Callisto
Recommendations for the control of zoonoses

Haemostasis
Laboratory evaluation and interpretation

Cytology
Bone marrow sampling and interpretation

Curettage & diathermy in cats with SCC

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Gastrointestinal toxicity after vincristine or cyclophosphamide, portable glucose meters, veterinary app & book reviews, FECAVA news ... and more
Contents

The CALLISTO project approach to prioritising efforts on the control of pet-related zoonotic diseases  4 - 7  
Gad Baneth

Curettage and diathermy: a treatment for feline nasal planum actinic dysplasia and superficial squamous cell carcinoma  8 - 17  
Robyn H. Jarrett, Elizabeth J. Norman, Isobel R. Gibson and Paul Jarrett

Glucose measurement using portable blood glucose meters in dogs and cats  18 - 30  
Labrini V. Athanasiou, Constantina N. Tsokana and Manolis N. Saridomichelakis

Gastrointestinal toxicity after vincristine or cyclophosphamide administered with or without maropitant in dogs: a prospective randomised controlled study  31 - 41  
Sarah L. Mason, Iain A. Grant, James Elliott, Peter Cripps and Laura Blackwood

Laboratory evaluation and interpretation of haemostasis in small animals  42 - 56  
Reinhard Mischke

Practical bone marrow cytology in the dog and cat  57 - 71  
Mathios E. Mylonakis, Aristodimos Hatzis
Icons

Each scientific article is classified with one or more icons. These refer to the species (in green) of animal or the veterinary discipline (in blue) relevant for the article.

- Dogs
- Cats
- Dogs and Cats/Small animals
- Rabbits
- Less common pets

- Anaesthesia
- Bacterial Diseases
- Behaviour
- Cardiovascular
- Dental
- Dermatology
- Diagnostic imaging
- Digestive System
- Ear Nose Throat
- Genetics
- Internal Medicine
- Neurology
- Oncology
- Ophthalmology
- Orthopaedics
- Practice Management
- Surgery
- Urogenital
The CALLISTO project approach to prioritising efforts on the control of pet-related zoonotic diseases

Gad Baneth  

SUMMARY

The companion animal multisectorial interprofessional and interdisciplinary strategic think tank on zoonoses (CALLISTO) project studied infectious diseases transmitted between companion animals, man and food-producing animals and formed recommendations for the research and management of these diseases in Europe.

Prioritisation of diseases for which intervention should be considered was carried out by a new method based on questionnaires and scoring system adapted from a method developed for the World Organization for Animal Health (OIE). The CALLISTO prioritisation method was used to identify the most important pathogens and diseases based on their impact on public health and agriculture in Europe.

CALLISTO overviewed the role of companion animals as a source of infectious diseases for man and food-producing animals in Europe, identified knowledge gaps, and proposed targeted actions to bridge those gaps. The project’s final report included twenty-four recommendations divided into five main categories which ranged from suggesting education and communication activities to the development of new vaccines that protect against zoonotic pathogens.

Key words: Zoonoses; Dog; Cat; CALLISTO project; vector-borne diseases

Introduction

An increasing number of pet animals are kept in close interaction with humans. As companion animals share the same environment with people and can be carriers of microorganisms pathogenic for humans, there is a crucial need to understand the role of companion animals as sources of zoonotic infectious diseases [1,2,3]. Such understanding could promote the employment of better preventive measures to reduce the burden of zoonotic diseases in humans. CALLISTO (Companion Animal multisectorial interprofessional and interdisciplinary Strategic Think tank On zoonoses) is an EU Framework 7-funded project that investigated zoonotic infectious diseases transmitted between companion animals, man and food-producing animals and formed recommendations for the research and management of these diseases in Europe [4,5]. One of the main tasks that the CALLISTO project had was to prioritise the pet-associated zoonotic diseases for which intervention should be considered.

* This paper was presented at the FECAVA symposium on zoonosis on 7 November 2014. Eur J Comp An Pract, Summer 25(2); p xx-yy. Go to http://www.ejcap.org to see the online presentation of this paper.

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CALLISTO

CALLISTO was coordinated by the Federation of Veterinarians of Europe (FVE). Its mission was to provide an overview of the current situation with regard to the role of companion animals, as a source of infectious diseases for people and food animals, to identify knowledge and technology gaps for the most important zoonoses and to propose targeted actions to reduce the risk of zoonotic diseases transferred via companion animals. Stakeholders and the general public were informed of CALLISTO’s discussions to contribute to the uptake of the proposed actions and to promote risk-awareness in healthy human-animal relationships.

The project included seven expert advisory groups including groups on viral, bacterial and parasitic diseases, epidemiology, communication, sociology and welfare (table 1). The groups discussed and reviewed the role of companion animals as a source of infectious diseases for man and farm animals, including available information on disease incidence and geographical distribution in Europe; a list of priority diseases; risk assessment; and recommendations for targeted actions to be implemented in order to decrease the burden of pet-associated zoonoses and farm animal infectious diseases.

CALLISTO was organized in three main cycles of 12 months each. Cycle I focused primarily on the development of the overview of the current situation and crucial gaps in the existing knowledge. Cycle II linked the results of cycle I towards the formulation of action recommendations in cycle III by extending the activities of CALLISTO towards risk assessments. Cycle III concluded with translating the results of CALLISTO into priority areas for actions, including recommendations for intervention strategies, areas for further research, and priorities and recommendations for key target groups, messages and media educational and advocacy programs.

Infections transmitted by arthropod vectors comprise some of the most important pet-associated diseases that the CALLISTO project has studied, in addition to other contagious diseases transmitted by water, food or by other means, such as leptospirosis (Fig. 1). The main vector-borne diseases related to domestic animals reviewed by CALLISTO included bartonellosis caused by *Bartonella henselae* and transmitted between cats by fleas, leishmaniosis caused by *Leishmania infantum* and transmitted by sand flies (Fig. 2), Crimean-Congo haemorrhagic fever virus transmitted by ixodid ticks, and various additional infections.

<table>
<thead>
<tr>
<th>Group</th>
<th>Name</th>
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<tbody>
<tr>
<td>1</td>
<td>User community</td>
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<tr>
<td>2</td>
<td>Policy actions</td>
</tr>
<tr>
<td>3</td>
<td>Viral infections</td>
</tr>
<tr>
<td>4</td>
<td>Bacterial infections</td>
</tr>
<tr>
<td>5</td>
<td>Parasitic infections</td>
</tr>
<tr>
<td>6</td>
<td>Epidemiology and underlying factors</td>
</tr>
<tr>
<td>7</td>
<td>Sociology and welfare</td>
</tr>
</tbody>
</table>

Fig. 1 – Icterus in a dog with leptospirosis.

Fig. 2 – Exfoliative dermatitis in a dog with *Leishmania infantum* infection.
The CALLISTO project approach to prioritising efforts on the control of pet-related zoonotic diseases

**Methods and Results**

CALLISTO completed its 3rd cycle at the end of 2014 and its members and advisory groups have summarized and prioritized their recommendations for actions and interventions. A substantial part of these recommendations address the management and prevention of vector-borne diseases. The recommendations were published in a final report available on the CALLISTO website [4], as well as presented in a collection of scientific publications [5,6].

The prioritisation of diseases transmissible from companion animals to humans and farm animals was carried out by a new method developed by CALLISTO’s expert advisory group VI on epidemiology and underlying factors. This method was based on questionnaires and a scoring system adapted from a method developed for the World Organization for Animal Health (OIE) [6,7]. The CALLISTO prioritisation method was used to identify the 5 most important pathogens for each category: viral, bacterial/fungal and parasitic, in total 15 pathogens, based on their impact on public health and agriculture in Europe. The 15 selected ‘paradigmatic pathogens’ are shown in table 2.

**Table 2 – Pathogens selected by CALLISTO as causing the most important diseases transmitted from companion animals to humans and farm animals in Europe.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Pathogen name</th>
</tr>
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<tbody>
<tr>
<td>Viral</td>
<td>Crimean-Congo haemorrhagic fever virus</td>
</tr>
<tr>
<td></td>
<td>West-Nile virus</td>
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<tr>
<td></td>
<td>Foot and Mouth Disease virus (not zoonotic)</td>
</tr>
<tr>
<td></td>
<td>Rabies</td>
</tr>
<tr>
<td></td>
<td>Bluetongue virus (not zoonotic)</td>
</tr>
<tr>
<td>Bacterial</td>
<td>Campylobacter jejuni</td>
</tr>
<tr>
<td></td>
<td>Leptospira interrogans sensu lato</td>
</tr>
<tr>
<td></td>
<td>Salmonella enteritica</td>
</tr>
<tr>
<td></td>
<td>Bartonella henselae</td>
</tr>
<tr>
<td></td>
<td>ESBL1-producing organisms</td>
</tr>
<tr>
<td>Parasitic</td>
<td>Echinococcus granulosus sensu lato</td>
</tr>
<tr>
<td></td>
<td>Leishmania infantum</td>
</tr>
<tr>
<td></td>
<td>Toxoplasma gondii</td>
</tr>
<tr>
<td></td>
<td>Giardia species</td>
</tr>
<tr>
<td></td>
<td>Toxocara canis/cati</td>
</tr>
<tr>
<td>Other</td>
<td>Bite wound infections</td>
</tr>
</tbody>
</table>

1 ESBL: extended spectrum beta-lactamase

**Discussion**

The range of topics and situations which CALLISTO dealt with was very broad and included a large number of animal species considered as pets or companion animals, a wide geographical area and a variety of different pathogens and means of transmission. It considered both zoonotic diseases as well as non-zoonotic infections transmitted from pet animals to domestic farm animals with important economic impact. The viral diseases prioritized included three zoonoses of which two, Crimean-Congo haemorrhagic fever and West Nile virus, ranked highest. Rabies had a somewhat lower score because it is present mostly in sylvatic life cycles involving wild life with the fox and raccoon dog as main reservoir species, and the role of dogs and other companion animals is of considerably smaller importance in most of Europe [8]. Of the bacterial infections, all of the five highest ranking pathogens, *Campylobacter jejuni*, *Leptospira interrogans sensu lato* (sl), *Salmonella enteritica*, *B. henselae* and extended spectrum beta-lactamase (ESBL)-producing organisms, are zoonotic. In addition, bite wound infections involving a plethora of bacteria were also considered as an entity of great importance which was addressed separately by CALLISTO[9]. Of the prioritized parasitic pathogens, all five, *Echinococcus granulosus sl*, *L. infantum*, *Toxoplasma gondii*, *Giardia* spp. and *Toxocara canis / cati*, are zoonotic; the first four also cause disease in farm animals. *Echinococcus multilocularis* which causes alveolar echinococcosis in humans was discussed in depth by the parasitology expert advisory group, but was not included in the list, despite its importance as cause of severe human disease, because it is estimated that human infection with this parasite in Europe is related mostly to wildlife canines and to a lesser extent to domestic dogs. It has been estimated that the contribution of domestic dogs to environmental contamination with *E. multilocularis* eggs ranges between 4% and 19% in central Europe [10].

The CALLISTO final recommendations were divided into five main categories (table 3) and further prioritised using a star ranking system from one to three stars with increasing weight for the higher number of stars[4,5]. These twenty-four recommendations ranged from suggesting education and communication activities, such as creating opportunities for education of physicians, veterinarians, pet owners and relevant professionals in companion animal zoonoses in a One Health perspective, to the development of new vaccines that protect against zoonotic pathogens.
Table 3 – The main categories of CALLISTO final recommendations.

<table>
<thead>
<tr>
<th>Category no.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Demographics &amp; tracing/movement of companion animals</td>
</tr>
<tr>
<td>2</td>
<td>Education and communication</td>
</tr>
<tr>
<td>3</td>
<td>Surveillance &amp; infection control</td>
</tr>
<tr>
<td>4</td>
<td>Risk assessment</td>
</tr>
<tr>
<td>5</td>
<td>New tools for diagnosis, prevention and therapy</td>
</tr>
</tbody>
</table>

Conclusion

In conclusion, the CALLISTO project has overviewed the role of companion animals as a source of infectious diseases for man and food-producing animals in Europe, identified knowledge gaps, and proposed targeted actions to bridge those gaps and to disseminate the results of CALLISTO’s discussions to the public, animal owners, health professionals and relevant stakeholders.

Acknowledgments

The author acknowledges and thanks the Federation of Veterinarians of Europe (FVE) for initiating and coordinating CALLISTO, the large number of experts that contributed to the discussions and formation of CALLISTO’s recommendations, and the EU Framework FP7 programme for funding the CALLISTO project (project number 289316).

Conflict of interest statement

The author declares that he has no competing interests.

References

Curettage and diathermy: a treatment for feline nasal planum actinic dysplasia and superficial squamous cell carcinoma

Robyn H. Jarrett1, Elizabeth J. Norman, Isobel R. Gibson and Paul Jarrett

SUMMARY

Objective: To evaluate curettage and diathermy as a treatment for actinic dysplasia and superficial squamous cell carcinoma of the feline nasal planum.

Methods: Thirty-four cats clinically assessed to have actinic dysplasia and superficial squamous cell carcinoma involving less than 50% of the nasal planum were treated with a three-cycle curettage and diathermy procedure. Degree of dysplasia, response to treatment, adverse effects, owner perceptions, time to recurrence and proportion disease free at 1 year were evaluated.

Results: Lesions ranged from actinic keratoses to invasive squamous cell carcinoma. A complete response to treatment was obtained in all cats. The median follow-up time was 18.2 (IQR: 12.0–22.8) months. Two cats had a clinical recurrence of lesions at 161 and 192 days after treatment. The probability of remaining disease free after 12 months was 0.94 (95% CI: 0.85–1.0). Median time to recurrence was not reached. The procedure was well tolerated with a good cosmetic outcome and no significant post-operative complications.

Clinical significance: This study suggests that curettage and diathermy is an effective treatment for feline actinic dysplasia and for superficial squamous cell carcinoma involving less than 50% of the nasal planum. Curettage and diathermy is an easily mastered technique, requiring minimal equipment.

Introduction

Squamous cell carcinoma (SCC) is a common neoplasm of the head of cats, accounting for 50% of all cutaneous neoplasms in one study of feline skin cancer diagnoses in Perth, Australia, a region with high ambient UV radiation exposure (Burrows et al. 1994). It is more common in older cats, reflecting chronic exposure to UV radiation, with a predilection for the pinnae, nasal planum and eyelids of white cats or non-pigmented skin of dark-coloured cats (Macy & Reynolds 1981, Clarke 1991, Burrows et al. 1994). There can be a spectrum of lesions clinically and histopathologically from actinic dysplasia/keratosis (mild dysplasia), carcinoma in situ (full thickness epidermal dysplasia) to invasive SCC. Additional tumours may arise on the nasal planum as a consequence of the entire area being exposed to UV light. Numerous treatments have been described including hyperthermia, external beam radiotherapy, nasal planum resection, cryotherapy, photodynamic therapy, intrallesional
Curettage and diathermy: a treatment for feline nasal planum actinic dysplasia and superficial ...


Curettage and diathermy is used by dermatologists in human medicine to treat selected non-melanoma skin cancers with high success (Ahmed et al. 2000, Reschly & Shenefelt 2010). The technique is considered suitable for use in actinic keratoses, carcinoma in situ and selected SCC and basal cell carcinomas of less than 1–2 cm diameter occurring in sites associated with a moderate to low risk of metastasis or recurrence (Goldman 2005). The tumour is vigorously scraped with the curette to remove friable tissue that sloughs easily until more resistant tissue is reached and pinpoint bleeding occurs in the wound bed (Sheridan & Dawber 2000). Diathermy of the entire base and circumference of the defect is then applied to a depth of 1–2 mm to extend the treated margin. Many authors recommend immediately repeating curettage and diathermy of the carbonised wound bed to further extend the treated margins for a total of two or three cycles (Sheridan & Dawber 2000) although the optimum number of cycles has not been well established (Goldman 2005). High cure rates of over 95% are reported for treatment of appropriately selected SCC lesions (Neville et al. 2007, Chren et al. 2011).

The success of curettage and diathermy in human patients suggests that this simple and rapidly performed technique could offer an appropriate and cost-effective treatment for cats with feline nasal actinic dysplasia and superficial SCC lesions. The primary aim of this study was therefore to evaluate the application of this technique to treat such cases; and a secondary aim was to assess owner acceptance of the procedure including post-operative care, comfort of the cat and cosmetic outcome.

Materials and methods

Cats were prospectively recruited for the study from veterinary practices in the Bay of Plenty, New Zealand between May 2005 and December 2007. This region (latitude 37° 40I S) has a sunny climate with an average of 2300 annual sunshine hours [National Institute of Water and Atmospheric Research, http://cliflo.niwa.co.nz (accessed 8 February 2011)]. The cats recruited in this study had unrestricted outdoor access.

The criteria for inclusion in the study were the presence of persistent erythema with crusting or superficial ulceration involving less than 50% of the area of the nasal planum, no evidence of metastasis as determined by palpation of the regional lymph nodes, agreement of the owners to participate in the trial and availability of the cat for follow-up. Cats subjectively assessed to have deeply invasive lesions of the nasal planum were excluded. Indicators for deep invasion were large, deeply and extensively ulcerated lesions. Cats with previously treated lesions were included provided they met the inclusion criteria.

Under general anaesthesia, pre-operative photographs were taken and the area of the lesion was estimated by measuring the two longest dimensions (length × width) with a surgical ruler. A single treatment with three cycles of curettage and diathermy was carried out. A 3-mm width bone curette (Surgicon, Sialkot, Pakistan) was used to curette the lesion. Friable tissue was removed by vigorous scraping of the deep and circumferential margins of the lesion until the curette met resistance against firm tissue and no further tissue could be removed. Diathermy of the entire curetted bed was then applied using a thermocautery unit with a 1–2 mm diameter tip (Fig 1) (Geiger TCU; Geiger Medical Technologies, IA, USA) at a recommended setting of four (Lane et al. 2006) which is a medium setting (range 1–10) for this unit. This setting produced charring (confluent blackening of the wound bed) but not excessive burning. Curettage was immediately repeated to remove the carbonised tissue until bleeding points became apparent at which point diathermy was applied again. The cycle was repeated one further time to give a total of three sequential cycles during the procedure. Typically curettage of the cauterised wound bed removed minimal further tissue (<1 mm).

The area of the post-operative defect was estimated by measuring the two longest dimensions (length × width).
Postoperatively cats were closely monitored for signs of discomfort and received buprenorphine (Temgesic; Reckitt Benckiser) (0.01mg/ kg subcutaneously as a single dose) if required. Owners were instructed to report signs of pain such as rubbing of the face or inappetence. Petroleum jelly (Vaseline®, Unilever, Australia) was applied daily for at least 1 week by the owner to encourage moist wound healing. The fragments of tissue obtained by curettage were fixed in 10% formalin. Haematoxylin and eosin stained sections were assessed by one author (IRG) and classified according to the degree of dysplasia as normal, actinic dysplasia, carcinoma in situ or SCC.

Follow-up evaluations of the cats involved weekly clinical examinations giving the owners an opportunity to discuss any concerns. These were continued until the lesions were healed or until no further healing was observed over two consecutive weeks or ulceration progressed. Photographs were taken at each visit. Complete re-epithelialisation of the curetted bed constituted successful treatment. Incomplete re-epithelialisation of over 50% of the curetted area constituted partial remission and re-epithelialisation of less than 50% of the curetted area was defined as unsuccessful treatment. Thereafter 6-monthly examinations and photography were undertaken until the censor date of December 2008 or until recurrence of the lesion. Owners were also instructed to contact one of the authors (RJ) if they noticed any lesion between scheduled re-examinations. Suspected recurrences were confirmed by histopathological examination of curetted tissue. Lesions were regarded as recurrent if they developed within or abutting the previously treated area. Owners of cats with recurrent lesions were offered all available treatment options including further curettage and diathermy for cases in which the recurrent lesion was superficial and involved less than 50% of the area of the nasal planum with no evidence of metastasis on lymph node palpation. A questionnaire was sent to the owners of all cats at 3 months postoperatively to determine their perception of the procedure and the outcome (Appendix 1).

All data were tabulated in Excel 2003 (Microsoft, Redmond, WA, USA). For cats that developed a second lesion in a new site at a later date, only data from the first lesion were included in the statistical analysis. Disease-free interval was calculated from the date of initial treatment until date of clinical assessment of recurrence. Cases that remained free of recurrence were censored at the date of the last follow-up evaluation or death of the cat. The follow-up time for all cats is reported as median and interquartile range (IQR). A Kaplan-Meier survival analysis was performed using SPSS 16.0 for Windows (SPSS Inc.; Chicago, IL, USA) to calculate the proportion disease free at 1 year with 95% confidence limits.

This study conformed to “The code of ethical conduct for the use of live animals for teaching and research” as approved under the New Zealand “Animal Welfare Act 1999.”

