Original Article

Prospective study evaluating the incidence of bacteraemia and bacteruria in afebrile and febrile neutropaenic dogs undergoing chemotherapy

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Abstract

The purpose of this prospective study was to determine the incidence and character of bacteraemia and bacteruria in afebrile and febrile neutropaenic dogs undergoing cytotoxic chemotherapy. Fifty-five neutropaenic dogs presenting to the University of Wisconsin Veterinary Medical Teaching Hospital were enrolled for a total of 57 neutropaenic episodes. The overall incidence of bacteraemia was 12.3% ($n = 7/57$). Three afebrile dogs and four febrile dogs were bacteraemic; this difference was not significant ($P = 0.6968$). The overall incidence of bacteruria was 7.5% ($n = 4/53$). Two afebrile dogs and two febrile dogs were bacteruric; this difference was not significant ($P = 1.0$). Of the positive blood cultures, the majority of organisms cultured ($n = 6/7$) were gram-positive organisms with one gram-negative organism. Three of the positive blood cultures showed *in vitro* resistance to more than one antimicrobial agent. Clinical parameters (age, body weight, heart rate, rectal temperature, neutrophil count, haematocrit and platelet count) were not predictive of bacteraemia. The results of this study were not sufficient to justify the use of blood cultures as a first-line diagnostic test for neutropaenic patients. Blood cultures may have utility in individual case management for a minority of patients in guiding antibiotic choice in the case of resistant bacterial infections. Blood cultures may serve as a tool for antimicrobial de-escalation, although further study is needed.

Keywords: bacteraemia, bacteruria, neutropaenic, dogs, chemotherapy.

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Introduction

Chemotherapy-induced myelosuppression is an expected side effect of cytotoxic chemotherapy in veterinary patients; however, the majority of patients will remain afebrile without significant morbidity (Vail 2009; Gustafson & Page 2013). The incidence of febrile neutropaenia (FN) following chemotherapy in veterinary patients is unknown but likely low (Vail 2009; Gustafson & Page 2013), particularly when compared to human oncology patients. It is estimated that FN occurs at least once during the course of treatment in 10–50% of human patients with solid tumours and in >80% of patients with haematological malignancies (Klastersky 2004). This apparent discrepancy in the rate of FN between veterinary and human patients is likely related to the reduced intensity of chemotherapy protocols administered to veterinary patients, reflecting the different goals of treatment, with a greater emphasis on quality of life for veterinary patients (Vail 2009).

Blood cultures are routinely performed in human patients with FN secondary to chemotherapy prior to starting broad-spectrum antibiotics (de Naurois *et al.* 2010). Blood is sampled from two sites, including any indwelling intravenous (IV) catheters (de Naurois *et al.* 2010). Bacteraemia occurs in 20–30% of human patients with FN (Viscoli *et al.* 1994, 2006; Kern *et al.* 1999; Klastersky *et al.* 2007; Horasan *et al.* 2011), and is often associated with prolonged and profound neutropaenia (<100 neutrophils/μL) (Kern *et al.* 1999). The presence of bacteraemia in FN patients is associated with higher morbidity and mortality rates in patients with solid tumours and in

patients with haematological malignancies compared to FN patients without bacteraemia (Klastersky *et al.* 2007). In a large prospective, multi-institutional study, the mortality rate was 3% in non-bacteraemic patients as opposed to 10% in bacteraemic human patients (Klastersky *et al.* 2007).

The most common type of bacteria cultured from bacteraemic human patients with FN has changed over time and varies based on geographical location as well as regional microbiological resistance patterns. There has been a shift in the United States from a predominance of gram-negative organisms in the 1960's and 1970's to gram-positive organisms in the 1980's and onwards (Viscoli *et al.* 1994; Elting *et al.* 1997; Zinner 1999; Klastersky *et al.* 2007; Horasan *et al.* 2011; Villafuerte-Gutierrez *et al.* 2014). It is theorized that this increase in gram-positive organisms may be related to the increased use of indwelling IV catheters that become colonized with gram-positive skin flora bacteria and the administration of prophylactic antibiotics (Zinner 1999; Ramphal 2004).

Although the majority of human patients with FN do not have a clinical infection or bacteraemia, empirical antibiotic therapy within 1–2 h of presentation is recommended (Freifeld *et al.* 2011). Treatment of neutropaenic veterinary patients is not standardized; however, afebrile neutropaenic dogs are rarely hospitalized as they are often asymptomatic and the risk of acquiring a nosocomial infection is believed to outweigh the possible benefit of hospitalization (Vail 2009). Neutropaenic dogs with <1000 neutrophils/μL that are clinically well are prescribed a 5–7-day course of an oral broad-spectrum antibiotic that spares the anaerobic gastrointestinal flora, such as trimethoprim-sulfa or a fluoroquinolone (Vail 2009). Conversely, hospitalization and empiric broad-spectrum IV antibiotic therapy are commonly recommended for febrile neutropaenic dogs, as they are clinically ill and at risk of developing sepsis (Gustafson & Page 2013).

