Ventricular fractional shortening in 108 dogs with malignant lymphoma undergoing chemotherapy with a cyclic combination protocol including doxorubicin

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Key words
Fractional shortening, doxorubicin, malignant lymphoma, dog

Summary
Objective: The aim of this study was to evaluate whether changes in the left ventricular fractional shortening (LVFS) can be detected in dogs with malignant lymphoma undergoing a cyclic combination chemotherapy protocol including doxorubicin. Hypothesis: Left ventricular fractional shortening as a stand-alone measurement will not show a significant change during the cyclic combination protocol. Material and methods: In this retrospective study, the records of dogs with malignant lymphoma treated between April 2001 and October 2010 were reviewed. Inclusion criteria comprised: a diagnosis of malignant lymphoma, a cyclic combination chemotherapy (including L-asparaginase, vincristine, cyclophosphamide, doxorubicin and prednisolone), and an echocardiographic examination by an experienced examiner before treatment and after each doxorubicin administration. Results: One hundred and eight dogs were included and a total of 446 LVFS measurements had been performed. Patients were divided into four groups according to the number of doxorubicin administrations. Median LVFS did not change significantly during the cyclic combination protocol in all groups. All median LVFS values remained above the lower reference value of 25%. Conclusion and clinical relevance: The measurement of LVFS did not show a significant change during the cyclic combination protocol treatment including doxorubicin in this population of dogs. Therefore either this cyclic combination protocol does not cause a systolic dysfunction or LVFS is not sensitive enough to detect early changes. Newer methods that are more sensitive then LVFS might be necessary to detect such changes.

Schlüsselwörter
Linksventrikuläre Verkürzungsfraktion, Doxorubicin, malignes Lymphom, Hund

Zusammenfassung
Introduction

Malignant lymphoma is one of the most common neoplastic diseases in dogs (10, 11). Different treatment protocols can be used for this and other neoplasias in dogs, most of those protocols include the anthracycline chemotherapy agent doxorubicin (12, 18, 19, 25, 28, 30–33). The potential cardiotoxicity of doxorubicin has been reported in humans (6, 24), dogs and cats (20). The cardiotoxic effects may occur at a cumulative dose beginning at 150–240 mg/m² (15, 29). They display as a potentially irreversible cardiomyopathy manifesting as arrhythmias, myocardial failure or both (6, 15). The exact mechanism of doxorubicin-induced cardiac toxicity is not completely elucidated, free radical formation and lipid membrane peroxidation may be involved (29).

One of the most commonly used echocardiographic methods to assess cardiac function is the M-mode based Left Ventricular Fractional Shortening (LVFS) measurement (reference value: 25–45%) (3, 7, 14, 17) (Fig. 1). LVFS is an easy measure and a coefficient of variation range of 8.7–14.9 is reported in the literature (4). However, the interpretation of LVFS offers only low specificity with regard to LV systolic performance due to its strong dependence on pre- and afterload (14). By using the M-mode echocardiography, the motion of the left ventricular free wall (LVPW) and the interventricular septum (IVS) in relation to one another and to time can be depicted. It can be used to measure left ventricular wall thickness and internal diameters during the diastolic (EDD) and systolic (ESD) phases of the cardiac cycle. The LVFS is the difference between the enddiastolic and endsystolic dimensions divided by enddiastolic dimension. By multiplying it with 100 the FS in percentage can be calculated: \[ \text{LVFS} = \frac{(\text{EDD} - \text{ESD})}{\text{EDD}} \times 100 \] (3, 14).

The aim of this study was to evaluate whether changes in the FS can be detected in dogs with malignant lymphoma undergoing a chemotherapy combination protocol including doxorubicin.