Results

Thirty-eight cats with nasal lesions were presented during the study period. Four of the 38 had extensive deep lesions suggesting highly invasive SCC and were not included in the study.

Thirty-four cats met the inclusion criteria. The age of 30 cats was known and was a mean of 11.6 years (range: 6–20 years). Fifteen cats were castrated males, 18 cats were spayed females; the gender of one cat was not recorded. Breeds included domestic shorthair (n=30), and domestic longhair (n=4). Eight cats had previously been treated with cryotherapy. The exact location of the previously treated lesions on the nose was unknown and therefore the lesions treated by curettage and diathermy may have been recurrent lesions or new lesions in these 8 cats.

Three cats were initially presented with two clinically distinct lesions. In two of these cats, curettage demonstrated that these lesions were contiguous and they were therefore treated as one lesion for the follow-up and statistical analysis. In the third cat, the two areas remained distinct after curettage so the endpoint used was recurrence in either area or the termination of the study.

The clinical presentation of the lesions included superficial erythematous scaling lesions, crusting and nodules with superficial ulceration. Histologically, 16 cats had actinic dysplasia, 9 cats had carcinoma in situ and 7 cats had SCC. Two cats with crusted lesions (approximately 3 mm² and 20 mm²) had no evidence of dysplasia or neoplasia on examination of the curetted tissues and were reported as normal. The average measured pre-operative area of the lesions in all cats was 34 mm² (range: 2–225) and the average post-operative defect was 83 mm² (range: 20–308).

The typical healing process observed was a moist eschar which started to slough from 1 week postoperatively. Over
buprenorphine prior to discharge. Seven owners reported mild sneezing; in one case this was considered to be due to an upper respiratory tract infection unrelated to surgery. No post-operative nasal stenosis was observed.

The 34 cats were followed for a median of 18.2 (IQR: 12.0–22.8) months. Three cats died of unrelated causes at 108, 118 and 192 days and were known to have no recurrence of nasal lesions at the time of death. Two cats were lost to follow-up after 2–3 weeks.

The next 2–3 weeks re-epithelialisation occurred in all cats. Thirty-one cats were followed weekly during the first weeks after curettage and diathermy. The mean time to complete healing was 25 days (range: 17–35). An example of this healing process is shown in the sequence of photographs (Fig 2). There were no significant post-operative complications. In particular there was no post-operative infection or haemorrhage. One cat was observed rubbing its face after the procedure and was administered postoperative

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**FIG 2.** Sequence of pre-operative (A) and post-operative photographs of the nasal planum of a cat with squamous cell carcinoma of the nasal planum treated with curettage and diathermy. (B) immediate post-operative, (C) 11 days post-operative, (D) 18 days post-operative, (E) 34 days postoperative, (F) 18 months post-operative.
Curettage and diathermy: a treatment for feline nasal planum actinic dysplasia and superficial ...

Follow-up after examinations at 219 and 247 days at which they had no recurrence of lesions. One cat was censored at the termination of the study with no recurrence at 345 days. Two cats developed local recurrence of lesions at 161 and 192 days after treatment and in both cases the recurrence was noted close to the scheduled appointment. One of these cats had been previously treated with cryotherapy 1 year prior to the study. At initial presentation one of these two cats had a 28 mm² erythematous ulcer with a histological diagnosis of actinic dysplasia and the other had a 6 mm² crusted ulcer with a histological diagnosis of SCC. At recurrence both cases had histological diagnoses of SCC confirmed. Each was treated again with curettage and diathermy. As only two cases experienced recurrence in the course of the study, median time to recurrence was not reached. The probability of remaining disease free 12 months after therapy was 0.94 (95% CI: 0.85–1.0) (Fig 3).

All owners completed the questionnaire. Fifteen (47%) owners assessed their cat’s post-operative pain as “no pain at all” or “little or no pain”, nine (28%) owners assessed it as “slight” and eight (24%) as “moderate.” Two owners were unable to accurately answer the pain question because of concurrent surgical procedures and were excluded from the analysis. Twenty-seven (79%) owners assessed the cosmetic outcome as “looks normal” or “almost normal.” The care of the cat during the healing process was assessed “no problems at all” or “fairly easy” by 31 (91%) owners. Eight cats had previous cryotherapy. Of these eight cats, five owners (62%) subjectively considered that curettage and diathermy was less painful than the previous treatment, four (50%) considered healing was faster than the previous treatment and five (62%) thought that curettage and diathermy gave a better cosmetic outcome.

Four cats had extensive deep ulceration and did not meet the study criteria, however, their owners would not contemplate radical nasal resection and a three-cycle curettage and diathermy treatment was performed. Two cats had a partial remission lasting 74 and 78 days after treatment. There was no response to a second treatment with curettage and diathermy in one of these 2 cats. In 2 cats treatment was successful. One of these 2 cats had a recurrence at 88 days and a second treatment with curettage and diathermy resulted in a complete remission lasting at least 574 days. The other cat had a recurrence at 147 days; there was no response to a second treatment.

Discussion

This study investigated a previously unreported treatment option for a common condition in cats living in areas with high sun exposure and found it to be highly successful, simple to perform and well tolerated by cats and their owners.

Curettage and diathermy used in this study to treat feline actinic nasal lesions resulted in a 94% non-recurrence rate.
Curettage and diathermy: a treatment for feline nasal planum actinic dysplasia and superficial and non-metastatic SCC because of the limited depth of penetration of diathermy. Careful selection of cases for treatment with this technique is thus important and would have contributed to the high success rate found in this study. This is not unexpected since many therapies studied report a higher success rate in early stage SCC lesions compared to more advanced lesions (Carlisle & Gould 1982, Withrow & Straw 1990, Peaston et al. 1993, Theon et al. 1995, Cunha et al. 2010). Alternative treatments, such as nasal planum resection (Lana et al. 1997) or combined intralesional carboplatin and radiotherapy (de Vos et al. 2004) would be expected to be superior for treatment of extensive invasive SCC of the nasal planum.

Only cats with lesions involving less than 50% of the area of the nasal planum were included in this study. This figure was chosen as a way of limiting the inclusion of advanced lesions, however, it is recognised that the depth of invasion and the histological grade would better indicate the suitability of cases for treatment with curettage and diathermy. Even if less than 50% of the area of the nasal planum is involved, the presence of signs which may indicate deep invasion, such as gross distortion of the nasal planum, suggest an individual case is unsuitable for treatment with curettage and diathermy. Should curettage reveal an unexpectedly deeply invasive tumour it does not preclude subsequent surgical excision and may help to delineate the extent of tumour. The unsuitability of curettage and diathermy for treatment of lesions involving more than 50% of the area of the nasal planum or with deep tissue involvement or nasal distortion was suggested by the early recurrence of disease in 3 of the 4 cats excluded from this study because of the presence of advanced lesions. Tumour destruction of the dermis can make curettage more difficult because it reduces the firmness of tissue to scrape against and the deep extent of the lesion is hard to determine (Goldman 2005). The effectiveness of curettage may therefore be reduced. While it would be tempting to extrapolate the results of this study to actinic keratoses and superficial SCC in other sites, it must be noted that differences in the resilience of underlying dermis may also affect the suitability of curettage and diathermy. The mobile nature of eyelids may make curettage difficult and requires further study.

Humans undergoing curettage and diathermy are not routinely prescribed post-operative analgesia. However, in our study one quarter of owners (24%) reported moderate pain was experienced by their cat and one cat required deep tissue involvement or nasal distortion was suggested by the early recurrence of disease in 3 of the 4 cats excluded from this study because of the presence of advanced lesions. Tumour destruction of the dermis can make curettage more difficult because it reduces the firmness of tissue to scrape against and the deep extent of the lesion is hard to determine (Goldman 2005). The effectiveness of curettage may therefore be reduced. While it would be tempting to extrapolate the results of this study to actinic keratoses and superficial SCC in other sites, it must be noted that differences in the resilience of underlying dermis may also affect the suitability of curettage and diathermy. The mobile nature of eyelids may make curettage difficult and requires further study.

Humans undergoing curettage and diathermy are not routinely prescribed post-operative analgesia. However, in our study one quarter of owners (24%) reported moderate pain was experienced by their cat and one cat required
immediate post-operative buprenorphine after veterinary assessment. Since these assessments were subjective these findings must be interpreted with caution. However, it is suggested that all cats undergoing therapy with curettage and diathermy should be given pre-emptive postoperative analgesia.

There are a number of reasons why the treatment may have failed in 2 cats in the present study, including differences in the biological behaviour of SCC or operator technique between cats. One of the two had previously been treated with cryotherapy for a nasal lesion and the exact site was unknown. It could be postulated that scarring from previous cryotherapy might limit curettage and therefore reduce the efficacy of therapy. However, the fact that 7 other cats in this study, which had good long-term responses to treatment with curettage and diathermy, had also had previous cryotherapy suggests there is no strong relationship between previous cryotherapy and treatment failure.

The curettage step provides the advantage of tissue for histopathological confirmation of the clinical diagnosis in contrast to cryotherapy during which biopsies are often not obtained in general practice (Clarke 1991). However, the tissue provided is fragmented and it can be difficult to orientate. Tumour depth and margins cannot be assessed and in this study the clinical severity of lesions did not always correlate with the histological diagnoses. In two cases the curedt fragments appeared normal histologically. The clinical behaviour of these two lesions was persistent and progressive, with erosion and crusting. The lesional tissue sloughed easily on curettage, which normal epithelium does not do, and was clearly delineated by normal-looking resilient tissue. While it cannot be confirmed that these lesions were actinic dysplasia or SCC lesions, this behaviour makes it clear that the tissue was not normal. Given the difficulty that can be experienced when examining tissue fragments which may have lost important structural relationships, the normal histopathology results in these two cases may represent false-negative results due to sampling issues. Examination of curetted tissue may thus not be 100% sensitive for diagnosis of some lesions.

The high cure rates obtained in our study and in studies of curettage and diathermy in non-melanotic skin cancers in humans (Sheridan & Dawber 2000) suggest that the curettage step does not increase the risk of deep dissemination of tumour cells. The action of curettage is to scrape rather than drive or incise. Subsequent diathermy of the wound bed effectively targets residual neoplastic cells.

This study had some limitations. Pre-operative staging was limited to palpation of local lymph nodes and pre-operative biopsies were not performed. This may have resulted in underestimation of the clinical stage of lesions presented but would not be expected to positively bias the success rate of therapy. There was no control group, and the characteristics of the studied population, although similar to those in other reports of feline nasal SCC, may have influenced the success rate. As an unblinded study there is the potential for observer and owner bias, however, all suspected recurrent lesions were confirmed histologically. Some questionnaire responses relied on the owners’ memory of events 3 months previously and owner perceptions of this period may have been positively influenced by a good final outcome. Weekly veterinary assessment during the recovery period, however, gave additional observations contemporaneously. It is possible that the 6-monthly revisit interval could have led to the time to recurrence being underestimated, even though owners were instructed to re-present cats earlier if they noticed any lesions. However, because all except two cats were disease free at their last assessment, the six monthly reassessment intervals did not lead to substantial inaccuracy in the estimate of the probability of remaining disease free at 12 months. Although the study was not long enough to reach the median survival time, cats were studied for a clinically relevant period of at least one year, and the study was sufficiently powerful to estimate the 1 year disease-free proportion within clinically useful confidence limits.

This preliminary study indicates that curettage and diathermy is an appropriate and effective technique for treatment of superficial SCC lesions involving less than 50% of the area of the nasal planum in cats. The majority (34/38) of cats that were presented had lesions which met the inclusion criteria and were considered suitable for treatment with a superficial therapy like curettage and diathermy and this may also be the case in other regions of the world where owners are well educated about skin cancers and the need for early intervention. Compared with other treatment modalities, curettage and diathermy offers several advantages including the need for only one anaesthetic and good owner acceptance. The technique is easily mastered and requires minimal equipment making
it potentially widely available and affordable with the added advantage of tissues being routinely available for histological assessment. As cats may develop multiple lesions in a lifetime, curettage and diathermy could facilitate early treatment of small lesions in general practice, and reduce morbidity in feline nasal SCC.

Acknowledgements

The authors thank the Companion Animal Society of New Zealand for a grant to assist with the research. We also thank the veterinary practices and their clients in the Bay of Plenty, New Zealand who recruited cats to this study.

Conflict of interest

The authors confirm that all co-authors have given their permission to be listed. The authors confirm that the Companion Animal Society of New Zealand gives permission to be acknowledged in the paper.

References


APPENDIX 1

Question / possible responses
1. How would you rate your cat’s pain following surgery?*
   1: No pain at all;
   2: Little or no pain;
   3: Slight pain;
   4: Moderate pain;
   5: Severe pain.

2. How would you describe the cosmetic outcome of the treatment?
   1: Looks normal;
   2: Almost normal;
   3: Slightly disfiguring;
   4: Disfiguring;
   5: Very disfiguring.

3. How difficult did you find the care of your cat during the healing process?
   1: No problems at all;
   2: Fairly easy;
   3: Some problems;
   4: Difficult;
   5: Very difficult.

4. Has this cat had previous treatment for cancer on its nose?
   1: Yes;
   2: No;
   3: Don’t know.
   (if ‘yes’ answer q5-9; if ‘no’ or ‘don’t know’, you do not need to answer any more questions. Thank you for your help!)

5. Did your cat have surgery (part of its nose cut off)?
   1: Yes;
   2: No;
   3: Don’t know.

6. Did your cat have its nose frozen (cryotherapy)?
   1: Yes;
   2: No;
   3: Don’t know.

7. Compared with the previous treatment for cancer of the nose, this latest treatment was:
   1: Less painful;
   2: About the same;
   3: More painful;
   4: Don’t know.

8. Compared with the previous treatment for cancer of the nose, the healing time for this latest surgery was:
   1: Quicker;
   2: About the same;
   3: Slower;
   4: Don’t know.

9. Compared with the previous treatment for cancer of the nose, the cosmetic outcome or final appearance of the nose, after healing was complete, for this latest surgery was:
   1: Better;
   2: About the same;
   3: Worse;
   4: Don’t know.

Questionnaire completed by owners of 34 cats 3 months after treatment of the cats’ nasal actinic dysplasia or squamous cell carcinoma with curettage and diathermy.

* Please circle the number against the words that best describe the cat’s pain.
Glucose measurement using portable blood glucose meters in dogs and cats

Labrini V. Athanasiou¹, Constantina N. Tsokana and Manolis N. Saridomichelakis

SUMMARY
Portable blood glucose meters (PBGMs) are small electronic devices that measure the concentration of glucose in whole blood. Due to the technological advances, the measurement of glucose concentration is carried out using a small blood volume and is a relatively simple, quick and inexpensive procedure. PBGMs are frequently used in companion animal medicine, especially for the diagnosis and treatment monitoring of dogs and cats with diabetes mellitus and hypoglycaemia. The main factors effecting the precision of the measurement are: a) the device (manufacturer), the consumables (reagent strips) and their storage conditions; b) environmental conditions (temperature and possibly altitude); c) blood collection technique (site of sampling, cleanliness at the site of sampling, use of anticoagulants); d) patient factors (haematocrit, blood triglycerides, creatinine, uric acid and protein concentrations, drug administration); and, e) operator error. As a consequence of all these factors, readings of glucose concentration by PBGMs may differ from those of chemistry analysers, and this should be taken into account when shifting from one method to the other. Furthermore, because the results depend on the PBGM, the same device should be used and all measurements should be made under the same environmental conditions, using the same blood sampling technique for serial measurements of blood glucose to be comparable. Finally, all the above mentioned limitations of glucose measurement by PBGMs should be taken into consideration and the results should be interpreted along with the clinical signs and any other laboratory findings for optimal diagnostic and therapeutic decision making.

Keywords: cat, dog, portable blood glucose meters.

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Introduction
Glucose, the principal source of energy for mammalian cells, is derived from the diet, hepatic glyconeogenesis and hepatic and muscle glucogenolysis, and its concentration in blood is controlled by hormones, including insulin, glucagon, cortisol, catecholamines and growth hormone (Feldman and Nelson, 2004; Knieriem et al., 2007). Glucose metabolism and blood concentrations are abnormal in many disease states (Table 1). For this reason, hyperglycaemia and hypoglycaemia are common laboratory findings in dogs and cats. Measurement of blood glucose concentration is essential whenever the clinical picture includes hyperglycaemia or hypoglycaemia (Table 2), whenever any of the possible underlying causes

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Glucose measurement using portable blood glucose meters in dogs and cats

In all critical care patients, and for general health screening purposes (Villiers and Blackwood, 2005; Wiedmeyer and DeClue, 2008; Ford and Lynch, 2013; Surman and Fleeman, 2013). Furthermore, repeated measurements of blood glucose concentration are necessary to monitor and adjust the treatment of diseases that cause hyperglycaemia and hypoglycaemia and they are needed on a long-term to lifelong basis in dogs and cats with diabetes mellitus and insulinoma (Feldman and Nelson, 2004; Dietiker- Moretti et al., 2011).

The methodology of glucose analysis is currently based on either chromogenic or electrochemical reactions that are

Table 1. The most common causes of hyperglycaemia and hypoglycaemia in dogs and cats.

<table>
<thead>
<tr>
<th>Causes of hyperglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Diabetes mellitus (uncomplicated, ketoacidotic, hyperosmolar)</td>
</tr>
<tr>
<td>• Acute pancreatitis</td>
</tr>
<tr>
<td>• Glucagonoma</td>
</tr>
<tr>
<td>• Hyperadrenocorticism</td>
</tr>
<tr>
<td>• Pheochromocytoma in dogs</td>
</tr>
<tr>
<td>• Acromegaly (especially in cats)</td>
</tr>
<tr>
<td>• Hyperthyroidism in cats</td>
</tr>
<tr>
<td>• Dioestrus in dogs</td>
</tr>
<tr>
<td>• Sepsis</td>
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<tr>
<td>• Neoplasia</td>
</tr>
<tr>
<td>• Brain trauma</td>
</tr>
<tr>
<td>• Stress (especially in cats)</td>
</tr>
<tr>
<td>• Recent meal (especially if rich in monosaccharides, disaccharides or propylene glycol)</td>
</tr>
<tr>
<td>• Medication: glucocorticoids, progestagens, a2-adrenergic agonists, thiazide diuretics, dextrose-containing fluids, total parenteral nutrition</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Causes of hypoglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Insulinoma</td>
</tr>
<tr>
<td>• Hypoadrenocorticism</td>
</tr>
<tr>
<td>• Pituitary dwarfism</td>
</tr>
<tr>
<td>• Sepsis, canine babesiosis</td>
</tr>
<tr>
<td>• Neoplasia (other than insulinoma)</td>
</tr>
<tr>
<td>• Liver disease (glycogen storage disease, acute liver insufficiency, portosystemic shunts, cirrhosis)</td>
</tr>
<tr>
<td>• Neonatal and juvenile hypoglycaemia</td>
</tr>
<tr>
<td>• Hunting dog hypoglycaemia</td>
</tr>
<tr>
<td>• Prolonged fasting of animals in poor body condition</td>
</tr>
<tr>
<td>• Renal failure (most commonly acute renal failure)</td>
</tr>
<tr>
<td>• Toxics: ethylene glycol, alcohol, xylitol</td>
</tr>
<tr>
<td>• Medications: insulin, orally administered hypoglycaemic agents</td>
</tr>
<tr>
<td>• Laboratory error (delay in serum separation), severe polycythaemia</td>
</tr>
</tbody>
</table>

Table 2. Clinical signs of hyperglycaemia and hypoglycaemia in dogs and cats, irrespective of the underlying cause.

<table>
<thead>
<tr>
<th>Clinical signs of hyperglycaemia</th>
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<tbody>
<tr>
<td>• Polyauria-polydipsia</td>
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</table>

<table>
<thead>
<tr>
<th>Clinical signs of hypoglycaemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Due to neuroglycosopenia</td>
</tr>
<tr>
<td>• reduced level of consciousness, disorientation</td>
</tr>
<tr>
<td>• seizures</td>
</tr>
<tr>
<td>• blindness</td>
</tr>
<tr>
<td>• ataxia, paresis</td>
</tr>
<tr>
<td>• muscle weakness</td>
</tr>
<tr>
<td>Due to sympathetic stimulation</td>
</tr>
<tr>
<td>• nervousness, restlessness</td>
</tr>
<tr>
<td>• mydriasis</td>
</tr>
<tr>
<td>• muscle tremors</td>
</tr>
<tr>
<td>• polyphagia, increased bodyweight</td>
</tr>
</tbody>
</table>

Figure 1. After venous blood sampling, the needle has been removed from the syringe, and the reagent strip of the portable blood glucose meter is loaded using the drop of blood that has been created on the adaptor of the syringe.
mediated by an enzyme, such as glucose oxidase, glucose dehydrogenase and hexokinase (Kaneko et al., 2008; Johnson et al., 2009; Surman and Fleeman, 2013). These enzymatic methods have been applied in different equipment and devices, including in-house and reference laboratory automated chemistry analysers, point-of-care analysers, colorimetric reagent strips, portable blood glucose meters (PBMGs), and continuous glucose monitoring systems (CGMSs) (Wiedmeyer and DeClue, 2008; Surman and Fleeman, 2013).

