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A longitudinal study of ABC transporter expression in canine multicentric lymphoma

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ABSTRACT

Canine lymphoma is typically treated with a doxorubicin-based multidrug chemotherapy protocol. Although this is often initially successful, tumour recurrence is common and frequently refractory to treatment. Failure to respond to chemotherapy is thought to represent drug resistance and has been associated with active efflux of cytostatic drugs by transporter proteins of the ATP-binding cassette (ABC) family, including P-glycoprotein (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2). In this study, ABC transporter mRNA expression was assessed in 63 dogs diagnosed with multicentric lymphoma that were treated with a doxorubicin-based chemotherapy protocol. Expression of *ABCB1, ABCB5, ABCB8, ABCC1, ABCC3, ABCC5* and *ABCG2* mRNA was quantified in tumour samples ($n = 107$) obtained at the time of diagnosis, at first tumour relapse and when the tumour was no longer responsive to cytostatic drugs while receiving chemotherapy. Expression data were related to patient demographics, staging, treatment response and drug resistance (absent, intrinsic, acquired). ABC transporter expression was independent of sex, weight, age, stage or substage, but T cell lymphoma and hypercalcaemia were associated with increased *ABCB5* and *ABCC5* expression, and decreased *ABCC1* mRNA expression. Drug resistance occurred in 35/63 (55.6%) dogs and was associated with increased *ABCB1* mRNA expression in a subset of dogs with B cell lymphoma, and with increased *ABCG2* and decreased *ABCB8*, *ABCC1* and *ABCC3* mRNA expression in T cell lymphomas. ABC transporter expression in the pre-treatment sample was not predictive of the length of the first disease-free period or overall survival. Glucocorticoids had no effect on ABC transporter mRNA expression. In conclusion, drug resistance in canine multicentric lymphoma is an important cause of treatment failure and is associated with upregulation of *ABCB1* and *ABCG2* mRNA.

Introduction

Canine multicentric lymphoma (cML) is in essence not a single disease, but rather a collection of histologically distinct subtypes of lymphoid neoplasia (Valli et al., 2011), and is the most common haematopoietic malignancy in the dog. Canine lymphoma is comparable with non-Hodgkin lymphoma in human beings (Teske, 1994) and is routinely treated with a doxorubicin-based multidrug chemotherapy protocol (Garrett et al., 2002; Sorenmo et al., 2010). Despite a high initial response rate, tumour recurrence (relapse) is common and is thought to result from drug resistance (DR).

Resistance to anticancer drugs is associated with multiple genetic and epigenetic changes, but active efflux of drugs by transporter proteins of the ATP-binding cassette (ABC) superfamily is an important mechanism (Lage, 2008). In humans, expression of P-glycoprotein (P-gp; ABCB1), multidrug resistance related protein 1 (MRP1; ABCC1) and breast cancer resistance protein (BCRP; ABCG2) in tumour cells is associated with decreased sensitivity to cytotoxic agents in vitro, the clinical phenotype of DR and poor treatment outcome in multiple types of cancer (Gottesman et al., 2002). Recently, other ABC transporters, including ABCB5 (Yang et al., 2012), ABCB8 (Elliott and Al-Hajj, 2009), ABCC3 (Kool et al., 1999) and ABCC5 (Wijnholds et al., 2000; Nambaru et al., 2011) have also been implicated in human DR.

In canine neoplasia, P-gp (Bergman et al., 1996; Lee et al., 1996; Page et al., 2000; Tashbaeva et al., 2007; Mealey et al., 2008; Honscha et al., 2009; Hifumi et al., 2010), MRP1 (Ma et al., 2002; Honscha et al., 2009; Hifumi et al., 2010) and BCRP (Honscha et al., 2009; Mealey, 2012) have been studied, but there are no data on ABCB5, ABCB8, ABCC3 and ABCC5 expression.

Several studies in dogs with cML have documented that glucocorticoid use prior to starting cytotoxic chemotherapy has a negative effect on treatment outcome (Price et al., 1991; Piek et al., 1999;

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Table 1
Chemotherapy and prednisolone dose protocol.

Day	1	8	22	33	43	57	60	71	80	92	113	127–148
L-asparaginase (10 IU/m^2 IM)	+											
Doxorubicin (30 mg/m^2 IV)		+	+		+			+		+		
Chlorambucil (25 mg/m^2 PO over 2 days)				+					+			
Vincristine (0.5–0.7 mg/m^2 IV)						+						
Cyclophosphamide (200–250 mg/m^2 PO)							+				+	
Chlorambucil (2 mg/m^2 daily PO)												+
Day	8	15	22	29	43	57	71	85	99	113		
Prednisolone (mg/m^2 once daily)	50	40	30	25	20	15	10	5	5 EOD	Stop		

EOD, every other day; IM, intramuscular; IV, intravenous; PO, per os.

Gavazza et al., 2008; Marconato et al., 2011). This finding was explained by assuming glucocorticoid-induced ABC transporter expression and although dexamethasone is able to induce P-gp expression in human hepatocytes, enterocytes (Martin et al., 2008) and lymphocytes (Manceau et al., 2012), there are no data that support this hypothesis in dogs (Zandvliet et al., 2014). The aim of the present study was to measure ABC transporter mRNA expression in cML throughout disease progression and to correlate this to DR, prognosis and glucocorticoid use.

