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Contrast-enhanced ultrasound evaluation of testicular interstitial cell tumours in conscious non-sedated dogs

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ABSTRACT: Testicular tumours are the most common neoplasms of the genital system in male dogs. The three main types reported in dogs are interstitial cell tumour, seminoma and Sertoli cell tumour. Interstitial cell tumour is related to the presence of single or multiple nodules inside the testicular parenchyma, and it is detected by palpation or is often an incidental finding during ultrasonography examination. Contrast-enhanced ultrasound allows characterisation of the perfusion of the testicular lesion and reveals the micro-vascularisation; however, perfusion parameters may be strongly influenced by sedative drug administration, so our aim was to evaluate qualitative and quantitative perfusion of a single type of tumour (interstitial cell tumour) with contrast-enhanced ultrasound in conscious dogs to exclude any influence of pharmacological agents on vascular flow. Thirty dogs with focal testicular lesions found by palpation and/or by ultrasound (B-mode and Doppler) examination were selected; contrast-enhanced ultrasound was performed only in subjects that presented testicular focal lesions. After orchietomy, testes were submitted to histological evaluation; 2-minute clips recorded during contrast-enhanced ultrasound were analysed only in the case of dogs with interstitial cell tumours ($n = 12$). Contrast medium showed wash-in at around 25–30 seconds, at the same time as the surrounding tissue: lesions were hyperenhancing, homogeneous or inhomogeneous with rim enhancement and contained prominent inner vessels; however, enhancement of small regions was absent. Quantitative analysis demonstrated significantly higher PI% ($P = 0.005$), regional blood volume ($P = 0.02$) and regional blood flow ($P = 0.007$) values in lesions than in surrounding tissue; no differences were found for time-to-peak and mean transit time. In conclusion, the contrast-enhanced ultrasound pattern observed in conscious non-sedated dogs with interstitial cell tumour was similar to the pattern described in a previous study in dogs after intramuscular administration of medetomidine (10 $\mu\text{g}/\text{kg}$) and butorphanol (0.2 mg/kg).

Keywords: dog; canine; ultrasonography; contrast agents; testicular tumour

Testicular tumours are the most common neoplasms of the genital system in male dogs, representing more than 90% of all canine male genital tumours (North et al. 2009). According to the World Health Organization classification, the three main types reported in dogs are interstitial cell tumour (ICT), seminoma (SEM) and Sertoli cell tumour (SCT) (Kennedy et al. 1998). In a previous study on 232 tumour-affected dogs, ICT was the most common tumour (Grieco et al. 2008).

ICT is related to the presence of single or multiple nodules inside the testicular parenchyma and is either detected by palpation or is often an incidental

finding made in screening ultrasonography (Grieco et al. 2008). Examination of the testis with B-mode and Doppler ultrasound allows the description and, if possible, the characterisation of focal lesions. Due to the small size of microbubbles (1–7 μm), the use of ultrasonography contrast agents (CA) allows the assessment of the circulation in the micro-vessels and capillaries of testicular lesions. Contrast-enhanced ultrasound (CEUS) has gained in popularity relative to computed tomography (CT) and magnetic resonance imaging (MRI) during the last years due to its lower costs and because it is often unnecessary to use sedation or anaesthesia; how-

ever, CT and MRI provide important information for the detection of metastasis (Brunereau et al. 2012; Piscaglia et al. 2012).

The contrast agents used in CEUS are composed of gas-filled microbubbles which are injected into the bloodstream. Following injection, the bubbles greatly increase the amplitude of the scattered signals not only from large vessels but also from the microvasculature, providing real-time assessment of vascular perfusion of target organs and/or lesions (Tang et al. 2011). In veterinary medicine, CEUS has been used to provide accurate quantification of liver, splenic and kidney vascularisation in healthy dogs (Ziegler et al. 2003; Nyman et al. 2005; Nakamura et al. 2009; Macri et al. 2016) and to differentiate between malignant and benign hepatic renal, and splenic nodules in dogs and cats based on perfusion patterns (O'Brien et al. 2004; Haers et al. 2010).

