an action similar to phenobarbital on the $GABA_A$ receptor, even though the substances use different binding sides. Imepitoin connects to the benzodiazepine receptor whereas phenobarbital uses the barbiturate receptor. In contrast to phenobarbital, however, imepitoin is a low-affinity partial $GABA_A$ receptor agonist[3]. This may explain why patients do not develop tolerance, which is usually seen in complete receptor agonists such as phenobarbital[4,5]. Therefore, imepitoin can be abruptly discontinued if indicated, without any risk of withdrawal seizures, even though mild behavioural changes and muscular signs may be observed[6,7].

The anticonvulsive efficacy of imepitoin seems to be slightly lower than that of phenobarbital[4,8]. A study investigating efficacy of imepitoin as monotherapy in dogs with idiopathic epilepsy found a reduction in seizure frequency in 75% of dogs using an average maximum dose of 20 mg/kg BID. In dogs that responded, seizure frequency was reduced by 49.8%[8]. The same study compared the efficacy of imepitoin (n=12) and phenobarbital (n=44) in dogs with idiopathic epilepsy. In both treatment groups, the seizure frequency prior to initiating anticonvulsive therapy was 1.6 seizures per month. The frequency was reduced to 0.71 per month in the imepitoin group and to 0.59 in the phenobarbital group. This difference was significant ($P<0.05$). In addition, the percentage of dogs with a reduction of seizure frequency by more than 50% was 33% in the imepitoin group and 64% in the phenobarbital group. This difference, however, was not significant[8]. Another field study resulted in a reduction of seizure frequency from 2.3 to 1.1 per month in the imepitoin group compared to 2.4 to 1.1 in the phenobarbital group[3].

However, there might be fewer side effects with imepitoin compared to phenobarbital. No side effects were observed in dogs after any single dose of imepitoin, while phenobarbital induced sedation, ataxia, polydipsia and polyuria even though the exact phenobarbital dose used for that comparison is difficult to extract from this publication[5]. Another pharmacological safety study identified no statistically significant side effects at dosages up to 90 mg/kg BID for 26 weeks of imepitoin treatment in healthy Beagles[3]. In contrast, polyphagia was seen in 58% of dogs as main side effect of long-term imepitoin monotherapy[8]. This effect seemed to be permanent in only 21% of dogs[8]. In addition, but less frequently, the following transitory side effects have been seen with high doses of imepitoin: hyperactivity, polyuria, polydipsia, somnolence, salivation, vomiting, ataxia, diarrhoea, third eyelid protrusion, impaired vision as well as hypersensitivity to noise[7].

Therapy is initiated at a dose of 10 mg/kg BID and it can be increased up to 30 mg/kg BID if necessary. In the author's experience, most dogs require a dose higher than 10 mg/kg BID for appropriate seizure control. Serum level checks are not recommended since plasma levels do not correspond with seizure frequency[8]. Effectiveness is solely based on reduction of the seizure frequency, which can be assessed within a few days since full effect is expected within 2 days after initiating treatment[9]. Therefore, imepitoin seems to be a good choice for patients with high seizure frequencies where a significant reduction of seizures is desired within a few days.

Because of the characteristics of imepitoin as described above, clinicians and owners may consider switching anticonvulsive treatment from phenobarbital and/or potassium bromide to imepitoin. However, changing anticonvulsive therapy in well-controlled cases is strongly discouraged, since any change includes the risk of increasing seizure frequency. In cases where impaired liver function or unacceptable side effects require switching from phenobarbital to imepitoin the following protocol has been recommended: both medications are given simultaneously for a transitional period of 3 months. The phenobarbital dose is reduced by 25% every month before it is completely withdrawn at the end of the 3-month-period[10]. The imepitoin treatment protocol is summarised in Figure 2.

Potassium bromide

Bromide is the oldest of the anticonvulsives discussed here, and has been used for more than a hundred years[11]. It can be used as monotherapy but it is more commonly indicated as add-on medication in case phenobarbital alone cannot control seizure activity sufficiently. In such patients, adding potassium bromide results in seizure freedom in about 25% of dogs [12].

Potassium bromide is not metabolised by the liver but it is excreted unchanged by the kidneys. It is therefore used

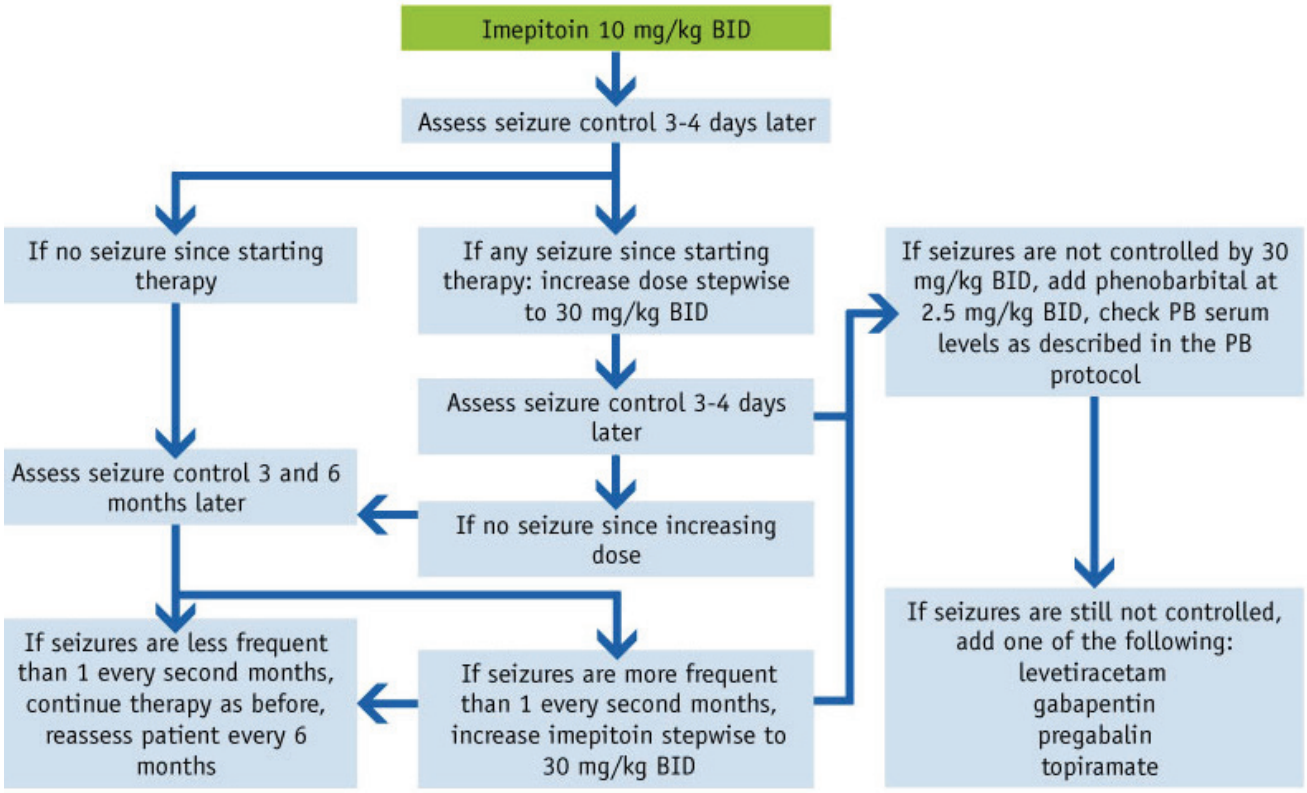

Figure 2: Imepitoin treatment protocol for dogs with epilepsy

in dogs with impaired liver function prior to initiating anticonvulsive therapy or where phenobarbital-induced hepatotoxicity prohibits further use of this medication. Potassium bromide is usually started at a dose of 20 mg/kg BID even though the extremely long half-life (3-4 weeks) allows once-daily medication of 40 mg/kg. This long half-life is one of the major disadvantages of potassium bromide: a delay of 3 to 4 months is required before steady-state serum levels can be measured and full efficacy can be expected. The desired blood level in potassium bromide monotherapy is 2000-3000 µg/ml (if combined with phenobarbital 1000-2000 µg/ml might be sufficient). It is possible to estimate the final serum level by measuring the value after about one month after starting therapy, when 50% of the final serum level should be reached. If after one month, blood levels are below 1000 µg/ml (monotherapy) or below 500 µg/ml (combination with phenobarbital), the potassium bromide dose should be increased by 25%. Once steady-state has been achieved, further serum level checks should be performed every 6 months.

In selected cases with high-seizure frequency achieving effective serum levels much earlier can be desired. In those dogs, loading the patient with potassium bromide can be done by giving 70 mg/kg twice daily for 5 days. This protocol should result in a serum level of at least 1000 µg/ml at the end of the loading period. The loading period may even be shortened to 2 days by giving 660 mg/kg divided in 10 doses over two days, administered with a small amount of food to prevent gastric irritation [13]. However, such dosages may result in significant sedation of the patient.

It is important to keep the salt intake constant in patients on potassium bromide, since increased chloride intake can reduce bromide levels. Bromide and chloride are subject a common competitive reabsorption mechanism in the kidneys. Therefore, the more chloride ions appear in the primary urine the less bromide ions can be reabsorbed. Owners should be instructed accordingly. Human food or treats containing salt should be avoided – as should trips to the beach! There is anecdotal evidence that milk products in the diet may inhibit intestinal bromide absorption. This could be taken into consideration in cases where appropriate dosage and good owner compliance fail to reach sufficient bromide blood levels.

Side effects of potassium bromide include:

- polyphagia
- polydipsia/polyuria
- sedation
- increased excitability

- gastrointestinal disturbances: gastritis, obstipation
- pancreatitis
- dermal reactions

In addition to the common side effects, signs of bromide intoxication, called bromism, can be seen in about 2% of dogs [14]. The signs of bromism are: reduced mentation, generalised ataxia, para- or tetraparesis and hyporeflexia. Bromism can be treated by intravenous infusion of 0.9% sodium chloride solution using the competitive reabsorption mechanism in the kidneys to flush out potassium bromide. Usually the signs of bromism are completely reversible once the serum level has been decreased. However, this therapy may result in an unpredictable decrease in the serum level potentially resulting in increased seizure activity. Therefore, treatment of bromism should be performed under direct control of the clinician.

Human products

Levetiracetam

Levetiracetam is a human medication, which is thought to exhibit not only anticonvulsive but also true antiepileptic properties, as it may prevent the so-called kindling-effect, whereby seizure activity may "set up" the brain for further seizures by changing neuronal circuits[15]. Levetiracetam is used as add-on medication at an oral dose of 10-20 mg/kg three times a day (TID). In addition, it has been proposed as temporary medication in dogs with a tendency to develop clusters of seizures. In these cases, levetiracetam can be given as a single dose of 40-60 mg/kg orally when a cluster starts, followed by 20 mg/kg every 8 hours until the patient has been seizure-free for 48 hours. After 2 days without seizures, levetiracetam can be discontinued [16].

Levetiracetam has a short elimination half-life of 3-4 hours. Therefore, effectiveness can be assessed within a few days without any need for serum level checks. Levetiracetam can be used in dogs with known impaired liver function, as only a small percentage is metabolised by the liver. The majority (70-90%) is excreted unchanged by the kidneys[16]. Side effects are rare but may include sedation and ataxia.

Gabapentin, Pregabalin

Both have been recommended as add-on therapy in dogs with seizures. They are excreted unchanged by the kidneys, without any hepatic metabolism. Therefore, it can be used in dogs with impaired liver function. In a study of dogs not responding to phenobarbital, potassium bromide or a combination of both, seizure frequency was reduced by 57% after adding pregabalin[17].

Gabapentin is given at a dose of 10 mg/kg TID, whereas pregabalin is initiated at a dose of 2 mg/kg BID to TID. The dose of pregabalin can be increased to 4 mg/kg BID to TID if necessary. Due to a short half-life, efficacy can be assessed with a few days. Serum level checks are not indicated. Side effects of both medications are usually mild and may include sedation, dizziness and hyperexcitability.

Topiramate

Adding topiramate resulted in a response rate of 50% in dogs that did not respond to a combination of phenobarbital and potassium bromide[18]. Therapy is initiated with an oral dose of 2 mg/kg BID, but it is increased to 5 mg/kg BID 2 weeks later. Side effects such as sedation and ataxia might be seen in half of dogs treated with topiramate. The severity of those side effects usually decreases after the first weeks to months of therapy.

Rarely used

Primidone: Primidone is converted by hepatic cytochrome-P-450 enzymes into phenobarbital and phenylethylmalonamide. It is thought that phenobarbital is the major active metabolite. Studies have shown that primidone is not more effective than phenobarbital alone but it appears to be more hepatotoxic than phenobarbital. Therefore, primidone is no longer recommended[19].

Zonisamide: Zonisamide has been used as add-on medication for non-responders, but is by far the most expensive medication currently used in dogs. It is unlikely to be used extensively in the future until a generic drug becomes available.

Felbamate: Felbamate was associated with severe side effects in dogs such as blood dyscrasia and hepatotoxicity and is therefore no longer recommended.

Table 1: Comparison of characteristics of drugs licensed for anticonvulsive therapy in dogs

	Phenobarbital	Potassium bromide	Imepitoin*
Use for monotherapy or as add-on	licensed for monotherapy, can be used as add-on	licensed for add-on to phenobarbital, can be used as monotherapy	licensed for monotherapy, can probably be used as add-on to phenobarbital
Anticonvulsive efficacy compared to phenobarbital if used as monotherapy	-	not known	probably slightly less
Starting dose	2.5 mg/kg BID	20 mg/kg BID or 40 mg/kg SID	10 mg/kg BID
Time delay for anticonvulsive action	10 days	3-4 months	2 days
Serum level checks	1st: after 2 weeks 2nd: after 3 months follow-up: every 6 months	1st: after 3-4 months follow-up: every 6 months	Not recommended
Desired serum level	20 – 30 µg/ml	monotherapy: 2000 – 3000 µg/ml add-on therapy to phenobarbital: 1000 – 2000 µg/ml	-
Side effects	**common:** permanent polyphagia, polydipsia and polyuria, temporary sedation **less common:** hyperexcitability, hepatotoxicity, ataxia, pancreatitis, anaemia, neutropenia, thrombocytopenia	**common:** permanent polyphagia, polydipsia, polyuria, sedation **less common:** pancreatitis, bromism, gastritis, obstipation, dermal reactions	**common:** polyphagia **rare and mainly temporary:** hyperactivity, polyuria, polydipsia, somnolence, salivation, vomiting, ataxia, diarrhoea, third eyelid protrusion, impaired vision, hypersensitivity to noise
Risk of seizures after sudden withdrawal of the medication	yes	not known	no
Interference with other tests	thyroid testing may falsely indicate hypothyroidism	false increase of chloride measurements	
Additional instructions for owners	Do not restrict access to water, keep amount of food the same as before treatment	keep salt intake constant, avoid beaches, do not restrict access to water, keep amount of food the same as before treatment	keep amount of food the same as before treatment, give tablets before feeding to increase bioavailability
Estimated monthly costs for a 20 kg dog based on the starting dosage, use of a drug licensed for dogs**	14 €	20 €	19 €

*most data are based on studies involving a relatively small number of dogs **German prices

Concluding summary

The recent introduction of imepitoin into the veterinary market has provided a promising alternative to the currently available anticonvulsive medications for dogs. It is, however, not expected that any new medication will replace all the other ones. It is more likely that treatment has to be tailored to the individual patient (see Table 1).

Which medication should be used first when treatment is initiated in a given patient? There might not be a clear answer to that question, even though the author feels that phenobarbital is still the medication of choice until imepitoin has proven its advantages in clinical practice. The topic could be presented to the pet owner as follows: "There are currently two different medications available. There is one, phenobarbital, that has been used for many years and we are aware of all facets of this medication. And there is new one, imepitoin, which is probably equally effective but slightly more expensive than phenobarbital. It appears that side effects such as sedation, polyphagia, polydipsia and polyuria are significantly less severe with imepitoin than with phenobarbital therapy. However, we do not have large scale, long-term experiences with that medication. Which one do we want to use?"

Disclaimer

The author declines any actual or potential conflict of interest including any financial, personal or other relationships with other people or organisations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work.

Abbreviations:

BID	twice daily
BW	body weight
SID	once daily
TID	three times daily

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REPRINT PAPER (N)*

Effect of ovariohysterectomy at the time of tumour removal in dogs with benign mammary tumours and hyperplastic lesions: a randomised controlled clinical trial

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ABSTRACT

Background: Non-malignant mammary tumours (NMT) are common in intact female dogs. Little is known about the clinical significance of these tumours, and the effect of ovariohysterectomy (OHE) on their development.

Hypothesis: Ovarian hormone ablation through OHE decreases the risk of new tumours and thereby improves long-term prognosis for dogs with NMT.

Animals: Eighty-four sexually intact bitches with NMT.

Methods: Dogs were allocated to undergo OHE (n=42) or not (n=42) at the time of NMT removal in a randomised clinical trial. Tumour diagnosis was confirmed histologically in all subjects. Information about new tumour development was collected via follow-up phone calls and recheck examinations. Separate survival analyses were performed with the endpoints new tumour development and death. Cause of death was classified as related to or unrelated to the mammary tumour. In addition to OHE-status, the influence of age, body weight, breed, tumour size, tumour number, tumour duration, type of surgery, and tumour histology was investigated.

Results: New mammary tumour(s) developed in 27 of 42 (64%) intact dogs and 15 of 42 (36%) ovariohysterectomised dogs (hazard ratio 0.47, P=0.022). Nine of the 42 dogs (21%) which developed new tumours were euthanised due to the mammary tumour. Survival was not significantly different between the two treatment groups. In the intact group, nine dogs subsequently developed ovarian-uterine diseases.

Conclusion: OHE performed at the time of mammary tumour excision reduced the risk of new tumours by about 50% among dogs with NMT. Survival was not significantly affected. Adjuvant OHE should be considered in adult dogs with mammary tumours.

Key words: Canine; Non-malignant mammary tumours; Cancer; Spaying

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List of abbreviations: BMT, benign mammary tumour; HR, hazard ratio; NMT, non-malignant mammary tumour; NT, new tumour; OHE, ovariohysterectomy; PI, principal investigator; TVE, time-varying effect.

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Introduction

Mammary tumours are the most common tumours in entire bitches.[1-5] Close to 60% of these tumours are benign.[6] Only 1% of mammary tumours smaller than 1 cm are malignant and 50% of tumours larger than 3 cm are benign.[7] The clinical implications of benign mammary tumours (BMT) are still largely unknown and are commonly considered as a disease of limited clinical importance. This is in contrast to malignant mammary tumours which have the potential for metastasis and a fatal outcome.[8-10] However, BMT is a recognised risk factor for developing additional mammary tumours later.[11-12] Mammary tumours in dogs develop as a histological continuum from benign to malignant with the malignant tumours representing the end stage of this continuum.[7] It therefore seems plausible that a subset of dogs with benign tumours could be at risk of developing malignant, and thus potentially fatal, tumours. In comparison, in human medicine it has been found that women who have had a benign breast tumour or atypical mammary hyperplasia have increased risk of subsequently developing breast cancer, and this risk increases with increasing atypia.[13] Factors leading to the subsequent development of malignant neoplasms have not been studied in dogs with BMT.

One well-recognised risk factor for mammary tumour development is exposure to ovarian hormones during the first two years of life, as demonstrated by the significantly reduced risk of mammary tumours in dogs that undergo ovariohysterectomy (OHE) during this time-period.[14] OHE performed after the age of four has been found to have a limited to no protective effect against the development of mammary tumours.[14-16]

New tumours in other mammary glands are common in dogs with a previous diagnosis of mammary tumours.[7,17] Statistically, one in four dogs develops a new mammary tumour after surgical removal of BMTs, [7,18] and importantly these later tumours can be malignant even if the initial tumour was benign[17-18] thus having the potential to pose a risk of metastasis and premature death for the affected dog. Several studies have shown that a significant portion of benign epithelial tumours and hyperplastic mammary lesions express oestrogen and progesterone receptors, implying a continued hormonal dependence.[19-20] We therefore hypothesised that ovarian hormone ablation through OHE would decrease the risk of new tumours and thereby improve the long term prognosis for dogs with non-malignant tumour(s) (NMT). A recent meta-analysis on the protective effect of OHE on the risk of mammary tumour development using Cochrane principles[21] concluded that the level of scientific evidence is currently too weak to reach firm conclusions on this issue. Therefore, a well-controlled randomised study to answer the questions regarding the effect of OHE in dogs with mammary tumours is clearly needed. The purpose of this study was to determine the effect of OHE on new tumour development and long term survival in dogs with NMT. A secondary objective was to determine the association between the initial and subsequent tumours regarding histology and tumour localisation.