Materials and methods

Sources of samples

Tumour samples were obtained from privately owned dogs presented to the University Clinic for Companion Animals, Faculty of Veterinary Medicine, Utrecht University for the treatment of cML. The animals in this study represent a subset of dogs previously reported (Zandvliet et al., 2013) and included all high-grade lymphoma cases for which a pre-treatment and (when applicable) at least one follow-up sample was available for PCR.

Breed, sex, age and weight at the time of diagnosis were recorded for each dog. The World Health Organization (WHO) staging system for canine lymphoma (Owen, 1980) was used to determine stage and substage. The minimum diagnostic dataset obtained for each dog included a haematological and clinical chemistry profile, a cytological bone marrow aspirate, cytological examination of a lymph node aspirate and immunocytochemistry for CD79a and CD3 (Dako).

The study was evaluated and approved by the Research Scientific and Ethical Committee at Utrecht University (approval number AVM/CT03-04; date of approval 5 January 2006) and, prior to entering the study, informed consent was obtained from all owners.

Treatment

None of the 63 dogs that entered the study had received any form of prior treatment for cML. All dogs were treated with the same doxorubicin-based chemotherapy protocol and randomised to receive additional treatment with prednisolone (31 dogs) or without prednisolone (32 dogs) (Table 1). Following relapse, rescue treatment was offered, but not standardised, and included treatment with doxorubicin, high-dose cyclophosphamide, vincristine and prednisone (COP), mitoxantrone, L-asparaginase, lomustine (CCNU) and prednisolone.

Response criteria

Treatment response was based on physical examination and evaluation of peripheral lymph nodes by palpation. Relapse was confirmed by cytological examination of a lymph node aspirate. Complete response (CR), partial response, stable disease, progressive disease, disease-free period (DFP), progression-free survival (PFS) and overall survival time (OST) were defined as proposed in the Veterinary Cooperative Oncology Group (VCOG) consensus document (Vail et al., 2010). All deaths during the study were considered disease or treatment-related, unless a clear cause of death that was not lymphoma or chemotherapy-related had been established.

All dogs that were alive at the end of the study were censored at the time of analysis. Intrinsic DR was defined as failure to obtain a first CR while under treatment with chemotherapy. Acquired DR was defined as failure to re-induce CR whilst receiving treatment with any type of cytostatic agent (excluding glucocorticoids) following a previous CR.

Collection of tumour samples

Tumour samples were obtained from a neoplastic lymph node through fine needle aspiration (FNA) and collected into an RNAse-free container that was frozen in liquid nitrogen and stored at –70 °C until further processing. Samples were collected prior to receiving chemotherapy (sample 1), at first tumour relapse (sample 2) and when cML was no longer responsive to cytostatic drug therapy following more than two CRs (sample 3). In total, 107 tumour samples were collected, including 63 pre-treatment samples (sample 1), 32 first relapse samples (sample 2) and 12 end of treatment samples (sample 3) (see Supplementary Fig. S1).

RNA isolation and cDNA synthesis

Total RNA was isolated using the SV-total RNA isolation kit (Promega), including a DNAse treatment, and eluted in Milli-Q water. RNA was quantified spectrophotometrically at 260 nm (ND-1000, Nanodrop Technologies). The purity of the samples was assessed by absorbance at 260 nm (A260) and 280 nm (A280); samples with A260/A230 >1.8 and A260/A280 >2 were included in the study. First strand cDNA was synthesised from 1 μg total RNA using the iScript cDNA Synthesis kit (Bio-Rad) containing both oligo (dT) and random hexamer primers in a final volume of 20 μL. cDNA was stored at –20 °C until analysis.

Quantitative real-time PCR

Intron spanning forward and reverse primers were designed for canine *ABCB1, ABCB5, ABCB8, ABCC1, ABCC3, ABCC5* and *ABCG2* (Table 2), and tested for efficiency

Table 2
Oligonucleotide primers.

Gene	GenBank	Forward primer (5′→3′)	Reverse primer (5′→3′)	T_a (°C)
ABCB1	NM_001003215	CTATGCCAAAGCCAAAGTATC	GAGGGCTGTAGCTGTCAATC	57.5
ABCB5	XM_539461	TTGGTAGGAGAAAAAGGAGCAC	CTAAGATCAGGATTTTTGGTTTTC	59.5
ABCB8	XM_539916	CCTGCTTGAGCGCTTCTATGA	GACTGGCTCCTGGCTGATGA	61.0
ABCC1	NM_001002971	CGTGACCGTCGACAAGAACA	CACGATGCTGCTGATGACCA	60.9
ABCC3	DQ225112	CATCCTGGCGATCTACTTCC	CAGGATCTCACTCATCAGCTTG	62.0
ABCC5	NM_001128100	TGTACGCCTGCCATTCC	CAGCGATCATTCCAACACA	57.0
ABCG2	NM_001048021	GGTATCCATAGCAACTCTCCTCA	GCAAAGCCGCATAACCAT	60.0
B2M	AB745507	TCCTCATCCTCCTCGCT	TTCTCTGCTGGGTGTCG	61.2
GAPDH	NM_001003142	TGTCCCCACCCCCAATGTATC	CTCCGATGCCTGCTTCACTACCTT	58.0
SDHA	DQ402985	GCCTTGGATCTCTTGATGGA	TTCTTGGCTCTTATGCGATG	61.0
TBP	XM_849432	CTATTTCTTGGTGTGCATGAGG	CCTCGGCATTCAGTCTTTTC	57.0
YWAZ	XM_843951	CGAAGTTGCTGCTGGTGA	TTGCATTTCCTTTTTGCTGA	58.0

T_a, annealing temperature.

by using a dilution series of cDNA. The efficiency of the primers was 95–105% and, based on melting curve analysis, only one PCR product was generated.