Automated chemistry analysers are used by most commercial diagnostic laboratories as they determine blood glucose concentrations in plasma or serum samples using colorimetric endpoint or kinetic assays. The former are more precise than turbidimetric endpoint methods, since turbidity decreases light transmittance, which is particularly important for dogs and cats with diseases that simultaneously affect glucose and lipid metabolism, including diabetes mellitus. The latter detect changes in the optical density of the specimen over time. Other widely used methods for the determination of blood glucose concentrations involve enzyme-catalysed reactions coupled to a colour detection system, oxidation-reduction methods, and methods that result in the generation of an electrical current (Hagvik, 2007; Johnson et al., 2009). Although automated chemistry analysers are usually very accurate, their major disadvantages when used for the determination of blood glucose concentrations are the relatively large volume of blood and the prolonged time to obtain results. For these reasons, they are usually preferred when additional biochemical parameters, besides glucose, are to be measured in a non-emergency setting (Surman and Fleeman, 2013).

Point-of-care analysers usually operating with dry chemistry slides or strips, require only small amounts of whole blood, serum or plasma, and provide rapid results. Besides glucose, they can also measure, using different slides or strips, other parameters that are helpful for the estimation of the acid-base and electrolyte status of the patient. For these reasons they are especially valuable in emergency cases. However, point-of-care analysers are relatively expensive and can only be used by trained personnel (Cohn et al., 2000).

Colorimetric reagent strips, which are read visually or with a reflectance meter, are available for estimating glucose concentration in fresh whole blood (Sherwood et al., 1983). Currently, they are rarely, if ever, used because they are the least precise of the available methods.

Portable blood glucose meters were originally developed to permit human diabetic patients to self-monitor blood glucose concentrations using a drop of capillary blood (Ginsberg, 2009a). Following an enzymatic reaction (glucose oxidase, glucose dehydrogenase, glucose oxidase-peroxidase) that is coupled to a chromogen or to an electron-donating mediator system, blood glucose concentration is measured via reflectance photometry (photometric methods) or via an electrode (electrochemical methods), respectively (Cohn et al., 2000; Johnson et al., 2009; Surman and Fleeman, 2013; Ford and Lynch, 2013). PBMGs are commonly used to measure blood glucose concentrations in dogs and cats. They require a small volume of venous or capillary blood, they have a relatively wide working range (from 10-20 to 500-600 mg/dL, depending on the device), they provide immediate results, they are of low cost and they are easy to handle, which permits their use even by animal owners after a short period of training (Cohen et al., 2009; Johnson et al., 2009; Dobromylskyj and Sparkes, 2010). These attributes make PBMGs the ideal way to measure blood glucose concentrations in emergency patients with hyperglycaemia or hypoglycaemia, in all critical care patients and for the longer-term monitoring of diabetic dogs and cats in the hospital or at home (Alt et al., 2007; Rios and Ward, 2008; Cohen et al., 2009). The accuracy of PBMGs is critical because the glucose readings that will be obtained will dictate the course of clinical action (Cohn et al., 2000).

Continuous glucose monitoring systems are electronic devices that measure glucose concentrations in the interstitial fluid with the aid of a small flexible sensor that is inserted into the subcutaneous tissues. The measurement is based on the glucose oxidase-mediated reaction between glucose and oxygen (Rios and Ward, 2008; Wiedmeyer and DeClue, 2008; Moretti et al., 2010; Dietiker-Moretti et al., 2011; Fleeman, 2011; Surman and Fleeman, 2013). Glucose concentrations in the blood and the interstitial fluid are usually highly correlated, and the calibration of the CGMS using peripheral blood samples with known glucose concentrations permits the device to accurately measure blood glucose (Rios and Ward, 2008; Wiedmeyer and DeClue, 2008; Moretti et al., 2010; Dietiker-Moretti et al., 2011; Hoenig et al., 2012; Surman and Fleeman, 2013). CGMSs typically record glucose concentrations every 3-5 minutes for up to 72 hours and are mainly used to regulate insulin treatment of stable diabetic dogs and cats (Wiedmeyer and DeClue, 2008; Moretti et al., 2010; Dietiker-Moretti et al., 2011; Fleeman, 2011; Surman and Fleeman, 2013). Additional indications may include monitoring of dogs and cats with diabetic ketoacidosis, of critical care patients, of dogs with juvenile hypoglycaemia as well as...
the intra- and post-operative monitoring of surgical patients at risk of hypoglycaemia or hyperglycaemia (Wiedmeyer and DeClue, 2008); however, the accuracy of CGMS for these purposes has not been fully evaluated and is debatable (Surman and Fleeman, 2013).

**Indications for use of portable blood glucose meters in dogs and cats**

**Emergency cases**

Hyperglycaemia or hypoglycaemia in dogs and cats (Table 1) may be an emergency, as in the case of ketoacidotic or hyperosmolar diabetes mellitus or hypoglycaemia-induced seizures (Dobromylskyj and Sparkes, 2010; Koenig, 2013). In such cases, the rapid diagnosis of abnormal blood glucose concentrations is essential for the initial therapeutic decision making. In all dogs and cats with clinical signs compatible with a diabetic emergency or with hypoglycaemia (Table 2) measurement of blood glucose concentration with a PBGM is strongly advised as one of the initial diagnostic steps. If the blood glucose readings of the PBGM are above or below the reference range, the result can be confirmed using an automated chemistry analyser, since a complete serum biochemical profile will be necessary to identify the full range of biochemical abnormalities that accompany diabetic emergencies or to investigate the cause of hypoglycaemia (Koenig, 2013). In addition, PBGMs are indispensable for monitoring the emergency treatment of hyperglycaemia (e.g. regular insulin administration) or hypoglycaemia (e.g. dextrose infusion) which is based on serial measurements of blood glucose concentrations (Feldman and Nelson, 2004; Koenig, 2013).

Non-diabetic critical care dogs and cats, with or without one of the known causes of hyperglycaemia (Table 1), may present disturbances of glucose metabolism leading to increased blood glucose concentrations (Knieriem et al., 2007). Possible underlying mechanisms include increased adrenal secretion of glucocorticoids and catecholamines and insulin resistance, in addition to the possible hyperglycaemic effects of various therapeutic interventions (Knieriem et al., 2007). Increased blood glucose concentrations have been correlated with the severity of head trauma in dogs and cats (Syring et al., 2001) and with a poor outcomes in dogs with congestive heart failure (Brady et al., 2004) and perhaps in dogs with sepsis (Hardie et al., 1985). Intensive insulin therapy has been shown to reduce the incidence of complications and to improve prognosis in human critical care patients with hyperglycaemia (Van den Berghe et al., 2003; Krinsley, 2004). Although there is a paucity of similar studies in companion animals, the use of regular insulin has been suggested in hyperglycaemic dogs and cats with head trauma or sepsis in an effort to control blood glucose concentration in a range between 85 and 130 mg/dl (Knieriem et al., 2007). Obviously, if this therapeutic intervention is adopted, repeated measurements of blood glucose concentrations with PBGMs is the only practical way to monitor treatment. At least in theory, a possible alternative could be the use of a CGMS with real-time display of interstitial fluid glucose concentration (Wiedmeyer and DeClue, 2008).

**Monitoring diabetic patients**

Blood glucose curves (BGC) are essential for monitoring and adjusting insulin treatment of dogs and cats with diabetes mellitus (Alt et al., 2007; Martin and Rand, 2007; Rios and Ward, 2008; Affenzeller et al., 2011; Dietiker-Moretti et al., 2011; Smith et al., 2012; Roomp and Rand, 2013). They can be generated in either hospitalized patients or at home (Alt et al., 2007; Rios and Ward, 2008; Dietiker-Moretti et al., 2011; Smith et al., 2012; Roomp and Rand, 2013; Ford and Lynch, 2013). Most diabetic pets are administered insulin twice daily; thus the BGC typically lasts for 10-12 hours and blood sampling for measurement of glucose concentration is typically performed every 1-2 hours during this period, although sampling every 3-4 hours may be carried out in cats at home (Moretti et al., 2010; Affenzeller et al., 2011; Dietiker-Moretti et al., 2011; Roomp and Rand, 2013). Since an important objective of the BGC is to determine the minimum glucose concentration or nadir (Martin and Rand, 2007; Dietiker-Moretti et al., 2011), more frequent sampling (usually every 30 min) is practised by the authors during the period of maximum insulin activity (between 2 and 6 hours after insulin administration) and/or when a trend of progressively declining blood glucose concentration is noticed. Therefore, at least 7 and up to 20 or more blood glucose measurements may be performed during a BGC. Obviously, PBGMs and not automated chemistry analysers are used to measure blood glucose concentrations when a BGC is conducted at home by the owner (Alt et al., 2007; Dobromylskyj and Sparkes, 2010; Ford and Lynch, 2013), and PBGMs are clearly preferred in the hospital environment due to the small blood volume that is required (avoidance of blood loss anaemia) and the option of using capillary instead of venous blood (less stressful sampling). On the other hand, current data suggest that CGMSs may be preferable over PBGMs for BGCs, mainly because of their superiority in terms of recording...
Glucose measurement using portable blood glucose meters in dogs and cats

EJCAP 25(2), Summer 2015  P 22

glucose nadir, zenith and daily fluctuations, and the option for continued monitoring of glucose concentrations for up to 72 hours, and of the avoidance of repeated patient restraint for blood sampling (Rios and Ward, 2008; Wiedmeyer and DeClue, 2008; Moretti et al., 2010; Affenzeller et al., 2011; Dietiker-Moretti et al., 2011). However, the cost of purchasing the equipment may not be prohibitive, the accuracy of the measurements may be lower compared to PBGMs, especially when blood glucose concentrations are in the hypoglycaemic range, necessitating periodic use of a PBGM and therapeutic decision-making may not significantly differ depending on the use of CGMS or a PBGM (Moretti et al., 2010; Dietiker-Moretti et al., 2011; Fleeman, 2011).

Home monitoring of blood glucose concentrations of diabetic dogs and cats decreases the medical cost (although it does not decrease the frequency of re-examinations), is feasible for most owners and it allows their active participation in the medical care of their pet (Roomp and Rand, 2009; Smith et al., 2012). Besides the traditional BGC that can be performed at home, current research suggests that especially in diabetic cats it is possible and safe to implement close control of hyperglycaemia with a combination of long-acting insulin administration and dietary modifications, provided that the owners are willing and able to measure blood glucose concentration with a PBGM at least 3 times per day and optimally 5 times per day, on an everyday basis for a few weeks up to 4 months (Roomp and Rand, 2009; Roomp and Rand, 2013; Ford and Lynch, 2013). The intense protocol has the advantage that in more than 80% of newly diagnosed cats diabetic remission can be achieved and insulin administration can be discontinued, on a temporary or even permanent basis (Roomp and Rand, 2009; Roomp and Rand, 2013).

Elective (e.g., neutering of female dogs) and non-elective surgery of diabetic dogs and cats poses a challenge in terms of controlling blood glucose concentration to avoid severe hyperglycaemia and hypoglycaemia. Lack of food intake, influence of the anaesthetic and the surgical stress on glucose metabolism and inability to monitor for clinical signs suggestive of abnormal blood glucose concentrations are the main considerations on the day of surgery until the diabetic animal wakes and resumes eating. The typical recommendation during this period is to monitor blood glucose concentration every 30-60 min and to establish close control (60-250 mg/dl) with the administration of regular insulin and the appropriate fluid (normal saline 0.9%, normal saline 0.45% and dextrose 2.5%, dextrose 5%) (Feldman and Nelson, 2004). Obviously, PBGMs are indispensable for the measurement of blood glucose concentrations and guiding treatment during this period.

Insulinoma

The diagnosis of insulinoma is based on the normal or increased serum insulin concentration in the face of hypoglycaemia (<50-60 mg/dl) (Koenig, 2013). In some dogs and cats with this tumour, blood glucose concentration fluctuates between low-normal and subnormal levels necessitating supervised fasting and repeated measurements in order to identify a hypoglycaemic serum sample where insulin concentration can be measured (Ford and Lynch, 2013; Koenig, 2013;). PBGMs are advised to monitor blood glucose concentration during the fasting period, although confirmation of hypoglycaemia in an automated chemistry analyser is needed before the serum sample is submitted to the laboratory for measurement of insulin concentration (Cohen et al., 2009)

Factors affecting glucose measurement by portable glucose meters

Different devices, employing different analytical methodologies are characterized, not only by variable method-specific interferences (e.g. the effect of blood oxygen tension on glucose oxidase method), but also by important differences in their inherent random (imprecision) and systematic (bias) error (Jensen and Kjelgaard-Hansen, 2006; Ginsberg, 2009a; Johnson et al., 2009). In human medicine, the need for an internationally accepted reference method, like isotope dilution gas chromatography-mass spectrometry (Hagvik, 2007), to evaluate the accuracy and to calibrate blood glucose measuring devices, including PBGMs, has long been recognized. Until such a goal is achieved and is applicable to dogs and cats, it is recommended to consistently use the same device for the same patient, especially if monitoring the trends in blood glucose concentration over time is clinically important (Johnson et al., 2009; Paul et al., 2011).

Human or veterinary use? Blood or plasma?

In general terms, the accuracy of blood glucose concentrations that are measured by PBGMs differ depending on whether the equipment has been designed for human or for veterinary use and for the former it also depends on whether they have been calibrated to measure whole blood or plasma-equivalent glucose concentrations (Roomp and Rand, 2013). More specifically, veterinary PBGMs, like AlphaTrak
Table 3. Selected commercially available portable blood glucose meters (PBGMs) and their accuracy for measurement of blood glucose concentration in dogs and cats

<table>
<thead>
<tr>
<th>PBGM</th>
<th>Designed for</th>
<th>Tested in</th>
<th>Blood glucose concentration</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>low</td>
<td>normal</td>
<td>high</td>
</tr>
<tr>
<td>Accu-Chek</td>
<td>H</td>
<td>D</td>
<td>U</td>
<td>U</td>
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<tr>
<td>Accu-Chek Active</td>
<td>H</td>
<td>D</td>
<td>U</td>
<td>U</td>
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<tr>
<td>Accu-Chek Compact</td>
<td>H</td>
<td>C</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>Accu-Chek Easy</td>
<td>H</td>
<td>D</td>
<td>U</td>
<td>U</td>
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<tr>
<td>Accu-Chek Simplicity</td>
<td>H</td>
<td>D</td>
<td>NC</td>
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<tr>
<td>Accu-Chek Simplicity</td>
<td>H</td>
<td>C</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>Ascensia Breeze</td>
<td>H</td>
<td>C</td>
<td>NC</td>
<td>NC</td>
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<tr>
<td>AlphaTrak</td>
<td>D/C</td>
<td>C</td>
<td>U</td>
<td>U</td>
</tr>
<tr>
<td>AlphaTrak</td>
<td>D/C</td>
<td>D</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Ascensia Elite</td>
<td>H</td>
<td>C</td>
<td>U</td>
<td>U</td>
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<tr>
<td>Contour</td>
<td>H</td>
<td>D</td>
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<tr>
<td>Elite</td>
<td>H</td>
<td>D</td>
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<tr>
<td>ExacTech RSG</td>
<td>H</td>
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<td>FreeStyle</td>
<td>H</td>
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<td>NC</td>
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<tr>
<td>Glucometer DEX</td>
<td>H</td>
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<td>NC</td>
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<tr>
<td>Glucometer DEX</td>
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<tr>
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<tr>
<td>SureStep</td>
<td>H</td>
<td>C</td>
<td>U</td>
<td>U</td>
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</tbody>
</table>

C: cats; D: dogs; H: humans; NC: non-consistent overestimation and/or underestimation of blood glucose concentration; O: overestimation of blood glucose concentration; U: underestimation of blood glucose concentration; U+, NC+: bias increased at higher blood glucose concentrations

Sources: (1) Cohen et al., 2009; (2) Johnson et al., 2009; (3) Cohn et al., 2000; (4) Wess and Reusch, 2000a; (5) Zini et al., 2009 (6) Wess and Reusch, 2000b (7) Wess and Reusch, 2000c (8) Dobromylskyj and Sparkes, 2010
Glucose measurement using portable blood glucose meters in dogs and cats

Dietiker-Moretti et al., 2011; Ford and Lynch, 2013), give plasma-equivalent glucose concentrations that can be lower or higher but are strongly correlated with those expected with an automated chemistry analyser and the differences are not large enough to negatively effect clinical decisions or patient outcome (Cohen et al., 2009; Johnson et al., 2009; Roomp and Rand, 2009; Ford and Lynch, 2013; Roomp and Rand, 2013; Surman and Fleeman, 2013;). Other PBGMs for veterinary use include the g-Pet (Woodley Equipment) and the i-Pet (UltiCare); their accuracy seems to be adequate based on the manufacturers’ results but, at the time of writing, there are no independent studies published in the peer-reviewed literature to support this (Surman and Fleeman, 2013). On the contrary, most human whole-blood PBGMs, which are the most widely available devices, systemically underestimate glucose concentrations in dogs and cats (by as much as 16-40% or by approximately 18-36 mg/dl at normal and low glucose levels and to a greater extent for hyperglycaemic samples) and human plasma-equivalent PBGMs give intermediate values (Cohen et al., 2009; Roomp and Rand, 2009; Ford and Lynch, 2013; Roomp and Rand, 2013; Surman and Fleeman, 2013). The reason for this is suspected to be the different distribution of glucose between plasma (approximately 50% in humans, 87.5% in dogs and 93% in cats) and red blood cells (approximately 50% in humans, 12.5% in dogs and 7% in cats) along with the fact that the porous membrane of PBGMs test strips separates red blood cells from plasma and the analysis is performed only in the latter (Johnson et al., 2009; Roomp and Rand, 2009; Surman and Fleeman, 2013). To further complicate this issue, the human PBGM Wavesense Presto (AgaMatrix) has been shown to give overall higher glucose concentration values, to overestimate low and to underestimate high glucose concentrations in cats (Hoenig et al., 2012) and in another study of six different human PBGMs on cats, both higher and lower glucose values than those obtained by a chemistry analyser were obtained (Dobromylskyj and Sparkes, 2010).

Blood volume, stability of strips

Another important attribute of the device is the blood volume that is needed to obtain a reading, especially when lancing is employed to obtain a drop of capillary blood (Ford and Lynch, 2013; Roomp and Rand, 2013). Veterinary PBGMs, like AlphaTrak, that require small blood volumes (e.g. 0.3 μL) are preferable to those requiring larger volumes (e.g. 0.6-4 μL for most human PBGMs or 1.5-1.6 μL for the g-Pet and the i-Pet meters), because the chances of accurate readings are increased, especially if the device is used by the owners (Cohen et al., 2009; Ford and Lynch, 2013; Roomp and Rand, 2013; Surman and Fleeman, 2013).

Some human PBGMs can also measure blood b-hydroxybutyrate concentrations using different strips (Roomp and Rand, 2013). In diabetic animals that develop ketosis blood b-hydroxybutyrate increases earlier than urine acetoacetic acid (which is predominantly measured with urine dipsticks) and for this reason such devices have the potential for earlier diagnosis of this complication of diabetes mellitus (Roomp and Rand, 2013).

Different reagent strips covered with a specific enzyme are used by all PBGMs. There is variation between strips, which may lead to some inaccuracy in blood glucose readings. Furthermore, slight variation in the size of the reaction wells of the strips as well as in their enzyme coverage (the amount of enzyme used and its dispersion on the well) may also influence the results (Ginsberg, 2009a). To account for the variation among different batches of strips, most PBGMs are calibrated for each new batch using a control solution, a batch-specific calibration strip or a batch specific code (Cohen et al., 2009; Johnson et al., 2009).

The stability of the strips is guaranteed and the corresponding expiry date provided by the manufacturer is valid when they are stored in their tightly sealed package under the
recommended storage conditions. A shorter life span may result from high temperature, high humidity or a strip package that has been left open. In such cases, at least for humans, the divergence of the readings is unpredictable and underestimation or overestimation of blood glucose concentration may equally occur (Bamberg et al., 2005). Therefore, strips should be stored properly and the package with the unused strips should be tightly closed immediately after the removal of a strip. Also, use of expired strips is strongly discouraged; some PBGMs automatically detect the expiration date and they alert the operator.