Materials and methods

Study population and randomisation

The study was designed as a randomised controlled clinical trial. Intact female dogs with mammary tumours and no previous history of mammary malignancy were eligible for inclusion. A complete reproductive and health history was collected as part of the screening and initial enrolment. Randomisation to either tumour removal or tumour removal and concomitant OHE, was performed prior to surgery. In addition, the dogs were stratified based on tumour size (< 3 cm, ≥ 3 cm) and age (< 9 years, ≥ 9 years) to ensure equal distribution of these two prognostic factors between the treatment groups. A block randomisation scheme was used within each stratum. The allocation sequence was computer generated, and the treatment allocation was not known to owners or the investigator until the enrolment had been completed.

All owners of participating dogs signed a written consent form where they were given relevant information about the project and agreed to the randomisation procedure. There was one fixed price for all participants irrespective of type of mammary tumour surgery or if adjunctive OHE was performed or not. The study was approved by the Institutional Animal Care and Use Board at the Norwegian School of Veterinary Science. Complete staging, including blood work (complete blood count and serum biochemistry profile), cytological investigation of enlarged draining lymph nodes, and 3-view thoracic radiographs were performed prior to surgery. Dogs with distant metastasis or other serious diseases were excluded. All tumours were recorded (number and localisation) and size was determined as the largest diameter measured by calliper. Surgery was performed according to standard surgical practices [22,23] and involved excision of at least 1 cm of gross normal appearing tissue surrounding the tumour dependent

on size and number of the mammary tumours treated. No prophylactic mastectomies of normal glands were performed. Age, weight and breed of the dogs were recorded at the time of surgery. OHE was performed concomitantly with tumour surgery.

Based on results from the histology, 2 separate groups were created: a carcinoma group consisting of dogs with at least one malignant epithelial tumour, and a non-malignant group which included dogs with hyperplastic nodular lesions, benign tumours regardless of tissue of origin, and carcinoma in situ. This paper reports on this latter group of dogs. Both tumours and hyperplastic lesions in the non-malignant group are termed non-malignant tumours (NMT) because they were clinically indistinguishable from each other - appearing as discrete well defined "tumour like" lesions. Cases with malignant epithelial tumours continued to be enrolled in the study until an adequate number of dogs were enrolled in the malignant group. The results from this group will be reported separately.

After surgery, the owners of the dogs were instructed to monitor for any signs of new mammary tumours and notify the principal investigator (PI) if any signs of recurrence or new tumours were noted. In addition, the owners were contacted by the PI (VK) every six months by phone to ensure information regarding recurrence or new tumour development (localisation and time) was communicated. In addition, other health issues were recorded. Dogs with reported/suspected new tumours were requested to return for clinical examination and confirmation. Surgical excision was recommended for all dogs with new tumours; however, no financial study support was provided for any of these subsequent treatments. Complete necropsies were performed regardless of cause of death in dogs who died or were euthanised.

Tumour histopathology

All tumours were fixed in 10% neutral buffered formalin and submitted for histopathological examination. The slides were stained with haematoxylin and eosin. To minimise misdiagnosis and the potential to introduce bias, the slides were evaluated by two independent pathologists (MHG and JT) who performed the evaluations blinded to each other's diagnoses. Dogs diagnosed with only NMT by at least one of the pathologists, and subsequent agreements, were included. The tumours were further classified according to the type of tissue present (epithelial, myoepithelial and/or connective tissue). A complete histopathological description was provided for each tumour and included information regarding the degree of tumour differentiation, presence of cellular atypia, carcinoma in situ, and tumour margins.[22]

Outcome variables

The two main outcome variables in the analysis were time to 1st new mammary tumour and time to death/euthanasia. The cause of death/euthanasia was also recorded.
New tumours were defined as any tumour arising in the mammary tissue after excision of the original tumour(s). Tumours occurring in the same gland or close to the original tumour were also classified as new tumours, rather than recurrences. All subsequent tumours detected by owner, during rechecks and/or at necropsies were recorded. If the dogs were not available for recheck examination, information about new tumour development was based on the owner's description. The localisation of new tumours were recorded with reference to the closest associated mammary gland and in relation to the originally removed tumours - in terms of adjacency and whether the new tumours were located ipsi- or contralateral to these. If available for histology, the new tumour was examined by the same pathologists and categorised as benign or malignant.
Cause of death (including euthanasia) was classified as mammary tumour specific or non-mammary tumour specific. In order to be classified as mammary tumour specific, the owners decision to euthanise had to be directly related to the tumour, or its metastases, and had to be confirmed by necropsy or by diagnostic imaging. Cause of death in dogs with mammary tumours in which these tumours did not cause clinical problems was classified as non-mammary tumour specific.

Explanatory variables

In addition to the intervention variable (spayed or not at the time of tumour removal: OHE/non-OHE), the following variables were examined for potential influence on new tumour development and overall survival: age at time of surgery as continuous and categorised (< 9 years, ≥ 9 years) variable, bodyweight (< 22 kg, ≥ 22 kg), breed (pure, mixed), tumour duration (< 6 months, ≥ 6 months), number of tumours (multiple, single), tumour size (< 3 cm, ≥ 3 cm), extent of surgery (lumpectomy/simple mastectomy, regional mastectomy/radical mastectomy), and tumour histology. Tumour histology was categorised into 1) hyperplasia and cysts, 2) benign tumours (adenomas, complex adenomas, benign mixed) without atypia, and 3) benign tumours

with atypia, necrosis or with carcinoma in situ. In dogs with multiple tumours, the tumour with the most atypical histological changes determined the category.

Statistical analysis

Descriptive statistics

The distribution of potential risk factors by each OHE group was calculated and compared for the variables age, bodyweight, breed, tumour size, tumour histology, number of tumours, type of surgery, and tumour duration to ensure similar group characteristics at baseline, using the chi-squared test. When categorised, the cut-off values were determined by the median derived from all dogs. For age and tumour size however, the pre-determined cut-off values for the stratification procedure were used. Dogs lost to follow-up or still alive without any of the events, were censored at the date of last known status. Dogs that developed ovarian or uterine disease requiring OHE were censored at the date of such surgery. The Kaplan-Meier method was used to compute survival curves and estimate remission and survival time of dogs by OHE group. Differences in survival between different groups were tested using the log-rank test.

Uni- and Multivariable analyses

To evaluate the effect of OHE on the two events 1) new tumour development and 2) death/euthanasia, adjusting for other possible risk factors, separate Cox proportional hazards models were built for each of the events. Two outcomes were modeled for death/euthanasia; death of any cause (overall survival) and death attributable to mammary tumour (tumour-specific survival). Time at risk was defined as months from surgery date to the event or censoring. All the clinical variables were initially screened for effect on tumour development or survival by applying univariable Cox proportional-hazards models with adjustment for age and using a cut-off of $P < 0.10$ for offering variables to the multivariable model. Predictors were retained in the final model if $P < 0.05$ or if assumed to have great biological interest. Finally, an estimate of the baseline hazard was derived which was conditional upon the set of co-efficients used in the model.

Validation of the model

The assumption of proportional hazards was evaluated based on Schoenfeld residuals for the variable OHE in each of the three models. If this assumption was violated and graphical

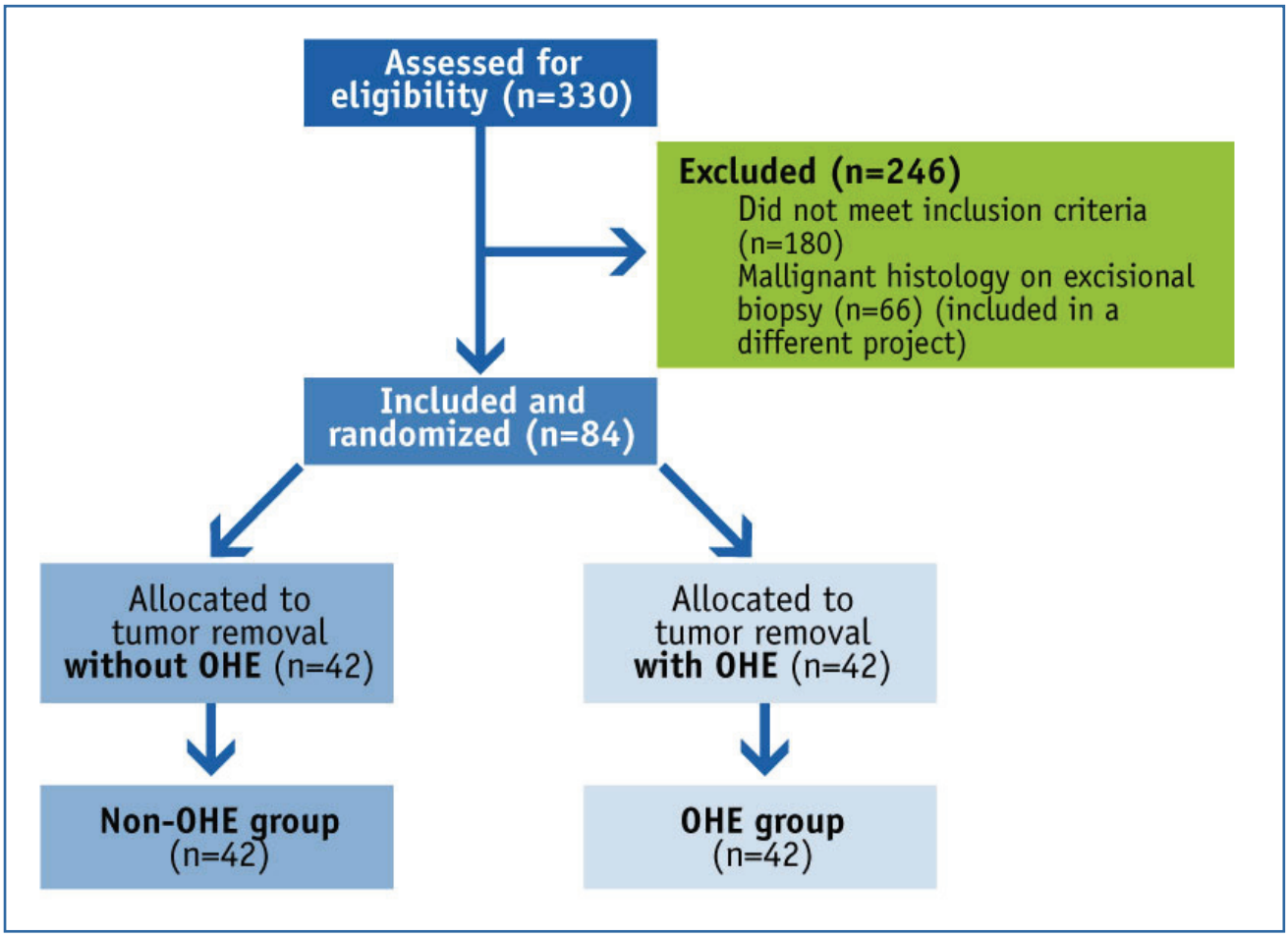

Figure 1: Flow diagram of the enrolment and randomisation procedure in a randomised controlled clinical trial investigating the effect of adjuvant ovariohysterectomy (OHE) in dogs with non-malignant mammary tumours.

assessment indicated a time-varying effect (TVE) of this variable, an interaction term between this variable and time (on either a linear or logarithmic scale) was included in the model. The importance of the assumption of independent censoring was evaluated by sensitivity analyses based on both complete positive and complete negative correlation between censoring and outcome. The amount of explained variation was evaluated by an overall r2 statistic for proportional hazard models. Plots of the deviance residuals, score residuals, and scaled score residuals against time at risk were used to identify outlying observations with influence on the model, and the model was fit with and without any outlying observations.[24] All analyses were performed using the software package Stata.[a]

[a] *Stata 11; Stata Corporation, 4905 Lakeway Drive, College Station, TX 77845, USA*

Results

Study population, randomisation and follow-up

Of 330 dogs initially screened for eligibility, 84 dogs had non-malignant tumours and were included in the study (Fig. 1). Histopathologically, 108 (51%) of a total of 210 tumours were classified as complex or mixed tumours. Forty-four (21%) tumours were adenomas or variants thereof, and four (2%) were diagnosed as carcinoma in situ. The remaining 54 (26%) tumours were diagnosed as different types of hyperplastic or dysplastic lesions. All dogs were evaluated, staged, surgically treated and whenever feasible rechecked at the Department of Companion Animal Clinical Sciences at the Norwegian School of Veterinary Sciences in the period from February 2005 until May 2012. The median follow-up time for all dogs, including the censored ones was 31.5 months (range 3.5-87.5). For the dogs that were still alive at the end of the study (n = 24) the median follow-up was 34 months (range 19.0-77.9).
Forty-two dogs underwent OHE concomitantly with tumour surgery and 42 remained sexually intact. For the non-OHE group the median follow-up time was 31.2 months (range 3.5-87.5) and for the OHE group 31.8 months (range 4.2-78.2). There was no difference between the treatment groups in terms of signalment, tumour characteristics and type of surgery, see Table 1. Postsurgically, all owners were able to provide follow-up information on their dog by regular phone-interviews. In addition, 1 or more clinical rechecks, necropsy or both were performed in 36 dogs in the non-OHE group, and in 34 dogs in the OHE group during the follow-up period.

Uni- and multivariable analysis

Effect of OHE on time to new tumour development:
Dogs from the non-OHE group developed significantly more new tumours (P = 0.022) than the dogs from the OHE group. Of the 42 dogs that developed new tumours, 27 (64%) and 15 (36%) were from non-OHE and OHE group, respectively. Twenty-six of the 27 dogs in the non-OHE group with new tumours, and all the 15 dogs in the OHE group had their tumours confirmed clinically by a veterinarian. Only in one of the dogs reported to have a new tumour was this information based solely on the owners description. Median time to new tumour development was 20.8 months (range 3.8-80) and 19.6 months (range 2.3-47.2) for the non-OHE and the OHE group, respectively. The Kaplan-Meier survival curves according to OHE status with new tumour as endpoint (Fig. 2) illustrate a protective effect of OHE. The OHE variable was found to be statistically significant (P = 0.022) in the univariable screening with a protective effect of OHE (HR 0.47, 95% CI 0.25-0.89). None of the other variables analysed were statistically significant in the univariable screening.
In the final Cox proportional-hazards model OHE-status was the only variable that had a statistically significant effect on the hazard of new tumour with a hazard ratio of 0.47 (95% CI 0.25-0.90, P = 0.022) for the OHE group. However, age at time of surgery was added to the model because of its possible biological importance. The hazard ratio for age was 1.12 per one-year increase (95% CI 0.94-1.33, P = 0.27).
The protective effect of OHE against new tumour development was found to vary with time. It increased on a logarithmic time scale (log-time). Thus, an interaction between OHE-status and log-time was included in the model. The protective effect of OHE appeared approximately five months after surgery and increased up to 48 months after surgery when it reached a more stable level (Table 2).

Effect of OHE on survival time
By the end of the observation period (May 31, 2012), 60 (30 non-OHE and 30 OHE) of the 84 dogs had died; of these 33 underwent necropsy. Figure 3 illustrates the Kaplan-Meier survival curves by OHE-status with death/euthanasia of any cause as the endpoint. There was no statistically significant difference in overall survival between the two groups. Median time to death was 31.2 months (range 3.5-87.5) and 31.0 months (range 4.2-78.2) for the non-OHE- and the OHE group, respectively. However, the hazard of dying regardless of cause was influenced by the dogs' age at the time of surgery with a hazard ratio of 1.4 per one-year increase in age (95% CI 1.19-1.62, P < 0.001). It did not differ significantly between the

Table 1. Distribution of patient characteristics by treatment group in 84 dogs with non-malignant mammary tumours randomised to ovariohysterectomy (OHE group) or to remain intact (non-OHE group) at the time of tumour removal.

	Non-OHE Group (n=42)	OHE Group (n=42)	P value
Age			**0.28**
≥ 9.0 years	19	24	
< 9.0 years	23	18	
Bodyweight			**0.38**
≥ 22 kg	18	22	
< 22 kg	24	20	
Small breed vs other			**1.00**
Small breed (< 10 kg)	7	7	
Other breed (≥ 10 kg)	35	35	
Pure vs mix breed			**0.29**
Pure breed [a]	35	31	
Mix breed [b]	7	11	
Tumour duration [c]			**0.38**
≥ 6 months	17	21	
< 6 months	25	21	
Multiple tumours			**0.23**
Yes	27	31	
No	15	11	
Tumour size			**0.83**
≥ 3 cm	20	21	
< 3 cm	22	21	
Type of surgery			**0.12**
Local surgery [d]	14	21	
Extensive surgery [e]	28	21	
Tumour type [f]			
Hyperplasia	4	6	0.78
Benign	31	30	
Benign with atypia [g]	7	6	

[a] *Comprised of 30 different breeds with English setter (n=8), German shepherd (n=6), German pointer (n=5), and Dachshund (n=5) as the most common.*
[b] *Dominated by middle sized dogs (median weight=22 kg, range: 3-53)*
[c] *Time from the tumour was first discovered by owner to the time of project inclusion*
[d] *Lumpectomy or simple mastectomy*
[e] *Regional or radical mastectomy*
[f] *Cases with multiple tumours and more than one histopathological diagnosis are grouped according to the most neoplastic characterisation of the tumours*
[g] *Includes benign tumours with necrosis, sclerosis and carcinoma in-situ*

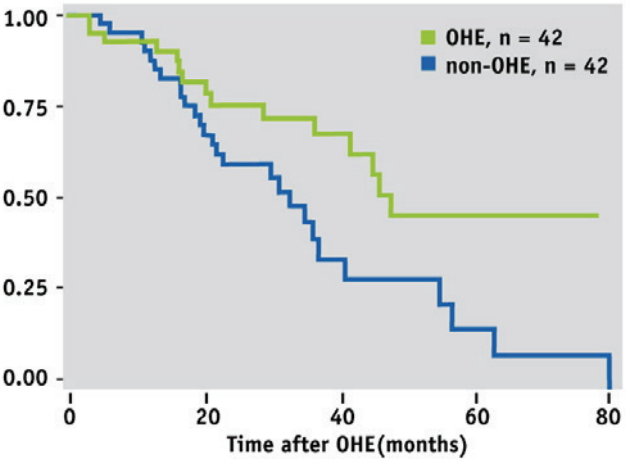

Figure 2: The Kaplan-Meier curve shows time to new tumour development in 84 dogs with non-malignant mammary tumours randomised to ovariohysterectomy or to remain intact at the time of surgery (P = 0.022). Median time to new tumour development was 19.6 and 20.8 months for the OHE- and the non-OHE group respectively.

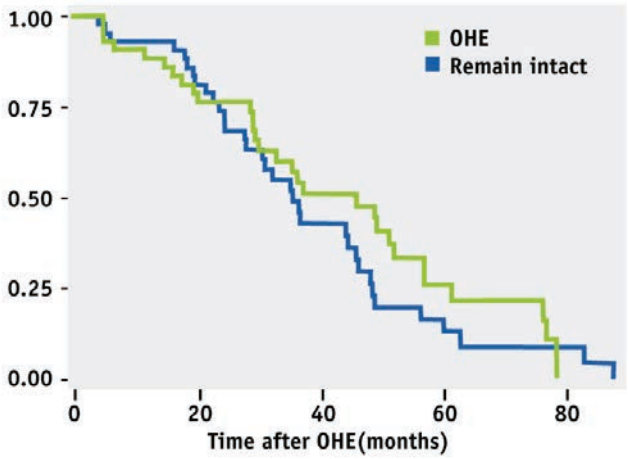

Figure 3: Kaplan-Meier survival curve on time to death from any cause after removal of non-malignant mammary tumours in 84 dogs randomised to ovariohysterectomy or to remain intact at the time of surgery (P = 0.19). Median survival time was 31.0 and 31.2 months for the OHE- and the non-OHE group respectively.

other investigated variables, including the OHE variable when applying a Cox proportional-hazard model with adjustment for age. The same result was seen when the endpoint was death from a mammary tumour.

Nine of the 42 dogs (21%) which developed new mammary tumours were euthanised because of clinical problems related to it (metastatic disease (n=6), large or ulcerated tumour compromising normal function (n=2), development of inflammatory carcinoma (n=1)). Six dogs belonged to the non-OHE group, and three dogs to the OHE group (95% CI: 0.098-1.59, P = 0.19). In Figure 4, clinical outcome by OHE-status is summarised as new tumour development, death due to mammary tumours and death related to other causes. Of deaths related to other causes, 19 were due to other tumour types and 32 were related to non-neoplastic causes (multifactorial causes ("old"; n=10), Chronic degenerative joint disease (n=8), heart disease (n=4), acute abdomen (n=3), trauma (n=3), pyometra (n=2), renal failure (n=1), and idiopathic epilepsy (n=1). The assumption of proportional hazards was valid for the OHE variable in the model when death/euthanasia was the endpoint.

Diagnosis and localisation of subsequent tumours

In total, 42 dogs (50%) developed new tumours during the follow-up-period. Only four dogs had the new tumours surgically excised, but histopathological analysis was available in a total of 27 dogs because an additional 23 dogs were returned for euthanasia and necropsy. Sixteen dogs had at least one malignant new tumour and 10 dogs were found to have only benign new tumours. The distribution of

Table 2. The time-varying effect of ovariohysterectomy (OHE) on the hazard of new tumour development at different time points estimated from a survival model in a randomised trial among dogs with non-malignant mammary tumours.