The majority of dogs treated for FN improve with supportive care and survive to discharge (Sorenmo *et al.* 2010; Britton *et al.* 2014). In an evaluation of 70 dogs with FN, Britton *et al.* reported a mortality rate of 8.5%, which included one dog that received an overdose of carboplatin chemotherapy and another that was euthanized due to disease progression and poor prognosis (Britton *et al.* 2014). The incidence of bacteraemia in veterinary patients remains unknown, as blood cultures are not routinely obtained. Britton *et al.* found evidence of clinical infections in 25.7% of dogs with FN, with pneumonia (50%), urinary tract infections (27.8%) and skin infections (16.7%) being the most common sources of infection; only one of the two dogs with blood cultures had confirmed bacteraemia (Britton *et al.* 2014).

Similarly, the incidence of drug-resistant bacteraemia in veterinary neutropaenic patients is unknown. Multi-drug–resistant organisms, which in the strictest sense, are defined as possessing *in vitro* resistance to more than one antimicrobial agent, are a significant public health threat (Magiorakos *et al.* 2012). In a recent consensus statement from the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) (http://ecdc.europa.eu/en/activities/diseaseprogrammes/ARHAI/Pages/public_consultation_clinical_microbiology_infection_article.aspx), a standardized definition for multi-drug–resistant organisms was proposed as non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories (Magiorakos *et al.* 2012). In veterinary medicine there currently is not an official consensus for the definition of multi-drug–resistant organisms.

The incidence and characterization of bacteraemia cultured from veterinary patients with chemotherapy-induced neutropaenia would provide useful information to improve initial treatments for patients on an emergency basis, and to provide prognostic information for clinicians and owners. The primary goals of this prospective observational study were to describe the incidence of bacteraemia and bacteruria in dogs undergoing chemotherapy that develop neutropaenia, and to compare the incidence of bacteraemia and bacteruria between febrile and afebrile groups. We hypothesized that there would be a higher incidence of positive blood and urine cultures in febrile than afebrile patients. Secondary objectives were to characterize the types and resistance patterns of bacteria present in culture-positive dogs, and

to evaluate for clinical markers predictive of bacteraemia that could provide useful information to clinicians in the initial triage of neutropaenic patients.

Materials and methods

Animals

The study was approved by the Institutional Animal Care and Use Committee.

Client-owned dogs that presented to the University of Wisconsin Veterinary Medical Teaching Hospital (UW-VMTH) from May 2011 to May 2015 that were neutropaenic following chemotherapy administration were prospectively enrolled in this study. Neutropaenia was defined as <2000 neutrophils/μL on a complete blood count (CBC) performed by the VMTH Clinical Pathology Laboratory. To be included in the study, dogs must have received chemotherapy for treatment of naturally occurring neoplasia or other medical condition requiring the administration of chemotherapy, such as meningoencephalitis of unknown aetiology (MUE). A CBC was recommended at 7 days following administration of chemotherapy, with the exception of carboplatin, for which a CBC was recommended at 10–14 days following administration given the delayed nadir associated with carboplatin chemotherapy (Vail 2009). Owner informed consent was required before patient enrolment. Dogs could be enrolled in the study more than once.

Upon enrolment, dogs were divided into two groups based on the rectal temperature at presentation. The febrile group included dogs with a rectal temperature of ≥102.5°F, and the afebrile group included dogs with a rectal temperature of ≤102.4°F. If the dog was lacking clinical signs of illness (e.g. lethargy, vomiting, diarrhoea, hyporexia) just before study enrolment, and there was historical evidence of excitement-related hyperthermia on prior non-neutropaenic (>2000 cells/μL) examinations at the VMTH, a cut-off rectal temperature of ≤103.0°F was used instead of ≤102.4°F for inclusion in the afebrile group.

The medical records were reviewed for breed, age, weight, sex, type of neoplasia or medical condition for which the dog was receiving chemotherapy, chemotherapy protocol, including the agent and dose most recently received, the length of time since administration, CBC results and historical antibiotic use. Owners were questioned as to the dogs' clinical signs at home since the time of chemotherapy administration. Abnormal clinical signs, such as general malaise, lethargy, hyporexia, anorexia, vomiting and diarrhoea, were assumed to be chemotherapy-related if observed within 3–7 days of chemotherapy administration for gastrointestinal signs, and within 4–10 days for neutropaenia-related signs (Vail 2009). If the dog was hospitalized after documentation of neutropaenia, the length of hospital stay and treatments administered were recorded. If the dog was discharged after documentation of neutropaenia, information regarding oral antibiotics prescribed, when appropriate, was recorded. If the dog died or was euthanized while hospitalized or shortly after discharge, the death was considered to be neutropaenia-related unless another cause was identified.

Sample collection

While wearing sterile gloves, blood was collected aseptically and simultaneously (Cockerill *et al.* 2004; Kirn & Weinstein 2013) from two aseptically prepared venous sites for aerobic and anaerobic blood cultures. Venipuncture from jugular veins was not performed in dogs with thrombocytopenia (<175 000 cells/μL).