Quantitative real-time PCR was performed in duplicate using 50 ng cDNA, 7.5 pmol each gene-specific primer and IQSybrGreen Supermix (Bio-Rad) in a final volume of 25 μL and analysed in a MyIQ single colour real time PCR detection system (Bio-Rad). Following 95 °C for 3 min, 40 cycles were run at 95 °C for 20 s, annealing at the specific temperature for 30 s and 72 °C for 30 s.

The reference genes *GAPDH*, *TBP*, *YWAZ*, *SDHA* and *B2M* were selected based on a previous report on canine reference genes (Peters et al., 2007) and primers were tested for efficiency and specificity. RNA transcription of target genes was normalised against the geometric mean of the five reference genes. Gene expression analysis was performed using the $\Delta\Delta$CT method.

Statistical analysis

All parameters were described as frequencies or medians with ranges. Differences between groups were tested for significance with the χ^2 or Fisher's exact test, and the Kruskal–Wallis test or the unpaired Student's *t* test, after being tested for normality. The Kaplan–Meier product limit method was used for estimating DFP, PFS and OST. Differences in gene expression between groups were calculated using REST 2009 software (Pfaffl et al., 2002; Vandesompele et al., 2002). Cluster analysis, using Pearson's correlation coefficient for genes and Spearman rank for samples, was performed using the R v.2.15.3 statistical package[1]. All other statistical analyses were performed using the IBM SPSS Statistics V22.0 software package. The level of significance was set at $P < 0.05$.

Results

Clinical data

Sixty-three dogs were enrolled in the study, including seven mixed-breed dogs and 56 purebred dogs, representing 30 breeds (Table 3). The initial treatment failed to induce a CR in 10 dogs; 27 dogs had a CR and relapsed ≤ 150 days; 18 dogs had a CR and relapsed after > 150 days and eight dogs had no relapse for the duration of the study (median follow-up 463 days; range 49–1720 days). CR following initial treatment was 53/63 (84%) and resulted in a first median DFP of 185 days (95% confidence interval, CI, 157–213 days) and median PFS of 176 days (95% CI 154–198 days). The 10 dogs with intrinsic DR were treated with glucocorticoids and five received additional rescue chemotherapy, but none obtained a CR.

Relapse occurred in 45/53 (84.9%) dogs that had an initial CR and treatment was continued in 43/45 (95.6%) with a monotherapy of glucocorticoids ($n = 9$) or chemotherapy ($n = 34$). Rescue chemotherapy had a second CR rate of 18/34 (53%) and a median second DFP of 122 days (95% CI 105–139). Rescue therapy resulted in a lower CR rate (53% vs 84%; $P = 0.024$) and shorter median DFP (185 vs 122 days; $P = 0.016$) than the initial treatment.

For analysis of OST, 10 dogs were censored (three were alive without relapse, seven died in CR from cML-unrelated causes), resulting in a median OST of 273 days (95% CI 215–331 days).

Drug resistance

Thirty-five of the 107 samples were designated as DR (10 intrinsic, 25 acquired). Intrinsic DR was more frequent amongst T cell (6/15) than B cell lymphomas (4/40; $P = 0.010$).

ABC transporter mRNA expression

Expression of *ABCB5* mRNA was detected at low levels (mean Ct 32.3, median Ct 32.9) in 69/107 (64%) samples, whereas expression of mRNA of the other six ABC transporters was detected in all 107 samples.

[1] See: http://www.r-project.org (accessed 2 November 2014).

Table 3
Clinical data for dogs ($n = 63$) included in the study.

Variable		
Dog breeds	Mixed breed	$n = 7$
	German shepherd	$n = 5$
	Bernese mountain dog	$n = 4$
	Boxer	$n = 4$
	English Cocker spaniel	$n = 4$
	Rottweiler	$n = 4$
	Bordeaux dog	$n = 3$
	Flat coated retriever	$n = 3$
	Golden retriever	$n = 3$
	Border collie	$n = 2$
	Bull mastiff	$n = 2$
	Great Dane	$n = 2$
	Tibetan terrier	$n = 2$
Age (years)	6.6 (1.5–14.0)	
Weight (kg)	36.0 (7–82)	
Sex (male:female)	38:25 (60%:40%)	
WHO stage		
I	$n = 1$ (2%)	
II	$n = 0$ (0%)	
III	$n = 21$ (33%)	
IV	$n = 18$ (29%)	
V	$n = 23$ (37%)	
WHO substage	a: $n = 48$ (76%)	b: $n = 15$ (24%)
Immunophenotype [a]	B: $n = 40$ (73%)	T: $n = 15$ (27%)
Hypercalcaemia	$n = 7$ (11%)	
Neoplastic cells in:		
Peripheral blood	$n = 12$ (19%)	
Bone marrow	$n = 23$ (37%)	
Prednisolone included	Yes: $n = 31$	No: $n = 32$
Treatment response	First ($n = 63$%)	Rescue ($n = 34$%)
Progressive disease	$n = 3$ (5%)	$n = 5$ (15%)
Stable disease	$n = 5$ (8%)	$n = 4$ (12%)
Partial response	$n = 2$ (3%)	$n = 7$ (21%)
Complete response	$n = 53$ (84%)	$n = 18$ (53%)
Disease-free period (days)	185 (157–213)[b]	122 (105–139)[b]
Overall survival time (days)	273 (215–331)[b]	

Continuous variables are reported as median (range), discrete variables as absolute (n) and relative (%) values.
[a] In eight cases not performed or inconclusive.
[b] 95% Confidence interval.
WHO, World Health Organization.