Months after OHE	5 [a]	12	24	36	48	60
Hazard ratio	1.00	0.61	0.41	0.32	0.27	0.24

[a] *OHE starts to be protective at this time (ratio >1.00)*

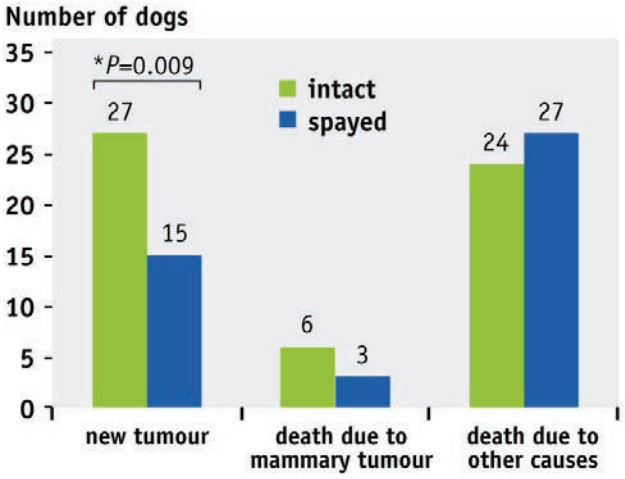

Figure 4: Distribution of postsurgical clinical outcome (new tumours, death due to mammary tumours, and death due to other causes) in 84 dogs with non-malignant mammary tumours randomised to ovariohysterectomy or to remain intact at the time of tumour removal (42 in each treatment group). Significant results are indicated by an asterisk (*)

these dogs by OHE status is showed in Figure 5.
Of the 12 dogs which initially had one single tumour, seven dogs (58%) developed new tumours ipsilaterally, of which

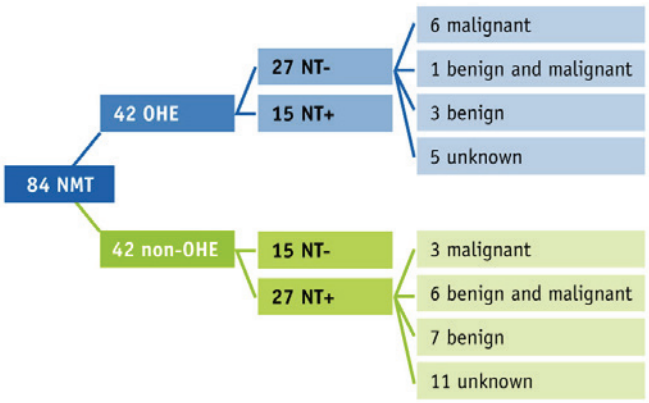

Figure 5: Distribution of new tumour development (NT) in 84 dogs after surgical removal of non-malignant mammary tumours with or without concomitant ovariohysterectomy (OHE).

five appeared in the adjacent gland. The other five dogs (42%) developed new tumours in the contralateral chain, of which four were found in the most adjacent of the contralateral glands.

Thirty dogs with new tumour development had multiple tumours initially; 22 cases with bilateral distribution. In the remaining dogs where the initial tumours were unilateral (n = 8), five developed new tumours in the contralateral mammary chain.

Impact of OHE on other health issues

Six dogs in the intact group developed pyometra for which 3 were treated with OHE (censored at that time) and 3 were euthanised. Moreover, clinically significant uterine/ovarian tumours were found in 2 other dogs; 1 dog developed uterine leiomyosarcoma and was ovariohysterectomised because of this (censored at that time). A 2nd dog was euthanised as a result of an acute abdomen due to torsion of a cystic ovary. Also, on necropsy, an ovarian granulosa cell tumour and a vaginal leiomyofibroma were revealed in one of the dogs.

Discussion

This study shows a significant risk reduction for new mammary tumour development in dogs treated with OHE adjuvant to NMT removal. Based on previous publications,[14-16] it has been assumed that OHE later in life has little effect on mammary tumour risk. However, none of these former studies were prospective, randomised, or stratified according to malignancy nor was the development of new tumours used as endpoint in the analysis. These findings strengthen the similarities between women and dogs in term of the role of hormonal exposure and breast cancer risk; in women the breast cancer risk is associated with the cumulative life time exposure of breast tissue to hormones, our results suggest a similar situation in the dog, the effect of hormones are continuous and additionally contribute to an increased risk after the age of 2.

The hazard ratio of 0.47 (OHE/ non-OHE) for new tumour development reflects that bitches spayed at the time of tumour removal, on average have approximately half the risk of developing new tumours at any given time compared to the bitches remaining intact. A hazard can therefore be interpreted as an instantaneous risk, and the term risk will be used in the following discussion because it is probably a more familiar concept to most readers.

The advantage of OHE on time to new tumour development did not become apparent until five months after the OHE intervention. This somewhat delayed effect and then increasing benefit over time makes sense from a biological point of view and probably reflects the time needed for ovarian hormones in mammary tissue to be washed out in the dogs undergoing OHE. Also, the fact that most of the dogs in the non-OHE group had time to experience oestrus during the five months period might explain the increasing benefit of OHE over time. The temporarily increased endogenous hormonal exposure might have accelerated new mammary tumour development in the non-OHE group compared to the OHE group. This may also explain why this benefit becomes apparent after five months. Normal mammary epithelial tissues and most benign tumours express hormone receptors. With time, continued exposure to hormones is likely to contribute to early subclinical stages of mammary tumourigenesis, resulting in additional mammary tumours in the subset of dogs that remained sexually intact.

Relatively few (n = 9) dogs died due to new mammary tumours, and all of them occurred late relative to the initial tumour surgery. Consequently, the increased rate of new tumour development in the intact group did not translate into a significant difference in overall survival time between the two treatment groups. In addition to the increased risk of new mammary tumours, nine of the intact dogs also experienced significant uterine and ovarian diseases necessitating emergency surgery or even euthanasia in some cases.

In this study, cause of death was only attributed to mammary tumours if the presence of mammary tumours and metastasis was the direct cause of death/euthanasia. The majority of the dogs in this study were euthanised due to other causes; however, many (n = 32) of these dogs did have documented

new mammary tumours at the time of euthanasia. Because of the complexities of issues affecting decisions regarding euthanasia, survival may represent a "soft" and biased endpoint in this study. In contrast, the development of new tumours represents an objective and clearly defined endpoint and our results show that OHE significantly decreases the risk for new tumours in dogs with NMT.

Alternatively or in addition to OHE, prophylactic mastectomies of normal mammary glands could be considered in order to prevent new mammary tumours. In order to make good decisions regarding which glands to prophylactically remove, the likely localisation of such new tumours must be known. According to a previous study, dogs with one mammary tumour were likely to develop new tumours ipsilaterally.[17] The majority of dogs with mammary tumours however, have more than 1 tumour. In the present study, those dogs were also included when determining the localisation of new tumours. Our results showed that most new tumours developed adjacent to a previously affected gland; however, almost half of the dogs (n = 5/12) with a single mammary tumour, developed a new tumour in the contra-lateral chain. These findings are in conflict with Stratmann et al's findings,[17] and reflect that the exact localisation of new tumours may be somewhat unpredictable. Clearly, a bilateral radical mastectomy would effectively prevent any new mammary tumours in dogs. This is however an aggressive procedure and may be difficult to justify as 36% of the intact dogs and 64% of the spayed dogs did not develop additional mammary tumours. In contrast, most dogs recover quickly from OHE. Furthermore, the benefits are 2-fold: OHE significantly reduces the risk for new tumours and prevents uterine/ovarian diseases later on. As many as nine of the 42 dogs (21 %) in the non-OHE group developed uterine-ovarian diseases, of which four were euthanised. The previously published data regarding the incidence of pyometra in dogs with mammary tumours are unclear. Some studies suggest that a significant percentage of bitches with mammary tumours have concomitant utero-ovarian disease, and that they are likely to experience clinical signs (pyometra, mucometra) in the future if OHE is not performed.[25] Others, however, did not find any increased risk of pyometra in dogs with mammary tumours.[26] In this study, 6/42 dogs (14%) developed pyometra.

Non-malignant mammary tumours are generally associated with a long postsurgical survival, thus allowing new tumours and other diseases to develop. As age advances, the risk of uterine diseases increases.[26,27] Some owners choose not to do more surgery in an old dog if it already has been put through 1 or more surgeries earlier. Otherwise treatable uterine and ovarian diseases, may for this reason lead to the decision to euthanise the dog rather than "putting an older dog through another surgery". OHE at the time of tumour removal may decrease the risk of new tumours, but also protect the dog from later uterine/ovarian diseases which again might save some dogs from being euthanised due to the negative influence such concurrent diseases might have on the owner's decision to pursue additional surgery in an old dog. Our study shows that OHE confers a double benefit in these dogs.

Methodological considerations

This study's main strength lies in the design. Prospective randomised clinical trials are considered the most reliable form of acquiring scientific evidence because they reduce spurious causality and bias by ensuring comparable groups. Thus, any significant differences between groups in the outcome event can be attributed to the intervention (OHE) and not to some other unidentified factor. Unfortunately, this study was not published in time to be included in the meta-analysis on the effect of OHE on mammary tumour risk performed by Beauvais et al. (2012). [21] In fact, the present study is the first investigating this question using this design. An additional strength of this study is the long follow-up (median 31.5 months, range 3.5-87.5). Few studies include more than two years of follow-up.

The main limitation of this study is that the postsurgical information for some dogs was retrieved solely by phone (17%). However, most of these owners had detected the initial tumours by themselves, thus confirming their ability to recognise such tumours. Moreover, we were only able to obtain the histopathological tumour diagnosis in 26 of the 42 dogs with new tumours. The fact that only 33 dogs were necropsied is also a limitation, but owner contact by phone ensured reliable information regarding why the dog was euthanised. Furthermore, these limitations are not likely to introduce bias between the groups and thus affect the outcome as these sources of bias were evenly distributed between the two intervention groups. Finally, the small sample size is a potential weakness when considering multiple variables because the power of detecting an effect that really is present decreases with increasing number of variables. However, the significant effect of the OHE-status variable was maintained in the multivariable analysis, indicating that the power of detecting an effect of this variable was indeed acceptable.

In conclusion, OHE at the time of tumour removal reduced the risk for subsequent mammary tumours by 47% in bitches with NMT in this study. This protective effect was evident from five months after the OHE intervention and increased until a steady state was reached after approximately four years. No significant effect of OHE on overall or tumour-specific survival was detected for this sample of dogs. Adjunctive OHE should be considered in the treatment of adult dogs with mammary tumours.

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The study was carried out in Norway at the Norwegian School of Veterinary Science.
Selected preliminary results were presented as a poster entitled "Ovariohysterectomy at the time of tumour removal decreases the risk of new tumours in dogs with benign mammary tumours" at the 2nd World Veterinary Cancer Congress, Paris, March 2012.

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REPRINT PAPER*

Anaesthetic arthrography of the shoulder joint in dogs

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SUMMARY

Objective: to evaluate the use of intra-articular anaesthesia combined with positive contrast arthrography (collectively called 'anaesthetic arthrography' [AA]) in the shoulder in order to identify and confirm the source of pain in lame dogs.

Methods: anaesthetic arthrography was performed in 30 dogs with shoulder joint lameness with a mixture of contrast medium (iohexol: 240 mg i/ml) and a local anaesthetic (mepivacaine 2%: 20 mg/ml). The effect of intra-articular anaesthesia was evaluated by an objectified visual scoring system and the arthrograms were evaluated for their diagnostic value.

Results: this study showed that AA was positive in 28 out of 30 dogs. Dilution of the contrast medium with a local anaesthetic produced an arthrogram of good quality for each shoulder joint. In 16 out of 18 cases of osteochondritis dissecans, a cartilage flap could be identified following arthrography. In all cases the flap was not identified from plain radiographs. Biceps brachii tendon pathology was diagnosed on arthrography in seven dogs but was missed in two dogs. Calcification at the caudal rim of the glenoid cavity was diagnosed in three dogs, but was of clinical importance in only two dogs. In addition one infection was diagnosed during synovial aspiration.

Conclusion: anaesthetic arthrography of the shoulder is a simple, safe, and reliable diagnostic test to confirm shoulder joint pain and simultaneously identify a lesion. This procedure may be of particular importance in cases of occult shoulder joint lameness when clinical findings and plain radiographs are inconclusive.

Keywords: Dogs, intra-articular anaesthesia, diagnosis, arthrography, shoulder joint

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Introduction

Disorders of the shoulder joint are a common cause of forelimb lameness in dogs. A thorough history and general physical work-up as well as a comprehensive orthopaedic and neurological examination are often required to identify the shoulder as the source of lameness. However, it is often difficult to localise pain to the shoulder joint and to

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distinguish it from elbow pain [1, 2]. In contrast to more distally located joints, the shoulder joint is more challenging for the evaluation of joint effusion and pain because of the large muscular coverage. In order to localise the problem to the shoulder joint, intra-articular anaesthesia may be useful. In a study on the use of diagnostic intra-articular anaesthesia of the canine elbow joint, it was demonstrated that this was easily applied and a useful technique. Eighty-six percent of the dogs with an elbow joint lesion showed a significant improvement of lameness after injection with the local anaesthetic mepivacaine [3].

The complete assessment of the shoulder joint often requires imaging methods such as ultrasonography, contrast arthrography, computerised tomography (CT), magnetic resonance imaging (MRI) and arthroscopy [4-6]. In the dog's shoulder joint, positive contrast arthrography enhances visualisation of important intra-articular structures including the articular cartilage, the synovial membrane outline and the biceps brachii tendon [7, 8]. Therefore, arthrography is a useful technique that provides additional information for the diagnosis and treatment decision of intra-articular shoulder problems.

In humans, the combination of arthrography and injection of a local anaesthetic (anaesthetic arthrography, AA) has been described in order to confirm the intra-articular position of the needle, to localise the pain source and to aid in surgical planning [9, 10]. The combination of both diagnostic procedures could also be useful in canine shoulder problems.

The aim of this study was to evaluate the diagnostic effect of intra-articular anaesthesia on lameness thought to be associated with shoulder pain, and the imaging quality of the arthrogram with a combination of a local anaesthetic and a non-ionic contrast medium.

Materials and methods

Dogs

Over a 6-month period, all dogs with unilateral forelimb lameness with suspicion for a shoulder problem had intra-articular injection with a combination of a non-ionic contrast medium (iohexol; Omnipaque®, 240 mg I/ml, GE Healthcare, Belgium) and a local anaesthetic (mepivacaine 2%; Scandicaine®, 20 mg/ml, AstraZeneca, Belgium). In total 30 dogs had confirmed shoulder pathology and were selected for this study. All dogs included in this study were presented with forelimb lameness and had a complete clinical examination and survey radiographs of both shoulders and elbows.

Preparation

The dogs were sedated with a low dose of acepromazine (0.01 to 0.02 mg/kg) and methadone (0.1 to 0.2 mg/kg) or with medetomidine (based on body surface area) followed by antagonisation with atipamezole as described in a previous study [11]. The choice between both sedation protocols was based on age and temperament of the dogs and on a potential selection for same day surgery.

Survey Radiography

Before AA was performed, survey radiographs of the shoulders were made in a mediolateral projection. The following criteria were evaluated: (i) presence of a radiolucent area or flattening on the caudal aspect of the humeral head, (ii) irregularity of the supraglenoid tubercle and osteosclerosis of the medial ridge of the intertubercular groove, (iii) presence of calcification of the caudal rim of the glenoid cavity and (iv) osteoarthritis. Also radiographs were evaluated for any other signs of lesions such as calcification in the supraspinatus tendon region. Radiographs of the elbows (mediolateral 90° flexed, mediolateral extended and craniocaudal) were additionally evaluated to detect any elbow pathology.

Anaesthetic arthrography (AA)

Positive-contrast arthrograms were performed using 3 to 6 ml of a solution of iohexol and mepivacaine (Scandicaine 2%®, 20 mg/ml, AstraZeneca, Belgium), at a 1:1 ratio (100 to 120 mg I per ml). The dogs were positioned in lateral recumbency with the affected shoulder uppermost and held in a neutral position. After clipping and aseptic preparation with chlorhexidine scrub and alcohol, the joint was punctured craniolaterally between the acromion and the caudal part of the greater tubercle in a caudomedial direction (Fig. 1a and 1b). A 25 mm, 22-gauge needle with a 5 ml syringe was used for medium sized to large dogs (15-60 kg) whereas small dogs (5-15 kg) were punctured with a 25 mm, 24-gauge needle with a 2 ml syringe. Synovial fluid was aspirated and a direct smear was made for cytological analysis using haematoxylin eosin staining. Synovial smears were judged to be inflammatory, non-inflammatory or septic on the basis of the number and types of cells (normal white cell counts ranging between 0 and 3000 WBC/mm^3,

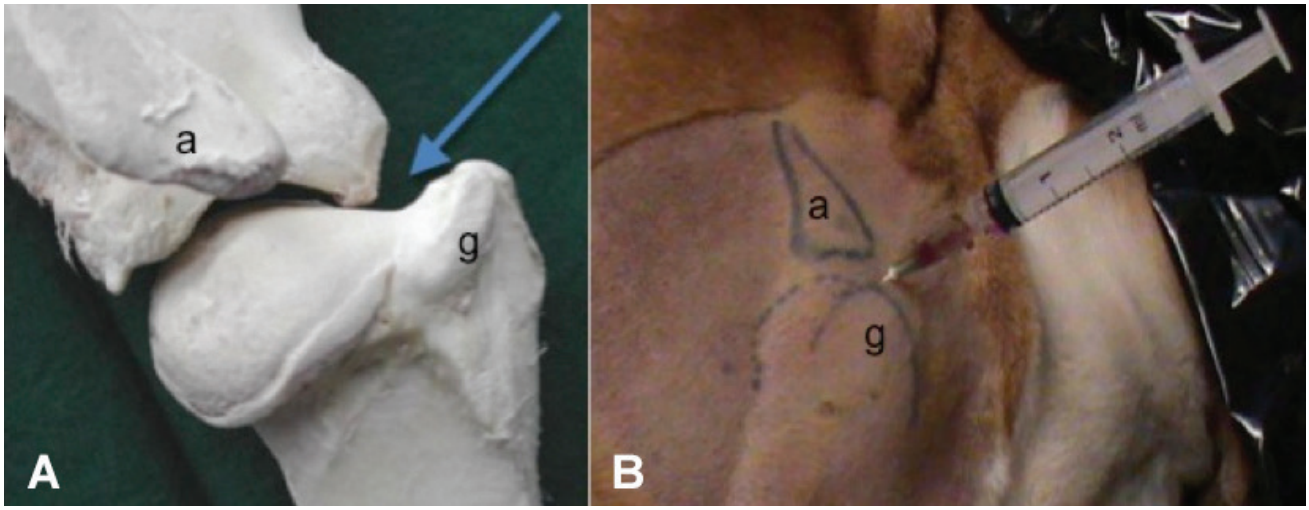

Figure 1 Lateral aspect of a right shoulder joint - craniolateral puncture site. The needle is inserted between the acromion (a) and the greater tubercle (g) in a caudomedial direction.

inflammatory between 3000 and 5000 WBC/mm^3 and septic joint inflammation when >5000 WBC/mm^3).

After aspiration of synovial fluid the contrast medium combined with mepivacaine was injected into the joint. After withdrawal of the needle, the injected joint was flexed and extended to allow the contrast to spread evenly in the joint. A medio-lateral radiographic projection was taken shortly after injection. The dog was stimulated to walk as soon as possible. When the dog had been given medetomidine, antagonisation with atipamezole was performed.

The following radiographic criteria were evaluated by one experienced clinician on arthrography: (i) presence of a cartilage flap on the caudal aspect of the humeral head, (ii) thickened articular cartilage over the defect, (iii) joint mice, (iv) decreased or irregular filling of the synovial sheath surrounding the biceps brachii tendon, (v) calcification of the caudal rim of the glenoid cavity and (vi) joint distension.

Ultrasonography

Ultrasonography [(a)] was performed for the evaluation of the biceps brachii tendon and its attachment or other pathology if needed. This was performed by different clinicians with different experience.

[(a)] MyLab 30, Esaote, Firenze, Italy

Intra-articular anaesthesia evaluation

The effect of intra-articular anaesthesia was evaluated after 2, 3, 5, 10, 15, 20, 25 and 30 minutes by two experienced clinicians. The dog was kept walking continuously and was videotaped before and after intra-articular anaesthesia for later independent evaluation. Intra-articular anaesthesia was noted as positive when lameness improved with at least 2 grades on a scale of 0 to 10 (numerical rating scale: 0/10 sound at all times; 10/10 continuous non-weight bearing lameness) [12] compared with the lameness grade before IA. When lameness had not improved after 30 minutes, the intra-articular anaesthesia was considered negative. This was evaluated twice by two observers, once at the time of the intra-articular anaesthesia and once at a later time by scoring the recorded video sequences. Observers were not blinded to details of the case at the time of initial evaluation, but observers were unaware of case details at the time of review of recorded video sequences.