Material and methods

Patients and echocardiographic examinations

In this retrospective study, records of dogs treated for malignant lymphoma with doxorubicin as part of a combination chemotherapy protocol between April 2001 and October 2010 were reviewed. Inclusion criteria were a diagnosis of malignant lymphoma, staging of the patient, the cyclic combination chemotherapy including doxorubicin and echocardiography. Malignant lymphoma was diagnosed by aspiration cytology, differentiation of T and B lymphoma was performed by flow cytometry (8). Complete staging was performed with radiography of thorax and abdomen, abdominal ultrasonography and complete blood work. Echocar-

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Fig. 1 Right parasternal long axis view. Depicting a M-Mode the cursor is placed along the long and short axis view at end diastole and end systole. IVSd = intraventricular septum diameter in diastole, IVSs = intraventricular septum diameter in systole, LVIDd = left ventricular inner diameter in the diastole, LVIDs = left ventricular inner diameter in the systole, LVPWd = left ventricular posterior wall diameter in diastole, LVPWs = left ventricular posterior wall diameter in systole, FS = left ventricular fractional shortening.
diography examinations were conducted prior to initiation of doxorubicin treatment (baseline) and following each doxorubicin administration no later than 7–10 days after administration. Patients were excluded if the LVFS could not be adequately measured because of paradoxal septum movement or inadequate image quality. All dogs were treated with a cyclic combination protocol including doxorubicin (30 mg/m²) as a 30-minute continuous rate infusion as well as vincristine (0.7 mg/m² IV), cyclophosphamide (200 mg/m² IV), L-asparaginase (400 IE/kg SC) and prednisolone (50 mg/m² PO q 24 h for 3 days) (28) (Table 1). The dogs were classified according to the WHO staging system and the anatomical classification described by Owen (21).

All echocardiographic examinations were performed without sedation in right and left lateral recumbency in accordance to the recommendations of the echocardiography committee of the Speciality of Cardiology of the American College of Veterinary Internal Medicine (34). Two different ultrasound machines were used: a Vivid 7 (General Electric Healthcare) machine equipped with a 4–8 MHz sector transducer and a Vivid E9 (General Electric Healthcare) machine equipped with a 5–6 MHz sector transducer. All data and measurements were documented and stored on the Anidata® Veterinary management software for later evaluation. M-mode echocardiographic indices derived from a right parasternal long-axis view were used to calculate the FS (Fig. 1). All measurements were performed on three cardiac cycles and the results were averaged.

In this study 25% was defined as the lower cut off value as some authors choose to discontinue the administration of doxorubicin when the FS is below 25% (29).

**Statistical analysis**

Statistical analysis was performed using a commercially available computer software: SPSS version 17® and MedCalc® version 11.5. Data is presented as median values and range including minimum and maximum values. Not all 108 patients completed the cyclic combination protocol with four doxorubicin administrations. For statistical purposes the patients were therefore divided into four groups according to the number of doxorubicin treatments that they received prior to reaching an end point of study (defined as death, loss to follow-up or beginning of a new protocol). Groups 1, 2, 3 and 4 included patients that received one, two, three or four doxorubicin treatments respectively. Repeated measures Analysis of Variance (ANOVA) test was chosen in order to examine statistically significant differences in LVFS values within each group (Table 3). A P value < 0.05 was considered statistically significant.

**Results**

**Patient characteristics:** One hundred and eight dogs met the inclusion criteria. They had a median body weight of 30.8 kg (range: 5.6–56 kg) and a median age of 7.1 years (range: 2.4–13.9 years). Forty-six dogs were female (42.6%) and 62 were male (57.4%) with 25 dogs of each gender being castrated. The median number of doxorubicin treatments was 3 (range: 1–4). Ten out of the 108 dogs could be clinically staged as 3a, six dogs as 3b, 30 dogs as 4a, 24 dogs as 4b, 15 dogs as 5a and 23 dogs as 5b. According to the anatomical classification five dogs had mediastinal lymphoma, 95 dogs multicentric lymphoma, three dogs intestinal lymphoma and in five dogs an atypical lymphoma was diagnosed. In 86 of the 108 dogs the lymphoma was classified as B cell lymphoma and in 20 dogs as T cell lymphoma. Two dogs had an unclassified lymphoma.