A previous study described an inhomogeneous lesion with hyperenhancing pattern as an important feature in the diagnosis of testicular neoplasms, but only SEM was characterised by prominent and persistent inner vessels in a hypo-isoechoic background (Volta et al. 2014). All dogs in that study were sedated and it remains unclear whether or not the cardiovascular system and consequently CEUS perfusion parameters were strongly influenced by the administration of sedative drugs (Leinonen et al. 2011; Restitutti et al. 2013; Stock et al. 2014; Rossi et al. 2016).

Our goal was to evaluate qualitative and quantitative perfusion of a single type of tumour (ICT) in conscious dogs with CEUS to exclude any influence of pharmacological agents on vascular flow.

MATERIAL AND METHODS

The study was conducted between February 2015 and March 2017 at the Veterinary Teaching Hospital of the Department of Veterinary Sciences, University of Messina, Italy. Dogs ($n = 30$) with focal or diffuse testicular lesions found by palpation and/or by ultrasound (B-mode and Doppler) examination were selected; CEUS was performed only in subjects that presented testicular focal lesions. After orchietomy, testes were submitted to histological evaluation and we recruited only dogs with a histological diagnosis of ICT. All treatments, housing and animal care were in compliance with

EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Written informed consent was obtained from owners. Prior to inclusion in the study, a complete general physical examination, serum chemistry profile and complete blood cell count were performed.

To avoid adverse reactions to the micro-bubble contrast agent, dogs were excluded if they had evidence of cardiac disease or a history of anaphylactic reactions to vaccines or other medications.

Conventional ultrasonography and CEUS examinations were performed by the same investigators (Q.M. and M.C., respectively), using a scanner (MyLab™ 40 VET, Esaote, Genova, Italy) equipped with contrast-tuned imaging technology (CnTI™, Esaote, Genova, Italy) in lateral recumbency without any sedative drug administration. Hair over the testes was clipped. A coupling gel was applied to the skin. B-mode and colour Doppler ultrasonography of testes was performed with microconvex (5.0 to 8.0-MHz) and linear (10 to 12-MHz) transducers. Transverse and longitudinal planes were used to fully assess testes. The parameters assessed in the ultrasonography of the testes masses included size and shape, echo pattern (homogeneous or heterogeneous) and location. Testes masses with uniform echogenicity or mixed echogenicity with hyperechoic and hypoechoic areas were recorded as “homogeneous” or “heterogeneous”, respectively. The presence of a rim or inner vessels was also evaluated using colour Doppler ultrasonography.

CEUS examination was performed immediately following B-mode ultrasonography, using a linear (5.0–7.5-MHz) transducer with contrast agent capability. The mechanical index (MI) was set from 0.08 to 0.09; a single focal zone was placed at the deepest part of the lesion. The CA was a sulfur hexafluoride signal enhancer (SonoVue®, Bracco Imaging, Milan, Italy) and it was prepared in accordance with the manufacturer's recommendations. Each vial (which contained 25 mg of freeze-dried powder) was reconstituted by injection of 5 ml of 0.9% sodium chloride; vials were then shaken vigorously for 20 seconds. An aliquot (0.03–0.04 ml/kg of body weight) of the contrast medium was rapidly injected via a three-way valve and 18–20 G catheter inserted in the cephalic vein. The contrast injection was immediately followed by a 5 ml saline flush.

Each dog received two bolus injections of contrast agent 8–10 minutes apart from each other.

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The activation of the timer was performed simultaneously with the contrast agent dose inoculation. The first injection was not used for interpretation, but to optimise the system settings.

Good-quality video clips obtained during CEUS were stored digitally on a hard disk and were subsequently analysed by the same operator (M.F.).

The focal lesion in the testes were classified as hyperenhancing (brighter than surrounding tissue either homogeneously or inhomogeneously, or with rim enhancement or with prominent inner vessels), iso-enhancing (no longer visible during contrast ultrasound) or hypo-enhancing (hypoechoic relative to the surrounding tissue) compared to the surrounding testes tissue, as described by Volta et al. (2014).