Altitude, temperature and oxygen concentration

The accuracy of blood glucose measurements can be influenced by environmental factors, such as altitude and temperature. In addition to data provided by the manufacturers, the influence of these factors on the human applications of PBGMs has been assessed under extreme conditions (Gautier et al., 1996). Glucose oxidase, but not glucose dehydrogenase, biosensor strips are sensitive to blood oxygen concentration. A decrease in blood oxygen concentration that may occur at high altitude, can lead to overestimation of the glucose value and vice versa. For the same reason and because of the higher oxygen concentration in capillary compared to venous blood, glucose oxidase strips may be more accurate when used with the former samples which have been used for their calibration. Furthermore, the accuracy of PBGMs using such strips may be compromised in patients receiving supplemental oxygen (Cohen et al., 2009; Ginsberg, 2009b). The influence of temperature on glucose measurements is unpredictable. For this reason, temperature sensors have been incorporated into modern PBGMs to warn operator against extreme temperatures (Fazel et al., 1996; Oberg and Ostenson, 2005).

Blood glucose concentration measurement by PBGMs can be performed with venous or with capillary blood samples and the latter can be obtained from different sites (ear pinnae or carpal/metacarpal pads). When capillary blood sampling is selected, the reagent strip of the PBGM is directly touched to the drop of blood on the animal’s skin, whereas when venous blood is used a drop of blood from the syringe can be placed on the wrapper of the strip (Cohen et al., 2009) or a drop can be created on the adaptor of the syringe after removal of needle (Fig. 1). Different blood samples may effect readings due to differences in glucose and oxygen concentration, local blood circulation and possible contamination by natural or chemical substances. Apart from the higher oxygen tension, capillary blood also has higher glucose concentrations compared to venous blood, due to tissue utilization of glucose and this difference is higher postprandially than during fasting (Johnson et al., 2009). However, capillary and venous blood glucose concentrations seem to have a good correlation in companion animals (Alt et al., 2007; Cohen et al., 2009).

Contamination at sampling

The most frequently used site for capillary blood sampling in dogs and cats is the lateral margin of the ear pinna (Ford and Lynch 2013) or the inner surface of the pinna (Rios and Ward, 2008). Blood collection may be facilitated by shaving (lateral margin technique), warming the puncture site for 30-60 sec with a damp cloth, applying a thin layer of petroleum jelly, holding a cotton ball or a gauze under the pinna and pricking close to but not onto a visible vein (Ford and Lynch, 2013) (Fig. 2). Recently, the carpal (pisiform or wrist) non-weight bearing pads of dogs and the metacarpal pad of cats have been also used and validated as alternative sampling sites for blood glucose testing (Zeugswetter et al., 2010; Borin-Crivellenti et al., 2012; Ford and Lynch, 2013). Sampling may become easier if the site is pre-warmed and lightly pinched with two fingers for a few seconds before and after puncture until an adequate blood drop forms (Ford and Lynch, 2013) (Fig. 3). Irrespective of the site, puncture is typically performed with commercially available lancing devices with lancets of 25-32 gauge, depending on the patient (Ford and Lynch, 2013); some lancing devices create negative pressure which may facilitate ear pinna capillary blood sampling (Rios and Ward, 2008). One exception is for capillary blood sampling from the carpal pad in dogs, where a hypodermic needle may be more appropriate (Borin-Crivellenti et al., 2012).

The presence of contaminants at the site of sampling, even in trace amounts, can significantly raise blood glucose, has been shown in human medicine (Ginsberg, 2009a). Hand washing is suggested prior to blood sampling in humans and cleaning of the sampling site in dogs and cats can be proposed. However, the use of any disinfectant should be avoided, until any possible effect of the substance on glucose measurement is excluded.

Anticoagulants

The effect of three anticoagulants (EDTA, lithium heparin and fluoride oxalate) on glucose measurements in canine
Figure 2. Capillary blood sampling from ear pinna of a dog for measurement of blood glucose concentration with a portable blood glucose meter: a) the margin of the pinna has been shaved; b) the puncture site is warmed with a damp cloth; c) a gauge is held under the puncture site; d) the lancing device is applied to the puncture site; e) a drop of capillary blood appears immediately after lancing; f) the capillary blood is used to load the reagent strip, and g) within a few seconds the glucose reading appears on the screen of the portable blood glucose meter.
blood was determined for five different PBGMs. While no difference was found among EDTA, lithium heparin and blood without anticoagulant, the concentration of glucose in fluoride oxalate anticoagulated blood was underestimated by one of the devices whose manufacturer advised against the use of anticoagulants (Wess and Reusch, 2000b). However, in a different study evaluating feline blood samples without anticoagulant, with lithium heparin and with fluoride oxalate, no effect on glucose concentration was witnessed for six different human PBGMs (Dobromylskyj and Sparkes, 2010). This topic remains controversial but it is of minor clinical importance because PBGMs readings are typically obtained immediately after collection of capillary or venous blood samples without anticoagulant.

**Haematocrit**

The haematocrit can influence blood glucose measurements by PMBGs. These devices measure glucose in a mixture of whole blood and plasma, while plasma glucose is used for their calibration during the manufacturing process. The effect of haematocrit is related, not only to the presence of glucose in red blood cells, but also to the influence of erythrocytes on various mechanisms operating during PBGM measurement, such as the diffusion of plasma into the test strip reagent pad, the enzymatic reaction and the proper function of the electrodes of the device (Wiener, 1991). For example, haemoconcentration and the associated increased blood viscosity may lead to obstruction of the holes of the strip filter and to a decreased plasma diffusion rate and consequently to falsely lower glucose readings, whereas artificially increased glucose concentrations are expected in anaemic patients (Tang et al., 2000). In general, PBGMs employing glucose dehydrogenase are suggested for human patients with abnormal haematocrit values and efforts have been made to apply mathematical equations for the correction of glucose readings for haematocrit values (Musholt et al., 2011).

The effect of the haematocrit is expected to also apply in dogs and cats (Paul et al., 2011) and perhaps to become more important with the use of human PBGMs which have been evaluated within the narrower haematocrit range of humans. Although, a veterinary PBGM was more accurate than a human one in canine blood samples with normal or increased haematocrit, the reverse was true in anaemic samples (Paul et al., 2011). In any case, PBGMs seem to consistently overestimate blood glucose concentration in anaemic dogs (Johnson et al., 2009) and cats (Wess and Reusch, 2000a), although a recent study did not reveal an effect of haematocrit on the accuracy of six different human PBGMs in feline blood samples (Dobromylskyj and Sparkes, 2010).

**Metabolites**

Plasma concentration of certain metabolites (i.e. triglycerides, creatinine, uric acid, proteins) have also been reported to influence the measurement of blood glucose in humans with PBGMs (Ervin and Kiser, 1999), more notably when their concentration is altered during certain disease states. For example, high triglyceride concentration may decrease the amount of glucose in the capillary blood, whereas, uric acid at extreme concentrations erroneously increases glucose values (Ginsberg, 2009a). However, the latter effect is unlikely to be of clinical significance in companion animals due to the differences in purine metabolism compared to humans and the effects of the other metabolites on the accuracy of PBGMs in dogs and cats have not been studied.
Drugs

Various drugs, such as paracetamol, L-dopa, tolazamide, and ascorbic acid, interact with the electrode of PBGMs employing the electrochemical glucose oxidase system and alter glucose readings in human blood (Ginsberg, 2009a). To the author’s knowledge similar data are not available in the veterinary literature.

The importance of following manufacturer’s instructions is fundamental for the accurate measurement of blood glucose concentration with PBGMs in both the hospital and the home setting. Operator error can become a major source of misleading results and therefore adequate training of operators is of great importance.

Conclusion

Due to several factors, including the analytical method employed, results from different PBGMs are likely to differ. Even though such differences are not always clinically relevant and may not effect diagnostic or therapeutic decisions (Johnson et al., 2009), veterinary PBGMs should be preferred over human devices. If this is not feasible, human PBGMs that have been shown to be characterized by a systemic bias when used in dogs and cats must be carefully selected. Furthermore, it is always a good idea to compare the results of any newly purchased PBGM with that of the previous device and/or of a chemical analyser.

Moreover, the clinician must be aware of the potential limitations of PBGMs and the factors that can interfere with the result including: storage conditions, altitude, temperature, blood sampling method, haematocrit etc. For this reason, when consecutive measurements of blood glucose are necessary, as in diabetic animals, all these factors should remain as constant as possible so that any influence on the measured blood glucose concentration would be uniform.

Since the advent of the PBGMs, home monitoring of diabetic dogs and cats has become feasible. Also, the immediate availability of the results offers an important advantage of PBGMs over automated chemistry analysers in the critical care setting. In any case, training of the operator, being either a pet owner or a health care provider, is essential to obtain reliable results. Finally, as with all diagnostic tests and devices, the clinician should combine clinical judgment and experience with the measurement of blood glucose concentration by PBGMs and interpret the result in light of all the information available.

Conflict of interest

None of the authors of this article has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

References


Gastrointestinal toxicity after vincristine or cyclophosphamide administered with or without maropitant in dogs: a prospective randomised controlled study

Sarah L. Mason¹, Iain A. Grant, James Elliott, Peter Cripps and Laura Blackwood

SUMMARY

Objectives: To assess the prevalence of gastrointestinal toxicity in dogs receiving chemotherapy with vincristine and cyclophosphamide and the efficacy of maropitant citrate (Cerenia™, Zoetis) in reducing these events.

Methods: Dogs receiving chemotherapy with cyclophosphamide or vincristine were randomised to either receive maropitant or not in the period immediately after treatment and for 4 days afterwards. Owners completed a diary of adverse events following treatment.

Results: Adverse events occurred in 40/58 (69%) dogs in the vincristine group. Most of these adverse events were mild and included: lethargy (62%), appetite loss (43%), diarrhoea (34%) and vomiting (24%). Adverse events occurred in 34/42 (81%) dogs treated with cyclophosphamide. Most of these adverse events were mild and included: lethargy (62%), diarrhoea (36%), appetite loss (36%) and vomiting (21%). There was no difference in total clinical score, vomiting, diarrhoea, appetite loss or lethargy score between dogs treated with maropitant and non-treated dogs in either the vincristine or cyclophosphamide groups.

Clinical significance: Chemotherapy-related side effects are frequent but usually mild in dogs receiving vincristine or cyclophosphamide. Prophylactic administration of maropitant does not reduce the frequency of adverse events and maropitant should be administered only as required for individual cases.

Introduction


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The frequency and severity of these adverse effects in dogs are not, however, extensively documented. Gastrointestinal adverse effects (vomiting, diarrhoea, nausea and anorexia) are recognised in humans and dogs receiving chemotherapy (Mellanby et al. 2002, Jordan et al. 2005, Cave 2006, Tomiyasu et al. 2010, Elliott et al. 2012, Marrington et al. 2012). The frequency and severity of these adverse effects in dogs are not, however, extensively documented. Most studies evaluating vomiting and nausea in dogs receiving chemotherapy have focused on cisplatin (Sharma et al. 1997, de la Puente-Redondo et al. 2007, Vail et al. 2007), a severely emetogenic drug.

Two studies have specifically evaluated gastrointestinal adverse effects of vincristine and cyclophosphamide in dogs (Mellanby et al. 2002, Tomiyasu et al. 2010). Both studies were retrospective and may have underestimated the frequency of adverse effects. Gastrointestinal adverse effects were reported in 20 to 50% of patients, although most owners were satisfied with their dog's quality of life during treatment (Mellanby et al. 2002, Tomiyasu et al. 2010).

Toxicity may lead to patient morbidity, reduction in drug doses or treatment intensity, or withdrawal of treatment. While vomiting and diarrhoea are easily recognised, nausea can be challenging to diagnose and can manifest as ptalism, inappetence and lethargy. Nausea is therefore potentially under recognised by both owners and veterinarians.

Maropitant citrate (Cerenia™, Zoetis) is a selective neurokinin1 (NK1) receptor antagonist (Benchaoui et al. 2007). It is effective following stimulation of both central and peripheral neurotransmitter pathways by a wide range of emetics including apomorphine (Sedlacek et al. 2008), ipecac (Sedlacek et al. 2008), hydromorphone (Hay Kraus 2013), cisplatin (de la Puente-Redondo et al. 2007, Vail et al. 2007) and doxorubicin (Rau et al. 2010). Maropitant is licensed in the UK for the prevention and treatment of nausea induced by chemotherapy, as well as other conditions causing emesis. The objectives of this prospective, randomised study were to assess the prevalence and severity of gastrointestinal toxicity in dogs receiving vincristine and cyclophosphamide, and to evaluate the efficacy of maropitant in reducing the frequency of these adverse events. It was hypothesised that there would be reduced frequency and severity of gastrointestinal adverse events in the group treated with maropitant.

Materials and methods

Owners of dogs with lymphoma receiving vincristine or cyclophosphamide treatment as part of their chemotherapy protocol between July 2010 and January 2013 were invited to enrol in the study. Dogs with any stage of disease, in any anatomical location other than gastrointestinal were included in the study. Dogs were excluded if they had evidence of gastrointestinal involvement with lymphoma (based on the results of ultrasound, histology or cytology), or if they had vomiting, diarrhoea or inappetence at the time of enrolment. Dogs could be enrolled at any time during their treatment, and data were collected for an individual dog once following vincristine and once following cyclophosphamide treatment. In general, the aim was to enrol patients at the time of their first treatment with both of these drugs. An adverse event after one drug did not preclude the dog from being included in the study following treatment with the other. Dogs could receive other medications deemed clinically necessary by the attending clinician with the exception of alternative anti-emetics.

Maropitant was licensed in the UK for the prevention and treatment of nausea induced by chemotherapy, as well as other conditions causing emesis. The study was carried out in accordance with the University of Liverpool ethical guidelines and all owners gave informed consent.
Dogs were randomised into two groups based on coin toss, performed by an oncology clinician or nurse. Group Y dogs (treatment) received maropitant at a dose of 1 mg/kg subcutaneously immediately following chemotherapy and were discharged from the hospital with 4 days of maropitant to be given orally at home (2 mg/kg PO q24 hours). Group N dogs (non-treatment) received no anti-emetics. Owners of both group Y and group N dogs were advised to contact the hospital at any time following chemotherapy if they felt their dog was exhibiting gastrointestinal adverse effects. If clinically indicated, group N dogs came off study and received treatment with maropitant or another antiemetic at the discretion of the attending clinician, and dogs in group Y received additional anti-emetics as required. Dogs could be withdrawn from the study at any time by the owner or attending clinician. If the dog was hospitalised it was withdrawn from the study as owner recording of adverse events was not possible in these circumstances. The number of dogs recruited to the study was determined by the available funding.

Outcome measures were evaluated using an objective scoring system (Appendix 2) derived from the Veterinary Co-operative Oncology Group Common Terminology Criteria for Adverse Events document (VCOG 2011).

Owners were asked to complete a diary (Appendix 3) using the objective scoring system detailed in a guidance sheet. Briefly, the owner entered a score each day for the occurrence and severity of the following adverse events: lethargy, vomiting, diarrhoea and appetite loss. Diaries were included in the study if owners had completed a minimum of 4 days’ entries at home. Any score greater than 0 was considered an adverse event.

Statistical analysis was performed using a commercial software package (MINITAB V16). Mean and median scores and differences between treatment groups for vomiting, diarrhoea, inappetence, lethargy and total score were assessed. The likelihood of suffering an adverse effect on days 1 to 4 after treatment and differences between days 1 and 4 were also assessed. Dogs were excluded from statistical analysis if the diary was not returned/ completed, if the maropitant treatment was not recorded (NR) or if they were withdrawn from the study. Kruskall-Wallis test was used to assess significance of clinical scores between groups. Fisher’s exact test was used to compare distribution of events between groups. P<0.05 was considered significant.

Results

Vincristine

Eighty-four dogs were enrolled; four were excluded (missing treatment data) and there were 44 dogs in group Y and 36 dogs in group N. The median age of dogs in group Y was 8 (range 3 to 14) years and the median age of dogs in group N was 7 (range 1 to 13) years. There were 18 breeds of dog represented in group Y and there were 6 male, 4 female, 15 male neutered and 19 female neutered dogs. There were 23 breeds of dog represented in group N and there were 6 male, 3 female, 15 male

<table>
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<tr>
<th>Clinical score</th>
<th>Vincristine</th>
<th>No maropitant</th>
<th>P value Kruskal-Wallis test</th>
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<td>Maropitant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of dogs</td>
<td>Number of dogs having adverse effects</td>
<td>Median score</td>
<td>Number of dogs</td>
</tr>
<tr>
<td>Total score</td>
<td>37</td>
<td>26</td>
<td>5 (range 0 to 36: IQR 0 to 13.5)</td>
</tr>
<tr>
<td>Lethargy score</td>
<td>37</td>
<td>23</td>
<td>1 (range 0 to 17: IQR 0 to 5)</td>
</tr>
<tr>
<td>Vomiting score</td>
<td>37</td>
<td>8</td>
<td>0 (range 0 to 6: IQR 0)</td>
</tr>
<tr>
<td>Diarrhoea score</td>
<td>37</td>
<td>13</td>
<td>5 (range 0 to 7: IQR 0 to 1)</td>
</tr>
<tr>
<td>Appetite loss score</td>
<td>36</td>
<td>17</td>
<td>5 (range 0 to 18: IQR 0 to 5.7)</td>
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</table>

IQR Interquartile range
neutered and 12 female neutered dogs. There were no significant differences between the groups with respect to age (P=0.27). The breed distribution was too wide to assess the effect of breed on outcome. As collie breeds are known to be more likely to be sensitive to vincristine the distribution of this breed was assessed and was not significantly different between groups (P=0.8).

In total, 63/82 (77%) completed diaries were included (2/84 were returned blank). Nineteen diaries were not returned or were incomplete and they were excluded from the analysis (6 from group Y, 11 from group N, 2 NR). Owners were no less likely to fail to return or complete the diary if the dog was in the non-treatment group than if the dog was in the treatment group (38/44 dogs versus 25/36 dogs P=0.07).

Five dogs were withdrawn from the study because of adverse effects (one from group Y and four from group N). Dogs were no more likely to be withdrawn from the study if they were in the non-treated group (P=0.12). Two of these dogs did not have completed diaries as they were hospitalised (one from group Y and one from group N). The other three dogs each received a single dose of maropitant and were treated as out-patients. Fifty-eight dogs were included in the final analysis (37 from group neutered and 12 female neutered dogs. There were no significant differences between the groups with respect to age (P=0.27). The breed distribution was too wide to assess the effect of breed on outcome. As collie breeds are known to be more likely to be sensitive to vincristine the distribution of this breed was assessed and was not significantly different between groups (P=0.8).

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![Figure 1](image-url)
Y and 21 from group N). Including these excluded dogs in the data analysis did not significantly change mean or median vomiting or total score for vincristine treated dogs.

In total 40/58 (69%) dogs had a documented adverse event. Of the adverse events, 36 (62%) dogs had at least one episode of lethargy. Fourteen (24%) dogs had at least one episode of vomiting; one dog in group N was reported as having eaten a toy prior to the single vomiting episode and was included in the analysis. Twenty (34%) dogs had at least one episode of diarrhoea. Twenty-five (43%) dogs had at least one episode of reduced appetite; one of the dogs with appetite loss in group Y was reported as always having had a picky appetite by the owner and a second owner did not fill out the appetite loss section of the diary and was excluded from this part of the analysis. There was no significant difference in scores for any parameter between groups (Table 1). The data are summarised in Fig 1.

Ten (17%) owners made use of the box for comments. Concerns reported by owners are summarised in Table 2. There was a trend for scores to decrease slightly by 1 day after treatment. However, there was no significant difference between scores on day 1 and day 4 for all dogs for any variable (data not shown).

Table 2. Comments reported in the questionnaire by owners of vincristine treated dogs

<table>
<thead>
<tr>
<th>Owner’s comment</th>
<th>Number of dogs reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Restlessness</td>
<td>1</td>
</tr>
<tr>
<td>Drinking and urinating more</td>
<td>1</td>
</tr>
<tr>
<td>Polyphagia</td>
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</tr>
<tr>
<td>Loose/soft stool</td>
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</tr>
<tr>
<td>Flatulence</td>
<td>2</td>
</tr>
<tr>
<td>Difficulty squatting</td>
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</tr>
<tr>
<td>Difficulty climbing stairs</td>
<td>1</td>
</tr>
<tr>
<td>Urinary incontinence</td>
<td>3</td>
</tr>
<tr>
<td>Swollen limb</td>
<td>1</td>
</tr>
<tr>
<td>Weepy eye</td>
<td>1</td>
</tr>
<tr>
<td>Forgetfulness</td>
<td>1</td>
</tr>
<tr>
<td>Lameness</td>
<td>1</td>
</tr>
<tr>
<td>Nervousness</td>
<td>1</td>
</tr>
<tr>
<td>Shivering/shaking</td>
<td>3</td>
</tr>
</tbody>
</table>

Cyclophosphamide

Overall, 58 dogs were enrolled and there were 22 dogs in group Y and 30 dogs in group N. In six cases treatment (Y/N) was not recorded. The median age of dogs in group Y was 7 (range 1 to 13) years. The median age of dogs in group N was 7.3 (range 1.5 to 13) years. Thirteen breeds of dog were represented in group Y and there were 2 male, 1 female, 12 male neutered and 7 female neutered dogs. Sixteen breeds of dog were represented in group N and there were 4 male, 3 female, 11 male neutered and 12 female neutered dogs. There were no significant differences between the groups with respect to age (P=0.17).