Arthroscopy

All dogs had arthroscopy on the same day or within a week after the AA. Shoulder arthroscopy was performed in a standardised manner for all dogs using a craniolateral approach (2.7 mm, 30° fore-oblique arthroscope for medium to large dogs and 1.9 mm or 2.4 mm arthroscope for small dogs)[b] [13]. The joint was explored using a standard compartmental lateral approach. At the time of arthroscopy, fixed digital and video images of each structure were obtained for subsequent evaluation and data recording. Arthroscopic findings were assessed by one experienced clinician. The surgeon performing the arthroscopy was aware of the results of the diagnostic workup. Findings included: (i) presence or absence of synovitis and condition of the glenohumeral ligament and m. subscapularis, (ii) cartilage lesions, (iii) flaps and joint mice, (iv) partial or complete rupture of the biceps brachii tendon and (v) calcification of the caudal rim of the glenoid cavity. In doubtful cases a probe was used to

assess the biceps brachii tendon and cartilage.
[b] Richard Wolf, Knittlingen, Germany

Results

Patients

Thirty dogs were successfully injected in the shoulder joint with the combination of mepivacaine and iohexol. All except two were medium to large breed dogs including the Bernese Mountain dog (n=5), Border Collie (n=4), Belgian Malinois (n=3), mixed breed (n=3), Golden Retriever (n=2), Cane Corso (n=2), and one Great Dane, Borzoi, German Shepherd Dog, Epagneul Breton, Rottweiler, Beauceron, Hovawart, German Wire haired Pointer, English Springer Spaniel, and Pyrenean Mountain dog. The small dogs were an 8 kg Pug and one small mongrel breed (13 kg). The ages ranged from 7 to 112.8 months (mean ± SD: 38.3 ± 36 months) and weight from 8 to 76 kg (mean ± SD: 31.6 ± 13.3 kg). The degree of lameness upon presentation varied from 4 to 8 on a scale of 10 (mean ± SD: 6.3 ± 1.2) with 0 indicating that the dog is sound and 10 indicating the complete lack of use of the limb [11, 12].

Radiographs

In table 1 the results of the plain radiographs of the shoulder are described. Shoulder radiographs showed flattening, a small indentation or a radio-lucent area of the humeral head in 16 out of 18 cases of OCD (Fig. 2), clear irregularity of the supraglenoid tubercle and/or sclerosis at the medial ridge of the intertubercular groove in 7 out of 9 cases with partial rupture of the biceps brachii tendon, calcification of the caudal rim of the glenoid cavity in 3 cases, and osteoarthritis in 9 cases.
In 5 dogs elbow radiographs were considered abnormal (Table 1). In 3 dogs an abnormal shape of the medial coronoid process was found. In one dog (case 29; Fig. 3) this was diagnosed as a fissure on arthroscopy. Additionally, there were 2 cases of incongruity, 2 of sclerosis and one of osteoarthritis.

Intra-articular injection

Both sedation protocols were sufficient to allow intra-articular injection in the shoulder. Pain reaction when the needle entered the skin and joint was noted in 4% of the dogs sedated with acepromazine/methadone, whereas none experienced pain when sedated with medetomidine. No adverse reactions were observed associated with the intra-articular administration of the combined products.
In 28 out of 30 dogs, the intra-articular injection had a positive effect (Table 1), meaning that lameness improved with a minimum of 2 degrees on a scale of 0 to 10. The mean time ± SD before a clear positive effect was seen was 11 ± 3.5 minutes. The earliest effect was seen after 2 minutes with the last effect being observed after 15 minutes. In two dogs, no clear improvement of the lameness was observed. Those dogs were considered as false negatives because clear lesions were seen during shoulder arthroscopy with significant pathology of other joints being excluded.

The response to intra-articular anaesthesia was positive in 18 out of 18 dogs with an OCD lesion. The mean time before lameness decreased was 10.4 minutes.
Intra-articular anaesthesia was positive in 8 out of 9 dogs with a partial rupture of the biceps brachii tendon. In two dogs, the arthrogram showed only minor changes but the intra-articular anaesthesia confirmed the shoulder joint as being the painful joint. The mean time after which lameness decreased was 10.8 minutes. After intra-articular anaesthesia, flexion of the shoulder joint, which

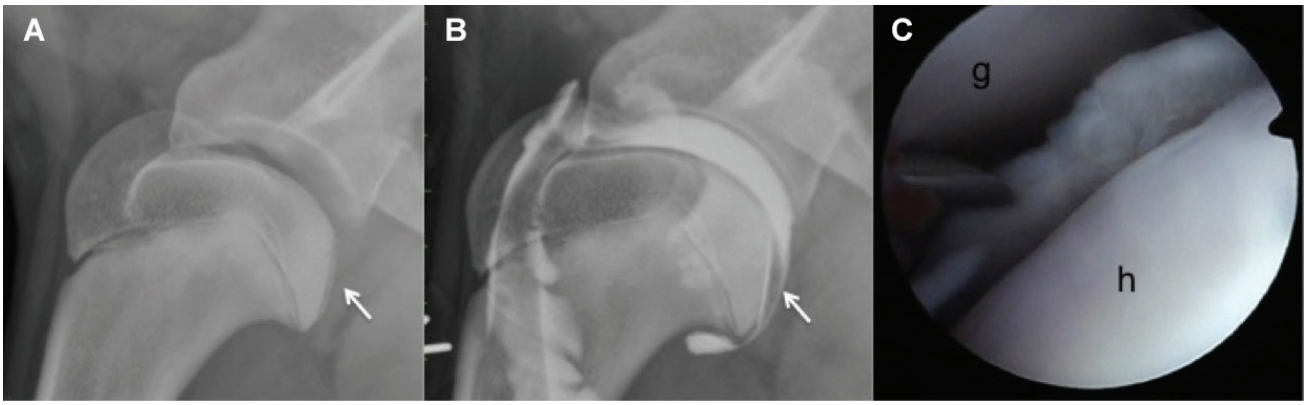

Figure 2 A) Mediolateral radiograph of case 10 (7-month-old Cane Corso) with osteochondrosis visible as a large defect of the humeral head (arrow). B) Arthrography with infiltration of contrast medium underneath the cartilage (arrow). C) Arthroscopic image of the same shoulder showing a cartilage flap (g = caudal glenoid; h = humeral head).

Table 1: Data of 30 patients with a shoulder disorder.

Case	Effect of IA	Radiography		Shoulder joint arthrography	Ultrasonography of the biceps brachii tendon	Arthroscopy
		Shoulder joint	Elbow joint			
1	P	Flattening of the humeral head	Abnormal shape coronoid process	Flap	Normal	OCD
2	P	OA, elongation caudal glenoid	Normal	Decreased or irregular filling of the biceps brachii tendon sheath, fragment caudal glenoid looks isolated	Normal	Partial rupture of the biceps brachii tendon, fragment not isolated
3	P	Flattening of the humeral head	Normal	Contrast line indicating a flap	Normal	OCD
4	N	Irregular supra-glenoid tubercle	Normal	Decreased or irregular filling of the biceps tendon sheath	Thick, heterogeneous with anechoic core lesion	Partial rupture of the biceps brachii tendon
5	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
6	P	OA, irregular supra-glenoid tubercle, sclerosis at the medial ridge of the intertubercular groove	Normal	Decreased or irregular filling of the biceps brachii tendon sheath	Thick, heterogeneous with loss of fibre	Partial rupture of the biceps brachii tendon
7	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
8	P	Minimal sclerosis at the medial ridge of the intertubercular groove	Normal	Normal	Normal	Partial rupture of the biceps brachii tendon.
9	P	OA	Normal	Irregular contrast outline	Normal	Infection
10	P	Flattening of the humeral head	Abnormal shape coronoid process, mini-mal OA	Flap	Normal	OCD
11	N	Calcification caudal glenoid, OA	Sclerosis	Two fragments of the caudal glenoid look isolated	Normal	Calcification caudal glenoid
12	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
13	P	Flattening of the humeral head	Incongruent	Flap	Normal	OCD
14	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
15	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
16	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
17	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
18	P	Sclerosis at the medial ridge of the intertubercular groove, irregu-lar supraglenoid tubercle	Normal	Decreased or irregular filling of the biceps brachii tendon sheath	Loss of normal fibre, anechoic, irregular attachment	Partial rupture of the biceps brachii tendon
19	P	OA, flattening of the humeral head	Normal	Flap	Normal	OCD

IA = intra-articular anaesthesia; P = positive; N = negative, OA = osteoarthritis; OCD = osteochondritis dissecans; MCD = medial coronoid disease.

Table 1: Continued ...

Case	Effect of IA	Radiography		Shoulder joint arthrography	Ultrasonography of the biceps brachii tendon	Arthroscopy
		Shoulder joint	Elbow joint			
20	P	Small indentation at caudal aspect humeral head, minimal OA	Normal	Thickening of contrast line/ no flap or subchondral defect detected	Normal	Small OCD
21	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
22	P	Calcification caudal glenoid	Normal	Decreased or irregular filling of the biceps brachii tendon sheath, fragment does not look isolated	Normal	Calcification caudal glenoid
23	P	OA, sclerosis at the medial ridge of the intertubercular groove, irregu-lar supraglenoid tubercle	Normal	Decreased or irregular filling of the biceps brachii tendon sheath	Loss of normal fibre, anechoic, irregular attachment	Partial rupture of the biceps brachii tendon
24	P	Flattening of the humeral head	Normal	Flap	Normal	OCD
25	P	Sclerosis at the medial ridge of the intertubercular groove	Normal	Decreased or irregular filling of the biceps brachii tendon sheath	Loss of fibres at the level of the supragle-noid tubercle	Partial rupture of the biceps brachii tendon
26	P	Large radio-lucent area humeral head	Normal	Thickened cartilage layer, no flap	Normal	Large OCD
27	P	Sclerosis at the medial ridge of the intertubercular groove, OA	Normal	Complete filling of the proximal part of the biceps brachii tendon sheath	Loss of fibre at the level of the supraglenoid tubercle	Partial rupture of the biceps brachii tendon
28	P	Inhomogenous subchondral bone	Normal	Flap	Normal	OCD
29	P	Minimal irregularity of the supraglenoid tubercle	Incongruent, sclerosis, abnormal shape coronoid process	Minimal decreased or irregular filling of the biceps brachii tendon sheath	Normal	Shoulder: Partial rupture of the biceps brachii tendon. Elbow: MCD (fissure)
30	P	OA, Mineralisation in caudal pouch	Normal	Filling defect in caudal pouch	Normal	Old OCD, joint mice

IA = intra-articular anaesthesia; P = positive; N = negative, OA = osteoarthritis; OCD = osteochondritis dissecans; MCD = medial coronoid disease.

is particularly painful in this condition, was less painful in all dogs. The one dog with a false negative result (case 4) was a 5-year-old Golden retriever with confirmed pathology on arthrography and ultrasonography and no significant pathology in the elbow joint. Arthroscopy revealed that the biceps brachii tendon was nearly completely ruptured; tenotomy was performed and lameness resolved within one month.

One of the two dogs with a calcification of the caudal rim of the glenoid cavity showed no positive response to the intra-articular anaesthesia although clinical improvement was seen after arthroscopic removal of the fragment. The dog with the shoulder infection showed an improvement of lameness within 15 minutes after intra-articular anaesthesia.

Arthrogram

All arthrograms were of good diagnostic quality (good filling of the joint and good opacity) but not all arthrograms provided a definitive diagnosis (Table 1). Osteochondritis dissecans (OCD) was diagnosed in 16 dogs by visualisation of a loose cartilage flap (Fig. 2). In two dogs (case 20 and 26) only a thickened cartilage layer

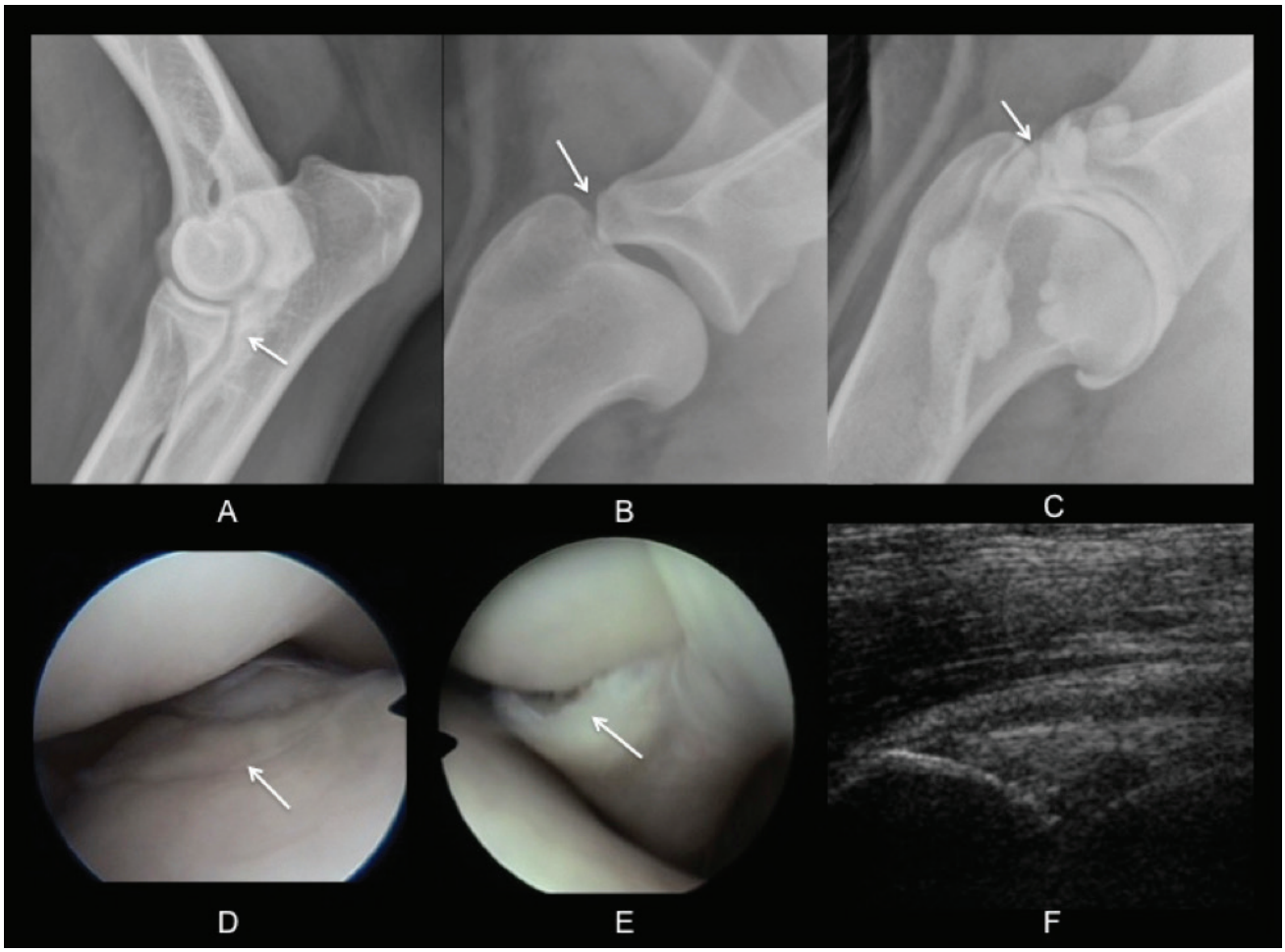

Figure 3 Images of a two-year-old Bernese Mountain dog (case number 29). A) Mediolateral radiograph of the elbow joint - discrete signs of sclerosis (arrow) and an abnormal shape of the medial coronoid process. B) Mediolateral radiograph of the shoulder joint of the same dog – minimal irregularity of the supraglenoid tubercle (arrow). C) Arthrography of the same shoulder – irregular outline of the biceps brachii tendon (arrow). D) Arthroscopic image of the elbow joint identified in A. A fissure of the medial coronoid process is identified (arrow). E) Arthroscopic image of the shoulder joint identified in B and C - partial rupture of the biceps brachii tendon at its attachment (arrow). F) Ultrasonographic image of the shoulder joint identified in B, C, and E. No clear abnormalities at the origin of the biceps brachii tendon are identified.

on the caudal aspect of the humeral head was detected. Biceps brachii tendon pathology was diagnosed in 7 dogs but was missed in two dogs (cases 8 and 29). A calcification at the caudal rim of the glenoid cavity was seen in 3 dogs (cases 2, 11 and 22; Fig. 4 and 5). Synovial fluid was collected from all dogs. In one dog there was evidence of septic joint inflammation (>5000 wbc/mm^3), although no bacteria were cultured.

Ultrasonography

Ultrasonographic examination confirmed the partial rupture of the biceps brachii tendon in 6 dogs but failed to demonstrate this lesion in three other dogs (cases 2, 8 and 29). Subsequent arthroscopy confirmed the existing pathology in those cases. In the other joints, ultrasound and arthroscopy demonstrated an intact tendon.

Arthroscopy

All dogs underwent arthroscopy in order to confirm and treat the lesions. Eighteen dogs with OCD were treated by removal of the OCD flap. Tenotomy was performed in all 9 dogs with a partial rupture of the biceps brachii tendon. In 2 out of the 3 dogs with a calcification of the caudal rim of the glenoid cavity arthroscopic treatment was performed by 'fragment' removal. The calcification of the third dog (case 2; Fig. 4) was found unseparated from the caudal rim of the glenoid cavity and therefore left untreated. In the same joint, a partial rupture of the biceps brachii tendon was diagnosed which was treated successfully with a tenotomy. The dog with the suspected infection had no underlying primary lesions and was treated by arthroscopic joint lavage followed by four weeks of a broad-spectrum antibiotic. Synovitis was diagnosed visually in all joints ranging from mild to severe.

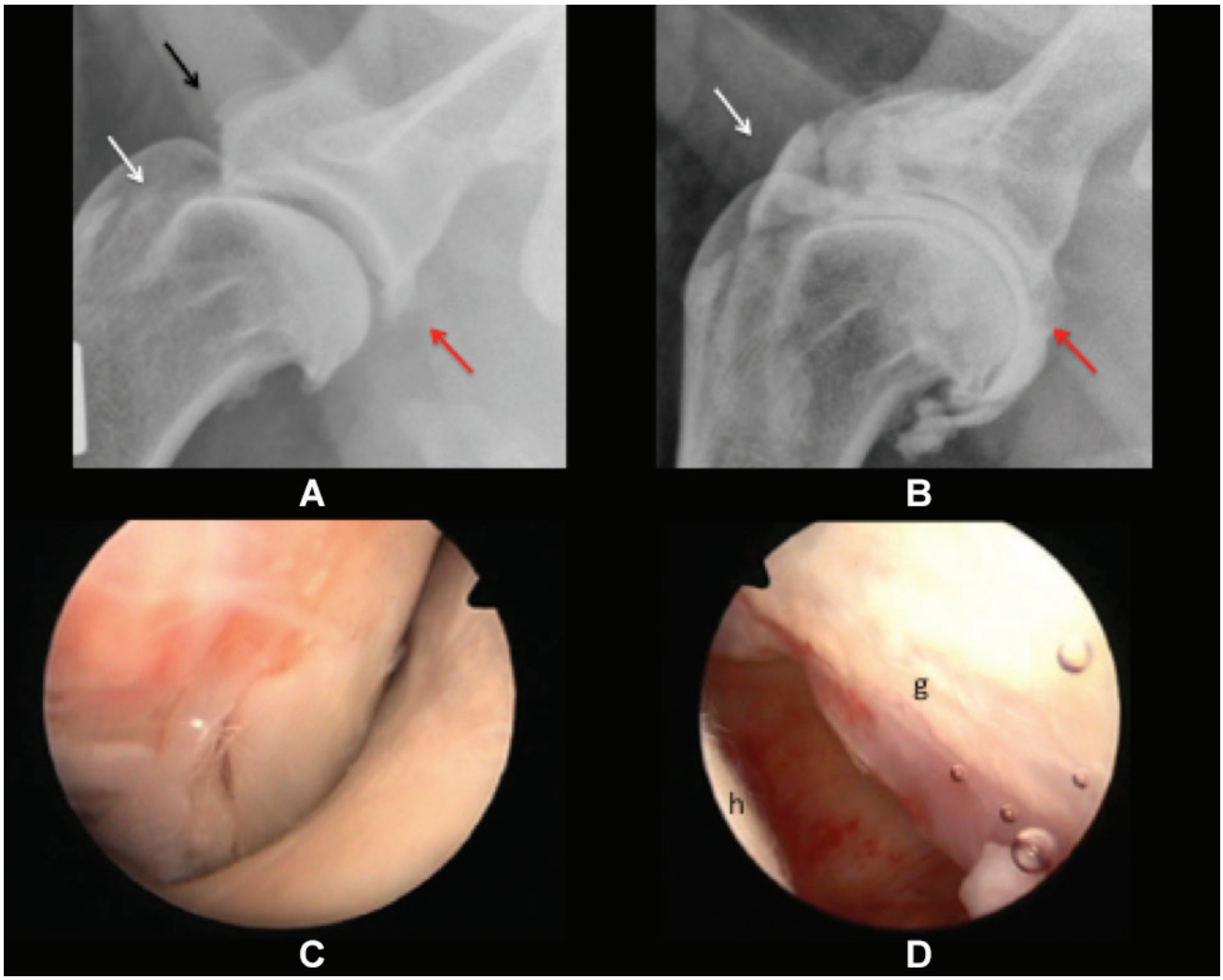

Figure 4 Images of an eight-year-old Belgian Malinois with a partial biceps brachii tendon rupture (case 2). A) Mediolateral radiograph of the shoulder joint - osteosclerosis (white arrow) at the medial ridge of the intertubercular groove, periosteal reaction of the supraglenoidal tubercle (black arrow) and osteoarthritis. The glenoid cavity is elongated and may suggest the presence of a calcified body (red arrow). B) Arthrogram of the same shoulder joint - unclear delineation of the biceps brachii tendon and its sheath (white arrow). The calcified body at the caudal rim of the glenoid cavity seems to be surrounded by the contrast medium (red arrow). C) Arthroscopic image of the same shoulder joint - partial rupture of the biceps brachii tendon. D) Arthroscopic image showing the calcification at the caudal rim of the caudal glenoidal cavity (g) which is not visible as a separated fragment. A small part of the fibrillated cartilage of the humeral head (h) is visible.