ABC transporter expression and dog characteristics

Expression of ABC transporters in pre-treatment samples (sample 1) was independent of sex, age, weight, WHO stage or substage. T cell immunophenotype and hypercalcaemia were associated respectively with a 5.9-fold ($P = 0.021$) and 11.3-fold ($P = 0.022$) higher *ABCB5*, 3.7-fold ($P < 0.001$) and 9.2-fold ($P = 0.016$) higher *ABCC5*, and 0.3-fold ($P < 0.001$) and 0.4-fold ($P < 0.001$) lower *ABCC1* expression compared with B cell lymphomas and normocalcaemic dogs.

ABC transporter expression and drug resistance

There were no significant differences in ABC transporter expression between DR ($n = 72$) and DR (intrinsic and acquired; $n = 35$; $P \geq 0.05$). No significant changes in ABC transporter expression were found between sampling times (Fig. 1).

ABC transporter expression, drug resistance and immunophenotype

B cell lymphomas had 2.5-fold higher ($P = 0.02$) *ABCB1* expression in sample 3 than sample 1 and 0.2-fold lower ($P = 0.03$) *ABCG2* expression in intrinsic DR compared with no DR (Figs. 2 and 3; see Supplementary Tables S2 and S3). Among T cell lymphomas, sample 2 had 0.4-fold ($P = 0.01$) lower *ABCC3* expression than sample 1, whilst acquired DR was associated with 3.4-fold higher ($P = 0.02$) *ABCG2*, 0.3-fold lower ($P = 0.02$) *ABCB8*, 0.2-fold lower ($P = 0.04$) *ABCC1*