Post-processing quantitative analysis of video-clips was performed by use of image-analysis software (Qontrast™, Bracco, Milan, Italy). For each dog, one region of interest (ROI) was manually drawn around the entire tumour; a second ROI, if possible as large as the lesion's ROI and at approximately the same depth, was drawn in the normal parenchyma.

Time intensity curves (TICs) were generated for each ROI. Analysis of tissue perfusion was based on video signal intensity (SI) changes over time using CEUS. The SI of a white band in the grey scale bar (8 bit) was defined as maximal (100%). Other pixels in the image were then assigned SI values based on this reference. The following parameters were computed and considered within the selected ROI during the period of enhancement: peak intensity (PI%), time-to-peak (TTP) measured from T_0 (injection time), mean transit time (MTT), regional blood volume (RBV) and regional blood flow (RBF). Peak intensity is defined as the percentage increase in SI – from 0 to a maximal intensity of 100 – reached during transit of the contrast agent at a specific time point. The RBF is defined as the ratio between the regional blood volume (RBV, proportional to the area under the curve) and mean transit time (MTT, the circulation time of contrast agent in the tissue/lesion/structure under investigation).

All obtained data were statistically processed and were expressed as mean (M) ± standard deviation (SD) and ROI data from the lesion and surrounding tissue were compared with each other using Student's *t*-test. Values were considered significant when $P < 0.05$.

RESULTS

One each of the Boxer and Cocker Spaniel breeds, two each of the German shepherd and Hound breeds, and six mixed breed dogs were included in the study. The age of the twelve enrolled dogs was 9.8 (±1.4) years (range: seven to 12 years). Blood count and serum biochemistry were within normal ranges.

Out of 60 testes analysed, 20 presented focal lesions in CEUS analysis, and in 12 conscious dogs in which ICT was confirmed histologically, the procedure was suitable for qualitative and quantitative analysis. In all cases the procedure was well-tolerated and no adverse effects were noted.

B-mode ultrasonography revealed the presence of non-prominent, predominantly hyperechogenic focal lesions of the parenchyma, sometimes containing small anechoic areas, and with diameters of around 1 cm for all twelve dogs.

Colour Doppler (Figure 1A) showed a prominent vascularisation of these lesions with inner vessels.

Contrast-enhanced ultrasound (Figure 1B) showed that wash-in occurred at around 25–30 s, at the same time as the surrounding tissue: lesions were hyper-enhancing and inhomogeneous with enhanced rims, and contained prominent inner vessels; however, there was also no enhancement of small regions, corresponding to the anechoic areas detected in B-mode.

The mean PI% was 12.8 ± 9 in lesions and 6.4 ± 4.3 in non-pathologic tissue. Mean RBV was 748 ± 417 in lesions and 357 ± 263 in non-pathologic tissue. Mean RBF was 12.68 ± 10 (range 2–33) in lesions and 6 ± 4.7 (range 2–20) in surrounding tissue. Mean TTP was 44 ± 11 (range 25–62) in lesions and 44 ± 10.8 (range 33–62) in surrounding tissue. Mean MTT was 54.94 ± 15.59 (range 32–77) in lesions and 51.73 ± 13.56 (range 38–85) in surrounding tissue. Quantitative analysis revealed significantly higher PI% ($P = 0.005$), RBV ($P = 0.02$) and RBF ($P = 0.007$) in lesions than in surrounding tissue; no differences were found for TTP and MTT (Figures 2A and 2B).

DISCUSSION

In human medicine, CEUS helps in the differentiation of ICT from other testicular tumours, and, in particular, is useful in highlighting its earlier