Discussion

In general, clinical and radiographic examination is often sufficient to localise lameness in dogs. However, full assessment of the shoulder joint may require other diagnostic tests and more advanced imaging methods such as ultrasonographic examination, contrast arthrography, CT, MRI or arthroscopy. Arthrography of the shoulder joint in the dog is a well-described technique which, to the authors' observation, is infrequently used in clinical practice. In addition, the combination with intra-articular anaesthesia has not been reported to our knowledge. Therefore, this study of dogs with shoulder problems was initiated to investigate the value of the combination of intra-articular anaesthesia with arthrography, described in human medicine as anaesthetic arthrography. The dogs included in this study were mostly medium to large sized dogs except for one Pug (8 kg) and one small mongrel breed (13 kg). The diagnosis of shoulder abnormalities in this study cohort was based on clinical and imaging findings and further confirmed by arthroscopy. OCD was the most frequent diagnosis, followed by a partial rupture of the biceps brachii tendon and a calcification at the caudal rim of the glenoid cavity. In contrast to what has been reported in the literature, shoulder instability was not seen in this study [14]. In our clinic, measurements of abduction angles are not routinely performed because in the author's opinion shoulder instability is a very rare finding, which

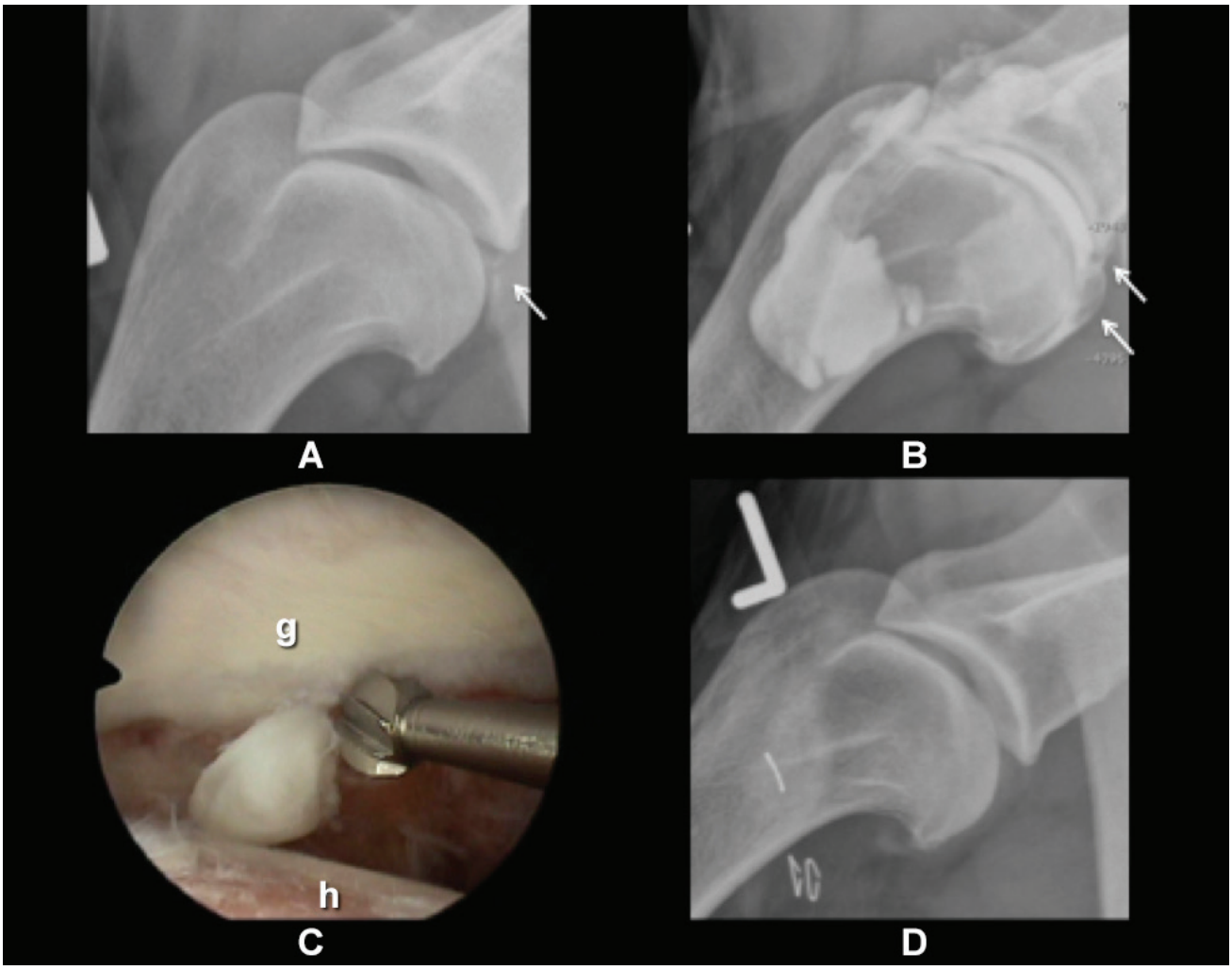

Figure 5 Images of a five-year-old mixed breed dog (case 11) with a calcification at the caudal rim of the glenoid cavity. A) Mediolateral radiograph of the shoulder joint - large calcification with small separate fragment (arrow). B) Arthrogram - The contrast is surrounding two bony fragments (arrows), which are impinging the cartilage of the humeral head. The cartilage appears to be thinner at that location. C) Arthroscopic image of the same shoulder joint - the small fragment of the caudal glenoid cavity (g) which is loosened using a 2 mm hand burr. The humeral head shows local erosions corresponding to the arthrographic finding (h). D) Mediolateral radiograph of the same shoulder joint after removal of the calcifications.

is occasionally identified after traumatic injury or in small breed dogs. For clarity, this study was limited to the evaluation of the effect of anaesthetic arthrography on intra-articular causes of shoulder pain and did not investigate other causes of shoulder pain such as shoulder instability or extra-articular processes.

All dogs required sedation in order to allow an intra-articular injection. A recent study of the effect of sedation on lameness prior to intra-articular anaesthesia showed that no significant effect on lameness was observed after sedation with two different protocols and that further clinical evaluation was possible [11]. The acepromazine-opioid protocol was shown to be the preferred sedation method because the dogs were able to walk before and after sedation, which allowed a direct evaluation of the intra-articular anaesthesia. One limitation of this sedation protocol was the need for additional assistance to restrain the dog. In smaller practices, this could be a limiting factor with fewer co-workers. In such a case, the medetomidine-atipamizole protocol provides an alternative by allowing the evaluation of intra-articular anaesthesia after antagonisation [11].

Different sites for aspiration of the shoulder joint have been documented [15]. The site mentioned in our study is also described as the first puncture site for shoulder arthroscopy [16]. Alternatively, the shoulder may be punctured distal to the acromion. In either case, the needle should be inserted gently and correctly to prevent iatrogenic cartilage damage.
In this study, mepivacaine was used as the local anaesthetic due to its favourable properties [17]. In order to perform an anaesthetic arthrogram, the contrast medium was mixed with mepivacaine. The contrast medium, iohexol, used in this study is a low osmolar non-

ionic monomeric agent, which causes minimal synovial inflammation and has a slow resorption [18]. The volume and concentration of iohexol was based on contrast studies performed on normal bitches with a body weight ranging from 23 to 37 kg [19]. To prevent the arthrogram from being too radiopaque, a dilution of the contrast to 100 to 120 mg iodine per ml is required. Since most contrast products have a concentration of 200 to 300 mg iodine per ml, a 1:1 combination with mepivacaine provided the ideal dilution and volume. No adverse reactions to the intra-articular injection of the combined products were observed except for minor pain reactions in 4 % of the dogs, which may be caused by the insertion of the needle, the increased intra-articular pressure while injecting the product or by hitting the subchondral bone. No iatrogenic lesions of the cartilage and no abnormal synovitis were seen during later arthroscopy.

The results of previous studies [8, 20] indicate arthrography of the shoulder joint is a valuable diagnostic method in dogs with obscure lameness when plain radiographs are negative or inconclusive. This technique was found to be accurate in evaluating the status of the articular cartilage and, more importantly, it is particularly useful for separating surgical from nonsurgical candidates in cases of bilateral OCD [21]. All arthrograms obtained in this study were of good diagnostic quality and gave more information than the plain radiographs (Table 1), which indicates that dilution with mepivacaine does not interfere with the image quality.

Ultrasonography was shown to be useful for the evaluation of the biceps brachii tendon. In three dogs (cases 2, 8 and 29), however, ultrasonographic examination did not demonstrate the partial rupture of the biceps brachii tendon. In those dogs, the AA had a positive effect and confirmed the presence of pathology within the shoulder. Care should be taken to interpret these findings because observers with a different level of experience performed the examination. In addition, ultrasonography was performed after the AA, which caused some minor accumulation of fluid and air bubbles with minimal disturbance of the evaluation of the biceps brachii tendon. Ultrasonography prior to AA, however, might improve the diagnosis of lesions of the biceps brachii tendon.

Diagnosis of shoulder OCD based on clinical and radiographic examination is usually straightforward. However, when manipulation of the shoulder does not produce a pain reaction or when radiographic findings are subtle (cases 20 and 28), the diagnosis may be difficult to obtain. In those cases, AA may prove to be helpful for further confirmation of the location of lameness and the identification of the lesion. In this study, two cases showed minimal clinical and radiographic changes. AA led to a decreased lameness and demonstrated a small defect in one joint and a large flap in the other joint.

The benefit of plain radiographs of the shoulder joint in cases of biceps brachii tendon lesions is low because the radiographic changes are not very specific. Osteosclerosis of the intertubercular groove has been described as a radiographic feature of bicipital injuries [22]. In addition, a periosteal reaction and osteosclerosis of the supraglenoid tubercle can often be demonstrated. In the described cases, 7 dogs showed a relatively apparent sclerosis and/or an irregular supraglenoid tubercle. In two dogs the radiographic changes were not clear (cases 2 and 8). Confirmation with ultrasonography, arthrography and arthroscopy is desired when tenotomy is considered as a possible treatment. Positive contrast arthrography has been described as an additional imaging tool [23] and was found to be more sensitive compared to ultrasonography for the diagnosis of lesions of the biceps brachii tendon [24]. In this series arthrography added additional information for the diagnosis of a biceps brachii tendon lesion except in one dog (case 8). Arthrography was more helpful than ultrasonography in two dogs (case 2 and 29). Finally, direct visualisation of the biceps brachii tendon via shoulder arthroscopy is recognised as superior to the other diagnostic procedures [25]. Clear lesions of the biceps brachii tendon were identified during arthroscopy in all 9 cases even when the arthrography or ultrasonography did not demonstrate obvious lesions. Anaesthetic arthrography localised lameness to the shoulder joint, which justified arthroscopy of that joint.

Calcifications of the caudal rim of the glenoid cavity are a rare cause of lameness in the canine shoulder. Often, this observation is classified as a clinically insignificant lesion even though it has been described as a possible cause of shoulder lameness [26]. Since the calcified bodies are not always the cause of lameness, diagnosis should be based on the localisation of pain to the shoulder and the exclusion of other shoulder and forelimb problems. In this study, three dogs had a fragmentation of the glenoid cavity (cases 2, 11 and 22). In two dogs (case 2 and 22), AA confirmed the shoulder joint as the painful joint but arthrography showed no other pathology in one dog and

concurrent biceps brachii tendon pathology in the other joint. In the latter dog (case 22), arthroscopy demonstrated that the calcification was a bony elongation of the glenoid cavity, suggesting that it was of no clinical importance. Treatment was therefore limited to the ruptured biceps brachii tendon, which could be considered as the primary cause of lameness since arthroscopic transection resolved lameness. In one dog (case 11), intra-articular anaesthesia was negative and arthrography showed no other shoulder pathology besides a calcified body at the caudal glenoid cavity. In addition, there was mild osteosclerosis of the ulnar trochlear notch of the elbow. In the latter case, diagnosis was only definitive when arthroscopy enabled the visualisation of the calcification. In the absence of other shoulder pathology, this calcification of the caudal rim of the glenoid cavity is considered as the primary cause of lameness, which was confirmed by the positive outcome after arthroscopic removal. However, occult elbow lesions may be present and should be ruled out as the cause of lameness as well. In this case, only shoulder pain could be elucidated and synovial fluid of the shoulder joint was abnormal, which led us to the shoulder. In this particular case, AA failed to reduce lameness, which reminds us of its limitations. In this study 4 other dogs had radiographic changes in the elbow. In all of these cases AA of the shoulder was positive, which confirmed the shoulder as the cause of lameness and the elbow lesions as being clinically irrelevant.

This study demonstrates that AA, or the combination of intra-articular anaesthesia with arthrography, is a fast, minimally invasive and very efficient diagnostic tool to localise intra-articular shoulder problems and provide additional diagnostic information with only one intervention. This diagnostic technique may be helpful when abnormalities are detected simultaneously in the shoulder and/or other joints - often the elbow[2] - or when plain radiographs do not confirm suspected pathology of the shoulder. A limitation of this study is that the examined group did not comprise shoulder lameness caused by shoulder instability or supraspinatus tendinopathy. Logically, those disorders would not respond to the intra-articular administration of a local anaesthetic since they are partially or complete located outside the joint cavity. In addition, both intra-articular anaesthesia and arthrography have their limitations and may provide eventually false negative results. In those cases, further diagnostic methods including CT, MRI, scintigraphy or direct arthroscopic visualisation should lead to the final diagnosis.

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REPRINT PAPER

An update on meningoencephalomyelitis of unknown aetiology in dogs

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SUMMARY

Inflammatory diseases of the central nervous system are common causes of neurological dysfunction in the dog and can be grouped into two broad categories; those of infectious and those of unknown aetiology. Meningoencephalomyelitis (MEM) of unknown aetiology describes a group of non-infectious inflammatory diseases of the central nervous system in which abnormal findings on magnetic resonance imaging and cerebrospinal fluid analysis indicate inflammatory central nervous system disease, but for which a histopathological confirmation has not yet been reached. This group includes granulomatous MEM and necrotising encephalitis; the latter can be further subdivided into necrotising meningo-encephalitis and necrotising leucoencephalitis. Steroid-responsive meningitis-arteritis may also be included in this category however, usually it does not present with signs of encephalitis or/and myelitis (except in the chronic form) and is it easier to diagnose without histopathological examination. In most cases of MEM of unknown aetiology, a presumptive diagnosis can be made by assessing the case presentation, the neurologic signs, cerebrospinal fluid testing, cross-sectional imaging of the central nervous system and appropriate microbiological tests. Definitive diagnosis is achieved with histopathological examination. The underlying cause for these diseases is unknown. The clinical signs in MEM of unknown aetiology are variable and depend on which area of the central nervous system is affected. MEM presents with an acute onset, it is progressive in nature, and it is associated with multifocal to diffuse neuro-anatomic localisation. Extra-neural signs are less common and usually include pyrexia and peripheral neutrophilia. The differential diagnoses for dogs presented with an acute onset of multifocal central nervous system signs include genetic abnormalities, metabolic disorders, infectious meningo-encephalitis, toxin exposure, stroke and neoplasia. The diagnostic approach includes a complete blood count, a comprehensive chemistry panel, urinalysis, survey radiographs of the thorax plus abdominal ultrasound to rule out systematic disease and metastatic neoplasia, computed-tomography or magnetic resonance imaging, cerebrospinal fluid analysis and microbiological tests. When neoplasia is suspected, computed-tomography-guided brain biopsy may be required to confirm the diagnosis. MEM of unknown aetiology responds adequately to immunosuppressive therapies, but the prognosis given should be guarded to poor with the exception of steroid-responsive meningitis-arteritis, for which it is good. Treatment protocols are based on prednisolone, but new immunosuppressive agents have now been added to the treatment protocol in order to control these diseases which seem to be effective. However, the gold standard protocol has yet to be established.

Key words: dog, inflammatory diseases, meningoencephalomyelitis, nervous system

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Introduction

Inflammatory diseases of the central nervous system are common causes of neurological dysfunction in the dog and can be grouped into two broad categories; those of infectious and those of unknown aetiology. Meningoencephalomyelitis (MEM) of unknown aetiology describes a group of non-infectious inflammatory diseases of the central nervous system in which abnormal findings on magnetic resonance imaging and cerebrospinal fluid analysis indicate inflammatory disease, but for which histopathological confirmation has not been reached. The group MEM of unknown aetiology includes the conditions granulomatous MEM and necrotising encephalitis; the latter can be further subdivided into necrotising meningo-encephalitis and necrotising leucoencephalitis. Steroid-responsive meningitis-arteritis may be also included to this category. MEM of unknown aetiology usually have an acute onset and rapid progression of clinical signs, although, sometimes, they present with a more chronic clinical course.[30]

The underlying causes of MEM of unknown aetiology are unknown. Proposed causes include infectious,[47] auto-immune,[26, 34] and neoplastic conditions. Moreover, for some breeds, there may be a genetic component for these disorders.[22] Numerous attempts have been made to identify infectious agents in affected dogs, but, to date, these have been unsuccessful. A possible explanation is that these disease processes may be triggered by an infectious agent that is rapidly eliminated, but which, nevertheless, has initiated a destructive immune response. As a result, treatment protocols applied are based, in principle, on immunosuppressing the animal.[38]

Granulomatous meningoencephalomyelitis

Granulomatous MEM is an inflammatory disease of the central nervous system which affects mainly dogs, although rarely it can affect cats.[7, 63, 44] The disorder may account for up to 25% of all central nervous system diseases in dogs.[62] The cause of granulomatous MEM is unknown although it has been suggested that a T cell-mediated delayed-type hypersensitivity may be a possible pathogenic mechanism for the disease.[26] This mechanism would lead to formation of peri-vascular cellular infiltrates of histiocytic cells mixed with lymphocytes, plasma cells and, occasionally other leukocytes, and involve the majority of the blood vessels in the white matter (predominantly), as well as the pia matter of the central nervous system.[14] Another possible hypothesis for the cause of granulomatous MEM is an altered host response to an infectious agent or a genetic disorder.[55] It has been reported that the disease may be triggered by vaccination against canine distemper and rabies,[24] which supports the hypothesis of an auto-immune type disease.

Clinical presentation

Most cases of granulomatous MEM occur in small breed dogs; most commonly in terriers, toy breeds and in Poodles, although any breed may be affected.[36] Most cases of the disease occur in young to middle-aged dogs with a mean age of presentation of ~5 years (range: 6 months-12 years). Granulomatous MEM occurs in animals of both sexes; however, there appears to be a higher incidence risk for the disease in female dogs.[37]

Clinical signs

Granulomatous MEM occurs as an acute-onset, progressive, multifocal neurologic disease that may be fatal if left untreated.[16,37] Clinically, the disease is characterised by three differing clinical presentations: multifocal, focal and ocular.