Dogs with anaemia (<25%) were not enrolled, and blood volume collected for culture was adjusted for weight (<5 kg, 6 mL blood from each site; 5–14 kg, 8–10 mL blood from each site; >15 kg, 16–20 mL blood from each site). The blood collected from each site was placed into paired aerobic and anaerobic blood culture media with antibiotic binding resin. One aerobic and one anaerobic blood culture bottle was filled from each collection site resulting in a total of four collection bottles. Before injection of collected blood, the top of each blood culture media bottle was cleaned with 70% isopropyl alcohol. New sterile needles were used for each culture bottle injection. Blood was sterilely injected first into the anaerobic collection bottle taking care to avoid

introduction of air. The blood was dispersed in the culture media by gently inverting the samples. The blood samples were processed for isolation and characterization of aerobic and anaerobic bacteria and antibiotic sensitivity in the VMTH Clinical Pathology Laboratory. Antimicrobial minimum inhibitory concentrations (MIC) were determined for gram-positive isolates using the Sensititre® antimicrobial susceptibility system (Trek Diagnostics Systems, Westlake, OH), according to manufacturer's instructions. MICs were determined for gram-negative isolates using the Vitek 2 Compact antimicrobial susceptibility system (bioMérieux, Inc, Durham, NC), according to manufacturer's instructions. Quality control was performed for both assays according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (Clinical and Laboratory Standards Institute, 2013, 2016). Quality control organisms included *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. MIC results were interpreted according to CLSI standards criteria.

A dog was considered bacteraemic if the same bacterium was cultured from both venipuncture sites or if there was significant growth of a bacterium from one venipuncture site, and the bacterium was not considered to be part of the animals' normal skin flora. Positive culture samples were considered to be contamination if only positive in one of four culture bottles, and if consistent with normal skin flora. Positive culture samples with common human skin flora were also deemed to be contaminants.

Dogs had urine sampled for routine urinalysis and aerobic bacterial culture. Baseline urinalysis prior to neutropaenic episode and study enrolment was not required. If the platelet count was within normal limits and if there was no history of urogenital neoplasia, urine was preferably obtained by cystocentesis or catheterization in male dogs. Thrombocytopenic dogs or those with previously diagnosed urogenital neoplasia had urine obtained via a midstream-voided sample. Alternatively, based on clinician preference, urine could be collected by a midstream-voided sample. Urine was

submitted to the VMTH Clinical Pathology Laboratory for routine urinalysis and aerobic culture and antibiotic sensitivity. Microscopic examination of the urine sediment was performed at $40\times$ to detect haemturia (>5 red blood cells per high-power field), pyuria (>5 white blood cells per high-power field) and bacteruria (presence of bacteria). The threshold for a true-positive urine culture was >1000 CFU/mL for cystocentesis samples and >100 000 CFU/mL for catheterized and midstream-voided samples (Carter *et al.* 1978; Comer & Ling 1981).

Statistics

Descriptive statistics were used to describe the groups; afebrile vs. febrile dogs, and bacteraemic vs. non-bacteraemic dogs. Data were evaluated for normality. Continuous variables were compared with a Mann–Whitney U-test and the results were reported as median (range), for afebrile vs. febrile dogs, and for bacteraemic vs. non-bacteraemic dogs. Categorical variables were compared with a chi-squared test or Fisher exact test (if expected value in a cell was <5) to analyse differences between afebrile and febrile dogs, and bacteraemic and non-bacteraemic dogs. Online statistical software programs were used for all calculations (Website for Statistical Computation, VassarStats: http://vassarstats.net/index.html). Values of $P < 0.05$ were considered significant for all analyses.

Results

Due to difficulty in enrolling febrile neutropaenic patients, and no significant difference between groups in preliminary calculations, data collection was discontinued after 30 afebrile and 27 febrile patients were enrolled. Fifty-five neutropaenic patients were enrolled for a total of 57 neutropaenic episodes. Two patients were enrolled twice, one in each group. Of the 30 afebrile dogs, four had rectal temperatures between 102.5°F and 103.0°F with a history of similarly elevated temperatures when neutrophil counts had been within normal limits. All four dogs were reported to be feeling well at home

without clinical signs of illness and were described as excited and active when examined.

Patient characteristics

The distribution of patient age and body weight was not significantly different between febrile and afebrile groups (Table 1). In the afebrile group there were 16 spayed females, 3 intact males and 11 castrated males. In the febrile group, there was 1 intact female, 10 spayed females and 16 castrated males. The distribution of sex was not significantly different between groups ($P = 0.0664$). Within the afebrile group, there were 7 golden retrievers, 3 Labrador retrievers, 16 other purebred dogs and 4 mixed breed dogs. In the febrile group, there were 2 golden retrievers, 1 Labrador retriever, 14 other purebreds and 10 mixed breed dogs. The distribution of dog breeds was not significantly different between groups ($P = 0.1272$).

The most common neoplasm was lymphoma ($n = 28$). Within the afebrile group, half of the dogs ($n = 15$) had lymphoma. Most of the dogs with lymphoma had large-cell multi-centric lymphoma ($n = 12$); however, two dogs had hepatosplenic lymphoma and one dog was diagnosed with stage V lymphoma or acute lymphoblastic leukaemia (ALL). Five dogs had mast cell tumours and three had osteosarcoma. Two dogs each had histiocytic sarcoma and high-grade soft-tissue sarcomas. One dog each had a mixed sarcoma of the left tibia and stifle, multiple myeloma and prostatic carcinoma.

Similarly, in the febrile group, close to half of the dogs had lymphoma ($n = 13$). Of the dogs diagnosed with lymphoma, the majority had multi-centric large-cell lymphoma ($n = 11$); however, one dog had small-cell multi-centric lymphoma and one dog had stage V lymphoma or ALL. Five dogs had mast cell tumours. One dog each had anal sac adenocarcinoma, anaplastic sarcoma of the liver and spleen, axial osteosarcoma (zygomatic arch), axillary sarcoma with a history of appendicular osteosarcoma, subungal melanoma, metastatic seminoma and prostatic carcinoma. One dog was being treated for a metastatic carcinoma of the left axillary lymph node, with a prior history of a removed mammary mass on which histopathology had not been performed.