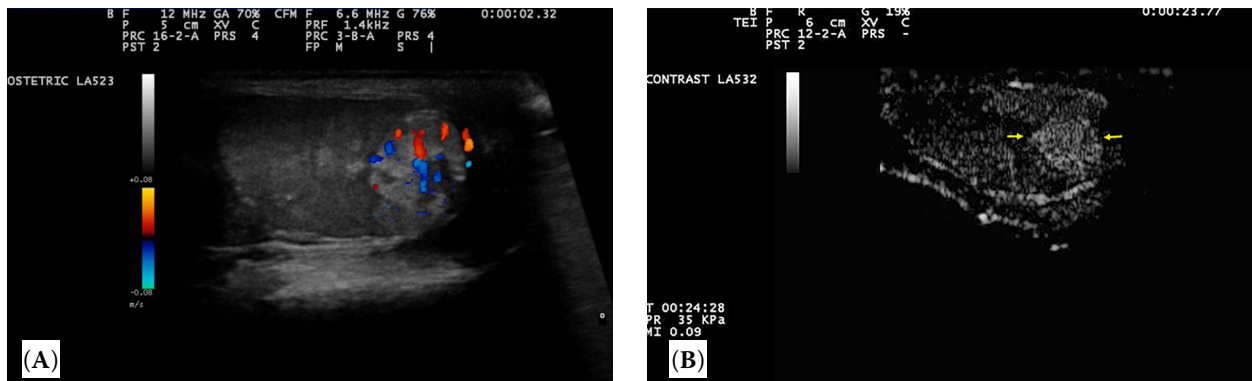


Figure 1. Colour Doppler and contrast-enhanced ultrasound ultrasonography of testis affected by interstitial cell tumour: vascular pattern of a focal lesion (A); the parenchyma of the testis is characterised by early wash-in of the parenchyma with inhomogeneous hyperenhancement of the focal lesion (arrows) (B)

and higher enhancement relative to seminoma (Cantisani et al. 2015). The first study performed in dogs revealed hyperenhancing and heterogeneous lesions with rim enhancement or inner vessels both in ICT and in SCT (Volta et al. 2014).

Similar to these previous results, in this study, we also observed a perfusion pattern of hyperenhancement in focal lesions of ICT together with rim enhancement, prominent inner vessels and a lack of any enhancement of small regions.

The mean peak intensity (PI%) reported here is lower than the mean values reported for various neoplasms by Volta et al. (2014); however, perfusion parameters of ICT lesions were significantly higher than the healthy parenchyma as also described in that study, suggesting hypervascularisation of neoplastic tissue.

Several factors may contribute to variability in quantitative analysis of CEUS results and the resulting perfusion variables. These factors can be divided