In total, 48/58 (82%) completed diaries were returned (20 from group Y, 22 from group N, 6 NR), 10 diaries were not returned or were incomplete and excluded from the analysis (2 from group Y, 8 from group N), as were the 6 diaries where treatment was not recorded. Owners were no less likely to fail to return or complete the diary if the dog was in the non-treatment group than if the dog was in the treatment group (22/30 dogs versus 20/22 dogs P=0.16).

No dogs were withdrawn from the cyclophosphamide study due to side effects. In total 34/42 (81%) dogs had a documented adverse event. Of the adverse events 26 (62%) dogs had at least one episode of lethargy, 9 (21%) dogs had at least one episode of vomiting; 15 (36%) dogs had at least one episode of diarrhoea; 15 (36%) dogs had at least one episode of reduced appetite. There was no significant difference in scores for any of the parameters between groups (Table 3). The data are summarised in Fig 2. 14 (30%) owners made use of the box for comments. Concerns reported by owners are summarised in Table 4.

There was no significant difference between scores on day 1 and day 4 for all dogs for any variable (data not shown).
FIG 2. Box and whisker plots showing clinical scores following treatment for vincristine treated dogs receiving maropitant (Y) or not (N). TS Total score, VS Vomiting score, ALS Appetite loss score, DS Diarrhoea score, LS Lethargy score.
in each case) but were mild and self-limiting in the majority of cases. The frequency of adverse events was higher than in previous retrospective studies, most likely because the animals were monitored more closely with a daily, objective recording system. Most of the dogs were receiving prednisolone and other drugs at the time of the study. It is possible that other drugs could have contributed to the noted adverse effects, or that they had comorbidities at the time of data collection. Maropitant is metabolised via cytochrome p450 enzymes in the liver and administration alongside chemotherapeutics has the potential to alter metabolism of the parent drug leading to altered plasma concentrations of the chemotherapeutic agent and its metabolites (Benchaoui et al. 2007).

However, the lack of difference in toxicity scores between treated and untreated groups suggests that if there is altered metabolism, it does not alter toxicity. It remains possible that co-administration of maropitant could increase the risk of gastrointestinal adverse effects by this mechanism, but directly ameliorate these so that no difference was observed.

Appetite loss and lethargy were assessed as signs suggestive of nausea, and neither nausea nor frequency of vomiting was decreased in dogs who received prophylactic maropitant. It is possible that nausea and vomiting associated with vincristine administration are due to effects other than NK1 stimulation. Vincristine administration can cause gastric hypomotility in dogs (Tsukamoto et al. 2011) and may cause gastrointestinal

### Discussion

To the authors’ knowledge, this is the first prospective study to assess the frequency of adverse effects following treatment with vincristine or cyclophosphamide. Adverse effects were common in the dogs studied following treatment with vincristine or cyclophosphamide (>60%
adverse effects via direct damage to mucosal epithelial cells or by stimulation of the vomiting centre or chemoreceptor trigger zone.

Some of the dogs received chemotherapy dose reductions, either due to previous gastrointestinal or haematological toxicity or known breed sensitivities while pending MDR typing. Unfortunately the study design did not permit assessment of the number of dogs receiving dose reductions due to data anonymisation and this may have impacted on observed frequency of adverse effects. However, the median vincristine dose administered to a random selection of patients within the study time was 0.7 mg/m². It is likely that the study patients in general received doses close to the target dose. In addition, the range reported reflects the range of dosages used in general practice.

The questionnaire return rate of 77 to 82% is good and may be because an easy to use objective scoring system with defined end points was used. However, it still highlights limitations of studies of this nature because of owner lack of compliance. Overall the use of a semi-objective scoring system may help to produce repeatability in terms of data recording (Malone et al. 2011).

There was no significant difference in gastrointestinal adverse effects in dogs receiving vincristine or cyclophosphamide with or without maropitant and the results do not support the use of maropitant as routine prophylaxis for dogs receiving vincristine and cyclophosphamide chemotherapy. There was a trend towards dogs receiving maropitant having less diarrhoea than untreated dogs. While not statistically significant this has been reported in a previous study evaluating the efficacy of maropitant when administered with doxorubicin (Rau et al. 2010) and was hypothesised to be due to inhibition of substance P in the gastrointestinal tract.

Limitations of this study include observer bias, as there was no placebo (the study was non-blinded because of funding limitations). Owners may have been inclined to over or under estimate adverse effects depending on their perception of the value of anti-emetics. Additionally, clients may have overestimated lethargy and appetite loss due to monitoring the animals more closely than normal, for the purposes of the study. The study is underpowered, partly due to the availability of funding and partly due to owner reluctance to participate because of fears that their pet may suffer adverse effects if allocated to the non-treated group. There is no way of ensuring that all dogs in the treatment group received the treatment and it is possible that some owners of dogs in group Y failed to administer the medication.

The exclusion of dogs with gastrointestinal signs at the time of treatment likely resulted in sub-stage b dogs being excluded from the study population and the benefit of maropitant in these dogs was therefore not assessed. There is likely to have been a degree of clinician bias when considering the exclusion criteria which may have resulted in fewer patients perceived at higher risk of adverse effects being recruited, increasing the effect of underpowering.

Standard of care in veterinary oncology is maintaining excellent quality of life while achieving the best response to treatment for each individual patient. Chemotherapy adverse effects can result in significant impairment of quality of life and the need for prophylactic management versus the risk of adding significant costs to the treatment regime due to overzealous prophylaxis needs to be assessed carefully for each patient. This is important as many clients elect to stop therapy once a specific financial limit is reached.

On the basis of the results of this study the authors’ standard practice and recommendation is to ask owners of dogs receiving vincristine or cyclophosphamide to keep a supply of maropitant at home and to administer this to their dog at the first indication of any adverse effects consistent with nausea (refusal of a meal or activity, single episode of vomiting). This is likely to be more cost effective for owners and further prospective studies are planned to assess the outcomes of this approach.

Acknowledgements

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Conflict of interest

Professor Blackwood is a member of the Zoetis Animal Cancer Experts (Europe) panel (formerly Pfizer).
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Priester, W. A. & Mantel, N. (1971) Occurrence of tumors in domestic animals - Data from 12 United-States and Canadian colleges of veterinary medicine. *Journal of the National Cancer Institute* 47, 1333


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Appendix 1: Twenty-five week non-continuous CEOP protocol

<table>
<thead>
<tr>
<th>Date</th>
<th>Week</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-asparaginase (400 U/kg or 10,000 U/m² SQ or IM)</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vincristine (0.7 mg/m² IV)</td>
<td>✔️</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide (200 to 250 mg/m² IV or PO)</td>
<td>✔️</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epirubicin (30 mg/m² IV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisolone (reducing dose)</td>
<td>40 mg/m² PO SID</td>
<td>30 mg/m² PO SID</td>
<td>20 mg/m² PO SID</td>
<td>10 mg/m² PO SID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Date
Week 11 13 15 17 19 21 23 25
Vincristine (0.7 mg/m² IV) ✔️ ✔️ ✔️ ✔️ ✔️ ✔️
Cyclophosphamide (250 mg/m² IV or PO) ✔️ ✔️ ✔️ ✔️
Epirubicin (30 mg/mg² IV) ✔️ ✔️ ✔️

PO Per os, SID Once daily ✔️ = Treatment is administered on this week

Appendix 2: Chemotherapy side effects scoring sheet

Please look at the table below which describes possible side effects of chemotherapy. The symptom or side effect is described on the left side of the chart and the severity of the symptom is assigned a score across the top of the chart. If your dog has no side effects, then a score of 0 (zero) should be entered for that heading.

<table>
<thead>
<tr>
<th>Symptom/score</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lethargy</td>
<td>Mild fatigue compared to normal</td>
<td>Moderate fatigue causing some difficulty performing food or toilet activities</td>
<td>Severe effects on normal food and toilet activities</td>
<td>Disabled, needs help to eat and toilet</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Less than three episodes in a day</td>
<td>3 to 5 episodes in 24 hours</td>
<td>More than 5 episodes in 24 hours</td>
<td>Severe</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>More than 2 stools extra a day</td>
<td>2 to 6 extra stools a day but still eating</td>
<td>More than 6 extra stools with normal eating/exercise</td>
<td>Severe requiring hospitalisation</td>
</tr>
<tr>
<td>Appetite loss</td>
<td>Ate normal food voluntarily but did not finish meal</td>
<td>Ate normal food on coaxing</td>
<td>Ate other (e.g. human food, treats) on tempting or coaxing</td>
<td>Did not eat food today</td>
</tr>
</tbody>
</table>

Insert the score that corresponds to the severity of your dog’s side effects into the diary provided. Office Use: Patient Study Number

Appendix 3: Side effects diary

The chart below forms a diary for you to document the side effects after today’s chemotherapy treatment with:

<table>
<thead>
<tr>
<th>Vincristine Vc</th>
<th>Cyclophosphamide Cx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day after chemotherapy</td>
<td>1</td>
</tr>
<tr>
<td>Lethargy</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td></td>
</tr>
<tr>
<td>Appetite loss</td>
<td></td>
</tr>
<tr>
<td>Other (describe below)</td>
<td></td>
</tr>
</tbody>
</table>

For side effects, please use the scoring sheet to grade your pet’s symptoms. Enter the score in the chart above under each of the side effect categories for days 1 to 7 after treatment. If no side effects are seen, enter 0 (zero) in the chart.

Please describe other side effects (if present):
SUMMARY

This review deals with indications for performing haemostasis tests, methodical aspects of blood sampling and handling of sample material, selection of appropriate tests and basic principles of test interpretation. Indications for laboratory evaluation of haemostasis in small animals include spontaneous bleeding or bleeding disproportionate to the degree of trauma, bleeding from multiple sites and diseases which are frequently associated with haemostatic disorders. Patient history and clinical findings can provide clues to the type of haemorrhagic diathesis. Important pre-analytical issues include: prevention of prolonged venous stasis, order of drawing and appropriate filling of the collection tubes and careful mixing of blood with anticoagulant.

Initial screening can be performed by global tests of haemostasis including viscoelastic testing using thrombelastography and rotation thrombelastometry and/or a ‘basic examination profile’ including platelet count, capillary bleeding time (optional in cases of suspected functional platelet disorders) and a combination of the tests prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin (clotting) time (optionally in cases of prolonged PT and APTT). This procedure allows a rational evaluation of the haemostatic system and specific indication for the use of further tests including individual coagulation factor activities, inhibitor activities, activation markers, examination of von Willebrand factor, and platelet function analyses. It is important (1) to use species and method specific reference values for interpretation and (2) to consider the limitations of tests when performed according to the standard test procedure optimised for human sample material (e.g. low sensitivity of PT test for canine and feline samples).

Keywords: Blood coagulation, individual coagulation factors, platelet count, platelet function analysis, preanalytics, screening test

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Introduction

Adequate haemostasis depends on normal structure and function of the blood vascular system, platelet numbers and normal platelet function, and an adequate coagulation system. The vascular component can rarely be diagnosed as the cause of haemostatic disorders, because standardised tests for vascular haemostatic dysfunction do not exist in veterinary medicine. In contrast, haemostatic disorders due to either quantitative and qualitative platelet defects or coagulation system disorders can be defined in detail by numerous specific tests.

A detailed history and a thorough clinical examination can deliver clues toward an underlying haemostatic disorder and its type (Hackner 1995; Herring and McMichael 2012). Bleeding patterns can indicate the cause of haemorrhage diathesis. Mucosal and cutaneous petechiae, purpura and ecchymoses are characteristic findings in disorders of primary - i.e. platelet-derived - haemostasis (thrombocytopenia, thrombocytopathy) (Figure 1) (Botsch et al. 2009; Wondratschek et al. 2010; O’Marra et al. 2011).

In contrast, disorders of blood coagulation (secondary haemostasis) such as haemophilia are associated with larger areas of haemorrhage, e.g. subcutaneous or intramuscular haematomas, body cavity and joint haemorrhages (Mischke 2013) (Figure 2). Bleeding from mucosal surfaces (e.g. epistaxis, oral cavity haemorrhage, haematemesis, haematuria, haematochezia, melaena) can be associated with disorders of primary or secondary haemostasis.

Global tests and/or a basic haemostasis examination profile with screening tests enable a rational assessment of the haemostatic function and in case of a haemostatic disorder, in many cases suggest its type or indicate specific tests necessary for final diagnosis (e.g. factor VIII and IX measurements for haemophilia diagnosis).

Indications for haemostasis testing

Spontaneous bleeding (without detectable cause), bleeding disproportionate to degree of trauma and bleeding from multiple sites are suspicious for haemostatic disorders and, therefore, are among the indications to perform laboratory tests for haemostasis (Table 1).

Sample material

Collection and handling

Haemostasis tests make higher demands on blood sampling techniques than most other parameters in the clinical laboratory (Lippi et al. 2012). For the measurements of platelet counts, K2EDTA blood is required. Blood coagulation tests are performed with plasma prepared from...
blood anti-coagulated with sodium citrate or citrate buffer. For whole blood platelet aggregation studies (with the Multiplate analyser) hirudin-anticoagulated blood shows better results (Kalbantner et al. 2010).

**Sample tubes for citrated blood**

To obtain citrated plasma, prefabricated sample containers are available from various manufacturers. The sample containers are prefilled with an adequate volume of 0.11 mol/l sodium citrate or citrate buffer solution to guarantee a mixture ratio of 9 parts of blood and one part of anticoagulant, when tubes are filled up to the graduation with blood. This results in a ratio between plasma and citrate solution of approximately 4.5 to 1 in a sample with normal haematocrit (e.g. 50 %). Underfilling of the vials by more than 10 % is critical, because human studies demonstrate that this leads to significantly prolonged coagulation times, especially of the activated partial thromboplastin time (APTT) (Reneke et al. 1998; Lippi et al. 2012).

Controlled over- or underfilling, respectively, of the tubes with blood may be indicated to achieve an adequate mixture ratio between the plasma content and citrate solution, when the haematocrit is markedly increased or decreased (e.g. the ratio between plasma and citrate solution is approximately 2.25:1 in a sample with a haematocrit of 75%, whereas overfilling of the tube to approximately 190 % of the declared volume results in an adequate ratio).

**Blood collection**

Blood collection should be carried out, if possible, without raising the vein or performing this only slightly and briefly (< 30 s) to avoid significant activation of the haemostatic and fibrinolytic system (Lippi et al. 2012). A sharp disposable needle with an adequate lumen (e.g. 1.1x30 mm [19G] for dogs) should be used. The first drops of blood should be discarded or used for other sample tubes to avoid contamination of the samples with tissue factor, because this may result in misleading results due to activation of clotting factors and/or platelets. The blood should flow carefully along the wall of the tube. If citrated blood is obtained with a syringe, aspiration should be carefully performed, since high vacuum can lead to activation of the coagulation system. Alternatively, samples for routine tests may be collected via an indwelling intravenous catheter (Maeckelbergh and Acierno 2008), which, in dogs, results in acceptable correlation with values from blood samples collected via direct venipuncture. Use of samples collected via an intravenous catheter to monitor routine coagulation tests in critically ill patients eliminates additional venous trauma and discomfort and may also reduce the volume of blood lost during sample collection (Maeckelbergh and Acierno 2008).

To guarantee intensive mixing of the collected blood with the anticoagulant (sodium citrate), tubes (or syringes) should be swirled carefully while the blood is being collected. After the blood collection is finished, the sample container must be very gently swirled and inverted several times. Haemolysis should be avoided (Lippi et al. 2012).

In vitro addition of haemolysate significantly interferes with coagulation times such as APTT and thrombin time in canine plasma (Moreno and Ginel 1999), although illustrations indicate only limited absolute effects and no clear concentration dependency. In addition, studies on human sample material showed only negligible differences of prothrombin time (PT) and APTT between 50 paired haemolysed and recollected non-haemolysed samples and between in vitro haemolysed samples and non-haemolysed controls (Laga et al. 2006), possibly making it unnecessary to reject haemolysed samples for routine tests.

**Citrated plasma preparation**

Centrifugation of the sample and subsequent immediate removal of the (platelet-poor) citrated plasma supernatant
using a pipette should be performed as quickly as possible, at the latest within 2 hours after blood collection. Immediately before centrifugation for 10 minutes at 1,500–2,000 x g, the sample should be checked for possible clots and coagulated samples must be discarded.

Storage stability, sample transport

Only minimal, mostly non-significant changes in routine coagulation tests (PT, APTT), the vast majority of the coagulation factors, antithrombin activity, and D-dimer concentration occur after storage of normal citrated plasma samples at room or refrigerator temperature for up to 48 hours (Furlanello et al. 2006). After 24 hours, the only significant difference was a reduction of fibrinogen concentration in samples stored at 24°C (mean values: initially 1.95 g/l after 24 hours: 1.87 g/l). These results contrast with another storage study, where mean plasma fibrinogen concentration in normal canine plasma declined from initial mean values of 3.2 g/l to 2.9 g/l and 2.3 g/l, respectively, after storage for 8 or 24 hours at 8°C (Piccione et al. 2010). Canine von Willebrand factor (vWF) in plasma stored at either 4°C or 22°C (and even in blood when stored at 22°C) is also relatively stable for up to 48 hours (Johnstone et al. 1991). Based on these findings, shipment via overnight express of citrated plasma to specialised laboratories seems possible and does not require cooling or freezing. However, the storage stability of coagulation proteins in citrated plasma has to be confirmed for pathological and feline sample material.

In citrated whole blood from 40 dogs showing various diseases stored at room temperature (comment: procedure is not recommended, plasma should be separated as soon as possible, see above), median APTT decreased from 14.2 seconds (baseline) to 12.6 and 12.0 seconds after a storage time of 24 or 48 hours, respectively, possibly indicating pre-activation of the contact phase (Maunder et al. 2012). This artificial shortening of APTT can mask mild factor deficiencies.

Platelet function is less stable and it is generally recommended to perform analyses (see below) within 3–4 hours after sample collection.

Global testing

Global tests assess the overall haemostatic function of a blood sample including the relationship between platelets and the coagulation system. These include simple tests such as the whole blood clotting time or the activated clotting time (ACT) (Bateman et al. 1999) and automatic viscoelastic point-of-care haemostatic assays, which are undergoing a revival (Kol and Borjesson 2010). These methods are easy to perform and can be used as ‘bedside’ tests. Generally, these methods have limited sensitivity with respect to the detection of changes of individual haemostatic components.

Thrombelastography and thrombelastometry

The two currently leading point-of-care haemostatic test systems are the thrombelastography (TEG®) and rotation thrombelastometry (ROTEM®). Blood is collected in a cup heated to 37°C. Either native blood or recalcified citrated blood can serve as sample material, in each case with or without the addition of activating agents (e.g. tissue factor, kaolin). A pin is suspended within the cup connected to a detector system (a torsion wire in the TEG and an optical detector in the ROTEM). The cup and pin are oscillated relative to each other through an angle of 4° 45’. The movement is initiated from either the cup (TEG) or the pin (ROTEM). When fibrin forms between the cup and pin, the transmitted rotation from the cup to pin (TEG) or the impedance of the rotation of the pin (ROTEM) are detected at the pin and registered as a graph. The different parts of the graph reflect different stages of the haemostatic process (clotting time, kinetics of fibrin formation, strength of the clot, and fibrinolysis). The nomenclature is slightly different between TEG and ROTEM (Figure 3a).

The method assesses the viscoelastic properties of clotting whole blood, reflecting interactions between cellular (platelets, erythrocytes, leukocytes) and plasma elements, and also of fibrinolysis under low shear conditions (Kol and Borjesson 2010). Different haemostatic disorders are indicated by characteristic tracings (Figure 3b), whereas the automated numeric analysis of the graph is less important. Thereby, viscoelastic tests give an impression of the total haemostatic potential of a sample. In contrast to clotting tests, the method informs about the strength/stability of the clot and a possible lysis. It seems especially suited for detecting hypercoagulable states (Wiinberg et al. 2008).