Twenty-two of the afebrile dogs (73.3%) had gross disease (partial response, stable disease or progressive disease) at the time of treatment for neutropaenia, and eight (26.7%) were in a complete clinical remission or had presumed microscopic disease. Eighteen of the febrile dogs with neoplasia (69%) had gross disease (partial response, stable disease or progressive disease) at the time of treatment for neutropaenia, whereas eight dogs (31%) were in a complete clinical remission or had presumed microscopic disease. One dog in the febrile group was receiving chemotherapy for suspected meningoencephalomyelitis of unknown aetiology (MUE) after showing only a partial response to corticosteroids.

When the two groups are combined, vinblastine ($n = 11$), vincristine ($n = 11$, one dog received concurrent vincristine and L-asparaginase) and oral or IV cyclophosphamide ($n = 11$) were the most commonly administered chemotherapies prior to the neutropaenic episodes. Within the febrile group, the most commonly administered chemotherapy was

Table 1. Presenting clinical data in afebrile and febrile patients. Data are presented as median and range

Variable	Afebrile patients ($n = 30$)	Febrile patients ($n = 27$)	P-value
Age	8.7 years (4.1–13)	9.5 years (4.3–15)	0.2811
Body weight	24 kg (7.7–60)	24 kg (4.0–41)	0.9715
Rectal temperature	102°F (100–103)	104°F (103–106)	<0.0001
Heart rate	109 bpm (80–160)	136 bpm (75–190)	0.0002
Neutrophil count	1129/μL (0–1910)	100/μL (0–1856)	<0.0001
PCV	39% (25–51)	36% (24–50)	0.4573
Platelet count	219 000/μL (29 000–660 000)	84 000/μL (11 000–385 000)	0.0094
Days hospitalized	0 days (0–3)	2 days (0–5)	<0.0001
Days since last chemotherapy	7 days (3–21)	7 days (4–15)	0.6271

CCNU ($n = 7$, one dog received concurrent CCNU and L-asparaginase) as opposed to the afebrile group in which only one dog received CCNU prior to the neutropaenic event. The specific chemotherapy that caused the neutropaenic episode was a new chemotherapy agent for 14 of the dogs (46.7%) in the afebrile group and for 20 of the dogs (74%) in the febrile group; the difference between afebrile and febrile groups was not significant when comparing dogs with lymphoma ($P = 0.0671$) or when comparing dogs with solid tumours ($P = 0.461$). Data regarding neoplasms, remission status and chemotherapy administered are summarized in Table 2.

In the 2 weeks prior to study enrolment six dogs in the afebrile and three dogs in the febrile groups had received antibiotics for other infections or conditions. In the afebrile group, four of the six dogs were receiving antibiotics at the time of trial enrolment, including one dog that had been administered a cefovecin injection 7 days before enrolment. In the febrile group, one of the three dogs was receiving antibiotics at the time of trial enrolment. All dogs that received antibiotics within 2 weeks prior to study enrolment had negative blood and urine cultures. The recent use of antibiotics between the groups was not significant ($P = 0.4761$).

Clinical presentation

Twelve dogs in the afebrile group (40%), and 26 dogs in the febrile group (96.3%) were reported to

Table 2. Summary of malignancy type, remission status, and chemotherapy received prior to neutropaenia

Variable	Afebrile patients		Febrile patients		*P*-value
	Number ($n = 30$)	Percent	Number ($n = 27$)	Percent	
Tumour Type					0.7879
Lymphoma	15	50.0%	13	48.1%	
Solid tumour	15	50.0%	13	48.1%	
MUE	0		1	3.7%	
Remission status					
Lymphoma					1.0
Clinical remission	5	33.3%	5	38.5%	
Gross disease	10	66.7%	8	61.5%	
Solid Tumour					1.0
Clinical remission	3	20%	3	23.1%	
Gross disease	12	80%	10	76.9%	
New chemotherapy					
Lymphoma					0.0671
Yes	6	40%	10	76.9%	
No	9	60%	3	23.1%	
Solid tumour					0.461
Yes	8	53.3%	9	69.2%	
No	7	46.7%	4	30.8%	
Chemotherapy agent					
Carboplatin	4	13.30%	5	18.50%	
CCNU	1	3.30%	6	22.20%	
CCNU + L-asparaginase	0	0%	1	3.70%	
Cyclophosphamide	7	23.30%	4	14.80%	
Doxorubicin	1	3.30%	1	3.70%	
Doxorubicin + Toceranib Phosphate	1	3.30%	1	3.70%	
Mitoxantrone	0	0%	1	3.70%	
Vinblastine	7	23.30%	4	14.80%	
Vincristine	7	23.30%	3	11.10%	
Vincristine + L-asparaginase	0	0%	1	3.70%	
Vinorelbine	2	6.70%	0	0%	

have at least one adverse clinical sign that was attributed to the previously administered chemotherapy, including lethargy, nausea, hyporexia or anorexia, vomiting, diarrhoea and epistaxis (n = 1). Febrile dogs were significantly more likely to have at least one adverse clinical sign than afebrile dogs (P < 0.0001).