An overall of 446 LVFS measurements were recorded and analysed, originating from four groups of patients – group 1: one doxorubicin treatment (n = 7), group 2: two doxorubicin treatments (n = 17), group 3: three doxorubicin treatments (n = 39), and group 4: four doxorubicin treatments (n = 45). The LVFS did not show a significant change in any of the four groups during the cyclic combination chemotherapy protocol including doxorubicin, and did not decrease below the defined cut-off value of 25% at any time point (Table 2, 3).

**Table 1** Schematic of the 12-week chemotherapy protocol used for the treatment of canine lymphoma as described by Simon et al. (28).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Week</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>L-Asparaginase</td>
<td>400 IU/kg SC</td>
<td>x</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.7 mg/m² IV</td>
<td>x</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>200 mg/m² IV</td>
<td>x</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>30 mg/m² IV&lt;sup&gt;a&lt;/sup&gt;</td>
<td>x</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>50 mg/m² PO q 24 h</td>
<td>x</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pretreatment with dexamethasone, <sup>b</sup>Administration of prednisolone on days 1–3, <sup>c</sup>Infusion over 30 min

IV = intravenous administration; PO = oral administration

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Table 2 Summary table showing left ventricular fractional shortening (LVFS) changes of the four groups during the cyclic combination chemotherapy protocol including doxorubicin.

Tab. 2 Zusammenfassende Darstellung der Veränderungen der linksventrikulären Verkürzungfraktion (LVFS) in den vier Gruppen während des zyklischen Kombinationsprotokolls mit Doxorubicin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Measurement</th>
<th>LVFS (min-max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (one DT)</td>
<td>median baseline LVFS (%)</td>
<td>33 (26–40)</td>
</tr>
<tr>
<td>(n = 7)</td>
<td>median LVFS post 1 DT (%)</td>
<td>30 (27–37)</td>
</tr>
<tr>
<td>Group 2 (two DT)</td>
<td>median baseline LVFS (%)</td>
<td>33 (20–49)</td>
</tr>
<tr>
<td>(n = 17)</td>
<td>median LVFS post 1 DT (%)</td>
<td>34 (25–43)</td>
</tr>
<tr>
<td></td>
<td>median LVFS post 2 DT (%)</td>
<td>31 (25–43)</td>
</tr>
<tr>
<td>Group 3 (three DT)</td>
<td>median baseline LVFS (%)</td>
<td>35 (24–50)</td>
</tr>
<tr>
<td>(n = 39)</td>
<td>median LVFS post 1 DT (%)</td>
<td>34 (25–50)</td>
</tr>
<tr>
<td></td>
<td>median LVFS post 2 DT (%)</td>
<td>34 (25–50)</td>
</tr>
<tr>
<td></td>
<td>median LVFS post 3 DT (%)</td>
<td>32 (22–43)</td>
</tr>
<tr>
<td>Group 4 (four DT)</td>
<td>median baseline LVFS (%)</td>
<td>32 (22–49)</td>
</tr>
<tr>
<td>(n = 45)</td>
<td>median LVFS post 1 DT (%)</td>
<td>32 (24–54)</td>
</tr>
<tr>
<td></td>
<td>median LVFS post 2 DT (%)</td>
<td>35 (18–54)</td>
</tr>
<tr>
<td></td>
<td>median LVFS post 3 DT (%)</td>
<td>32 (25–54)</td>
</tr>
<tr>
<td></td>
<td>median LVFS post 4 DT (%)</td>
<td>32 (20–48)</td>
</tr>
</tbody>
</table>

DT = doxorubicin treatment(s)

Discussions

In this study, no significant changes of LVFS could be demonstrated during a cyclic chemotherapy combination protocol including doxorubicin. None of the included patients, that were divided into four groups according to the number of doxorubicin treatment received, showed a significant change in median LVFS or a decreased median LVFS below the cut-off value of 25% during treatment.