The multifocal form is the most common; typically, it has an acute onset, with rapidly progressing multifocal neurologic signs over a 1 to 8 week period. The cerebrum, caudal brainstem, cerebellum and/or the cervical spinal cord may be involved.[7,14,36] The clinical signs reflect a multifocal syndrome, as a result of the scattered distribution of the lesions, and include incoordination, vestibular or proprioceptive ataxia, cervical hyperesthesia, head tilt, nystagmus, facial and/or trigeminal nerve paralysis, circling, visual deficits, seizures and depression.[1,2,8] Occasionally, fever and peripheral neutrophilia can accompany the neurological signs.[14,51]

The focal form of the disease is less common, although focal signs have been reported in up to 50% of cases.[37] This form represents a true mass lesion located most often in the cerebral hemispheres, brainstem or spinal cord.[16] An infrequently reported ocular form of granulomatous MEM appears to be related to lesions localised in the optic nerves and optic chiasm and can result in visual impairment and abnormal pupillary reflexes. A hyperaemic and oedematous optic disk may be seen on ophthalmic

examination; dilated vessels can be seen and focal haemorrhage may be present. Dogs with the ocular form of the disease may also, concurrently, show or develop the multifocal form.[7,16]

Generally, it has been reported that 50% of the dogs with granulomatous MEM have forebrain-type symptoms and 50% have both forebrain-type and brainstem-type symptoms. In addition, dogs with the acute form of the disease often show signs of central vestibular syndrome.[30] Cervical spinal pain is also common in patients and it can sometimes pre-exist the spinal cord signs.

Diagnosis

A tentative diagnosis may be suggested by the medical history, clinical presentation, results of the clinical examination, examination of cerebrospinal fluid and the findings from neuroimaging (magnetic resonance imaging, computed-tomography). Definitive diagnosis is based on histopathological examination of the lesion in the central nervous system tissue, which can be collected by computed-tomography-guided brain biopsy or other neurosurgical techniques (craniotomy or laminectomy).[30] In most dogs, examination of the cerebrospinal fluid reveals mild to pronounced pleocytosis, ranging from 50 to 900 leucocytes /µl. Mononuclear cells, mainly lymphocytes (60-90%) and monocytes (10-20%) can also be present (Fig. 1). While neutrophils typically comprise from 1-20% of leucocytes in the cerebrospinal fluid, on rare occasions they may be the predominant cell type. The protein concentration is usually mildly to moderately increased, ranging from 40 to 400 mg/dl.[17,45]

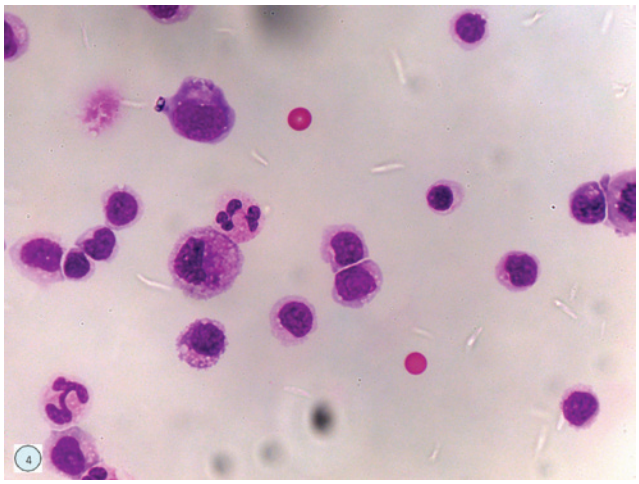

Fig. 1. Predominance of lymphocytes in cerebrospinal fluid sample from a dog with granulomatous meningoencephalomyelitis (modified Wright-Giemsa, ×60 objective).

The most common magnetic resonance imaging findings for the multifocal form include multiple hyperintensities on T2-weighted or fluid-attenuated inversion recovery (FLAIR) sequences scattered throughout the central nervous system white matter. These lesions typically assume an infiltrative appearance and have irregular margins. Lesions visible in magnetic resonance imaging often are distributed throughout both the grey and the white matter. The lesions display a variable intensity on T1-weighted images and variable degrees of contrast enhancement (Fig. 2). Meningeal enhancement is uncommon.[10] The focal form may be identified on magnetic resonance imaging or computed-tomography as a non-specific single space-occupying mass lesion.[52] In the optic form, optic nerves may be iso-intense on T2-weighted images and may be enhanced on T1-weighted images with contrast medium; the optic chiasm also may appear enlarged.[27] Computed-tomography may reveal evidence of brain inflammation, although it is not as sensitive as magnetic resonance imaging in delineating the parenchymal and meningeal lesions. Both focal and multifocal forms may be associated with contrast enhancement on computed-tomography and a mass effect may be observed by displacement of the surrounding brain tissue. The multifocal form is characterised by the presence of multiple poorly defined, enhancing lesions of the parenchyma and meninges.[41]

Differential diagnosis

The differential diagnosis includes infectious MEM (including canine distemper encephalomyelitis, toxoplasmosis, neosporosis and cryptococcosis) and brain tumours.

Differentiation from canine distemper encephalomyelitis can be based on vaccination history and the presence of systemic (respiratory, gastrointestinal) signs, although sometimes vaccinated dogs may also be affected.[6] Differentiation from toxoplasmosis and neosporosis can be based on the information gained from the history, clinical presentation, clinical pathology findings (non-regenerative anaemia, neutrophilia, lymphocytosis, eosinophilia and increased serum alanine transaminase, aspartate aminotransferase, creatinine kinase activity, especially in dogs with acute liver and/or muscle necrosis) and measurement of serum IgG and IgM (preferably) levels, as these clearly indicate infection by the above protozoa.[5] PCR could also aid differentiation as it is a highly sensitive method of detecting protozoal DNA.[53] Cryptococcosis

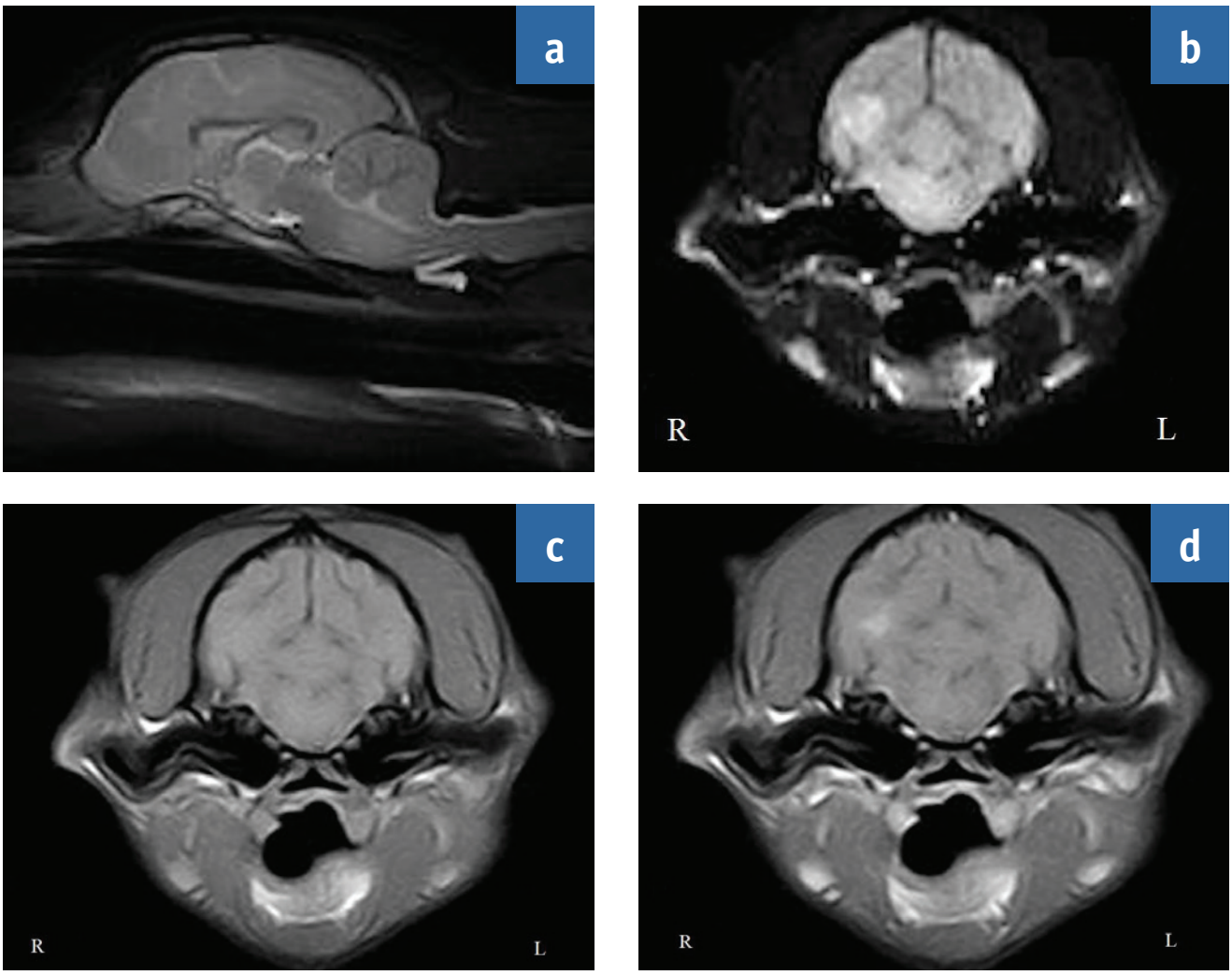

Fig. 2. Magnetic resonance imaging findings in multifocal granulomatous meningoencephalomyelitis: (a) sagittal, T2-weighted MR image at the level of midline, with focal hyper-intense area in the brainstem extending from pons cranially to medulla oblongata caudally; (b) transverse, fluid-attenuated inversion recovery MR image at the level of the pons, with hyper-intensity of previously described brainstem lesion and hyper-intense lesion in the caudal right occipital lobe, reflecting focal inflammation and oedema; (c) transverse T1W pre-contrast MR image at the same level as (b), with extremely mild hyper-intensity of the right occipital lobe lesion involving the grey and white matter, whilst the brainstem lesion is iso-intense to the surrounding tissue and cannot be visualised; (d) transverse T1W post-contrast MR image at the same level as (b), with the occipital lobe lesion enhancing and the brainstem lesion not enhancing (figure generously provided by Dr Nicolas Rousset from the Queen's Veterinary School Hospital of the University of Cambridge).

is rare and, in addition to the neurological signs, it is accompanied with other symptoms, e.g. nasal and ocular discharge and submandibular lymph node enlargement. Moreover, as with toxoplasmosis/neosporosis, serological testing and PCR will aid in the diagnosis of cryptococcosis. In addition to this, the demonstration of organisms on smears taken from the discharge and in culture samples will differentiate between the two diseases.[4] Finally, brain tumours can be differentiated from granulomatous MEM mainly by magnetic resonance imaging findings, although this may prove difficult in some cases. As a result, histologic examination of the lesions may be inevitable.

Prognosis

The prognosis of granulomatous MEM is poor without aggressive immunosuppression. Immunosuppressive treatment, mainly corticosteroids, is believed to markedly improve the clinical outcome.[13] However, most affected dogs will succumb to the disorder or are euthanised within a few weeks to months after diagnosis, despite the treatment. Dogs with focal granulomatous MEM have been reported to survive longer (3-6 months or even more) than those with the multifocal form, which die within a few days to weeks (median interval from diagnosis to death: 8 days). Dogs with focal forebrain-type signs had significantly longer survival times (>1 year) than dogs with

signs indicating localisation in other areas of the central nervous system (2 months). [37]

Necrotising encephalitis

There are two distinct subtypes of necrotising encephalitis (NE): necrotising meningo-encephalitis and necrotising leucoencephalitis. Both have similar clinical presentations and histopathologic features, as they cause bilateral, asymmetric cerebral necrosis. Necrotising meningo-encephalitis commonly affects the cerebral hemispheres and subcortical white matter, with profound inflammation extending from the leptomeninges through the cerebral cortex and into the corona radiate.[16] Conversely, necrotising leucoencephalitis is relatively sparing of the cerebral cortex and meninges and predominately affects perivascular cerebral white matter, including the centrum semiovale, the thalamocortical fibres, the internal capsule, the thalamus and, sometimes, the brainstem.[30]

The aetiopathogenesis for these disorders is not yet fully understood. It is believed that a combination of genetic, infectious (mainly Canine Herpesvirus-1) and environmental factors trigger the onset through the induction of immune mediated responses.[40,66] As far as necrotising meningo-encephalitis is concerned, it has been suggested that auto-antibodies against astrocytes and glial cells (anti-astrocytic and glial fibrillary acid protein antibodies) may be responsible for the disease, based on their presence in the cerebrospinal fluid of affected dogs. However, similar antibody levels occur in the cerebrospinal fluid of dogs with granulomatous MEM and brain tumours and sometimes even in a few clinically normal dogs.[50]

Clinical presentation

NE is breed-specific. Breeds affected include Pugs, Maltese Terriers, Chihuahuas, Yorkshire Terriers, Pekingese, West Highland White Terriers, Boston Terriers, Japanese Spitz, Miniature Pinschers, French Bulldogs, Lhasa Apso and Shih-Tzu.[12,15] Necrotising meningo-encephalitis more commonly affects Pugs and Maltese Terriers, whilst necrotising leucoencephalitis more commonly affects Yorkshire Terriers and French Bulldogs.[12]

Most cases of necrotising meningo-encephalitis occur in young dogs. The age of dogs at the onset of clinical signs ranges from 6 months to 7 years, with mean age being 2.5 years.[15] Necrotising leucoencephalitis occurs in animals aged 4 months to 10 years of age, with the mean age being 4.5 years.[29] Female animals are more frequently affected compared than males.[12]

Clinical signs

Dogs with NE commonly display cerebrothalamic-type clinical signs, because of the localisation of the lesions in the prosencephalon. In necrotising leucoencephalitis, mid-to-caudal brainstem-type clinical signs may also occur, due to additional lesions in that area of the brain. The clinical signs progress rapidly; most commonly, they include seizures, depression, circling, vestibulocerebellar-type signs, visual deficits, ultimately leading to death. [12] Generally, the signs vary and depend on the brain area that has been affected.[16] Cervical spinal pain is a common symptom that occurs, because of the localisation of the lesions in the meninges and/or the forebrain.[36]

Diagnosis

A tentative diagnosis of the NE may be suggested by the history, the clinical presentation, the clinical examination, the cerebrospinal fluid analysis and the neuroimaging findings (magnetic resonance imaging, computed-tomography). Definitive diagnosis is based on the histological examination of the lesions. Cerebrospinal fluid analysis, as in granulomatous MEM, reveals increased protein content and mononuclear (usually lymphocytic) pleocytosis. Magnetic resonance imaging lesions associated with necrotising meningo-encephalitis include asymmetric, multifocal prosencephalic-type lesions affecting the grey and white matter that appear hyper-intense on T2-weighted images and iso-intense to slightly hypo-intense on T1-weighted images, with slight contrast enhancement. Loss of grey/white matter demarcation may be noticed (Fig. 3). In necrotising leucoencephalitis, multiple, asymmetric bilateral prosencephalic lesions appear mainly in the subcortical white matter. The lesions are hyper-intense on T2-weighted and fluid-attenuated inversion recovery images and hypo-intense or iso-intense on T1-weighted images, with variable contrast enhancement. [64,68] A computed-tomography scan may also contribute to the diagnosis. Mainly in the acute stages of NE, focal hypodense lesions in the prosencephalon may be revealed, which may or may not be enhanced with contrast.[56]

Differential Diagnosis

Differential diagnosis includes neoplastic, infectious and immune-mediated disorders of the central nervous system.

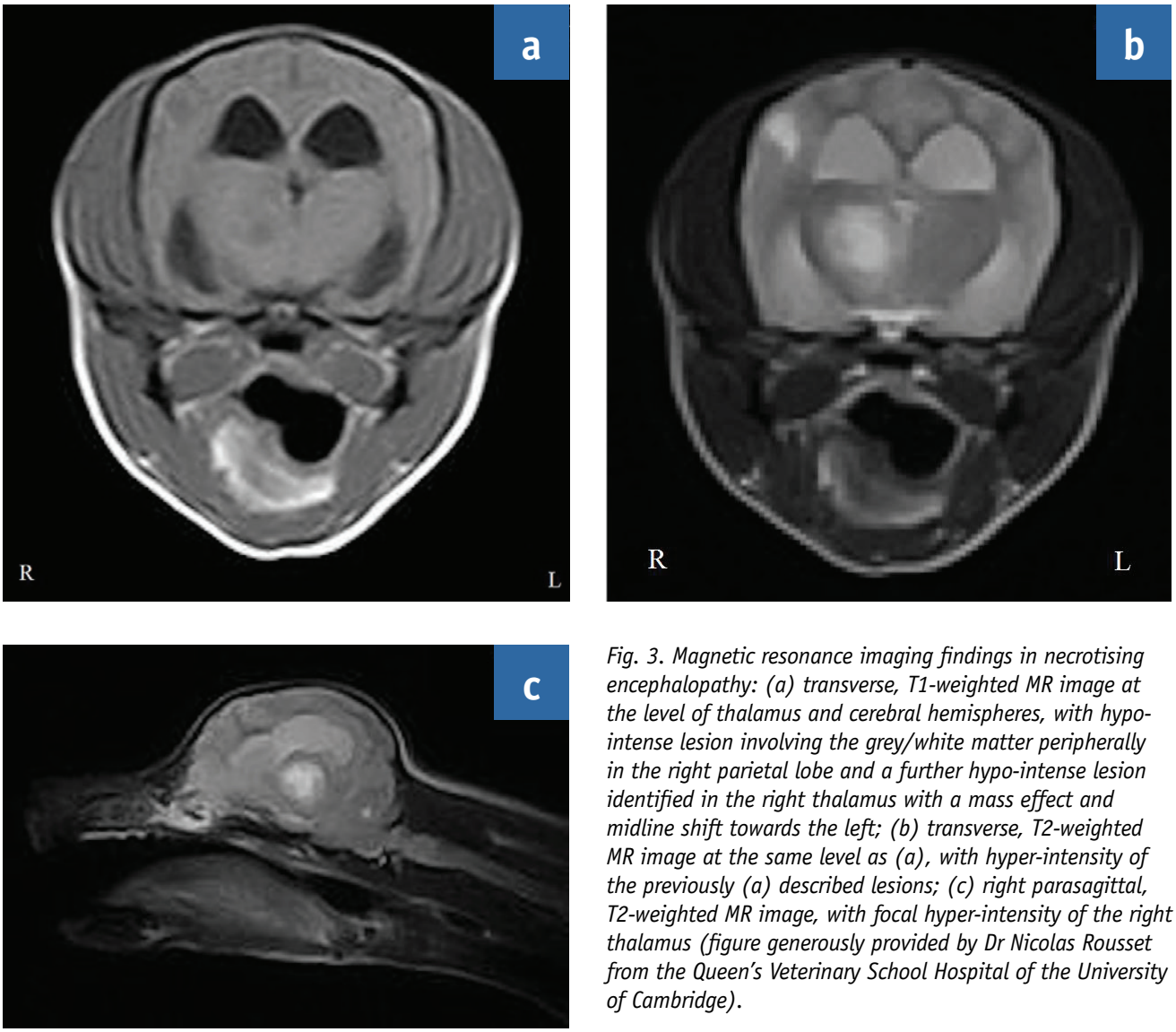

Fig. 3. Magnetic resonance imaging findings in necrotising encephalopathy: (a) transverse, T1-weighted MR image at the level of thalamus and cerebral hemispheres, with hypo-intense lesion involving the grey/white matter peripherally in the right parietal lobe and a further hypo-intense lesion identified in the right thalamus with a mass effect and midline shift towards the left; (b) transverse, T2-weighted MR image at the same level as (a), with hyper-intensity of the previously (a) described lesions; (c) right parasagittal, T2-weighted MR image, with focal hyper-intensity of the right thalamus (figure generously provided by Dr Nicolas Rousset from the Queen's Veterinary School Hospital of the University of Cambridge).

Prognosis

The prognosis should be guarded and depends on the severity of clinical signs and distribution of lesions in the central nervous system. The median interval from diagnosis to death has been estimated to be 93 days.[12]

Steroid-responsive meningitis-arteritis

Steroid-responsive meningitis-arteritis is a debilitating inflammatory disease of the canine central nervous system. Pathogenesis of the disease is unclear, but it has been proposed that it may be triggered by environmental factors, which activate an immune-mediated reaction.[57] Specifically, it has been suggested that a Th2 immune response is responsible for the disease; activated T cells produce large amounts of interleukin-4 (IL-4), which stimulates B cells to produce large amounts of immunoglobulin A. IgA then infiltrates into the meningeal vessels (mainly in the cervical area) causing vasculitis and meningitis.[48] The increased concentration of immunoglobulin A in blood and cerebrospinal fluid,[57, 60] remission of clinical signs after the administration of immunosuppressive doses of steroids[35] and the absence of identifiable infectious organisms[23, 42, 60] lend support to this hypothesis.

Moreover, it has also been suggested that repeated vaccinations against various pathogens may cause the disease by sensitising the dog to those antigens. This may account for the increased incidence of the disease in young animals.[30]

Steroid-responsive meningitis-arteritis may sometimes occur in combination with immune-mediated polyarthritis, identified as 'polyarthritis-meningitis syndrome'.