Upon presentation, the median rectal temperature for the afebrile group was 102°F (range 100–103°F) and 104°F for the febrile group (range 103–106°F); as expected this was significantly different between the groups (P < 0.0001). The median heart rate for the afebrile group was 109 beats per minute (bpm) (range 80–160 bpm) and for the febrile group, 136 bpm (range 75–190 bpm), which was also significantly different between groups (P = 0.0002). Data summarizing presenting clinical signs are summarized in Table 1.

Laboratory data

Afebrile dogs were significantly less neutropaenic than febrile patients, with median neutrophil counts of 1129 cells/μL (0–1910), compared to median neutrophil counts of 100 cells/μL (0–1856) for febrile dogs (P < 0.0001). Afebrile dogs had significantly more platelets than febrile patients, with median platelet counts of 219 000/μL (29 000–660 000/μL), compared to median platelet counts of 84 000/μL (11 000–385 000/μL) for febrile dogs (P = 0.0094). Haematocrit was not significantly different between groups (Table 1).

The overall incidence of bacteraemia was 12.3% (7/57). Three afebrile patients (10%) and four febrile patients (14.8%) were bacteraemic; the difference between groups was not significantly different (P = 0.6968). The majority (6/7) of positive blood cultures grew gram-positive organisms. All positive blood cultures yielded one organism. The following organisms were isolated from blood cultures in afebrile patients; *Pseudomonas aeruginosa*, *Streptococcus canis* and *Staphylococcus pseudintermedius*. The pseudomonas was resistant to ticarcillin and ticarcillin/clavulanic acid. The following organisms were isolated from blood cultures in febrile patients; *Streptococcus*

canis, Staphylococcus intermedius, Staphylococcus pseudintermedius and *Staphylococcus epidermidis*. The *S. epidermidis* infection (febrile group) was resistant to erythromycin, oxacillin and therefore all B-lactam agents. The *S. pseudintermedius* infection (febrile group) was resistant to all antibiotics tested (chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, minocycline, tetracycline, penicillin and TMS) except amikacin and oxacillin.

Forty-four urinalyses were analysed, 23 in the afebrile group and 21 in the febrile group. Thirteen samples were not submitted for routine urinalysis. Pyuria was documented in two patients, one in the afebrile group and one in the febrile group. Both patients with pyuria did not have bacteruria on urinalysis or urine culture.

Fifty-three urine cultures were analysed, 28 in the afebrile group and 25 in the febrile group. Four urine samples were not collected or misplaced, two in each group. Five samples were obtained via catheterization, 36 samples were obtained via cystocentesis and 12 samples were obtained via the midstream-voided procedure. Two afebrile patients (7.1%) and two febrile patients (8%) were bacteruric; one dog that was entered in the study twice was bacteruric at both enrolments; in the afebrile group and then in the febrile group 13 months later. The rates of bacteruria were not significantly different between the groups (P = 1.0). Three of the positive urine cultures were voided samples and one was obtained via cystocentesis.

None of the dogs with positive urine cultures were reported to exhibit lower urinary tract signs prior to study enrolment. The majority of these dogs (two of three) did have confirmed or possible urinary tract infections prior to study enrolment. The dog that was enrolled in the study twice had historic urinary tract infections prior to the first study enrolment (afebrile group) with no identified predisposing condition. The most recent infection had been documented 26 days prior to enrolment in the afebrile group and treated with a course of antibiotics; a urine culture was not rechecked following the antibiotic course. One of the afebrile dogs had a urinalysis analysed by the referring veterinarian 12 days prior to study

enrolment, which revealed several cocci bacteria. Contamination was suspected due to the lack of lower urinary tract signs; urinalysis was not repeated, and the dog was not treated with antibiotics. One of the febrile dogs did not have a history of urinary tract infections and no baseline urinalysis was performed prior to study enrolment.

The majority of the urinary infections were gram-positive organisms. The following organisms were isolated from urine samples in afebrile patients; *Enterococcus* and *Streptococcus* canis from the dog that was entered in the study twice. The following organisms were isolated from urine samples in febrile patients; methicillin-resistant *Staphylococcus pseudintermedius*, and a mixed infection with *Escherichia coli* and *Streptococcus* canis from the dog that was entered in the study twice. None of the dogs with bacteruria were bacteraemic.

Bacteraemic and non-bacteraemic groups did not differ significantly in age, weight, temperature or heart rate at presentation, neutrophil count, platelet count, haemtocrit, time since receiving chemotherapy or days of hospitalization. Data comparing bacteraemic and non-bacteraemic dogs are summarized in Table 3.

Outcome

Four dogs (13.3%) in the afebrile group were hospitalized for treatment. None of these dogs were bacteraemic or bacteruric. The decision for hospitalization was based on individual clinician recommendations and severity of dogs' presenting clinical signs and owners' preference; standardized treatment protocols were not utilized. All four dogs were reported to be lethargic with a decreased appetite or vomiting at the time of study enrolment, which was 3–17 days following chemotherapy administration. In addition, all four dogs had fewer than 300 neutrophils/uL on CBC, which likely influenced the recommendation for hospitalization. The remaining 26 dogs in the afebrile group were discharged with a course of oral antibiotics when the neutrophil count was <1000 cells/μL. The prescribed oral antibiotic agents varied by clinician preference, and included ciprofloxacin ($n = 5$), enrofloxacin ($n = 7$), amoxicillin/clavulanic acid ($n = 2$) and both enrofloxacin and amoxicillin/clavulanic acid ($n = 2$). Fourteen patients in the afebrile group did not receive oral antibiotics upon discharge. The afebrile dogs were hospitalized for a median of zero days (range 0–3).