There are a number of reports of cardiotoxicity associated with doxorubicin administration in the human and veterinary literature (2, 6, 16, 18, 20, 23, 24, 37). Cardiotoxic effects including pericarditis/myocarditis, left ventricular dysfunction and arrhythmias (2) have been described as well as a cardiomyopathy leading to congestive heart failure and considered to be dependant on the cumulative dose (6). Cardiotoxicity may be observed during or after the chemotherapy (2). The exact range of onset of cardiotoxicity is unknown. One study in human medicine considered effects occurring within 3 months of the completion of chemotherapy early (2).

Mauldin et al. (16) reported clinical cardiac abnormalities in 37 of 135 dogs treated with doxorubicin. These abnormalities included arrhythmias and congestive heart failure. On necropsy, dogs showed a noninflammatory myocardial degeneration, myocytolysis, vacuolation, fibrosis and intramural coronary arteriosclerosis. Cardiac histopathologic findings in doxorubicin toxicity are characterized by vacuolar degeneration of myocytes being most severe in the interventricular septum and less frequently seen in the right ventricle and rarely in the left and right atrium. The damaged myocytes show distension of the sarcoplasmic reticulum and sarcoplasmic vacules (15, 23, 37).

Some authors recommend avoiding doxorubicin treatment if the LVFS is below 20%. Echocardiography controls are recommended starting at a cumulative doxorubicin dose of 90 mg/m². Furthermore, it has been recommended to consider discontinuation of doxorubicin administration if the LVFS measurement falls below 25% (unless no other reasonable alternative treatment is available) (1). One patient (number 98), an extremely calm Golden
Retriever, demonstrated an FS of 18% after the second doxorubicin administration. The end-diastolic and endsystolic diameters (EDD and ESD, respectively) were within normal reference values (7) (EDD: 3.36 cm, ESD: 2.75 cm) and the dog did not show signs of systolic dysfunction, therefore treatment was continued. Subsequently, the same patient demonstrated a normal FS of 28% and 25% during follow-up examinations. One explanation for these findings may be the reported variability of the FS measurement between 8.7 to 14.9% (4). In this study, a maximum of four consecutive administrations of 30 mg/m² doxorubicin (a cumulative dose of 120 mg/m²) were evaluated. Two more doxorubicin doses were administered to dogs experiencing a recurrence of the disease with an ultimative cumulative dose of up to 180 mg/m². This fifth or even sixth doxorubicin administration may occur at an unscheduled time point according to the patient’s general state and state of remission. As the time period between the forth and next renewed doxorubicin administration is highly variable, these treatments were not evaluated in this study.

The measurement of LVFS is one of the most commonly used and well known measurements of cardiac assessment and is relatively rapid and easy to perform (3, 7, 14, 17). Unfortunately, this measurement depends on pre- and afterload and is not a very specific index of myocardial contractility (14).

Several explanations for the findings of this study are possible. LVFS measurement may not be sensitive enough to detect doxorubicin-induced effects in this chemotherapy protocol. Alternatively, the cumulative dose reached in this study (150 mg/m² or more) may not be high enough to cause significant changes in the LVFS measurements. Thirdly, the method of doxorubicin administration may play an important role. Some reports of toxicity were based on older protocols administering doxorubicin as a bolus rather than a continuous rate infusion as used in this protocol, or on experimental protocols with intracoronary administration (9, 26, 35).