Basic examination profile

The basic examination profile for haemostasis usually includes platelet count, PT (syn.: thromboplastin time)
Laboratory evaluation and interpretation of haemostasis in small animals

It may be expanded by thrombin (clotting) time and/or fibrinogen concentration measurements when PT and APTT are prolonged, to further differentiate whether the final stage of blood coagulation (thrombin-fibrinogen interaction, fibrin formation) is affected. Additional measurement of capillary bleeding time is indicated in cases where platelet dysfunction or von Willebrand’s disease are suspected.

Platelet count

The platelet count can be estimated in a peripheral blood smear which, for example, can be used to verify thrombocytopenia measured by an automated blood cell counter. In addition, microscopic evaluation provides valuable information on platelet morphology (size, activation status, aggregates). Each platelet in an oil immersion lens field (1000 x total magnification) represents approximately 15 x 10⁹ platelets/L (McConnell 2000). Thus, for example, a median of more than 10 platelets/oil immersion field represents an adequate platelet count in dogs. Large platelets (shift platelets or macrothrombocytes may be seen in conditions in which there is excessive platelet destruction and a regenerative platelet response with the exception of primary immune-mediated thrombocytopenia (Dircks et al. 2009). They may also be seen in infiltrative diseases of the bone marrow and as a frequent breed variant in Cavalier King Charles Spaniels (Cowan et al. 2004) (Figure 4). Small platelets (microthrombocytes) may be seen in iron deficiency anaemia.

Automated cell counters often provide inaccurate platelet counts in cats because of the small size of red blood cells in cats and the tendency of feline platelets to form aggregates (Tasker et al. 2001) (Figure 5). Spurious thrombocytopenia occurs in Cavalier King Charles Spaniels, caused by the high frequency of large platelets. These dogs usually have a normal haemostatic function, which seems to be best reflected by plateletcrit determined...
Laboratory evaluation and interpretation of haemostasis in small animals

by the IDEXX VetAutoread Hematology Analyzer (IDEXX Laboratories, Westbrook, USA) performing quantitative buffy coat analysis or by the Advia 2120 Haematology system (Siemens Healthcare Diagnostics, Eschborn, Germany) (Tvedten et al. 2008, Tvedten et al. 2012). False counting can also occur if small or large platelets are not detected by the machine because of inappropriate machine settings or calibration. Commercial kits (e.g. ThromboPlus, Sarstedt, Nümbrecht, Germany) simplify visual platelet counting using a counting chamber (Figures 6, 7). Healthy dogs have platelet counts of 150–500 x 10^9/l and healthy cats of 180–550 x 10^9/l. Breed may have an influence (Lawrence et al. 2013) which is currently inappropriately considered. Usually platelet number must decrease below 60 x 10^9/l to cause a significantly increased bleeding risk.

Further tests to verify the cause of thrombocytopenia

Thrombocytopenia is one of the most important causes of haemorrhagic diathesis. Possible mechanisms include reduced production due to bone marrow diseases (e.g. hypoplasia, leukaemia, myelodysplasia) or increased turnover (loss, distribution abnormalities [splenomegaly, septicaemia] or consumption [immune-mediated, disseminated intravascular coagulation/DIC, vasculitis]). Various laboratory tests help to verify the underlying mechanism and disease. Bone marrow examination does not only assess thrombopoietic activity (reference values in adult female dogs: 10.4 ± 2.67 platelet precursors per low power field and in male dogs: 7.84 ± 2.04, P = 0.0008) (Mischke et al. 2002), but may also offer further clues on the underlying disease (Figure 8). Increased mean platelet volume can indicate increased thrombopoietic activity, but may be low in primary immune-mediated thrombocytopenia despite high thrombopoietic activity (Dircks et al. 2009). Reticulated platelets are an alternative parameter of thrombopoietic activity. Flow cytometric detection of platelet-associated antibodies is helpful, but this test does not differentiate between primary and the more frequently occurring secondary immune-mediated thrombocytopenias (Dircks et al. 2009).
Capillary bleeding time

The (capillary) bleeding time is the time until standardised capillary bleeding ceases and is a useful in vivo screening test for function of primary haemostasis. It is indicated in patients with suspected platelet function disorder or von Willebrand’s disease, i.e. in patients with haemorrhagic diathesis with normal platelet count and clotting tests. Furthermore, it is a useful pre-surgical screening test for ruling out defective primary haemostasis in an animal with no contemporary clinical evidence of bleeding.

Capillary bleeding time in dogs is preferably measured in the non-anaesthetised dog in the lateral position (Nolte et al. 1997). This method, according to the experience of the author, superior to the widely used buccal mucosal bleeding time (BMBT) (Jergens et al. 1987) with regard to feasibility, possibility of standardisation, and safety purposes (i.e. possibility to stop bleeding with a bandage in case of a severe haemostatic disorder).

The skin of the lateral side of a front toe is shaved and a hyperaemic agent is applied for 1 minute to the shaved area and wiped off (Figure 9). A sphygmomanometer cuff, which was earlier placed above the antebrachium, is pumped up to apply a pressure of 60 mm Hg. This is performed to increase and standardise blood flow in the puncture area. One minute after applying a pressure of 60 mm Hg, the skin is punctured twice 5 mm apart, with a sterile manual or semiautomatic blood lancet, close to the edge of the horny skin of the pad (Figure 10). The blood is carefully dabbed off every 15 seconds with a gauze pad until the bleeding stops (i.e. until there are no traces of blood noticeable on the swab). The average time from the two punctures is calculated as capillary bleeding time.

The reference values of this method in the dog are 1–2.5 minutes. The reference ranges for the BMBT in dogs are 1.7–4.2 minutes (Jergens et al. 1987) and in cats 1.0–2.4 minutes (Parker et al. 1988). Result depends significantly on the device (Aumann et al. 2013). Prolonged capillary bleeding times can be caused by severe thrombocytopenia, platelet function disorders or von Willebrand’s disease. In patients with normal platelet count, capillary bleeding time serves as a screening test of platelet function disorders and von Willebrand’s disease. Severe von Willebrand’s disease can be associated with extremely prolonged haemorrhage from puncture sites and may require application of a bandage.

Blood coagulation tests (group tests)

Coagulation tests measure the time taken for citrated platelet-poor plasma to clot when coagulation is initiated by the addition of calcium and activating agents.

Blood coagulation tests are usually performed with coagulometric methods, but can also be performed with chromogenic assays. Coagulation times can be measured by manual techniques (tilt tube, hook technique) with visual detection of the time point of clot formation, but these techniques require considerable practice to obtain reliable results. Preferably, semi-automated or automated coagulometers are used, which are based on different electromechanical (e.g. Schnitger and Gross coagulometer based on the hook technique, ball coagulometer) or photometric techniques that detect a change in the intensity...
of light transmission when clot formation occurs. In addition, point-of-care analysers with test cartridges for PT and APTT are available for animals (e.g. VetScan VSpro Coagulation Analyzer; Abaxis Europe, Darmstadt, Germany). A recent study demonstrated an acceptable correlation with conventional PT and APTT assays (Dixon-Jimenez et al. 2013).

In order to test the plasmatic coagulation system rationally, the screening tests PT and APTT are widely used. These tests evaluate the whole plasma coagulation system with the exception of factor XIII-dependent fibrin cross-linking (Figure 11). Each of these ‘group tests’ records several coagulation factors of the extrinsic and common or intrinsic and common pathway, respectively. When PT and aPTT are prolonged, the complementary measurement of the thrombin time, the third group test, is indicated. Measurements of these parameters in canine and feline plasma are typically performed using tests optimised for the determination of human plasma, but species specific features suggest test modifications and/or must be considered when interpreting test results.

**Prothrombin time**
Prothrombin time imitates coagulation activation via the extrinsic pathway and measures the coagulation factors of the extrinsic and common pathway. Clotting is initiated by adding calcium and tissue thromboplastin, which provides tissue factor and substitutes the negatively charged phospholipid surface provided by platelets in vivo. Prothrombin time measured in canine or feline plasma following the manufacturers’ test instructions (‘PT standard test’) is significantly shorter than in human beings and consequently, insensitive for indicating decreased coagulation factor activity. In specialised laboratories, the use of an optimised test (e.g. 100 µl 1:20 diluted sample, 100 µl fibrinogen solution [to guarantee an adequate fibrin clot formation], incubation for 2 minutes, 100 µl Ca-thromboplastin reagent) is preferable to the standard test for use in dogs and cats (Mischke et al. 1996; Mischke and Nolte 1997). Apart from the modified test, commercial PT test modifications such as Hepato Quick or Normotest are suitable screening tests of the extrinsic system in dogs and cats (Mischke 2002).

In general, reagents based on animal-derived thromboplastins seem better activators of animal samples than synthetic/recombinant human thromboplastins. High inter-batch stability indicates that the preparation of batch-specific reference values is not required. Calculation of percentage activity values or PT ratios may minimise differences between different lots of one defined reagent, if an adequate and consistent standard material is available. However, even after converting the result into a prothrombin ratio or into a percentage of normal activity (compared to a reference curve prepared with a canine or feline pooled plasma), it is impossible to get a reagent-independent result (Mischke 2010).

![Figure 11. Classical presentation of the blood coagulation system indicating areas examined by the three group tests, prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT). Abbreviations: F = (coagulation) factor; HMWK = high molecular weight kininogen; PL = phospholipid; Ca = Calcium ions.](image-url)
Prolongation of the PT standard test indicates a severe deficiency in clotting factors, because factors must decrease to less than 30% of normal to cause prolongation of PT, whereas the optimised test can also detect milder factor deficiencies. The PT is frequently prolonged in acquired vitamin K deficiency, liver disease, specific factor deficiencies and DIC. Factor VII deficiency causes prolongation of the PT with no change in APTT and thrombin time. Due to the short half-life of factor VII, the PT is selectively prolonged in the early stages of acquired vitamin K deficiency.

**Activated partial thromboplastin time**

APTT imitates coagulation activation via the contact phase and identifies coagulation factor abnormalities of the intrinsic (high-molecular weight kininogen, prekallikrein, factors XII, XI, IX and VIII) and common pathway (factors X, V, II, and fibrinogen).

Clotting is initiated by adding a surface activator (such as ellagic acid, silica or kaolin), phospholipid (which substitutes the negatively charged phospholipid surface provided by platelets in vivo), and excess calcium. The reagents used in the APTT vary greatly in composition and therefore in sensitivity and specificity. Dilution of the plasma does not increase the sensitivity of this test when using electro-optical detection of fibrin formation (Johnstone 1984).

With sensitive APTT reagents (e.g. Hemos IL SynthAFAX, Instrumentation Laboratory, Bedford, USA), APTT test optimised for human plasma can even detect mild individual factor deficiencies in canine and feline plasma (Mischke 2000). Haemophiliacs usually have prolonged APTTs, dogs with haemophilia A of approx. 1.5 to 2.5 times normal. With less sensitive reagents even significant factor deficiencies to values < 30 % may result in false negative (normal) results. Haemophilic carriers with residual activities of usually 40–60 % factor VIII or IX cannot be detected with sufficient certainty. Optimal APTT ratio for heparin treatment (i.e. corresponding to 0.3 to 0.7 IU/ml) must be assessed by APTT-reagent and heparin-preparation specific calibration.

**Thrombin time (thrombin clotting time)**

Thrombin time measurements are indicated in cases of combined prolongation of PT and APTT to assess, whether the final fibrin generation process is involved in the haemostatic disorder. In addition, thrombin time is used to control heparin therapy.

Thrombin time measures the clotting time of citrated plasma after the addition of exogenous thrombin. The result depends on the availability of an adequate concentration of fibrinogen and possible inhibitors of the thrombin-fibrinogen-interaction (heparin) and fibrin polymerisation (fibrinogen degradation products, abnormal serum proteins). Commercial test kits are suitable to measure thrombin time in dogs and cats. Thrombin time in dogs and cats gives very similar results to humans. The thrombin time is prolonged, if the fibrinogen concentration is less than 0.7 g/l. In samples containing a normal fibrinogen concentration, fibrinogen degradation products cause prolongation at concentrations of ≥ 0.1 mg/ml (Mischke and Wolling 2000). High thrombin concentrations are required to monitor animals receiving therapeutic doses of unfractionated heparin (Mischke and Jacobs 2001).

**Reference values**

The use of animal species-, method-, and reagent-specific reference values is important for the interpretation of results of group tests and also of other haemostasis test procedures. These reference values are best established in the laboratory itself and should be based on a sufficient number of healthy animals of the respective species (at least 40).

**Interpretation of test results of the basic programme**

Combined interpretation of results from platelet count and group tests and, if necessary, capillary bleeding time, often allows a tentative diagnosis to be made, which partially needs confirmation or further differentiation by further tests, especially individual factor analyses (Table 2).

**Further and specific tests**

In individual cases, additional specific tests are necessary to confirm diagnosis or to accompany therapy.

**Blood clotting factors**

Determination of plasma activities of individual clotting factors is important for the diagnosis and monitoring of haemostatic disorders, particularly hereditary individual clotting factor deficiencies such as haemophilia A and B.
Factors II, V, VII, and X are measured with a modified PT test, factors VIII, IX, XI, and XII with a modified APTT. The tests are performed with a diluted sample and addition of a plasma that is deficient in a single individual factor. Therefore, the coagulation time of the test depends on the residual activity of the requested coagulation factor. Coagulometric tests that incorporate human deficient plasma are typically used for the determination of clotting factor activities in dogs and cats. Due to the fact that particularly factor V and VIII:C activities in canine and feline plasma are several times as high as in human plasma, accurate measurement of coagulation factor activities in canine or feline plasma, using human deficient plasma, requires higher sample dilution (i.e. 1:20) than typically used for human plasma (Mischke 2001).

Differences in activities between human and canine or feline plasma and non-parallelism of the standard curves emphasize the necessity of using species-specific standard curves for accurate determination of clotting factor activity. Standard curves are prepared with different dilutions of species-specific pooled plasma whose activity is usually defined as 100 %. Factor activities of 30 % are usually sufficient for a physiological haemostatic function. Haemophilic patients have usually < 10 % of the respective coagulation factor.

The fibrinogen concentration is usually determined in an extended coagulation profile and also with regard to its role as an acute phase reactant, mostly using the functional clotting method according to Clauss (Ameri et al. 2011). Commercially available standard curves for human fibrinogen or human standard material can be used for calibration, because calibration curves prepared with canine or feline specific fibrinogen standards show very similar results. The fibrinogen concentration ranges from 1.0 to 3.0 g/l in normal dogs and cats. Hyperfibrinolysis occurs frequently including animals with DIC. Hypofibrinogenemia occurs primarily because of increased fibrinolysis in DIC with dominant hyperfibrinolysis.

**Inhibitors**

The most important inhibitors of the blood clotting system are antithrombin (AT) and protein C. Antithrombin measurement is particularly necessary in cases of DIC receiving high dose heparin treatment to guarantee an adequate AT level. Heparin mainly functions by enhancing the inhibition of thrombin by AT and, thereby, increases AT consumption. Assessment of AT activity is also useful in animals suspected of having a hypercoagulable state. Common assays for AT activity are based on synthetic factor Xa- or thrombin-dependent chromogenic substrates and well suited for measurement of AT activity in animals (Mandell et al. 1991).

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**Table 2. Combined interpretation of test results from routine tests of haemostatic function and recommended further tests to differentiate between differential diagnoses**

<table>
<thead>
<tr>
<th>Platelet count</th>
<th>Bleeding time</th>
<th>PT</th>
<th>APTT</th>
<th>Thrombin time</th>
<th>Diagnosis / differential diagnosis</th>
<th>Further recommended tests to confirm diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓/↓/↓/↓</td>
<td>n or ↑/↑/↑+</td>
<td>n</td>
<td>n</td>
<td>n or (↓)</td>
<td>- Thrombocytopenia</td>
<td>-</td>
</tr>
<tr>
<td>n</td>
<td>↑/↑/↑+</td>
<td>n</td>
<td>n</td>
<td>n</td>
<td>- vWD</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>- Platelet dysfunction</td>
<td>vWF, Collagen binding activity, Platelet function tests</td>
</tr>
<tr>
<td>n or ↓</td>
<td>n</td>
<td>↑/↑+</td>
<td>↑↑</td>
<td>(↓) or n</td>
<td>- Anticoagulant rodenticide intoxication</td>
<td>Control of therapeutic effect of Vitamin K1</td>
</tr>
<tr>
<td>↓/↓</td>
<td>n</td>
<td>↑/↑</td>
<td>↑↑</td>
<td>↑↑</td>
<td>- DIC (final stage)/hyperfibrinolysis</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>↑</td>
<td>↑</td>
<td>n or (↓)</td>
<td>- DIC, Liver-induced</td>
<td>individual coagulation factors, activation markers, Control of therapeutic effect of Vitamin K1</td>
</tr>
<tr>
<td></td>
<td>n or ↓</td>
<td>n or ↑</td>
<td>↑/↑/↑+</td>
<td>n or (↓)</td>
<td>- Haemophilia, Contact factor deficiencies, DIC, etc.</td>
<td>individual coagulation factors</td>
</tr>
</tbody>
</table>

PT = prothrombin time; APTT = activated partial thromboplastin time; vWD = von Willebrand’s disease; vWF = von Willebrand’s factor; DIC = disseminated intravascular coagulation.

↓/↓/↓/↓ = mild to severe decrease/shortening; (↓) = reactive shortening; ↑/↑/↑+ = mild to severe prolongation; n = normal finding
Canine and feline pool plasmas are ideal standards. Subnormal AT levels (< 75 %) are associated with decreased heparin effectiveness. AT concentrations decrease in DIC. In one study, 85% of dogs (35 of 41) with confirmed DIC had decreased AT levels (Feldman et al. 1981). AT also decreases in hepatic disease owing to decreased synthesis. AT is similar in size to albumin and is lost in protein-losing nephropathies and protein-losing enteropathies.

Activation markers

Plasma markers which are useful to detect an activation of the clotting system in small animals (e.g. thrombosis, DIC) include D-dimer, soluble fibrin and thrombin-antithrombin complex concentrations. Because antibodies against human D-dimers cross react with canine and feline D-dimers, human tests are suitable. Immunoturbidimetric methods are useful reference methods (Boutet et al. 2009). Point of care cartridge tests are available and partly evaluated for canine samples (e.g. NycoCard D-dimer test and NycoCard READER II, Axis-Shield Point-of-Care Division, Oslo, Norway) (Dewhurst et al. 2008).

Healthy dogs have D-dimer concentrations of less than 0.25 µg/ml. Because generation of D-dimers requires actions of thrombin and plasmin, increased D-dimers are markers of intravascular clotting. A negative result more or less rules out thrombosis, but a positive result is relatively unspecific (Nelson and Andreasen 2003). Many DIC scores include increased D-dimer levels.

Von Willebrand’s disease

Von Willebrand diagnostics is indicated especially in animals with prolonged bleeding time that cannot be explained elsewhere (especially by thrombocytopenia). Furthermore, measurement of vWF is indicated in cases with suspected haemorrhagic disorder, which is not reflected by changes in results of platelet count and clotting tests. In most cases, measurements of vWF concentration is adequate. This can be performed with human test kits based on latex agglutination or ELISA test principles due to cross-reactivity of the antibodies. Specific assays (collagen binding assays, electrophoretic multimeric analysis) detect a selective loss of the functionally active large multimers to characterize a Type II von Willebrand’s disease (Burgess and Woods 2008).

Canine pooled plasma serves as the standard. VWF has a wide reference range (50–180 %). It is an acute phase reactant and may be increased in conditions such as strenuous exercise, age, azotaemia, liver disease, parturition and after application of the vasopressin analogue, 1-desamino-8-d-arginine vasopressin (DDAVP).

Platelet function analysis

In cases of prolonged capillary bleeding time despite normal platelet count and exclusion of von Willebrand’s disease, functional platelet tests can provide further information with respect to the underlying cause. Further indications exist in patients suffering from primary diseases frequently associated with platelet functional disorders (e.g. liver disease) or for monitoring and evaluation of anti-platelet drug treatment. Finally, global tests of primary haemostasis such as capillary bleeding time and automated platelet function analysis are indicated for the estimation of the overall function of primary haemostasis in thrombocytopenic dogs requiring surgery. To characterise functional platelet disorders, a wide variety of platelet function tests is available which, unfortunately, often lack standardisation. Apart from the ‘global tests’ cited above these include, for example, platelet aggregation, flow cytometric analyses, thromboelastography (esp. ‘TEG platelet mapping’ which is performed with special reagents) (Brainard et al. 2010) and thrombelastometry as well as mean platelet component. Whereas primary functional abnormalities of platelets (e.g. Glanzmann’s thrombasthenia; signalling defects, storage pool deficiency, and Chediak-Higashi syndrome) are extremely rare in animals (Boudreaux 2008) and in humans, acquired platelet dysfunctions are more frequent and occur associated with many diseases and disorders (e.g. hepatopathy, monoclonal gammopathy) and after application of anti-platelet agents (aspirin, clopidogrel) and other drugs (NSAIDs, dextran, antibiotics).