The three afebrile dogs with positive blood cultures were all reported to be asymptomatic at the time of the neutropaenic episode. All three dogs had greater than 1000 neutrophils/μL (1033 cells/μL, 1462 cells/μL and 1737 cells/μL) on CBC. One of the dogs was prescribed a 7-day course of ciprofloxacin following the positive culture results, and the other two dogs were not prescribed antimicrobials. All three dogs remained asymptomatic following the blood culture results. Blood cultures were not repeated in any of these dogs. The two afebrile dogs with bacteruria were both treated with appropriate courses of oral antibiotics.

Twenty-four dogs (89%) in the febrile group, including all bacteraemic dogs, were hospitalized for

Table 3. Clinical data comparing bacteraemic and non-bacteraemic patients. Data are presented as median and range

Variable	Non-bacteraemic patients ($n = 50$)	Bacteraemic Patients ($n = 7$)	*P*-value
Age	9.2 years (4.1–14.1)	8.1 years (4.3–15.1)	0.7479
Body weight	24 kg (4–60)	27.9 kg (21–37.9)	0.3565
Rectal temperature	102.6°F (100.1–106.4)	103.8°F (100.3–106.0)	0.4692
Heart rate	120 bpm (75–190)	130 bpm (92–180)	0.337
Neutrophil count	426/μL (0–1910)	918/μL (0–1737)	0.8995
PCV	39% (24–51)	35% (25–45)	0.37
Platelet count	190 000/μL (12 000–660 000)	168 000/μL (11 000–474 000)	0.8510
Days hospitalized	0 days (0–4)	3 days (0–5)	0.1261
Days since last chemotherapy	7 days (3–21)	8 days (6–21)	0.0652

treatment for 1–5 days. All of the hospitalized febrile dogs were treated with IV antibiotics that varied by clinician preference; they included ampicillin/sulbactam ($n = 2$), enrofloxacin ($n = 1$), ampicillin/sulbactam and enrofloxacin ($n = 10$), ampicillin and enrofloxacin ($n = 5$), metronidazole and enrofloxacin ($n = 1$) and ampicillin/sulbactam, enrofloxacin and metronidazole ($n = 5$). Of the three dogs that were discharged, one was prescribed oral enrofloxacin, and two were not prescribed oral antibiotics, as the neutrophil count for these patients was >1000 cells/μL. The febrile dogs were hospitalized for a median of 2 days (range 0–5). Febrile dogs were hospitalized significantly longer than afebrile dogs ($P < 0.0001$). The febrile dog with *Escherichia coli* and *Streptococcus canis* bacteruria was started on enrofloxacin and amoxicillin/clavulanic acid for febrile neutropaenia, and both bacteria cultured from the urine were susceptible to these antibiotics. The febrile dog with the methicillin-resistant *Staphylococcus pseudintermedius* bacteruria was not started on appropriate antibiotics for the bacteruria as euthanasia was elected due to progressive neoplasia on the day following the finalized susceptibility report.

Two dogs (3.6%) died ($n = 1$) or were euthanized ($n = 1$) while hospitalized for treatment of neutropaenia, and both were in the febrile group. The remainder of dogs in the febrile group, and all dogs in the afebrile group survived to discharge. The dog that died in the hospital, after 4 days of hospitalization, had stage V T-cell lymphoma, with progressive disease documented on a bone marrow aspirate prior to the CCNU chemotherapy administration responsible for the FN episode. The necropsy indicated that the cause of death was likely due to sepsis rather than progressive lymphoma, as there was severe perianal cellulitis, oedema and haemorrhage with an aggregate of cocci bacteria within the skeletal muscle near the left popliteal lymph node, indicating haemtogenous seeding. This dog had the resistant *Staphylococcus pseudointermedius* bacteraemia. The other dog was euthanized due to clinical decline in-hospital, with development of pneumonia and ventricular arrhythmias during the hospitalization period. The dog was being treated for lymphoma and was in a complete clinical remission before the chemotherapy

dose that resulted in neutropaenia. This dog was not bacteraemic or bacteruric, however, cultures were obtained prior to clinical evidence of pneumonia, and so the presence or absence of sepsis at the time of euthanasia remains unknown. Necropsy was not performed.

Discussion

The incidence of bacteraemia in febrile and afebrile dogs in this study was 14.8% and 10%, respectively. There was no significant difference in incidence between the groups ($P = 0.6968$). To the authors' knowledge this is the first report of such information in the veterinary literature. Our incidence of bacteraemia for FN is lower than what is documented for human patients with FN (20–30%) (Viscoli *et al.* 1994, 2006; Kern *et al.* 1999; Klastersky *et al.* 2007; Horasan *et al.* 2011). This discrepancy in the rate of bacteraemia in neutropaenic patients between humans and animals may reflect the differences in the goals for treating pets with cancer, with an emphasis on quality of life over quantity, and therefore the use of less intense protocols and doses of chemotherapy (Vail 2009).