Belham et al. (2) examined 67 human patients receiving doxorubicin chemotherapy. In this study six patients had pre-existing cardiac disease. The remaining 61 patients received an average dose of 293 ± 103 mg/m² doxorubicin at time of completion study. Ejection fraction (EF) based on the modified Simpson’s rule, Tei index, tissue Doppler imaging parameters and traditional systolic and diastolic parameters (including LVFS) were evaluated prior to and following chemotherapy. The best parameter to predict development of functional cardiotoxicity was the ejection fraction. However, slight changes in the LVFS were also noted in this study. In the veterinary literature, Sorenmo et al. (33) examined 20 dogs with hemangiosarcoma undergoing five to seven doxorubicin treatments at a dose of 30 mg/m². At necropsy, 11 dogs underwent histologic grading of cardiac damage secondary to doxorubicin toxicity. In five of these 11 dogs, moderate to severe fibrosis and mild to moderate vascular changes were found, two dogs had mild changes and four dogs did not show histologic changes. No clinical signs of dilated cardiomyopathy or congestive heart failure were documented in these dogs. LVFS was not evaluated in that study.

To the best of the authors’ knowledge this current study is the first study reporting serial FS measurements in dogs with malignant lymphoma undergoing chemotherapy treatment with a cyclic combination protocol including doxorubicin. Future studies should evaluate the efficacy of other testing methods such as cardiac troponin I (cTnI), tissue Doppler imaging (TDI) or modified Simpson’s method of disc in the analysis of chemotherapy-induced cardiotoxicity. The latter method’s variability is reported to be smaller (40).

Today several sensitive laboratory tests, such as the biomarker cTnI exist to evaluate myocardial damage which is increased in cases of myocardial ischemia, congestive heart failure, and myocarditis, but also in some extracardiac conditions such as gastric dilatation volvulus and snake bites (22, 38). cTnI is also elevated in cases of specific cardiac diseases such as the Doberman cardiomyopathy (39), bradyarrhythmias (36) or in boxers with arrhythmogenic right ventricular cardiomyopathy (1) and was reported to be increased in dogs undergoing doxorubicin therapy and experimental intracoronary administration of doxorubicin (25). Tissue Doppler imaging is commonly used for evaluation of cardiac function, systolic and diastolic functions, including tissue velocity imaging, strain, strain rate and speckle tracking (5, 13, 27). The Simpson’s method of disc has been evaluated in veterinary medicine and could be used to investigate the effects of doxorubicin on the cardiac muscles in animals (40).

There are several limitations to this study. One of them is the retrospective nature. Examination of the different dogs was performed by different investigators, no intra- and interobserver analysis or reproducibility evaluation was possible. Prospective studies evaluating intra- and interobserver variations as well as measurement reproducibility are warranted to verify the observations of the present study. Another limitation of this study is that the potential arrhythmogenic effect of doxorubicin was not evaluated. In this study all patients were treated with the cyclic combination protocol consisting of four different drugs. Although only doxorubicin is reported to have a cardiotoxic effect, it is not possible to rule out that another drug or a combination of drugs could be responsible for an effect on cardiac function. No other parameter but LVFS was evaluated in this study. A complete evaluation of cardiac function includes many parameters that are less pre- and after-

Conclusion and clinical relevance

In this population of dogs, no significant changes could be demonstrated in the LVFS during the cyclic combination protocol including doxorubicin. It is not recommended that LVFS be used as an exclusive method for the evaluation of cardiac contractility and function in dogs undergoing chemotherapy with a cyclic combination protocol including doxorubicin. Other methods to measure cardiac damage are available today and might prove to be more sensitive in detecting the cardiotoxic effect of doxorubicin including tissue Doppler imaging, Simpson’s method of disc and ultrasensitive cTnI measurements.
load-dependent parameters evaluating systolic and diastolic functions (such as enddiastolic and endystolic diameters, normalised diameter indexes, tissue velocity imaging, etc.) and different cardiac biomarkers. Due to the retrospective nature of this study not all of these parameters are available. In contrast to today's evaluation, only LVFS was considered to be necessary for the evaluation of cardioxicity and cardiac contractility evaluation in the past.

Conflict of interest
The authors confirm that they do not have any conflict of interest.

References