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Treatment of canine and feline sarcoma using MR-guided focused ultrasound system

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Abstract

Purpose In recent years, veterinary medicine has enhanced its applications beyond traditional approaches, progressively incorporating the Focused Ultrasound (FUS) technology. This study investigated the ability of FUS to precisely ablate naturally occurring canine and feline soft tissue sarcomas (STS).

Methods Six dogs and four cats with superficial tumours were enrolled in the study. The tumours were treated with a Magnetic Resonance guided FUS (MRgFUS) robotic system featuring a single element spherically focused transducer of 2.6 MHz. The tumours were then removed by surgery and sent for hematoxylin and eosin (H&E) staining.

Results The MRgFUS system was capable of inflicting well-defined overlapping lesions in the tumours. The anatomical sites of the treated tumours were the neck, leg, face, back and belly. Coagulative necrosis was evidenced by histopathology assessment in 80% of cases.

Conclusion Therefore, this technology can be a therapeutic solution for veterinary cancer and a model for advancing the knowledge on human STS.

Keywords MRgFUS · Robotic device · Superficial cancer · Sarcomas · Dogs · Cats

Introduction

Although veterinary medicine used to fall behind human medicine, it is now gaining more popularity as we realize the importance of clinical applications in companion animals. Clinical trials in companion animals provide alternative therapies to pets as well as essential data for accelerating human medicine. Pets experience similar environmental conditions, and thus, it is not surprising that they develop diseases similar to humans.

Cancer is a leading cause of death in dogs, especially over ten years of age, with an occurrence incidence up to 50% [1]. STS account for 8–15% of all cutaneous and subcutaneous canine tumours [2–4], and up to about 20% of all malignant skin tumours [5]. They typically arise from connective tissues such as fibrous tissue, fat, muscle, and lymphatic vessels. They form a group of locally invasive biologically heterogeneous tumours that share common clinical features and are usually enclosed within a pseudo-capsule of cancerous cells with unclear histological margins [6, 7].

Hemangiosarcomas and fibrosarcomas represent two prevalent types of canine STS [8]. The former occurs primarily in the spleen, heart, liver and skin/subcutis. The latter mainly occurs in the skin, subcutaneous space, and oral cavity. Other primary forms of STS are the peripheral nerve sheath tumours (also called neurofibrosarcomas), which can arise anywhere in the body, and histiocytic sarcomas, which usually spread aggressively to the lymph nodes, kidneys, liver and central nervous system [8]. Notably, some types seem to be more prevalent in specific dog breeds [8].

Though less common than in dogs, cancer also constitutes an ordinary medical condition in cats, with STS being among the most common tumour types [9]. Likewise to canine STS, the feline equivalent constitutes a group of poorly circumscribed heterogeneous tumours of mesenchymal origin regularly arising in cutaneous and subcutaneous tissues [9, 10]. Based on a retrospective study [11],

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the most common anatomical sites for these tumours are the trunk, followed by the limbs, head and neck. Feline injection-site sarcomas (FISS) are typical feline STS [10] occurring in one to ten of every 10,000 vaccinated cats, according to epidemiological data [12]. This type of sarcoma is biologically aggressive and characterized by a locally invasive nature [13].

Management and treatment of cancer in pets is quite similar to humans, with surgery followed by radiotherapy and chemotherapy. Aggressive radical resection is the method of choice for efficient management of feline and canine STS [12–14], while incomplete surgical margins were associated with an increased risk of local recurrence [14]. Radiotherapy and chemotherapy were proposed in the adjuvant setting for improving the outcomes of canines [5, 15] and felines [16–19], especially in cases of insufficient surgical excision.

Post-treatment local recurrence is a usual phenomenon in reportedly up to 62% of canines, which seems to relate to the failure to manage the disease effectively and the low survival rates recorded [6, 20–24]. Feline STS have also been associated with a high rate of local recurrence, especially following marginal tumour removal [25].