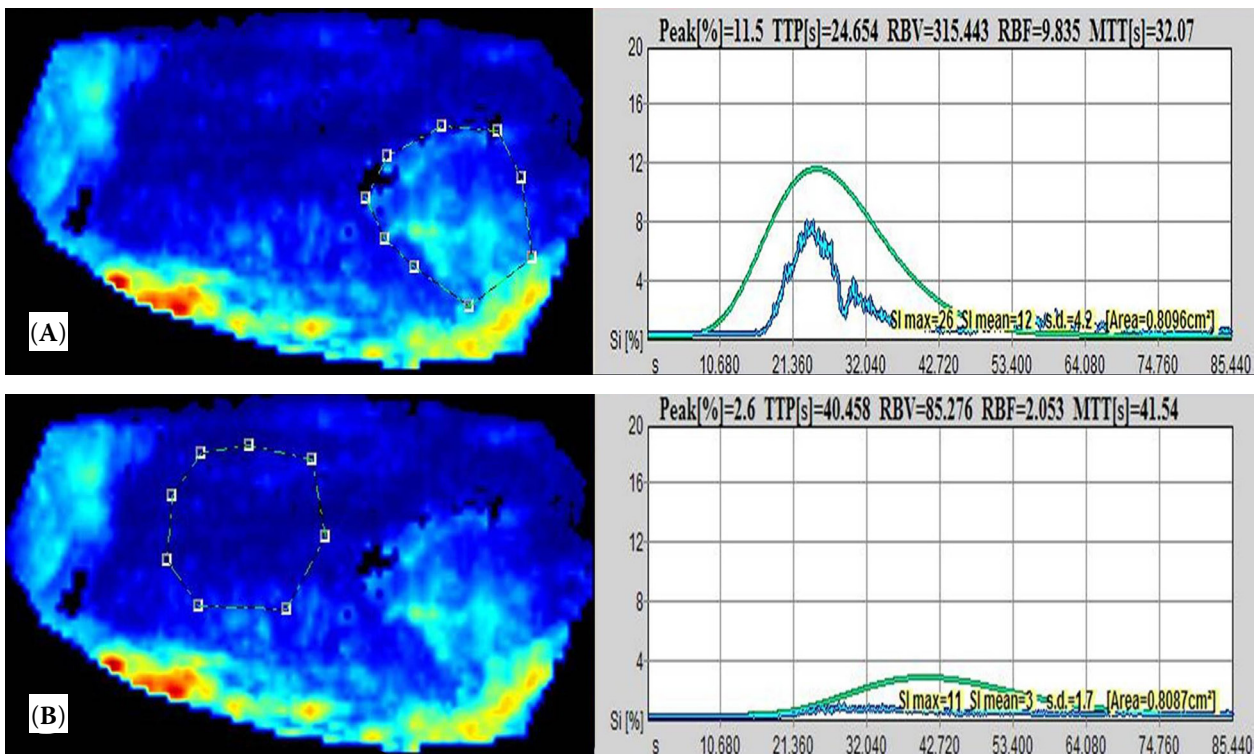


Figure 2. Contrast-enhanced quantitative analysis of the focal lesion in testis with interstitial cell tumour: the focal lesion is outlined by the position of the region of interest shown with respective time-intensity curves (A); the region of interest is positioned in normal tissue, with respective time-intensity curves (B)

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into three categories: factors related to technical variables, including scanner settings, transmission power, focal zone, dynamic range, gain setting, time gain compensation and transmission frequency; factors related to the contrast medium, including type, preparation, injection technique and dose; and patient-related factors, including physiologic differences (heart rate, blood pressure and respiratory rate), physiologic interactions of the patient with the microbubble contrast agents, variability of propagation and attenuation of ultrasonography waves in tissues and haemodynamic variability among species (Tang et al. 2011). To minimise the influence of these factors, imaging settings were consistent among all dogs and the same investigators performed the procedures on all dogs. A low mechanical index (0.08–0.09) was chosen to minimise microbubble disruption and allow accumulation of microbubbles in the microvasculature, which has been suggested to restrict variability in clinical applications of CEUS. Considering that the entire testes were only 3–4 cm in depth, a low MI was necessary to obtain a diagnostic image quality that avoided the pitfalls concerning insonation power (Dietrich et al. 2011). Experimentally, an increase in signal intensity of about 50% from an MI of 0.05 to 0.1 was found using SonoVue® (Tang et al. 2011). The mechanical index used was lower than that used (max. 0.15) by Volta et al. (2014), so this could be one of the factors responsible for the lower PI value.

There is no obligation to perform anaesthesia when using contrast-enhanced ultrasound, which is among the benefits of this potential diagnostic method in veterinary medicine; however, in uncooperative patients, the administration of a sedative is necessary. Dexmedetomidine elicits a decrease of the PI in the renal cortex but not in the spleen, liver and intestines according to Restitutti et al. (2013); in contrast, Rossi et al. (2016) observed a significant reduction in splenic enhancement with dexmedetomidine and stated that the use of this sedative should be contraindicated for splenic CEUS procedures in dogs; administration of butorphanol as a sedative agent without subsequent anaesthesia does not modify the perfusion of the spleen. The pharmacologic features of butorphanol include systemic analgesia without significant changes in myocardial contractility and vascular tone, while medetomidine provides for reduced perfusion in peripheral organs (Pawson 2008). In this study, a similar CEUS pattern to that described

in dogs after the intramuscular administration of medetomidine (10 µg/kg) and butorphanol (0.2 mg/kg) was observed in conscious non-sedated dogs with ICT. The lower peak intensity and RBF in awake dogs could be related to technical or methodological differences between the two studies, but is unlikely to be due to the pharmacologic action of the sedatives used in the recent study (Volta et al. 2014). In conclusion, CEUS seems to be a feasible imaging technique also in non-sedated dogs with ICT; the testes exhibit a hyperenhancing pattern with a rim or inner vessels.

Further studies employing contrast-enhanced ultrasound to study canine testicular tumours that include a larger number of animals are needed in order to standardise the technical variability.

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