Platelet function analyser (PFA 100)

The platelet function analyser PFA-100 (Siemens Healthcare Diagnostics, Germany) has been evaluated and is suitable for use in dogs (Callan and Giger 2001; Mischke and Keidel 2003). The instrument comprises a microprocessor-controlled instrument and disposable test cartridges containing a biologically active membrane. The instrument aspirates a blood volume under constant vacuum from a
Laboratory evaluation and interpretation of haemostasis in small animals

EJCAP 25(2), Summer 2015  P 53

sample reservoir in the test cartridge through a capillary and a microscopic aperture cut into the membrane at the end of the capillary (Figure 12). The membrane is coated with collagen and adenosine diphosphate (collagen/ADP cartridge) or collagen and epinephrine (adrenaline) (collagen/epinephrine cartridge). The presence of these biochemical stimuli, and the high shear rates developed under standardized flow conditions, result in platelet attachment, activation, aggregation, and slow formation of a stable platelet plug at the aperture. The ‘closure time’ is reported by the analyser.

The analysis should be performed between 30 min and 2 h after collection of the citrate buffer anti-coagulated blood which is the standard sample material (Mischke and Keidel 2002). It is advisable to use only one position, e.g. position A, of the two alternative measuring positions of the analyser, because spontaneous sedimentation can lead to artificial results in the second cartridge inserted into position B.

Reference values are as follows: collagen/ADP cartridge: 52–86 seconds (Callan and Giger 2001), 53–98 seconds (Mischke and Keidel 2003); collagen/epinephrine cartridge: 97–225 seconds (Callan and Giger 2001), 92–>300 seconds (Mischke and Keidel 2003).

The method can be regarded as a global screening test of primary haemostasis. Apart from platelet function, the result is significantly influenced by platelet count and haematocrit (Callan and Giger 2001; Mischke and Keidel 2003). Unfortunately, even slightly subnormal haematocrit values are associated with a prolongation of the closure time, which limits the clinical applicability of the analyser. Because measurements using the collagen/epinephrine cartridge exceed the upper limit of the measuring range already in a small percentage of healthy dogs (Mischke and Keidel 2003), this cartridge type is less suitable for tests of primary platelet function in dogs.

Platelet aggregation

The measurement of platelet aggregation is one of the most important in vitro methods of assessing platelet function. There are two main measurement principles of in vitro platelet aggregation following the addition of agonists. The turbidimetric method measures the increase of light transmission in platelet-rich plasma during aggregation, while impedance aggregometry measures the increase in the electrical resistance in blood resulting from platelet accumulation (Dyszkiewicz-Korpanty et al. 2005).

Although the turbidimetric method has the advantage that the platelet count can be adjusted to a defined value, the preparation of platelet-rich plasma requires additional equipment and time, large sample volumes, and experienced personnel. This preparation procedure can also alter the quality of the sample through loss of large platelets with increased or decreased reactivity. Impedance aggregometry on whole blood can more effectively evaluate lipaemic samples. It may also better reflect in vivo platelet functionality, given that platelets can interact with other blood cells. The electrical probes of the impedance method create an artificial surface and therefore may better mimic in vivo platelet aggregation which typically occurs on injured or inflamed vascular surfaces.

The novel impedance aggregometer Multiplate Analyser (Roche Diagnostics GmbH, Mannheim, Germany) seems to be well suited for use in dogs (Kalbantner et al. 2010). The test can be performed as recommended by the manufacturer for human sample material (300 µL isotonic sodium chloride solution, 300 µL of hirudin-antiocoagulated blood, 3 min incubation and stirring at 37°C, addition of 20 µL agonist solution) (Kalbantner et al. 2010). The

Figure 12. Schematic diagram of the cross-sectional view of the functional unit of the platelet function analyser PFA-100.
impedance change caused by the adhesion and aggregation of platelets on the electrodes is continuously detected.

To achieve optimal sensitivity, minimum effective (threshold) concentrations of agonists should be used which reliably induce high maximum aggregation values in healthy animals. In a canine study using the Born method, the following threshold concentrations (final test concentrations) were established: 25 µmol/l adenosine diphosphate (ADP), 10 µg/ml collagen and 1 IU/ml thrombin (Mischke and Schulze 2004). In an actual study using the Multiplate instrument, the following optimal agonist concentrations (sourced from Roche Diagnostics GmbH) were defined: 10 µmol/l ADP, 5 µg/ml collagen, and 1 mmol/l arachidonic acid (Kalbantner et al. 2010). Specific reference values should be established according to the method and laboratory used. Platelet aggregation is particularly suitable to detect decreased platelet function. Because low agonist concentrations are associated with a wide range of results, it is almost impossible to detect hyperaggregability in individual patients. Platelet counts influence the result.

**Conflict of interest statement**

The author of this paper does not have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

**References**


Practical bone marrow cytology in the dog and cat

Mathios E. Mylonakis¹, Aristodimos Hatzis

SUMMARY

In companion animal medicine, bone marrow (BM) aspiration cytology is a cost-effective diagnostic tool, which provides excellent morphological detail of cells and infectious agents. The major indications for BM aspiration include: unexplained clinical manifestations (e.g. fever, weight loss), persistent haematological or biochemical abnormalities (e.g. anaemia, leukocytosis), diagnosis and/or staging of malignancies (e.g. lymphoma, mast-cell tumour), diagnosis of important infectious diseases such as leishmaniasis (Leishmania infantum) and canine monocytic ehrlichiosis (Ehrlichia canis) and evaluation of canine iron stores. Complications associated with BM aspiration are rare and the equipment and supplies required minimal, which accounts for the popularity of this procedure in the clinical setting compared to BM core biopsy. This review focuses on the technical details pertaining to the collection of BM aspirate material, including the usual anatomic sites for sampling, the equipment required, the preparation of the animal, the aspiration technique and BM material processing for good quality slide preparation. The description of the cellular population normally anticipated in the BM of the dog and cat and the systematic approach to evaluating and interpreting the BM cytological findings are also highlighted. In the last part of this review, the current classification guidelines for the canine and feline BM malignancies are briefly outlined.

Keywords: Bone marrow, cat, cytology, cytological evaluation, dog

Indications for bone marrow aspiration

After birth and in health, haematopoiesis is normally confined to BM, which occupies the medullary cavities of trabecular bone (Figure 1). Bone marrow comprises red marrow that contains an abundance of haematopoietic cells (active marrow) and yellow marrow, predominantly adipose tissue. The distribution and relative proportion of red and yellow marrow are age dependent. After birth, the BM cavity is completely occupied by red marrow. With ageing, haematopoietic marrow is gradually replaced by yellow marrow so that during adulthood, red marrow is mainly confined to the vertebrae, skull, ribs, sternum, pelvis and the proximal humeri and femora. This trend, however, can change and with increased demand and appropriate stimuli haematopoietic tissue can expand. Bone marrow aspiration cytology is a cost effective diagnostic tool, providing excellent morphological detail of cells and infectious agents (Grindem et al. 2002). Though with BM core biopsy, the architectural pattern of tissue is better reflected and a range of features including BM cellularity, the presence of myelofibrosis and focal

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or neoplastic lesions are better appreciated (Raskin and Messick 2012), cellular detail is more difficult to assess; the latter along with a slightly more elaborate procedure for obtaining the sample and longer processing times might contribute to the fact that, in the clinical setting, BM aspirations are more frequently performed compared to core biopsies in the dog and cat (Grindem et al. 2002).

Indications for BM examination include haematological abnormalities that cannot be investigated by other means, investigation, staging and management of haematologic and other neoplasms (e.g. lymphoma, mast cell tumours, solid tumours), evaluation of unexplained clinical manifestations (e.g. fever of undetermined origin, weight loss, lameness), evaluation of a patient that does not respond to treatment after initial diagnosis (e.g. failure of response to therapy in a patient diagnosed with immune-mediated thrombocytopenia), response to therapy for patients with haemolymphoid neoplasms, diagnosis of diseases such as leishmaniasis (Leishmania infantum) and canine monocytic ehrlichiosis (Ehrlichia canis) and evaluation of iron stores in the dog (e.g. differentiation of iron deficiency anaemia from anaemia of inflammation) (Foucar 2001, Mylonakis et al. 2003, Mylonakis et al. 2004, Saridomichelakis et al. 2005, Raskin and Messick 2012).

Several variables should be taken into account before making the decision to perform a BM examination (Table 1). In conjunction with the clinical history and other laboratory findings, these include the age of the animal, the presence of single or multiple haematological abnormalities, the presence of single or multi-lineage dysplasia.

**Table 1: Decision-making variables for bone marrow (BM) examination in dogs and cats with haematologic abnormalities**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>• BM examination is not necessary in a young animal with neutropenia and anaemia, and laboratory tests supportive of parvovirus infection</td>
</tr>
</tbody>
</table>
| Single or multi-lineage aberrations | • Usually single abnormalities do not necessitate BM examination. Multiple abnormalities, especially if morphologic atypia is present, are strong indications for BM examination  
• BM examination is indicated in the majority of pancytopenic patients |
| Anaemia                | • BM examination is not indicated if underlying cause is inferred by the history, clinical examination or peripheral blood examination |
| Erythrocytosis         | • BM examination not required for secondary or relative erythrocytosis that can be explained by the clinical condition (e.g. hyperadrenocorticism) |
| Neutropenia            | • Isolated neutropenia, especially with morphological evidence of toxic changes in the blood smear examination, usually does not require BM examination  
• Marked chronic neutropenia, especially with absence of toxic morphology and no obvious underlying cause probably requires BM examination |
| Neutrophilia           | • Neutrophilia with toxic changes and obvious underlying aetiology usually does not require BM examination  
• Marked chronic neutrophilia, with appreciable dysplasia indicates the need for BM examination |
| Thrombocytopenia       | • Isolated thrombocytopenia may or (more commonly) may not require BM examination |
| Blasts                 | • BM examination is always indicated |
| Other abnormal cells   | • May or may not require BM examination |
| Single-multilineage dysplasia | • Multilineage dysplasia always indicates BM examination for possible myelodysplasia or leukaemia (in practice occasionally omitted in FeLV -positive cats). |

*From Foucar 2001 (modified)*
Practical bone marrow cytology in the dog and cat

EJCAP 25(2), Summer 2015  P 59

dysplasia or the detection of blast or other abnormal cells in peripheral blood. Marrow aspiration is redundant if the underlying cause of the abnormality is inferred or known from the history (e.g. a chemotherapy session preceding the development of neutropenia), the physical examination (e.g. melaena in an anaemic animal) or the blood smear evaluation (e.g. regeneration and spherocytosis in an anaemic dog, Babesia spp. trophozoites in a dog with fever) (Harvey 2012a). On the contrary, the presence of blast cells or pancytopenia, almost always necessitate BM examination. In any case of uncertainty, the clinician is advised to seek the assistance of a clinical pathologist or a specialized haematopathologist. Bleeding tendency is not a contraindication for BM aspiration if a superficial site is sampled and proper haemostatic measures are applied (Mylonakis et al. 2004).

Overall, BM aspiration is a very safe procedure with rarely appearing complications. Post-sampling tissue injury, haemorrhage, neuropathy or infection may occur, but they are avoided if adherence to technical details is exercised. The collection of BM from the sternum or the ribs bears the risk of inadvertently penetrating the thoracic cavity and lacerating intra-thoracic organs, especially if material is obtained from small-sized dogs (Harvey 2012a).

Sites, equipment and technique for BM aspiration

The most suitable BM aspiration sites in large sized dogs include the iliac crest and proximal humerus (greater tubercle), while in small-sized dogs and cats the humerus and the proximal femur (greater trochanter) are the most accessible (Raskin and Messick 2012). Alternatively, it was recently shown in healthy anaesthetized Beagles that sternal BM aspiration with a hypodermic needle was safe, providing cytological samples of equivalent quality to those from humerus or ilium (Defarges et al. 2013). Historically, aspiration of the proximal rib with special aspiration needles (Silverman-type) has also been found to provide cytological samples of high quality in the dog, although the technique is more risky and labour-intensive compared to the iliac crest and sternal sampling (Penny and Carlisle, 1970). Anecdotally, aspiration of the costochondral junction of the rib with a hypodermic needle in medium to large-sized dogs may also be a safe and practical technique for obtaining cytological material. A combined technique has been recently described and evaluated in adult dog cadavers, in which aspiration precedes core biopsy but both procedures are done with the same needle and at the same site (Reeder et al. 2013). Although there was no difference in cellularity, megakaryocyte count, myeloid/erythroid ratio (M/E ratio), iron stores, or the overall diagnostic quality, marrow core length was shorter, haemorrhage artefact was more common and the failure rate in obtaining diagnostic material was higher using the combined technique compared to the standard direct core biopsy method (Reeder et al. 2013).

Post-mortem BM aspiration should be performed within 30 minutes from the animal’s death, as cellular degeneration (especially of the myeloid lineage) begins rapidly after death, and the overall quality of the aspirate tends to be poor (Harvey 2012a, Grindem et al. 2014). Ideally, in a planned euthanasia, BM aspiration should be performed prior to the administration of the euthanasia solution (Harvey 2012a). If a delay is anticipated until post-mortem aspiration, the cadaver is placed under refrigeration but not frozen (Raskin and Messick 2012).

Figure 2: Equipment and supplies for bone marrow (BM) aspiration: aspiration needle (15-gauge, Illinois-type), 2% lidocaine solution, 10-ml syringe rinsed with 0.15 ml of 2-3% EDTA solution, microscope slides, scalpel blade No. 11, EDTA tube for BM storage, sterile gloves, antiseptic soap preparation.

Equipment and supplies for BM aspiration are minimal (Figure 2). Mild to moderate pain is experienced during the BM aspiration procedure (Guillot et al. 2011); therefore, sedation (e.g. butorphanol [0.2 mg/kg] and acetylpromazine [40-50 μg/kg] in one syringe, intramuscularly) may be required in uncooperative animals.* In the majority of clinical canine and feline cases, local anaesthesia (infiltration of subcutaneous and subperiosteal tissues with lidocaine 2% [0.1 ml/kg]) normally suffices.

* Editor’s remark: Please note that in some countries it may be considered unethical to perform this procedure without the use of general anaesthetic
Practical bone marrow cytology in the dog and cat

EJCAP 25(2), Summer 2015  P 60

or coverslip. The latter technique offers the advantage of allowing the clinician to aspirate a larger volume of sample increasing the likelihood of a successful aspiration procedure, since it helps decrease the effect of blood contamination during the selection and smearing process. Once several smears have been prepared, they are subject to rapid air-drying, and stained with a Romanowsky-type stain such as Giemsa, Wright-Giemsa and Diff-Quik (Mylonakis et al. 2005), usually requiring longer staining times compared with regular blood smears (usually 2 staining cycles for automated stainers). Additional stains, such as Pearls’ stain for iron, Periodic acid–Schiff (PaS) for fungi, can be performed on an individual basis. Smears containing adequate BM material will have deep blue-staining material on them. Submission to a diagnostic laboratory should ideally include numerous stained and unstained, unfixed slides.

Normal marrow cell population

The recognition of the cells normally appearing in the BM and their expected reference ranges is essential for the proper cytological evaluation of BM in diseased dogs and cats.

Erythrocytic lineage

The consecutive developmental stages of the erythroid (erythroid) cells include the rubriblasts, prorubricytes, rubricytes (basophilic and polychromatophilic), metarubricytes, and the polychromatophilic and mature

BM aspiration sample processing

BM aspiration samples should be processed as quickly as possible after collection, ideally within 2 hours; for short-term storage (up to 8 hours), they may be kept at room temperature or be refrigerated (Raskin and Messick 2012). Several smear preparation techniques have been described (Mylonakis et al. 2005, Harvey 2012a). The most commonly used is the squash technique: one drop of blood is expelled onto a glass slide, the latter is tilted to allow drainage of excessive blood and the slide-attached spicules are squashed by another perpendicularly-placed slide. Alternatively, BM aspirate material is expelled into a Petri dish and several spicules are picked with a plastic pipette, transferred to glass slides and squashed by a slide

Figure 3: Canine bone marrow aspirate: various developmental stages of the erythroid series are seen, including rubriblasts (rb), prorubricytes (prc), basophilic (br) and polychromatophilic (pr) rubricytes and metarubricytes (mr). A few band neutrophils (bn) and metamyelocytes (mm) are also present (Giemsa, x1000).
Practical bone marrow cytology in the dog and cat

EJCAP 25(2), Summer 2015  P 61

erythrocytes (Figures 3-5) (Harvey 2012a). The rubriblasts have an almost perfectly round nucleus, with a fine chromatin pattern and one or two visible nucleoli. The cytoplasm is intensely basophilic and this stage has the highest nucleus-to-cytoplasm (N:C) ratio of the erythroid precursors. The prorubricytes usually have no visible nucleoli, a slightly less basophilic cytoplasm and a lower N:C ratio compared to rubriblasts. The basophilic rubricytes are smaller than the prorubricytes, have a blue cytoplasm, lower N:C ratio and a very heterogeneous chromatin pattern (dark and light areas). The polychromatophilic rubricytes have reddish-blue cytoplasm, are slightly smaller than basophilic rubricytes and have a more condensed nucleus. The metarubricytes feature a very pyknotic nucleus and a cytoplasm that is polychromatophilic or orthochromic (red). The polychromatophilic erythrocytes have lost their nucleus and they mostly represent reticulocytes (stained with supravital stains), eventually forming mature erythrocytes. Normally, rubriblasts account for less than 5% of all erythroid cells in the BM (blast pool), the aggregate of prorubricytes and rubricytes for the 65-75% of all erythroid cells (proliferative pool) and metarubricytes for 20-30% of all erythroid cells (maturation-storage pool) (Grindem et al. 2014).

Granulocytic and monocytic lineages

The consecutive developmental stages of the granulocytic cells include the myeloblasts, the progranulocytes, the myelocytes, the metamyelocytes, the bands and the mature neutrophils (segmented neutrophils or segmenters) (Figure 6). The myeloblast, the myelocyte and the metamyelocyte stages of the granulocytic series cannot reliably be differentiated from monocytes (Grindem et al. 2014). The myeloblasts are large round cells (overall, larger than rubriblasts but smaller than megakaryoblasts) with a round to oval nucleus with fine chromatin and one or more visible nucleoli. The cytoplasm is less basophilic than that of rubriblasts and this stage has the highest nucleus-to-cytoplasm (N:C) ratio of the series. Cytoplasmic granules are not usually observed, but primary (non-specific) granules tend to form in late myeloblasts and therefore a few (<15) magenta-staining granules may be seen in the erythrocytes (Figures 3-5) (Harvey 2012a).

The myeloblasts are large round cells (overall, larger than rubriblasts but smaller than megakaryoblasts) with a round to oval nucleus with fine chromatin and one or more visible nucleoli. The cytoplasm is less basophilic than that of rubriblasts and this stage has the highest nucleus-to-cytoplasm (N:C) ratio of the series. Cytoplasmic granules are not usually observed, but primary (non-specific) granules tend to form in late myeloblasts and therefore a few (<15) magenta-staining granules may be seen in the erythrocytes (Figures 3-5) (Harvey 2012a).
cytoplasm (Harvey 2012a). The progranulocytes usually have no visible nucleoli and the cytoplasm is typically packed with several primary magenta staining granules, while the N:C ratio is lower compared to myeloblasts. The myelocytes are smaller than progranulocytes and have a round nucleus without visible nucleoli. These cells are devoid of primary granules, but secondary granules appear at this stage. They are not visible in neutrophils due to their neutral staining properties, but are easily seen in eosinophils and basophils. The metamyelocytes feature a kidney shaped nucleus, with indentations extending between 25% and 75% of the nuclear width and cytoplasmic characteristics similar to those of myelocytes. The band cells have a curved, rod-shaped nucleus with parallel sides and no area with a diameter less than half the diameter of any other nuclear area. The segmented granulocytes (mature neutrophils, eosinophils or basophils) demonstrate a nucleus with two or more lobes and areas with intense constrictions.

Normally, myeloblasts account for less than 5% of all myeloid cells (blast pool), the progranulocytes plus myelocytes for 15% of all myeloid cells (proliferative pool) and the aggregate of metamyelocytes, bands and segmenters for 80-85% of all myeloid cells (maturation-storage pool) (Grindem et al. 2014).

The monocytic series includes the monoblasts, promonocytes and monocytes and accounts for a small percentage of the BM cells. Monoblasts cannot be differentiated from myeloblasts by light microscopy, although they tend to have a more irregular nucleus, while promonocytes are similar to neutrophilic myelocytes and metamyelocytes. Monocytes are identical to those seen in the blood (Grindem et al. 2014).