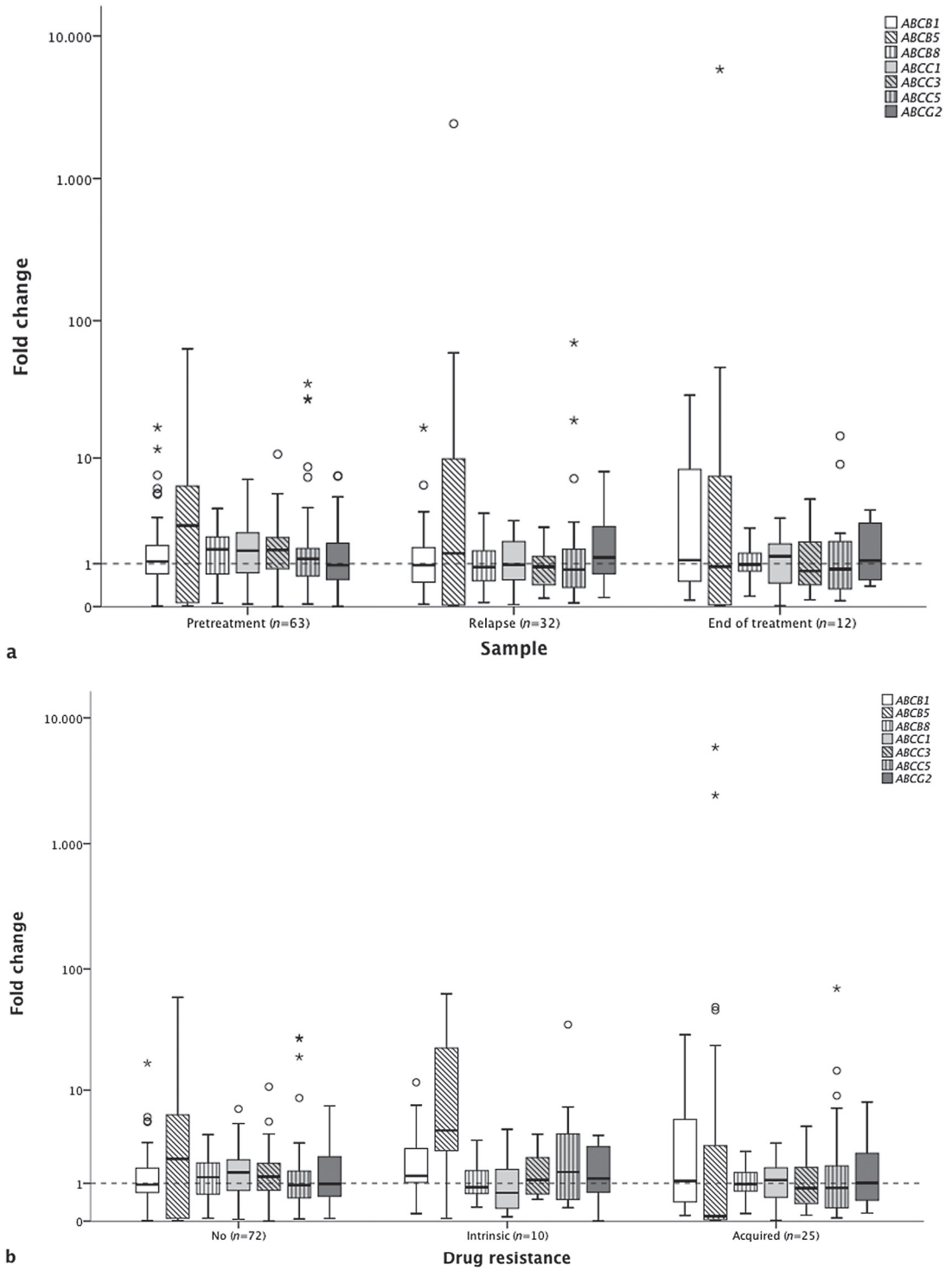

Fig. 1. Box and whisker plots representing fold-changes in mRNA expression for the ABC transporters *ABCB1*, *ABCB5*, *ABCB8*, *ABCC1*, *ABCC3*, *ABCC5* and *ABCG2* in 63 dogs with multicentric lymphoma throughout disease progression (a) and categorised by drug resistance (b).

and 0.3-fold lower ($P = 0.04$) *ABCC3* expression than no DR (Figs. 2 and 3; see Supplementary Tables S2 and S3). T cell lymphomas had 11.3-fold higher ($P = 0.03$) *ABCG2* expression in intrinsic DR, as well as 350-fold higher ($P < 0.001$) *ABCB5*, 4.9-fold higher ($P = 0.009$) *ABCG2* and a 0.09-fold ($P = 0.001$) lower *ABCC1* expression in acquired DR compared with B cell lymphomas (Fig. 3; see Supplementary Tables S2 and S3).

ABC transporter expression and prognosis

Pre-treatment mRNA expression levels for all ABC transporters, including *ABCB1*, were not significantly correlated with length of the first DFP or OST (Fig. 4). ABC transporter expression in the relapse sample (sample 2) was not correlated with the length of the second DFP or PFS.

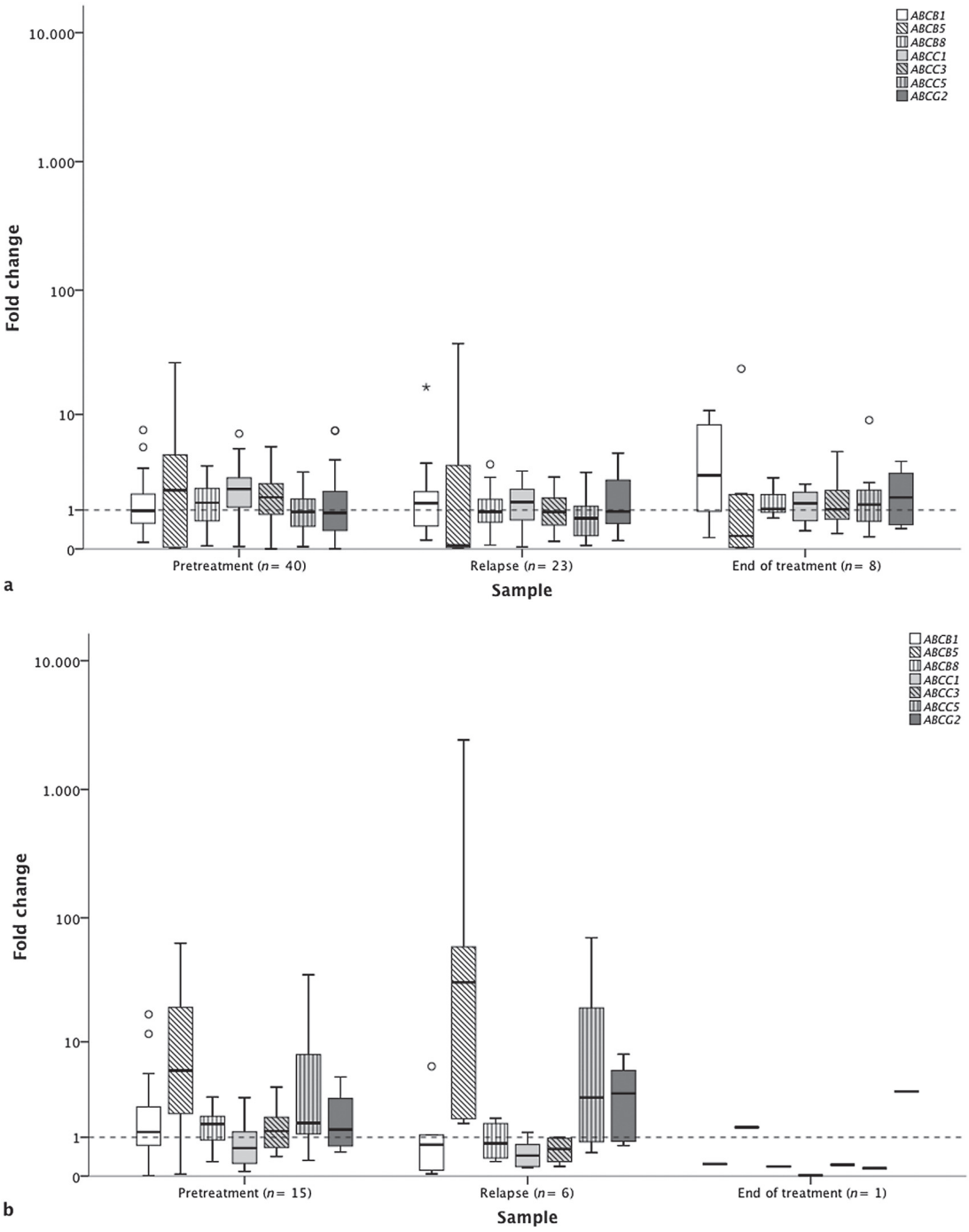

Fig. 2. Box and whisker plots representing fold-changes in mRNA expression for the ABC transporters *ABCB1*, *ABCB5*, *ABCB8*, *ABCC1*, *ABCC3*, *ABCC5* and *ABCG2* in 40 dogs with B cell lymphoma (a) and 15 dogs with T cell lymphoma (b) throughout disease progression.