Positive blood cultures grew predominantly gram-positive organisms (85.7%), similar to what is reported in the human literature (Viscoli *et al.* 1994; Elting *et al.* 1997; Zinner 1999; Horasan *et al.* 2011; Villafuerte-Gutierrez *et al.* 2014). In total, four staphylococcus organisms, two streptococcus organisms and one pseudomonas organism grew on blood cultures. Although gram-negative organisms are less commonly cultured in bacteraemic human patients with FN, there is an 18% mortality rate for patients with gram-negative infections as opposed to 5% for patients with gram-positive infections, which is attributed to the risk of endotoxemia and septic shock with gram-negative infections (Klastersky *et al.* 2007). Due to the small number of bacteraemic patients, we were unable to compare outcome data between patients with gram-negative and gram-positive bacteraemia.

Three of the blood cultures grew organisms (*Staphylococcus epidermidis* and *Staphylococcus pseudintermedius*, and *Pseudomonas aeruginosa*) with

variable antimicrobial resistance patterns. The *Staphylococcus epidermidis* grown was resistant to erythromycin and oxacillin, and was therefore resistant to all beta-lactam agents. *Staphylococcus epidermidis* is part of normal dog and human skin flora, and not usually pathogenic; however, it is the most frequently implicated coagulase-negative staphylococci infection in immune-compromised humans, and those with indwelling medical devices; it is usually hospital-acquired (Vengust *et al.* 2006; McCann *et al.* 2008; Rogers *et al.* 2009). It is estimated that 70% of the *Staphylococcus epidermidis* strains in the human hospital environment are resistant to methicillin, with the majority also being resistant to other classes of antibiotics (Santos Sanches *et al.* 2000). The *Staphylococcus pseudintermedius* was multi-drug–resistant including resistance to chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, minocycline, tetracycline, penicillin and TMS, with susceptibility for oxacillin (and beta-lactam agents) and amikacin. The dog with this resistant *Staphylococcus pseudintermedius* bacteraemia was the only dog that died, likely of sepsis, in this study. This dog was receiving three antibiotics (ampicillin/sulbactam, enrofloxacin and metronidazole) at the time of death, one of which (ampicillin/sulbactam) was appropriate to treat the resistant bacteraemia. The blood culture results were finalized on the day the dog died. The pseudomonas grown was resistant to ticarcillin and ticarcillin/clavulanic acid, which is common for this bacterium (Hariharan *et al.* 2006; Hillier *et al.* 2006; Mekić *et al.* 2011).

Three of the positive blood cultures were from afebrile, asymptomatic dogs. These positive blood cultures could represent transient bacteraemia that had not yet progressed to the point of causing clinical illness or could represent contamination by human flora during the collection procedure. Contamination is deemed unlikely given the stringent procedure followed by hospital staff and the species of organisms grown in this study. It is also possible that bacteraemia in the absence of adverse clinical signs can be observed in neutropaenic patients with impaired immune defences; further evaluation would be necessary to substantiate this claim.

The overall incidence of bacteruria was 7.5% (7.1% in afebrile dogs and 8% in febrile dogs). As the majority of dogs with documented bacteruria had historic evidence or suspicion for urinary tract infections, any correlation with neutropaenia is difficult to ascertain. In addition, two dogs were receiving immunosuppressive doses of prednisone, which may have predisposed them to development of urinary tract infections (Torres *et al.* 2005). The value of urine cultures for febrile neutropaenic dogs cannot be accurately assessed from these data.

A secondary goal of this study was to evaluate for the presence of clinical parameters indicative of bacteraemia. Unfortunately, no clinical markers to help discriminate between bacteraemic and non-bacteraemic dogs were identified. Certain risk factors have been associated with FN in dogs, but not correlated with the presence or absence of bacteraemia. Sorenmo et el found that dogs with a lower body weight, dogs undergoing treatment for lymphoma or dogs that received vincristine or doxorubicin chemotherapy had a greater likelihood of developing sepsis. In that study, sepsis was defined as neutropaenia and fever, and blood cultures were rarely performed (Sorenmo *et al.* 2010). In another retrospective study evaluating prognostic factors in dogs with FN, those with tachycardia at admission, complicating medical issues, G-CSF use and decreasing neutrophil count after hospitalization had significantly longer hospital stays. Hypotension and G-CSF use were associated with higher mortality in-hospital (Britton *et al.* 2014). The presence of hypotension was not routinely evaluated in our study, and none of the patients enrolled were receiving G-CSF. A larger study assessing more criteria may be able to identify useful clinical discriminators for the presence of bacteraemia.

In addition to assessing incidence of bacteraemia and characterizing the present bacterium in neutropaenic patients, this study also sought to assess the potential value of blood cultures as a first-line diagnostic tool in neutropaenic dogs. Collection of blood cultures is routinely performed in septic humans (Dellinger *et al.* 2013) and is recommended in septic veterinary patients (Boller & Otto 2015). Upon documentation of neutropaenia and prior to

the availability of blood culture results, broad-spectrum antibiotics are typically initiated in febrile, neutropaenic patients. The incidence of bacteraemia in our population of neutropaenic dogs was relatively low and the results of blood cultures do not change initial treatment decisions. Although not recommended in all neutropaenic dogs, antibiotic susceptibility for bacterial growth in blood cultures may have a role in individual case management in guiding changes for non-responding dogs that have multidrug–resistant bacterial infections. In this study, two of the seven bacteraemic dogs had blood cultures that identified bacterial resistance to a portion of the initially prescribed antibiotics. The results of the blood cultures, however, did not significantly affect the eventual outcome in either case.