Clinical presentation

Steroid responsive meningitis-arteritis usually affects

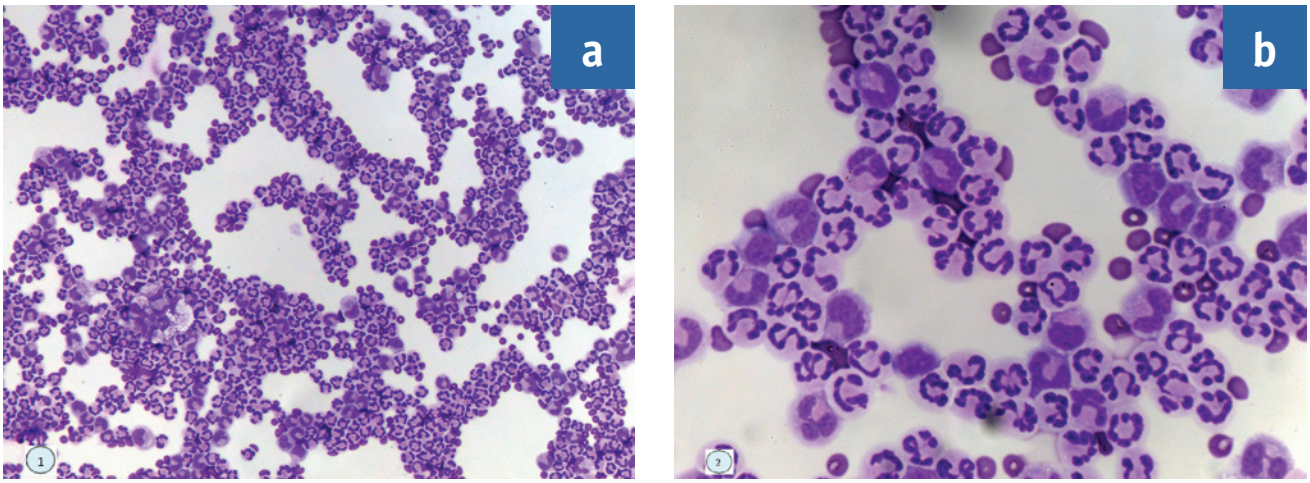

Fig. 4. Smear of cerebrospinal fluid sample from a dog with steroid responsive meningitis-arteritis, with predominance of neutrophils, of which a large number has with hyper-segmented nuclei, and a smaller number of macrophages (modified Wright-Giemsa, (a) ×20 objective, (b) ×40 objective).

medium to large breeds of dogs. Specific breeds with an increased risk for developing the disease are Beagles, Boxers, Weimaraners, Bernese Mountain Dogs and Nova Scotia Duck-Tolling Retrievers. The disease occurs mainly in dogs younger than 2 years of age, although it has been reported in dogs as old as 7 years.[12, 60]

Webb et al.[65] described that ~30% of dogs with immune-mediated polyarthritis had associated spinal pain; subsequently 50% of those were diagnosed with steroid responsive meningitis-arteritis. It is thought that dogs with 'polyarthritis-meningitis syndrome' experience spinal pain possibly due to both meningeal and intervertebral joint inflammation.

Clinical signs

Dogs present with cervical spinal hyperaesthesia (in over 90% of affected dogs), most commonly displayed as a low head carriage and an arched back.[60] Other clinical signs that may be present from time to time include a reluctance to move, a stiff gait, pain on opening the mouth, muscle rigidity and anorexia. Cervical spinal pain may be coupled by thoracolumbar pain, although, in some cases, only the latter is present.[30] Pyrexia and neutrophilic leucocytosis with a left shift occurs in approximately two-thirds of affected dogs.[12] The dog may present with clinical signs of an acute onset and progressive nature (acute form) or with a waxing and waning course over a period of weeks or months (chronic form).[30] Dogs that develop the acute form and remain untreated may self-limit the clinical signs within 12 to 18 months[39] or develop resistance to the disease with ageing.[49] Alternatively, they may develop the chronic form where, in addition to the meningitis, myelitis or encephalitis are also present.[60, 67] Therefore, in

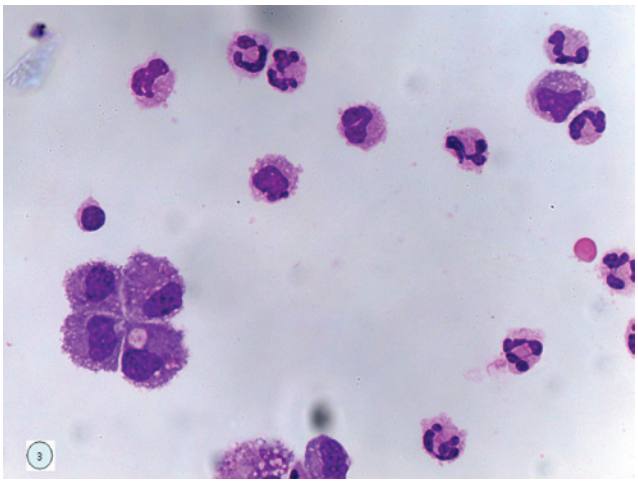

Fig. 5. Smear of cerebrospinal fluid sample from a dog with chronic form of steroid responsive meningitis-arteritis, with predominance of neutrophils and scarce macrophages. (modified Wright-Giemsa, ×60 objective).

the chronic form, neurological signs (e.g. ataxia, paresis) may occur.[58] Lastly, it has been reported that inflammation may spread from the meninges to the cerebral hemispheres, which would potentially be fatal for the animal.[67]

Diagnosis

Diagnosis is based on history, clinical presentation, findings on clinical examination, cerebrospinal fluid analysis and supported by haematological and biochemical findings. Cerebrospinal fluid analysis is characterised by an increased total nucleated cell count (reference range: <5-8 leucocytes/µl for cerebellomedullary cistern collection).[3,18] A predominance of neutrophils in the absence of bacteria is recorded in animals with the acute form of the disease (Fig. 4). However, as the disease progresses, a mixed pleocytosis is present with macrophages, lymphocytes and monocytes (Fig. 5). In association with this inflammatory response, an increase in the cerebrospinal fluid total protein

concentration can also be expected (reference range: <250 mg/l for cerebellomedullary cistern collection).[3,58] The cerebrospinal fluid total protein concentration in the chronic form may be within normal limits or slightly elevated.[58] Cerebrospinal fluid changes appear sensitive to immunosuppressive doses of steroid administration and will be suppressed if the patient has received treatment prior to cerebrospinal fluid sampling, although further investigation would be required to evaluate whether any particular cell lines are affected preferentially.[32]

Haematological findings may demonstrate evidence of a leucocytosis with left shift.[25] Serum biochemistry results may reveal a mild hypoalbuminaemia due to the inflammatory reaction, as albumin is a negative acute phase protein.[9] Hyperglobulinaemia has also been reported; increased concentrations of IgA are likely to be responsible for this.[60,62]

In both the acute and chronic form of the disease, IgA concentrations are increased in blood and cerebrospinal fluid. Though the increase in IgA in the cerebrospinal fluid is a common feature of many central nervous system diseases, the significant increase in serum concentrations is more characteristic, as in other central nervous system diseases serum IgA concentrations may be normal or only slightly increased.[60,62] Therefore, serum IgA measurements can be useful in the differentiation of steroid-responsive meningitis-arteritis from other neurological disorders. This can be especially helpful in the chronic form, which is not easily distinguished in terms of clinical presentation from other similar diseases. The specificity of this test varies from 88% [60] to 100%.[62] As part of the differential diagnosis process, one should be aware that increased serum IgA may also be present in animals with lymphoma, myeloma or histiocytosis.[59]

Evaluation of acute phase proteins can also be useful for diagnosing the disease. Acute phase proteins (e.g. C-reactive protein) blood concentrations are increased, as in other inflammatory diseases, and may lead to the early diagnosis of steroid-responsive meningitis-arteritis.[33] Finally, computed-tomography imaging may help localise changes in the central nervous system and help when monitoring the efficacy of treatment.[58]

Differential diagnosis

Spinal pain may be caused by any condition affecting muscles, vertebrae, facetal joints, nerve roots and meninges. The most common causes of spinal pain in a young adult dog include discospondylitis, cervical instability (cervical spondylopathy, atlanto-axial subluxation), intervertebral disk extrusion or protrusion pinching nerve root or meninges, trauma (fracture) and occasionally bacterial meningitis and vertebral or meningeal neoplasia.

A neutral lateral cervical radiograph should always be considered in any dog presenting with cervical pain, before manipulation of the atlantoaxial joint is performed due to the possibility of instability within this region. Preferably, radiographs should be taken with the animal in a conscious state, if such instability is suspected. Radiography may also reveal evidence of disconspondylitis, i.e. radiopaque irregular proliferative lesions located at the vertebral end plates (sclerosis) with associated lysis or evidence of disc disease. In case the initial radiographic evaluation does not reveal any abnormality, then specific diagnostic imaging procedures can be performed (myelography, magnetic resonance imaging).

Once these procedures have been performed to rule out instability, then it is safe to proceed with collecting a cerebrospinal fluid sample from the cerebromedullary cistern. Neutrophilic or mixed pleocytosis in the cerebrospinal fluid can be caused by a variety of conditions, such as protozoal and bacterial diseases. Therefore, in the presence of inflammatory cerebrospinal fluid disease, other causes should be excluded by appropriate testing, for example cerebrospinal fluid PCR testing for neosporosis. The differentiation of steroid-responsive meningitis-arteritis from bacterial meningitis is interesting; the latter is uncommon in dogs and can be diagnosed by culture of cerebrospinal fluid samples and the identification of microorganisms in direct observation of smears prepared from the sample.[35,43,57] Cerebrospinal fluid bacterial culture has a low sensitivity; thus, it may be vital to perform blood cultures which are more sensitive.[43] Other potential causes of inflammatory brain disease include granulomatous MEM and NE. These diseases can be confused with the chronic form of steroid-responsive meningitis-arteritis, when neurological deficits are present. However, these diseases cause brain dysfunction in addition to myelopathy, whereas in the chronic form of steroid-responsive meningitis-arteritis the spinal syndrome often occurs with no brain involvement. In such cases with brain involvement, the information from the history (i.e., progression from acute steroid-responsive meningitis-

arteritis) may be helpful in differentiation. Advanced imaging studies are recommended to evaluate brain involvement in the disease.

Prognosis

The prognosis is guarded to good. In acute forms and with appropriate treatment, the prognosis is excellent. In contrast, in the chronic form with symptoms of myelitis and/or encephalitis, the prognosis is guarded and fatality ranges from 5% to 100%.[11, 60]

Therapeutic approach

Management of granulomatous meningoencephalomyelitis and necrotising encephalitis

The cornerstone of management of granulomatous MEM and NE is immunosuppressive treatment, mainly with corticosteroids. Depending on the severity of signs and whether or not there is a clinical suspicion of infectious diseases, the clinician may initially administer anti-inflammatory dosages of steroids (prednisolone at 0.5-1.0 mg/kg, per os or intravenously, once daily) and wait for the results of serological examination and PCR. If the results do not provide evidence for an infectious disease or if index of suspicion for MEM of unknown aetiology is very high from the beginning (e.g. dog of Pug breed with magnetic resonance imaging lesions consistent with necrotising meningo-encephalitis), then the clinician can start the immunosuppressive therapy directly and gradually reduce the dose to the minimum that adequately controls the disease.[46] A proposed prednisolone protocol[46] which can be followed is as below:

- 1.5 mg/kg, per os or intravenously, twice a day for 3 weeks,
- 1.0 mg/kg, per os or intravenously, twice a day for 6 weeks,
- 0.5 mg/kg, per os or intravenously, twice a day for 3 weeks,
- 0.5 mg/kg, per os or intravenously, once a day for 3 weeks,
- 0.5 mg/kg, per os or intravenously, every two days indefinitely; the dose may be reduced to 0.25 mg/kg, per os or intravenously, every two days at a later stage.

Response to corticosteroids is variable and may be temporary, although dogs often show a good initial response to steroid monotherapy. A median survival time of 36 days (range: 2-1200 days) after corticosteroid treatment in 26 dogs with MEM of unknown aetiology has been reported.[21] Steroid monotherapy may adequately control the clinical signs, but it has been proved to be insufficient for some patients. In addition, long-term, high dose corticosteroid therapy may cause side-effects, including polyuria/polydipsia, polyphagia, weight gain, hepatotoxicity, gastrointestinal ulceration, pancreatitis and iatrogenic hyperadrenocorticism. For the above reasons, it is essential either to combine steroids with one or more immunomodulatory drugs or less often to use the latter as a monotherapy. Cytosine arabinoside, procarbazine, cyclosporine, lomustine, leflunomide and mycophenolate mofetil, all have been reported as additional and effective therapies. However, more clinical trials are needed to confirm their efficacy in controlling MEM of unknown aetiology.[46]

It is also advisable to use gastroprotectants in combination with the steroid therapy. Therefore, dogs should concurrently receive sucralfate (1-2 g per animal, per os, three times a day), ranitidine (2 mg/kg, per os, twice a day) or famotidine (0.5-1 mg/kg, per os, once a day).[31]

Management of steroid responsive meningitis-arteritis

Initially, immunosuppressive doses of steroids (prednisolone) can be used, these being gradually reduced within a period of months to the minimum dose that can control the disease. Approximately 50% of dogs with steroid responsive meningitis-arteritis relapse after discontinuation of treatment.[30] Furthermore, in dogs not treated appropriately, the chronic form of the disease develops. [58] For this reason, it is important that treatment should last at least six months, in order to reduce possibility of disease relapse.[30]

Another treatment protocol can be based on administration of prednisolone (4 mg/kg, per os or intravenously, once a day) initially, followed after 2 days by a reduction in the dose to 2 mg/kg for one to two weeks, followed by a further reduction to 1 mg/kg for another 2 weeks. At that point and then every 4 to 6 weeks, the dog is re-examined clinically and an evaluation of cerebrospinal fluid and a haematological examination are performed. When clinical and laboratory findings are normal, the dose can be gradually reduced to half that of the preceding regime, until a dose rate of 0.5 mg/kg per os every 48 or 72 hours is attained. Treatment is stopped 6 months after clinical and laboratory findings are normal. If the neurologic examination reveals abnormalities in the presence of

normal cerebrospinal fluid examination results, then it would be advisable to increase the dose of prednisolone or to combine it with another immunomodulatory drug.[11,60] A good combination medication would be azathioprine (1.5 mg/kg, per os, every 48 hours) which may be used in combination with steroids (e.g. alternating each drug every other day).[58]

If the diagnosis is uncertain and there is suspicion of infectious (possibly bacterial) meningitis, then an antibiotic which can penetrate the blood-brain barrier should be administered. Such antibiotics are clindamycin (12.5-25 mg/kg, per os, twice a day) and trimethoprim/sulphonamides. The use of antibiotics should be stopped when the cerebrospinal fluid culture yields no more microorganisms.[19]

During treatment, IgA concentrations in blood and cerebrospinal fluid remain increased despite regression of clinical signs.[58] In addition to this, measurement of acute phase protein concentration in the blood is useful for evaluation of the response to treatment. During treatment, almost all the concentrations of acute phase proteins decrease significantly compared to their initial values. In contrast, acute phase protein concentrations in cerebrospinal fluid are less reliable markers for evaluation of the response of steroid responsive meningitis-arteritis to the treatment.[33]

Overview of the main characteristics of meningoencephalomyelitis of unknown aetiology.

	Breed(s)	Age	Neuro- localization	Treatment	Prognosis
Granulomatous meningoencephalomyelitis					
	Any breed Terrier and Toy breeds	Usually young to middle age dogs	Multifocal (multiple lesions in brain and cervical spinal cord) Focal (one lesion in brain or spinal cord) Ocular form (Optic nerves and chiasm)	Immunosuppressive therapy	Poor (if no aggressive therapy)
Necrotising meningoencephalitis					
	Breed-specific Mainly Pugs and Maltese Terriers	Usually young dogs	Brain	Immunosuppressive therapy	Guarded to poor
Necrotising leucoencephalitis					
	Breed-specific Mainly Y. Terrier and French Bulldogs	Usually young dogs	Brain	Immunosuppressive therapy	Guarded to poor
Steroid-responsive meningitis-arteritis					
	Medium-large breed dogs Beagles, Boxers, Weimaraners, Bernese Mountain dogs	Young dogs (usually < 2 years)	Meninges of cervical spine (acute form) Spinal cord, brain (chronic form)	Immunosuppressive therapy	Good (acute form) to guarded (chronic form)

Conclusion

Meningo-encephalitis of unknown aetiology includes diseases of the central nervous system, with an immune-mediated background and distinct neuropathological findings. Treatment is based on the administration of immunosuppressive drugs. In addition to prednisolone, other immunosuppressive drugs appear to have a beneficial effect in the management of the diseases, improving their overall prognosis when used in conjunction with prednisolone. In the future, effective treatments will possibly be established after elucidation of the pathogenesis of these diseases.

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REPRINT PAPER*

Spindle toxins as chemotherapeutic agents in veterinary medicine

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SUMMARY

Spindle toxins belong to the most potent agents in the fight against cancer as their effect is based on direct interference with the cell cycle. Their main targets are the microtubules, which are essential for many physiological processes in the body. Microtubule-destabilising agents, such as vinca alkaloids, and microtubule-stabilising agents, such as taxanes and epothilones induce an arrest of the cell cycle at the transition to mitosis. This leads to apoptosis of the affected cell. In veterinary medicinee, vinca alkaloids (for example, vincristine) are used alone or in combination with other chemotherapeutic agents, and constitute part of the standard treatment of certain types of tumours. Members of the taxane family have only been used in a small number of trials due to the need for premedication in order to avoid hypersensitivity reactions. A new group called epothilones has not been in clinical use in dogs so far. As epothilones possess certain advantages compared to taxanes, these drugs are expected to play an important role in veterinary and human medicinee in the future. Water-soluble members of the epothilones, for example, do not cause hypersensitivity reactions; also they show activity even in chemo-resistant tumours and seem to cause less toxicity in human patients than taxanes. The aims of this article are to describe the mechanism of action of the spindle toxins, to introduce the different groups of drugs and to explain their importance for veterinary medicinee.

Key words: oncology, chemotherapy, taxanes, vinca alkaloids, epothilones

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Introduction

Microtubules play an important role in many different processes in the body of human beings and animals, especially during cell division (mitosis). Inhibition of mitosis leads to an interruption of the regular cell cycle and occasionally to cell death through apoptosis. Chemotherapeutic agents that impair the mechanism of action of microtubules are therefore among the most important substances in anticancer therapy (Jordan and Wilson, 2004). There are two groups of chemotherapeutic agents which exert an influence on microtubules: microtubule-destabilising and microtubule-stabilising agents. The former include vinca alkaloids, while taxanes and epothilones are among the latter. Vinca alkaloids are widely used in the anticancer treatment of humans, dogs and cats (Fischer et al., 1993; Myers et al., 1997; Milner et al., 2005). While the taxanes are employed with success among

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humans (Lee and Harris, 2008) and the epothilones are among the new, promising substances (Larkin and Kaye, 2007), in dogs, the taxanes have only been utilised in on a limited basis due to hypersensitivity reactions (Poirier et al., 2004b; Simon et al., 2006; Shiu et al., 2011). The epothilones, a relatively new group of microtubule-stabilising agents, have not been clinically applied in veterinary medicinee up to now. A study on the use among dogs with spontaneously occurring tumours is currently being conducted by the authors.

The aims of this article are to give an overview of the importance, mechanism of action and indication of this chemotherapeutic substance group, to introduce individual representatives and to examine applications of spindle toxins in veterinary medicinee.

Microtubules in the physiological context

Microtubules are found in all eukaryotic cells and are responsible for various mechanism of actions of a cell such as the formation of the spindle apparatus during cell division, the maintenance of cell shape, the intracellular transport of organelles and vesicles, for active cell movements and cell migration. These processes are enabled through exact regulation, which is controlled through microtubule-associated proteins, expression of tubulin isotypes and various post-translational modifications of tubulin (Nogales, 2001; Jordan and Wilson, 2004).

Microtubules consist of α- and β-tubulin heterodimers which are linked to form protofilaments.

Several of these protofilaments form the wall of a tubular microtubule. The plus end of the microtubule consists of β-tubulin, the minus end of α-tubulin. Two processes play an important physiological role in the dynamic mechanism of action of microtubules: treadmilling and dynamic instability. Treadmilling describes a process which maintains the microtubule in a state of dynamic equilibrium; a net flow of tubulin subunits from the plus end to the minus end takes place, without a change of the length of the microtubule. Dynamic instability is a term to describe the lability of these structures enabling them to erratically switch from a growth phase into a contraction phase (Nogales, 2001), as shown in Figure 1.

These dynamic processes of microtubules are essential in many processes in the body, particularly during different phases of mitosis. The dynamics are of great importance during prometaphase for the attachment of the spindle to the chromosome's kinetochore. The microtubules are able to grow to a great length but are also capable of quick shortening; they are therefore able to "search" the cytoplasm for chromosomes to which they can attach themselves. During metaphase the microtubules are responsible for the arrangement of chromosomes in the equatorial plane and subsequently for their synchronous separation during anaphase and telophase (Jordan and Wilson, 2004).