ABC transporter expression and use of prednisolone

The effect of prednisolone on ABC transporter expression was analysed in those dogs that relapsed following an initial CR, during the initial chemotherapy protocol (sample 2 for all dogs with a DFP < 150 days). Sixteen cases (seven with and nine without prednisolone in the first protocol) fulfilled these criteria and no significant changes were found in ABC transporter mRNA expression between these two groups ($P \geq 0.05$).

Cluster analysis of ABC transporter expression

Cluster analysis of ABC transporter gene expression in pretreatment samples (sample 1) showed two ABC transporter clusters (*ABCB1/ABCB5/ABCC5, ABCB8/ABCC1/ABCC3/ABCG2*) and two main groups of samples (Fig. 5a). Intrinsic DR samples clustered in one main group, but not in either of the two subgroups. In follow-up samples (samples 2 and 3), the ABC transporters clusters were identical, but no clustering of DR samples was noted (Fig. 5b).

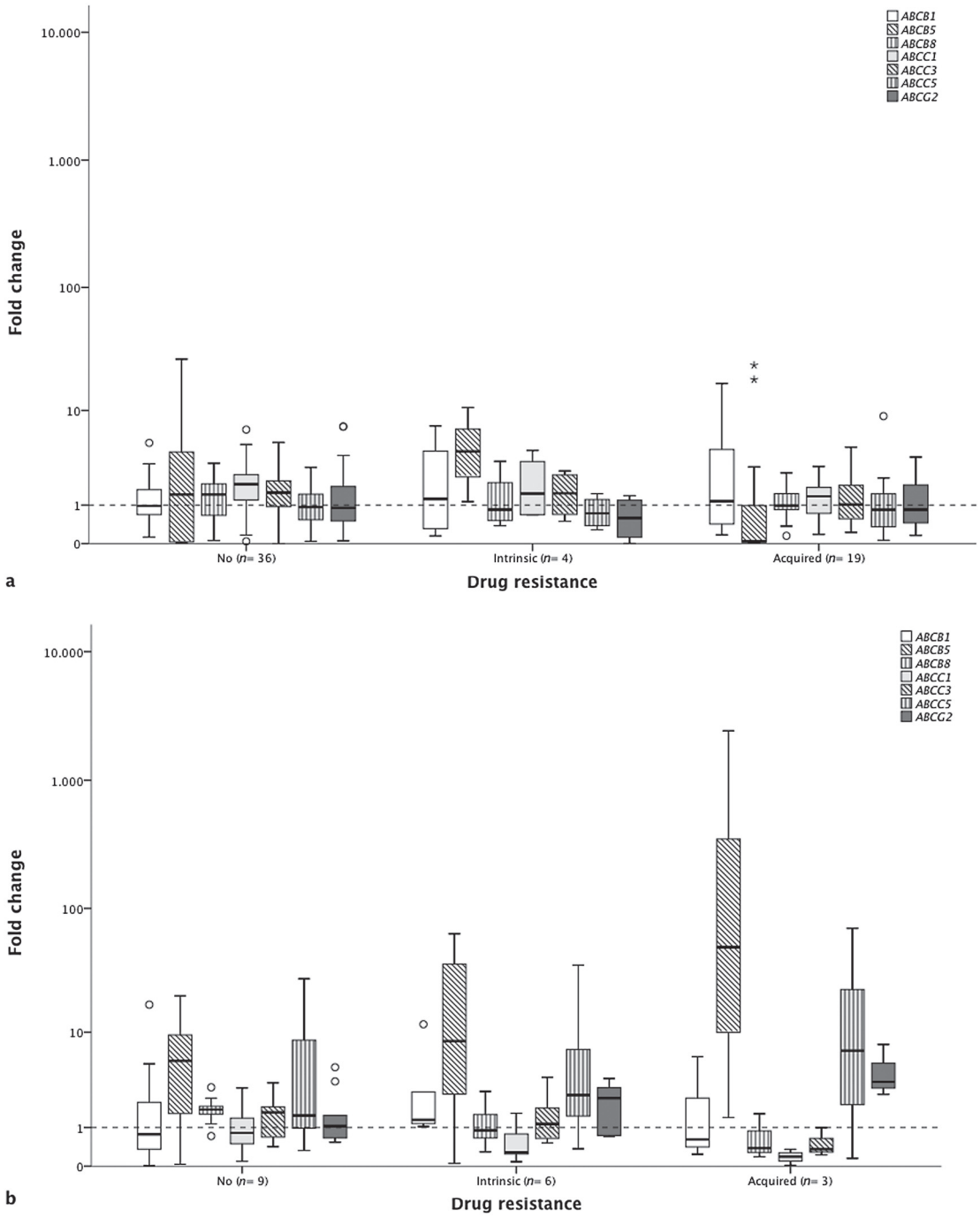

Fig. 3. Box and whisker plots representing fold-changes in mRNA expression for the ABC transporters *ABCB1*, *ABCB5*, *ABCB8*, *ABCC1*, *ABCC3*, *ABCC5* and *ABCG2* in 40 dogs with B cell lymphoma (a) and 15 dogs with T cell lymphoma (b) categorised by drug resistance.