The megakaryocytic series includes the megakaryoblasts, promegakaryocytes, and megakaryocytes (Figure 7). Megakaryoblasts are characterized by a single nucleus and deeply basophilic cytoplasm, and they are hardly differentiated from other blast cells. With maturation, megakaryoblasts undergo endoreduplicaton resulting in the formation of very large multinucleated cells. The ploidy level of megakaryocytes ranges from 4N (tetraploidy) to 32 N, with the majority of the cells present as 16N (Bain et al. 2010). Promegakaryocytes are defined as clearly larger cells compared to the erythrocytic or myelocytic precursors that display 2-4 distinct nuclei. Megakaryocytes are very large cells (50-200 μm in diameter) featuring a multilobulated nucleus. Based on their cytoplasmic characteristics they can be further classified into three distinct stages of maturation. Group 1 cells display strong cytoplasmic basophilia and a very high N:C ratio. Group 2 megakaryocytes display less intense staining basophilic cytoplasm, azurophilic granulation and a lower N:C ratio. Finally, Group 3 megakaryocytes feature abundant pale blue staining cytoplasm and numerous azurophilic granules. Overall, the majority of megakaryocytes (≥70-80%) in BM aspirates are mature (Queisser et al., 1971, Mylonakis et al. 2005).

Lymphocytes and plasma cells

Lymphocytes share a common precursor with myeloid cells. The lymphocytes appearing in the BM are identical to those in the blood. Small lymphocytes predominate, but occasional medium-sized or large-sized lymphocytes are seen. In the majority of dogs and cats, lymphocyte percentages do not exceed 5-10% of nucleated cells; however, as many as 15-20% lymphocytes have been noticed in healthy dogs and especially cats (Grindem et al. 2002).

Plasma cells (Figure 4) are normally larger than small lymphocytes and feature a round eccentric nucleus and densely basophilic cytoplasm. A clear cytoplasmic area adjacent to the nucleus representing the Golgi apparatus, further typifies plasma cells. Plasma cells containing round structures (Russell bodies) are called Mott cells. Both lymphocytes and plasma cells are unevenly distributed in the BM, and their percentages may substantially differ between different areas, but overall, plasma cells do not exceed 2% of BM cells in dogs and cats (Grindem et al. 2014).

Miscellaneous cells

Macrophages do not normally exceed 2% of cells in the BM of dogs and cats. They usually display cytoplasmic vacuolation and may contain phagocytized material such as nuclear debris, haemosiderin and less frequently erythrocytes or leukocytes. Osteoclasts (Figure 7) and osteoblasts are occasionally seen in BM aspiration smears especially in young growing animals. Osteoclasts are giant cells with separate multiple nuclei that may be confused with megakaryocytes; however, the latter have fused nuclei. Osteoblasts are reminiscent of large plasma
Practical bone marrow cytology in the dog and cat

EJCAP 25(2), Summer 2015  P 63

cells, having an eccentric nucleus and foamy basophilic cytoplasm. Mast cells lacking morphological atypia are very rarely seen in the BM of healthy dogs accounting for no more than one per 1,000 nucleated cells (Mylonakis et al. 2006). Vascular and connective tissue cells are uncommonly seen and tend to become more apparent in aplastic BM when the precursors of the other blood cells are depleted. Adipocytes are almost always present in BM aspirates. Mitotic cells, not always identifiable, may account for 2% of nucleated cells in the BM (Harvey 2012a).

Evaluation and interpretation of BM cytology

The BM cytological examination should always be performed in the context of haematological findings from a complete blood cell count and peripheral blood smear morphologic examination performed simultaneously or within hours of BM aspiration. A minimum of 3-4 smears should be examined as the cellularity can differ between the spicules or infiltration by a neoplastic population of cells can be patchy. The outcome of the aspiration procedure itself may offer some clues towards the overall BM quality. As a norm, easily obtained material indicates a normocellular or hypercellular BM, while repeated failure to obtain sufficient material (‘dry taps’) may be due to poor technique, hypocellular or fibrotic marrow, or a densely-packed marrow cavity (Grindem et al. 2014). A step-by-step cytological evaluation of BM is outlined in Table 2.

Low-power microscopy

The evaluation starts on the low power (x10 objective) for the assessment of smear quality (including the selection of

Figure 7: Megakaryocytic developmental stages in bone marrow aspirate smears. A: megakaryoblast (mkb) with single nucleolus, B: promegakaryocyte (pmkc) with four nuclei, C: basophilic megakaryocyte with multiple fused nuclei and an intracytoplasmic band neutrophil (bn), D: mature megakaryocyte with a cytoplasmic band neutrophil (bn) and two presumptive rubricytes (r). Inset of figure 7D: a multinucleated osteoclast is visualized (Giemsa, x1000).
areas suitable for high power microscopy), BM cellularity, megalakaryopoietic activity and the semiquantitation of iron stores. High quality smears have numerous well spread out BM spicules and several monolayers of intact, well stained cells. The cellularity of BM is evaluated by estimating the proportion of cells to adipose tissue in the flecks (Figures 8 and 9). It is an age-dependent variable with young animals having cellularity as high as 75% and aged individuals as low as 25% in health. Cellularity varies among smears and between flecks; therefore, several smears and flecks should be examined to reach a reasonably accurate estimation. Hypocellular BM is usually seen in aplastic disorders (e.g. myelosuppressive canine monocytic ehrlichiosis, viral feline leukaemia, oestrogen toxicity) or in myelofibrosis (Weiss 2003). A hypercellular BM indicates either erythroid or myeloid hyperplasia, myeloid, lymphoid or metastatic

<table>
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<tr>
<th>BM Feature</th>
<th>Specific observation or comment</th>
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<tr>
<td><strong>Low power (x 10 objective) light microscopy</strong></td>
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<tr>
<td>Quality of smear</td>
<td>• Adequacy of spicules</td>
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<td>• Monolayers with intact, well-stained cells</td>
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<td>• Selection of fields for high-power microscopy</td>
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<td>BM cellularity</td>
<td>• Age-dependent variable</td>
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<td>Megakaryocytic lineage</td>
<td>• Quantification</td>
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<td>• Sequence and maturation of developmental stages</td>
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<td></td>
<td>• Dysplasia</td>
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<td>Iron stores</td>
<td>• Applicable to dogs only</td>
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<td><strong>High power (x 100 objective) light microscopy</strong></td>
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<td>Myeloid/erythroid (M/E) ratio</td>
<td>• 500-cell differential or subjective differential count</td>
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<td>Erythrocytic lineage</td>
<td>• Sequence and maturation of developmental stages</td>
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<td>Percentage of blast cells</td>
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<td>• Leishmania spp, Ehrlichia spp, etc</td>
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<td>• Non-haematopoietic neoplastic cells</td>
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*From: Raskin and Messick 2012 (modified)*

Figure 8: Increased bone marrow cellularity in a dog with acute (non myelosuppressive) monocytic ehrlichiosis (*Ehrlichia canis*). Several megalakaryocytes (mkc) are scattered in the field (Giemsa, x100).
neoplasms, or myelodysplasia (Grindem et al. 2014). Quantitative assessment of megakaryopoiesis is largely subjective, due to the relatively low numbers and uneven distribution of platelet precursors in the BM smears, being more abundant within or near the BM particles or the smear edges (Grindem et al. 2014). Published normal canine megakaryocyte numbers vary considerably, mainly due to differences in smear preparation technique and in the way they are quantified (Mylonakis et al. 2005). Overall, 2–7 cells per spicule, or 2-4 per low-power field in squash preparations, represent a likely normal megakaryocyte count (Mylonakis et al. 2005, Silva et al. 2012, Grindem et al. 2014). More than 10-20 cells per low-power field may suggest megakaryocytic hyperplasia (Grindem et al. 2014), indicating a regenerative thrombocytopenia, usually due to increased destruction (e.g. immune-mediated thrombocytopenia) or utilization (e.g. disseminated intravascular coagulation) of platelets, reactive thrombocytosis (e.g. iron deficiency) or essential thrombocythaemia. When the percentage of mature megakaryocytes is less than 50%, a regenerative response or a maturation arrest is inferred (Grindem et al. 2014). Megakaryocytic hypoplasia is usually observed in the context of generalized BM suppression (Mylonakis et al. 2004), but uncommonly, selective aplasia of the megakaryocytic series may be seen, presumably due to immune-mediated mechanisms (Lachowicz et al. 2004). Engulfed neutrophils or other blood cells may temporarily be found superimposed on the cytoplasm of mature megakaryocytes, a phenomenon called emperipolesis (Figure 7). The significance of this finding in the dog and cat is currently unclear. Dysplastic changes of megakaryocytes (dysmegakaryocytopoiesis) include dwarf megakaryocytes (small megakaryocytes with one or two condensed nuclei and mature staining cytoplasm), large but hypolobulated or hyperlobulated megakaryocytes, and increased numbers of promegakaryocytes. Dysplasia may occur in primary (neoplastic) or secondary (non neoplastic) myelodysplastic syndromes mainly due to drugs, immune-mediated disease and various haematopoietic neoplasms (Harvey 2012b).

Stainable iron (hemosiderin) may appear within macrophages or extracellularly as grey to black aggregates in routinely stained cytological smears (Figure 10). Reliable assessment of BM iron stores necessitates the use of Prussian blue stain. Unlike dogs, healthy cats lack stainable iron in the BM. As a rule, diminished iron stores in the dog suggest iron deficiency, while increased stores may be associated with haemolytic anaemia, anaemia of chronic disease or increased age (Mylonakis et al. 2010, Harvey 2012a).

High power microscopy

Unlike megakaryocytic cells, a thorough morphologic examination of the remaining cell lineages, and the estimation of the differential counts and myeloid/erythroid ratio (M/E) require the use of x50 or x100 objectives. The M/E ratio (also referred to as granulocytic/erythroid ratio) is calculated by identifying 500 cells (originating equally from the marrow flecks and inter-fleck areas) as granulocytes (including segmenters) or nucleated erythrocytes (Moritz et al. 2010). In the clinical setting, subjective estimation of M/E ratio usually suffices. In the majority of healthy animals, the M/E ranges from 0.5 to 3 (Bienzle 2012, Harvey 2012a, Raskin and Messick 2012, Grindem et al. 2014). The interpretation of the M/E ratio should be made in conjunction with marrow cellularity
(Grindem et al. 2002).

In healthy dogs and cats, all stages of erythrocytic series are represented in the normal proportions, with the vast majority of cells (>80%) being in the mature stages (rubricytes and metarubricytes).

Isolated erythrocytic hyperplasia implies that the M/E ratio is decreased, the BM cellularity is normal or increased and the neutrophil count in the blood is normal and may be effective (increasing haematocrit) or ineffective (Harvey 2012b). Effective hyperplasia usually occurs in haemorrhagic or haemolytic anaemias (Figure 5), while ineffective hyperplasia may commonly appear in chronic iron deficiency anaemia and in non-regenerative immune-mediated haemolytic anaemia (Stokol et al. 2000).

Isolated erythrocytic hypoplasia denotes that the M/E ratio is increased, the BM cellularity is normal or decreased and the neutrophil count in the blood is normal or decreased (Harvey 2012b). When the M/E ratio exceeds 75, pure red cell aplasia or selective erythrocytic aplasia is defined (Harvey 2012b). Acquired erythrocytic hypoplasia or aplasia is usually the result of an immune-mediated response that may entirely inhibit the production of erythrocytes or result in a maturation arrest at any stage of the maturation process (Stokol et al. 2000, Harvey 2012b). It may also be associated with myeloid neoplasia, FeLV-infected cats and in a subset of animals given recombinant human erythropoietin (Mills 2012).

Dysplastic changes of erythrocytic cells (dyserthropoiesis) include increased numbers of rubriblasts, megaloblastic cells, multinucleated cells, abnormal nuclear shapes or fragmentation (Figure 5), nuclear and cytoplasmic asynchrony, maturation arrest and siderotic inclusions (Harvey 2012b). Dysplasia may occur in primary (neoplastic) or secondary (non-neoplastic) myelodysplastic syndromes due to drugs, various haematopoietic neoplasms, FeLV-infected cats, and in immune-mediated haemolytic anaemia (Harvey 2012b).

In healthy dogs and cats, all stages of myelocytic series are represented in normal proportions, with the vast majority of cells (>80%) being in the mature stages (metamyelocytes, bands and segmenters). Myelocytic (most commonly neutrophilic) hyperplasia implies that the M/E ratio is increased, the BM cellularity is normal or increased and the haematocrit is normal or increased, and may be effective (accompanying blood neutrophilia) or ineffective (persistent blood neutropenia) (Harvey 2012b). Effective hyperplasia (Figure 6) usually occurs in bacterial infections, immune-mediated diseases, paraneoplastic syndromes, early oestrogen toxicity and chronic myeloid leukaemia (Lucroy and Medewell, 1999, Lucroy and Medewell 2001). The percentage of myeloblasts is not usually increased out of proportion, with the exception of chronic myeloid leukaemia, in which myeloblasts may be up to 20% of all nucleated cells (Harvey 2012b).

Ineffective neutrophilic hyperplasia may commonly appear in myelodysplastic syndromes, acute myeloid leukemias and FeLV and FIV infections in cats. Granulocytic (most commonly neutrophilic) hypoplasia denotes that the M/E ratio is decreased, the BM cellularity is normal or decreased and the haematocrit is normal (Harvey 2012b).

Effective neutrophilic hypoplasia may commonly appear in myelodysplastic syndromes, acute myeloid leukemias and FeLV and FIV infections in cats. Granulocytic hypoplasia (most commonly neutrophilic) hypoplasia may commonly appear in chronic iron deficiency anaemia and in non-regenerative immune-mediated haemolytic anaemia (Stokol et al. 2000).

Isolated erythrocytic hyperplasia implies that the M/E ratio is decreased, the BM cellularity is normal or increased and the neutrophil count in the blood is normal and may be effective (increasing haematocrit) or ineffective (Harvey 2012b). Effective hyperplasia usually occurs in haemorrhagic or haemolytic anaemias (Figure 5), while ineffective hyperplasia may commonly appear in chronic iron deficiency anaemia and in non-regenerative immune-mediated haemolytic anaemia (Stokol et al. 2000).

Increased numbers of small lymphocytes, sometimes combined with plasmacytosis, may occur in immune-mediated haemolytic anaemia in the dog and cat and pure red cell aplasia, thymoma, cholangiohepatitis and pyrexia of unknown origin in the cat (Weiss 2005), in animals with chronic lymphocytic leukaemia or small cell lymphoma, in which mature-appearing lymphocytes exceed 30% of nucleated cells (Figures 11, a and B) (Workman and Vernau 2003). Increased numbers of medium-sized to large lymphocytes imply acute lymphoblastic leukaemia or stage V metastatic lymphoma (Figure 12) (Harvey 2012b).

Benign causes of increased percentages of plasma cells include infectious diseases including canine monocytic ehrlichiosis (particularly in the chronic phase of the disease), leishmaniasis (Figure 13) and feline infectious peritonitis and frequently immune-mediated haemolytic anaemia (Mylonakis et al. 2006, Harvey 2012a). Neoplastic proliferation of plasma cells, demonstrating or not morphologic atypia, defines multiple myeloma (Figure 14) (Weiss 2006a).
Figure 11: A) Canine chronic lymphocytic leukaemia. Small lymphocytes (sl) account for more than 30% of all nucleated cells (Giemsa, x1000). B) Feline small cell lymphoma: small lymphocytes efface the bone marrow (modified Wright-Giemsa, x500).

Figure 12: Canine acute lymphocytic leukaemia/large cell lymphoma. Large atypical lymphocytes with prominent nucleoli and increased mitotic rate efface the bone marrow (modified Wright-Giemsa, x 200).

Figure 13: Bone marrow smear from a dog with leishmaniasis (Leishmania infantum). Several macrophages with cytoplasmic amastigotes of L. infantum (M-Li) are seen. Small lymphocytes (sl) and plasma cells (pc) are also variably present (Giemsa, x1000).

Figure 14: Bone marrow smear from a dog with multiple myeloma: the predominant cell population consists of plasma cells (pc) with eccentrically placed nuclei and basophilic cytoplasm (Giemsa, x1000).

Figure 15: Neoplastic mast cells (arrows) showing no morphologic atypia, infiltrating canine bone marrow. Note the clustering of the cells. No circulating mast cells were identified in the examination of a peripheral blood film and buffy-coat of this patient (modified Wright-Giemsa, x500).
Abundant mast cells, especially when they present atypia and tend to form aggregates are strongly suggestive of metastatic mast cell tumour especially in the cat (O’Keefe et al., 1987) (Figure 15). Mast cell hyperplasia may occur in BM hypoplasia or aplasia of various aetiologies (e.g. myelosuppressive canine monocytic ehrlichiosis) (Mylonakis et al. 2006).

Macrophages may be increased in BM necrosis, granulomatous inflammation or histiocytic sarcoma (Weiss 2006a, Harvey 2012a). Infectious agents may be seen in the cytoplasm of macrophages (e.g. L. infantum, E. canis, Histoplasma capsulatum (Figure 16), Mycobacterium spp., Cytauxzoon felis) (Mylonakis et al. 2003, Saridomichelakis et al. 2005, Harvey 2012b).

Phagocytosis of blood cells and their precursors is rare in the BM of healthy animals and generally involves only mature erythrocytes (Harvey 2012b). Frequent BM erythrophagocytes may occur in immune-mediated haemolytic anaemias, in erythrocytic parasites (e.g. Babesia sp., haemoplasmosis), haematopoietic neoplasms, after blood transfusion, and in histiocytic neoplasia, including the haemophagocytic histiocytosis (Mylonakis et al. 2012), while idiopathic cases have also been reported (Weiss 2007).

**Bone marrow neoplasms**

Primary BM malignancies include acute myeloid leukaemias (aML) (i.e. neoplasms arising from the erythrocytic, myelocytic and megakaryocytic series), myeloproliferative neoplasms (MPN) (formerly termed chronic myeloid leukaemias), primary myelodysplastic syndromes (MDS), acute and chronic lymphoid leukaemias and multiple myeloma (McManus 2005, Juoperi et al. 2011, Harvey 2012b). Common metastatic marrow neoplasms include lymphoma, mast cell tumour, histiocytic sarcoma and various carcinomas (McManus 2005, Raskin and Messick 2012). The presence of blast cells in the BM in excess of 20% of all nucleated cells (aNC) is strongly suggestive of acute myeloid leukaemia (Figures 17 and 18), if an
exaggerated hyperplastic response has been ruled out (Harvey 2012b, Raskin and Messick 2012, Grindem et al. 2014). According to the classification established by the American Society for Veterinary Clinical Pathology Animal Leukemia Study Group, canine and feline acute myeloid leukaemias include aML-M1 (myeloblastic leukaemia without maturation), aML-M2 (myeloblastic leukaemia with maturation), aML-M4 (myelomonocytic leukaemia), aMLM5 (monocytic leukaemia), aML-M6 (erythroleukaemia) or aML-M6Er (erythremic myelosis), aML-M7 (megakaryocytic leukaemia) and acute undifferentiated leukaemia (Jain et al., 1991, McManus 2005). Blast cells less than 20% of aNC indicate MPN or primary MDS (McManus 2005). Myeloproliferative neoplasms include chronic myeloid leukaemia, eosinophilic leukaemia, basophilic leukaemia, primary erythrocytosis and essential thrombocythemia (Harvey 2012b). In MDS more than 10% of the cells in one or more BM cell lineages demonstrate dysplastic changes (Weiss and Aird 2001, Weiss 2006b, Harvey 2012b). In dogs and cats MDS have been classified in three subtypes, namely MDS with erythroid predominance (MDS-Er, M/E<1, blasts<20% aNC), myelodysplastic syndrome refractory cytopenias (MDS-RC, M/E>1, blasts<5% aNC) and myelodysplastic syndrome-excess blasts (MDS-EB, M/E>1, blasts 5-19% aNC) (McManus 2005, Harvey 2012b). Dogs and cats with MDS are prone to develop aML or lymphoid neoplasms (Harvey 2012b). Mature lymphocytes or lymphoblasts in excess of 30% of aNC support the diagnosis of chronic lymphocytic or acute lymphoblastic leukaemia, respectively, provided that a metastatic lymphoma has been ruled out (Workman and Vernau 2003). The diagnosis of multiple myeloma (Figure 14) is highly suggestive if 15% or more plasma cells occur in the BM (Harvey 2012b). Large cohesive cell clusters are usually associated with metastatic mast cell tumour, histiocytic sarcoma (Figure 19) or carcinomas (Grindem et al. 2014).

**Conclusion**

Bone marrow aspiration cytology is a cost-effective diagnostic tool, which provides excellent morphological detail of cells and infectious agents. Complications associated with BM aspiration are rare and the equipment required minimal, which accounts for the popularity of this procedure in the clinical setting of companion animal medicine.

**Conflict of interest statement**

The authors declare no conflict of interest.

**References**