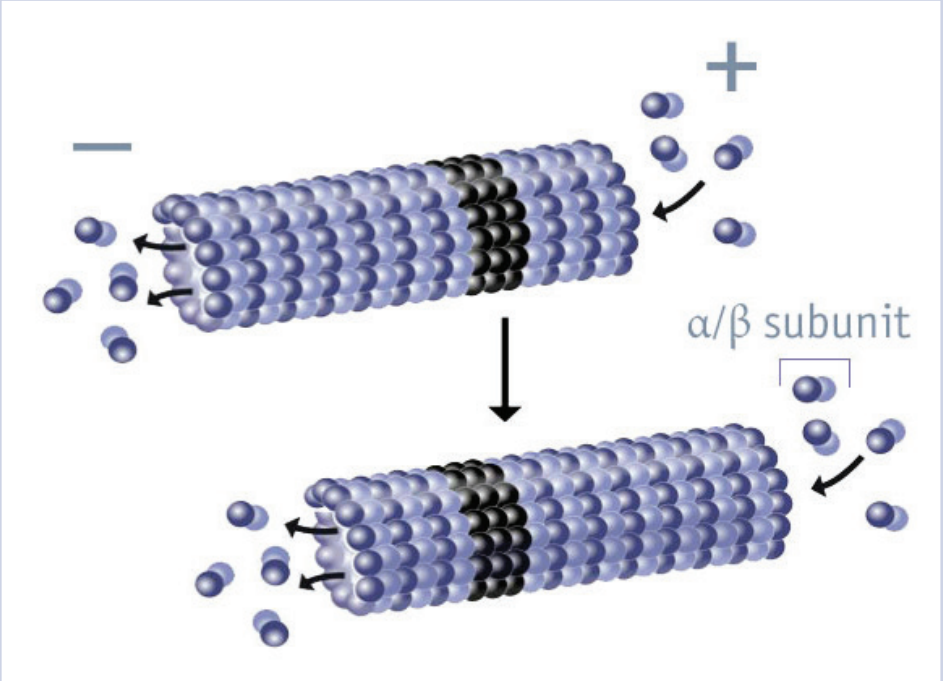

Figure 1: Microtubules consist of α and β tubulin subunits and are responsible for many different processes in the body, particularly for the construction of the spindle apparatus during cell division. The occurrence of different dynamic processes within a microtubule is essential for its functionality. The microtubule's capability for rapid changes in length with erratic prolongation and contraction is called dynamic instability.

Mechanism of action of spindle toxins

Spindle toxins disturb the balance of the dynamic processes of microtubules by either excessively stabilising the microtubules, i.e. promoting microtubule polymerisation, or by destabilising them, which happens through the prevention of polymerisation or an increased depolymerisation of microtubules (Honore et al., 2005). An interruption of the cell cycle occurs if the dynamic processes of microtubules are impaired during mitosis. Even a single chromosome which cannot be drawn to the opposite cell pole due to an incompletely functioning spindle apparatus is enough to prevent the transition into the anaphase. Stopping the cell cycle leads to apoptotic death of the affected cells, which corresponds to the direct cytotoxic effect of spindle toxins (Jordan et al., 1996).

Tumour cells divide frequently and are therefore quite sensitive to the antimitotic effect of spindle toxins (Jordan and Wilson, 2004). Another mechanism of action of spindle toxins is the antimetastatic effect, because the dynamic processes of microtubules are also of great importance for cell migration (Honore et al., 2005). Spindle toxins can also have an antivascular effect by impairing pre-existing blood vessels through inhibition of immature and rapidly dividing endothelial cells (Ferretti et al., 2005). The antiangiogenic effect of spindle toxins is based on the inhibition of proliferation and migration of endothelial cells as well as the inhibition of proangiogenic factors. The new formation of a tumour's blood vessels (angiogenesis) is prevented as a result (Bocci et al., 2002; Woltering et al., 2003; Pasquier et al., 2005; Rohrer Bley et al., 2011). Although spindle toxins consist of chemically very different substances and their effect on tubulin and microtubules are different, the underlying antineoplastic mechanisms of action of the vinca alkaloids, the taxanes and the epothilones are similar. The attack points of spindle toxins are binding sites at different locations of microtubules: vinca alkaloids bind to the vinca domain, taxanes and epothilones bind to the taxol domain, and various other binding sites are known for other substances (Dumontet and Jordan, 2010).

When used in high concentrations, the vinca alkaloids destabilise the microtubules by promoting depolymerisation and thus directly destroying the mitotic spindle apparatus (Jordan and Wilson, 2004). In low, but already clinically relevant dosages the vinca alkaloids are not able to depolymerise microtubules, but they inhibit the dynamic processes of regular cell division and lead to cell apoptosis (Jordan, 2002; Jordan and Wilson, 2004).

The mechanism of action of the taxanes is based on the induction of tubulin polymer formation, which is why they belong to the group of microtubule-stabilising chemotherapeutic agents. This blockage of spindle dynamics at the G2/M transition of the cell cycle (e.g. shortly before mitosis) leads to apoptosis of the cells and is responsible for their diverse antitumour activity (Schiff et al., 1979; Parness and Horwitz, 1981; Jordan, 2002). The epothilones also belong to the microtubule-stabilising agents and have a mechanism of action similar to the taxanes, even though they differ in their chemical structure. The stabilisation of microtubules by the epothilones leads to interruption of the cell cycle at the G2/M transition and ultimately to apoptosis of proliferating cells (Bollag et al., 1995; Wartmann and Altmann, 2002).

Application in veterinary medicinee

Vinca alkaloids

Vincristine

In veterinary oncology, vincristine is either used as a single substance or as part of a multiagent therapy protocol in a dosage of 0.5-0.75 mg/m^2 (Chun et al., 2007). Like most chemotherapeutic agents, vincristine should be administered strictly intravenously, because otherwise it can lead to severe tissue reactions leading to extensive skin necrosis (Chun et al., 2007). Vincristine is regarded as treatment of choice with canine transmissible venereal tumours. The response rate is high with 90-95% achieving a complete remission, which is usually of long-lasting duration, after only two to six doses (Amber et al., 1990; Nak et al., 2005). Vincristine is part of the most important multiagent therapy protocols (CHOP-based) in the treatment of canine and feline malignant lymphoma (Myers et al., 1997; Milner et al., 2005). CHOP-based protocols include the chemotherapeutic agents cyclophosphamide, doxorubicin, vincristine and prednisolone, and are regarded as the therapy of choice for most forms of canine malignant lymphoma.

Vinblastine

Vinblastine is primarily used in the treatment of aggressive mast cell tumours in dogs. This includes grade II tumours with a high mitotic index (> 5/ high power field) which were incompletely excised, appear at so-

called "high-risk locations" (e.g. mucocutaneous) or have already metastasised, as well as mast cell tumours of a high histological grade (grade III). The prognosis of these aggressive stages without additional chemotherapy is guarded to poor with a median survival time of four and a half to nine months (Bostock, 1973; Patnaik et al., 1984; Simoes et al., 1994; Murphy et al., 2004). If vinblastine was administered intravenously in a dosage of 2 mg/m^2, median tumour-free periods of over 17 months and median survival times of almost 46 months were achieved in dogs with surgically excised grade III tumours (Thamm et al., 2006).

Vinorelbine

The third representative of the vinca alkaloids, vinorelbine, was first used among dogs with various tumours, with neutropenia as dose-limiting toxicity (Poirier et al., 2004a). In a phase II study, vinorelbine was administered intravenously in a dosage of 15 mg/m^2 to dogs with cutaneous mast cell tumours, but achieved a non-satisfactory response rate of 13%, which was furthermore associated with other adverse effects (neutropenia, gastrointestinal toxicity) (Grant et al., 2008).

Peripheral neuropathy is described as a specific adverse effect of therapy with spindle toxins in humans. Severe mixed sensory and motor neuropathies are particularly pronounced among first-generation vinca alkaloids (e.g. vincristine); newer vinca alkaloids most often lead to mild sensory impairment (Carlson and Ocean, 2011). Sensory and partial motor neuropathies also occur with the taxanes and the epothilones (Lee and Swain, 2006; Carlson and Ocean, 2011). The underlying mechanisms of peripheral neuropathy have not yet been conclusively clarified. Impairment of neuronal microtubules leads to restricted axonal transport resulting in paraesthesias, dysaesthesias, numbness and pain in hands and feet (sensory neuropathy) as well as muscle weakness and decreased fine motor skills (motor neuropathy) (Lee and Swain, 2006; Argyriou et al., 2011). Animals under therapy with vinca alkaloids occasionally lick their paws excessively and show peripheral motor deficits, which is regarded as an indication of peripheral neuropathy (Chun et al., 2007). Constipation has been described as an adverse effect among cats (Chun et al., 2007). Neurotoxicity is usually observed among humans only after long-term administration (cumulative toxicity) and is reversible in most cases (Quasthoff and Hartung, 2002; Lee and Swain, 2006; Argyriou et al., 2011).

Taxanes

One of the first clinical applications of taxanes among canine patients occurred in 1999 in the form of a study with various primary and metastatic pulmonary tumours, in which paclitaxel or doxorubicin was administered by inhalation. Although no adverse effects were observed, a response could only be observed in 13.3% of cases treated with paclitaxel. Moreover, this form of therapy by means of inhalation is difficult to implement and fraught with high occupational safety-related risks for administering personnel (Hershey et al., 1999). A retrospective study from 2004 examined the application of paclitaxel among 25 dogs with various types of tumours. Despite premedication with corticosteroids as well as H1- and H2- antagonists, mild to moderate hypersensitivity reactions occurred in 64% of the dogs, and in some cases the animals had to be premedicated a second time. Moderately to severe adverse effects (grade III or IV neutropenia [24%] or thrombocytopenia [8%]; three dogs died from sepsis) appeared in almost half of the patients. The recommended dose for further studies was 132 mg/m^2. Only partial remissions and short response duration were observed in this study (Poirier et al., 2004b).

Another study in dogs with invasive mammary tumours compared surgical excision alone, with postoperative administration of doxorubicin or with docetaxel. No significant difference with regard to the recurrence-free period and survival could be ascertained between the three therapeutic options. Moreover, despite premedication among the majority of dogs, mild to moderate allergic skin reactions were observed after intravenous administration of 30 mg/m^2 of docetaxel (Simon et al., 2006).

Docetaxel was also employed in combination with cyclosporine A (per os) among dogs with various types of tumours in the course of a phase I study. The oral bioavailability of docetaxel alone is low, because it is a substrate of the P-glycoprotein pump on the luminal side of the enterocytes and is metabolised through the activity of the P450 isoenzyme CYP3A. Both processes drastically reduce the absorption of the chemotherapeutic agent. The combined administration of docetaxel and the P-glycoprotein/CYP3A modulator cyclosporine A clearly increased the bioavailability in dogs and did not lead to hypersensitivity reactions, but the chemotherapy was administered by means of a gastric tube (McEntee et al.,

2006b). A study with the same design was conducted among cats with various types of tumours. The dose-limiting toxicity was of gastrointestinal nature; acute allergic reactions were not observed. After two doses at an interval of three weeks, partial remission was achieved in one of 18 cats with adenocarcinoma on the paw pads; stable disease was achieved in two cats, one with a metastasised mast cell tumour and one with a sublingual squamous cell carcinoma (McEntee et al., 2006).

The intravenous administration of docetaxel to 21 cats with various tumours was tested in a phase I study in mid-2011. Fever, neutropenia and vomiting were described as the dose-limiting toxicity; the maximum tolerated dose was 2.25 mg/kg. Hypersensitivity reactions occurred only rarely (in four cats), but were of moderate intensity despite premedication (Shiu et al., 2010).

Since hypersensitivity reactions are triggered by the solvent Cremophor EL®, a new, water-soluble form of paclitaxel named Paccal® Vet (Oasmia Pharmaceutical AB, Sweden) was introduced in a press release in 2009. Preliminary results of a phase I/II study among 32 dogs with advanced tumour disease revealed a promising response rate of 74%. Adverse effects were mild, apart from dose-limiting neutropenia. Even without premedication, only one dog showed a slight hypersensitivity reaction. A phase III study on inoperable Grade II/III mast cell tumours also achieved a good response rate of 70% with a median progression-free period of 235 days (von Euler, 2009). In June of 2011, Oasmia Pharmaceutical obtained a restricted authorisation (minor use designation) from the American Food and Drug Administration (FDA) for Paccal® Vet for use in operable and inoperable canine squamous cell carcinomas.

Epothilones

The epothilones are used in various phase I to phase III studies in human patients. Ixabepilone (IxempraTM, Bristol-Meyers Squibb Company, USA) – a representative of the epothilones – has already been approved for treatment of metastasised breast cancer (Gradishar, 2009). In dogs, a prospective dose-finding study (phase I/II) by the authors with patupilone – a natural epothilone B – in dogs with various tumours is in progress. The substance is well-tolerated by dogs. However, results concerning the clinical efficacy are still pending. As shown in Figure 2, canine malignant lymphoma and haemangiosarcoma cell lines showed high in vitro sensitivity already to low dosages of patupilone.

Patupilone is a promising substance due to several advantages. In vitro experiments have shown that the binding to the β-tubulin subunit is stronger than with taxanes and other epothilones. In addition, it stabilises

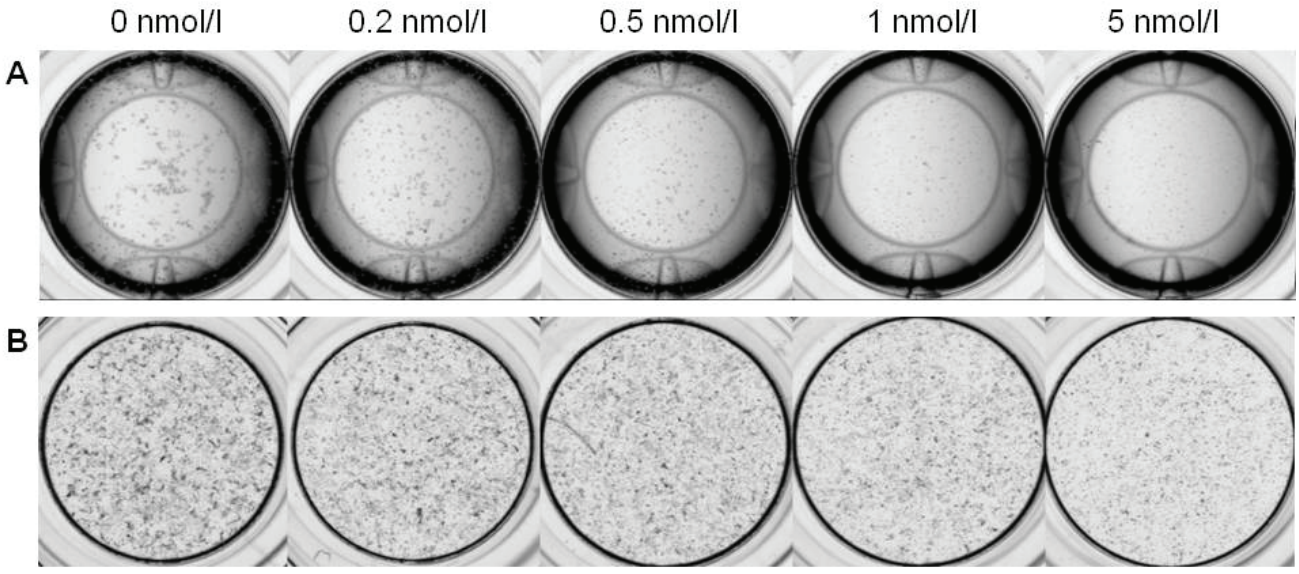

Figure 2: Canine lymphoma (A) and haemangiosarcoma cell lines (B) were treated with increasing dosages of patupilone (0-5 nmol/l). The metabolic activity of cells was measured 24, 48 and 72 hours after treatment by means of AlamarBlue® assay. This depicts a measurement for the proliferation of cells. The concentration by which the proliferation was inhibited by 50% – the so-called "50% inhibitory concentration" – was low with 0.2 nmol/l in the lymphoma cells and 1 nmol/l in the haemangiosarcoma cells (previously unpublished data from the authors). These concentrations are very low in comparison with described patupilone-sensitive human cell lines (Bley et al., 2009).

microtubules better than paclitaxel and seems to be able to competitively inhibit the binding of paclitaxel (Paik et al., 2010). Patupilone shows a greater efficacy in vitro, with a cytotoxicity that is threefold to 20-fold more potent than paclitaxel, and also shows an effect in paclitaxel-resistant cell lines (Bollag et al., 1995). Moreover, patupilone is significantly less sensitive to the formation of resistance towards chemotherapy induced by p-glycoprotein overexpression. P-glycoprotein overexpression only leads to a slight reduction of the effect of patupilone (fourfold to twelvefold), whereas with taxanes it leads to a 20,000-fold reduction of effect (Bollag et al., 1995). Patupilone is a close relative of ixabepilone, which has already been approved in human medicinee, and only differs from ixabepilone in its chemical structure through an oxygen atom instead of a nitrogen atom. But in contrast to ixabepilone it is water-soluble and therefore not dependent on solvents which can lead to hypersensitivity reactions (Rubin et al., 2005). In contrast to ixabepilone or the taxanes, patupilone has the ability to cross the blood-brain barrier. The effective level in tumour tissue and in brains of mice and rats treated with patupilone is consistently high for one week, whereas the plasma concentration rapidly decreases (O'Reilly et al., 2008). Various studies have shown that patupilone not only has a direct cytotoxic effect on tumour cells, but also an antivascular (Ferretti et al., 2005) and antiangiogenic effect (Bocci et al., 2002; Bley et al., 2009; Rohrer Bley et al., 2011). Because the cells treated with patupilone are blocked at the G2/M transition, i.e. at the time point during the cell cycle in which they react most sensitively to ionising radiation, a promising application of this chemotherapeutic agent lies in combination with radiation therapy (Sinclair, 1968; Bollag et al., 1995; Pawlik and Keyomarsi, 2004). In vitro and in vivo studies show a significant difference between irradiation alone and irradiation combined with patupilone. An additive to supra-additive effect on tumour growth with the combined treatment was seen in mice with human xenograft tumours from non-small cell lung cancer and colon adenocarcinoma (Hofstetter et al., 2005; Bley et al., 2009).

Conclusions

Chemotherapeutic agents which exert an influence on the microtubules and consequently impair cell division are among the strongest substances in the treatment of cancer. Since the dynamics of microtubules are essential for cell division, microtubule-stabilising as well as microtubule-destabilising agents show an anti-tumour effect. Representatives of the vinca alkaloids and the taxanes are approved for the therapy of various types of cancer in human patients, but some types of tumours show formation of resistance to taxanes. The epothilones are a new, promising group of spindle toxins. They show fewer adverse effects in humans and some are water-soluble, do not need solvents and therefore no hypersensitivity reactions are seen. In addition, they also have an effect on tumours with chemotherapy resistance. One representative, ixabepilone, has already been approved for treatment of metastasised breast cancer in Switzerland and the United States (Gradishar, 2009; Goodin et al., 2004). The approval for ixabepilone is still pending in Germany, but phase III studies are in progress. The vinca alkaloids – first and foremost vincristine and vinblastine – are currently being used in veterinary medicinee. Some tumours such as canine malignant lymphoma show clinical chemoresistance after chemotherapy, particularly after the CHOP protocol, which contains vincristine. The administration of taxanes in dogs can lead to hypersensitivity reactions despite premedication (Poirier et al., 2004b). Therefore, new therapeutic options are desired, particularly with drugs that are well tolerated and not a substrate of the p-glycoprotein pump (Marconato, 2011).

Dogs with spontaneously occurring tumours are regarded as suitable models for anticancer treatment in humans. Consequently, the application of new chemotherapeutic agents in veterinary medicinee is not only important for treatment optimisation in animal patients, but also for a more profound understanding of efficacy in human medicinee (Vail and MacEwen, 2000). For instance, canine malignant lymphoma, a tumour that is most frequently treated by means of chemotherapy in veterinary oncology, is comparable in many areas with non- Hodgkin's like lymphoma in humans with respect to histopathology, tumour genetics and tumour response to chemotherapy (Fournel-Fleury et al., 1997; Breen and Modiano, 2008). In the future, the administration of more tolerable (Paccal® Vet) or new representatives (epothilones) of the spindle toxins will need further evaluation for clinical use. The epothilones are currently being used in human patients with highly malignant tumours and with advanced stage disease, e.g. with metastasised mammary tumours (Gradishar, 2009) or glioblastoma multiforme (Fogh et al., 2010), and also seem to have high potential for use in veterinary medicinee.

Declaration

The authors hereby declare that they do not have any protected, financial, professional or other personal interests which could influence contents or opinions presented in the manuscript.

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