Discussion

The current study is the first large-scale prospective study on ABC transporter expression in cML and the first veterinary report on *ABCB5*, *ABCB8*, *ABCC3* and *ABCC5* expression. Although immunohistochemistry has been described for assessing ABCB1 transporter expression and DR in the dog (Bergman et al., 1996; Lee et al., 1996; Page et al., 2000), and remains the gold standard, it has two limitations in that it requires a histological biopsy and specific antibodies that are either not available or not validated in the dog for the non-classical ABC transporters.

Interpreting quantitative real-time PCR results can be challenging, because results are affected by the choice of reference genes and reference samples. Nevertheless, mRNA expression is predictive for CR rate, DFP, OST and the risk of chemotherapy-related adverse events in a number of human cancers (Burger et al., 2003; Kourti et al., 2007; Henderson et al., 2011; Srimunta et al., 2012).

FNA of lymph nodes is non-invasive, routinely performed in dogs with cML for diagnostic and prognostic purposes, and expression profiling on FNAs from dogs with cML has shown good correlation with the results obtained from tissue samples (Starkey and Murphy,

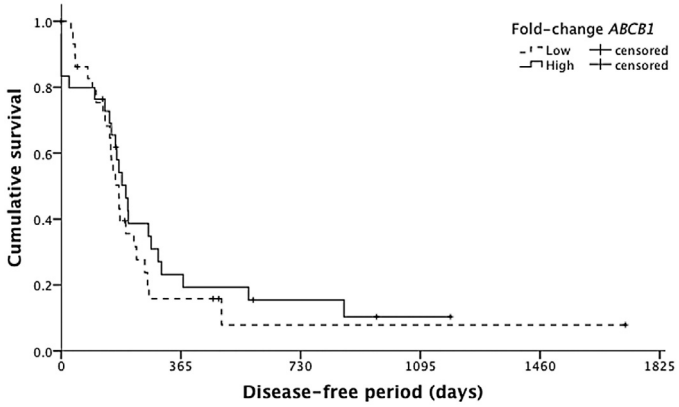

Fig. 4. Kaplan–Meier survival curve for progression-free survival in 63 dogs with multicentric lymphoma categorised by lower or higher than median *ABCB1* expression.

2010). However, cytology has limitations in diagnosing low-grade lymphomas, as well as in subtyping lymphomas.

Although the WHO classification scheme recognises many histological entities, only five histological subtypes account for almost 95% of all canine lymphomas (Valli et al., 2011). Since all cases in the current study were medium to high-grade lymphomas according to the updated Kiel classification, it can be assumed that most cases were 'diffuse large B cell lymphoma' and 'peripheral T cell lymphoma not otherwise specified'.

In the current study, DR, either innate or acquired, was the cause of treatment failure in at least 35/63 (56%) of dogs and was not associated with increased *ABCB1* expression. Although increased P-gp expression has been linked to DR (Bergman et al., 1996; Lee et al., 1996; Page et al., 2000), the current study, as well as previous studies (Steingold et al., 1998; Tomiyasu et al., 2010), failed to associate increased *ABCB1* mRNA expression with clinical DR.

Acquired DR was associated with increased *ABCB1* expression in a subset of B cell lymphomas and increased *ABCG2* expression in T cell lymphomas. Both ABCB1 and ABCG2 are capable of transporting doxorubicin and play a role in cellular sensitivity to doxorubicin in human beings (Lal et al., 2010). However, ABCG2-mediated doxorubicin resistance in humans results from a mutated ABCG2 (Honjo et al., 2001), while in the dog in vitro doxorubicin resistance has been observed with cloned wild type BCRP (Honscha et al., 2009).

Hypercalcaemia is typically associated with T cell lymphomas and both factors are associated with a more guarded prognosis (Teske et al., 1994; Ponce et al., 2004; Simon et al., 2006). The current study included 15 T cell lymphomas, of which seven were hypercalcaemic, and both T cell lymphoma and hypercalcaemia were associated with increased *ABCB5* and *ABCC5* mRNA expression. Although ABCC5 expression is associated with resistance to some nucleoside analogue drugs (Wijnholds et al., 2000; Nambaru et al., 2011), these dogs were not included in the current study treatment protocols. However, ABCB5 expression has been linked to doxorubicin resistance in vitro (Kawanobe et al., 2012) and DR in human leukaemias (Yang et al., 2012), and could explain a higher risk for treatment failure in T cell lymphomas treated with a doxorubicin-based chemotherapy protocol.

For a number of ABC transporters, decreased levels of expression were observed and this might be due to compensatory down-regulation of transporters with an overlapping substrate spectrum, as was previously described for ABCB1 and ABCC1 (Gromicho et al., 2011). An alternative explanation is that these ABC transporters may not play a role in canine DR, and as a result there is no selection for increased expression.