Although not assessed directly by our study, blood cultures may have potential roles in antibiotic de-escalation. When blood culture results are negative, clinicians' may more safely choose to limit the duration and/or spectrum of antimicrobials; such practices will be beneficial in reducing costs, medication administration and potentially mitigate development of antibiotic resistance. Excessive use of antibiotics can lead to development of antibiotic resistance and increase patients' risk of side effects from such agents. Due to concern for resistant infections in human medicine, if no infection is identified after 3 days of antibiotic therapy, if the neutrophil count is >500 cells/μL for 2 consecutive days and if the patient is afebrile for >48 h, then antibiotic therapy may be stopped (Hughes *et al.* 2002). Recent veterinary studies have demonstrated possible efficacy of shorter-course antimicrobials (Westropp *et al.* 2012; Clare *et al.* 2014). Further study on appropriate duration of antimicrobials in neutropaenic oncologic veterinary patients is warranted.

The majority of dogs treated for FN improve with supportive care and survive to discharge (Sorenmo *et al.* 2010; Britton *et al.* 2014). In an evaluation of 70 dogs with FN, Britton *et al.* reported a mortality rate of 8.5%, which included one dog that received an overdose of carboplatin chemotherapy and another that was euthanized due to disease progression and poor prognosis (Britton *et al.* 2014). In our study, the

mortality rate was comparable among dogs with FN; two dogs (7.4%) died or were euthanized as a result of a FN episode.

One strength of this study was the prospective design. In addition, this is the first veterinary study to assess the incidence of bacteraemia and bacteruria in a group of neutropaenic dogs undergoing chemotherapy. Currently at most institutions, blood cultures are not considered standard of care for neutropaenic dogs and little data are published as to the types of bacteria and possible resistance patterns obtained from positive blood cultures. The results of this study may not be sufficient to justify blood cultures as a first-line diagnostic test; however, the results do establish a foundation for additional studies.

Our study has several limitations. We fell short of the original goal of 60 neutropaenic episodes. Although 57 neutropaenic episodes were analysed, a larger number of dogs may have resulted in differences between febrile and afebrile groups, or between bacteraemic and non-bacteraemic groups. The two comparison groups for this study were based on rectal temperature, as this is an objective measurement that in the majority of cases distinguishes sick from clinically well neutropaenic dogs. The degree of fever, however, does not always positively correlate with the severity of illness related to neutropaenia, leading to possible misclassification of patients. Patients with gram-negative septicemia and endotoxemia often have low-grade fevers or normal temperatures (de Naurois *et al.* 2010). Britton *et al.* found that the median rectal temperature at admission of neutropaenic dogs that died in the hospital (103.4°F) was significantly lower than the median temperature of dogs that survived to discharge (104.5°F); the median temperatures of both groups were still above our cut-off of 102.5°F. Dividing groups based on rectal temperature may have led to inclusion of a small number of sick neutropaenic dogs in the afebrile group. Inclusion of several clinical and diagnostic criteria in dividing sick from well neutropaenic patients, as is done in human medicine, may be a more accurate method to guide treatment decisions and prognosis.

Given the reliance on medical records for information regarding clinical signs, it was difficult to

definitively attribute clinical signs to neutropaenia or gastrointestinal toxicity, which may have influenced interpretation. A total of four urine cultures were not completed, two dogs in the febrile group and two dogs in the afebrile group; although the results of these missing cultures are unlikely to have significantly changed the final results. The majority of the dogs with bacteruria had either a history of previous urinary tract infections or conditions that could predispose them to developing bacteruria. It is impossible to ascertain if these cases of bacteruria were secondary to neutropaenia. In addition, 12 of the 53 urine samples were obtained via midstream voiding, which does carry increased risk of bacterial contamination when compared to cystocentesis. Three of the positive urine cultures were midstream-voided samples. We utilized previously published CFU/mL thresholds (Carter et al. 1978; Comer & Ling 1981) to classify voided urine cultures as positive. However, given the possible bias and limitations related to the urine culture results, caution is advised in making any conclusions from the data in this population of dogs regarding bacteruria in neutropaenic dogs following chemotherapy. In addition, antimicrobials were administered to nine dogs within 2 weeks of enrolment, which may have influenced blood and urine culture results. This study lacked a control group of dogs with an underlying malignancy without neutropaenia.

Conclusion

The incidence of bacteraemia was 12.3% and was not significantly different between febrile and afebrile neutropaenic dogs undergoing chemotherapy. Gram-positive bacteraemia was more common than gram-negative bacteraemia, similar to trends in human medicine. Good clinical predictors for bacteraemia in neutropaenic patients were not identified. The incidence of bacteruria was 7.5% and was not significantly different between febrile and afebrile groups. Blood cultures and urine cultures are not imperative to initial treatment decisions, and although blood cultures may be helpful in a minority of patients with drug-resistant bacteraemia, neither blood nor urine cultures are proven important first-line diagnostic tools in the management of neutropaenic patients.

Conflicts of interest

None.

Contribution

None.

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