High levels of expression of *ABCB1*, *ABCB5*, *ABCC5* or *ABCG2* in cML samples were associated with DR, but failed to predict treatment response. Pre-treatment ABC transporter mRNA expression was not predictive for treatment response in our study and this is consistent with a recent prospective study on ABCB1 expression in cML (Dhaliwal et al., 2013). Relapsed lymphomas are often refractory to therapy, as was confirmed by the lower second CR-rate, but no significant correlations were found between ABC transporter

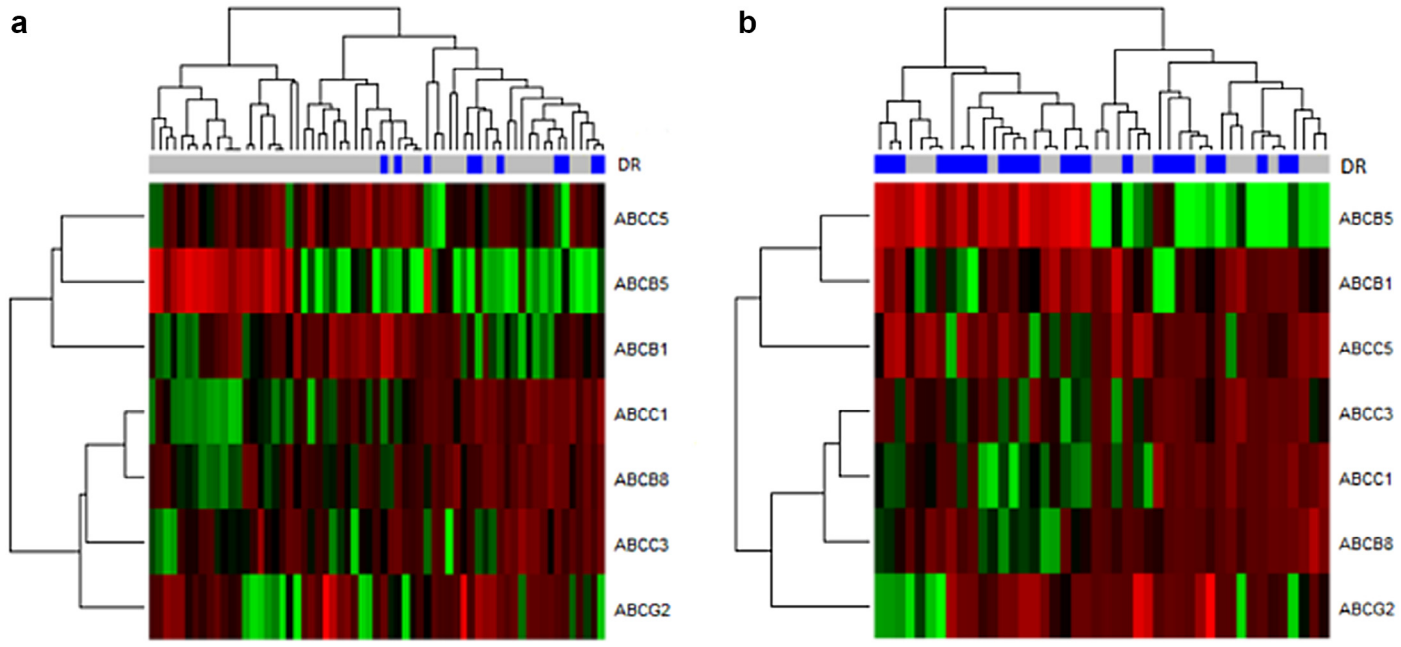

Fig. 5. Heat map generated by unsupervised hierarchical clustering of the ABC transporter genes (*ABCB1*, *ABCB5*, *ABCB8*, *ABCC1*, *ABCC3* and *ABCG2)* for sample 1 (a, $n = 63$) and samples 2 and 3 (b, $n = 44$). Each column represents a single tumour sample; each row represents the expression of a specific ABC transporter (green, increased expression; red, decreased expression). The top row depicts the presence of drug resistance (present, blue; absent, grey).

expression in the relapse sample (sample 2) and second treatment response. We conclude that, besides ABC transporters, other DR mechanisms, including increased cell growth and proliferation, resistance to apoptosis and enhanced DNA-repair (Lage, 2008), are likely to play a role in DR.

The use of prednisolone within a multidrug chemotherapy protocol had no effect on treatment outcome or ABC transporter expression and it seems unlikely that prednisolone induces DR through ABC transporter expression. This is in contrast with the situation in human beings (Wasilewska et al., 2006; Manceau et al., 2012), but was previously documented in a canine lymphoid leukaemia cell line (Zandvliet et al., 2014). Although the current study does not explain why pre-treatment with glucocorticoids prior to starting chemotherapy leads a poorer prognosis, it is unlikely to result from glucocorticoid-induced ABC transporter expression.

Cluster analysis confirmed two clusters of ABC transporters (*ABCB1/ABCB5/ABCC5* and *ABCB8/ABCC1/ABCC3/ABCG2*) and clustering by sample showed the clearest correlation with *ABCB5* mRNA expression, but was not associated with DR. So far, we have not found a biological explanation for the results of the cluster analysis.

Conclusions

DR in canine cML was associated with increased mRNA expression of *ABCB1* in a subset of B cell lymphomas and of *ABCG2* in T cell lymphomas. Glucocorticoids did not increase ABC transporter mRNA expression. Pre-treatment ABC transporter expression was not prognostic for treatment outcome in cML and other mechanisms leading to DR need to be evaluated.

Conflict of interest statement

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

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2014.11.002.

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