Alternative therapies are currently emerging over traditional approaches with the prospect of advancing the state-of-the-art in the field of veterinary sarcomas. Torrigiani et al. [26] proposed electrochemotherapy as a local ablative treatment that induces anti-tumour effects by enhancing the delivery of cytotoxic drugs into the malignant cells of canine STS. In the adjuvant setting, Iridium-192 brachytherapy has been proposed as an alternative therapy for managing FISS [27]. It should be noted that targeted and immune therapeutic approaches have recently been incorporated in the management of canine sarcomas [28]; however, they lack essential information regarding dosing, safety, and efficacy due to insufficient research.

FUS has arisen as an alternative option to develop new therapeutic solutions with the potential of treating many medical disorders non-invasively. Non-ionizing ultrasonic energy is delivered in deep tissue in a completely non-invasive manner to induce various thermal and mechanical effects [29]. By precisely focusing the ultrasonic waves into a mm-sized area, lethal temperatures are produced, causing instant death of malignant tissue [29]. FUS technology has already proven efficient as an alternative treatment option for oncological diseases [30].

MR thermometry has enabled more efficient delivery of ultrasound and quicker translation of the technology to clinical practices [31]. MR-guided procedures benefit from high-resolution imaging of the targeted anatomy [32], accurate delineation of the tumour margins [32] and most importantly, the ability to monitor the temperature elevation and tissue necrosis during therapy [31].

Preclinical FUS studies typically involve animal models, including experimental animals such as mice [33] and rats [34, 35], swine [36], bovines [37], sheep [38], rabbits [39] and non-human primates [40, 41]. Advantageously, in the last years, veterinary medicine has enhanced its applications beyond traditional approaches, progressively incorporating the FUS technology in the treatment of companion animals such as dogs and cats [42].

This is particularly important to the scientific community since family pets experience similar environmental conditions and, thus, are far more genetically homologous to humans than laboratory models. Naturally occurring tumours in dogs and cats, including STS, were qualified as clinical models for studying and enhancing the current knowledge on similar human tumours [8, 43–45]. Milovancev et al. [43] reported that canine STS present histological and immunohistochemical characteristics identical to their human counterparts. A similar conclusion was reached by Gustafson et al. [8] who presented the comparative pathology of the main types of canine sarcomas to their human equivalents. He revealed that the canine disease is related to the majority of human cancer histotypes. Moreover, FISS were found to possess histological and behavioral features comparable to human musculoskeletal sarcomas [44].

At the same time, pets can benefit from significant improvement of their life quality and welfare. Due to its non-invasive nature, FUS minimizes the risks of infection and eliminates the need for stitches and Elizabethan collar in veterinary oncology. The length of hospitalization is reduced, and the treatment costs are reduced for pet owners. Another essential benefit of the FUS technology is the ability to apply treatment multiple times, providing the previously reported high recurrence rates of canine and feline STS.

According to the FUS Foundation, veterinary trials and practices currently relate to oncological and pain relief applications and mainly involve canines. Otherwise, felines and equines are involved. Treatment of soft tissue canine and feline tumours (soft tissue sarcomas and mast cell tumours) has been documented in clinical practice since 2018. FUS therapy of bladder cancer, chronic wound, hepatocellular carcinoma, oral tumours and osteosarcoma are still at the stage of clinical trials. In contrast, therapy of brain tumours, lung cancer and sarcoids is yet to be translated to the clinic. Pain relief for elbow and hip dysplasia, osteoarthritis and soft tissue injuries was also translated to the clinical practices in 2018. Since then, half of the total treatments performed are related to osteoarthritis pain relief. From the rest, the majority of treated animals were related to soft tissue and oral tumours. Other proposed veterinary applications include managing epilepsy, kidney stones and spay.

Canine models were widely used to evaluate FUS protocols for transurethral [46] and transrectal [47] ablation of prostate

cancer. Recently, US-guided FUS ablation of an oral tumour in a male canine patient was attempted by Ranjan et al. [42]. Complete remission of the exposed tissue was evidenced by CT imaging and histological examination. Simultaneously, rapid multiplication of adaptive immunity cells was observed in the treated area, suggesting that FUS can trigger anti-tumour immune effects. Regarding adverse events, FUS-related thermal burns were observed and managed [42]. Therapeutic ultrasound has also been proposed in the management of canine primary glaucoma with promising results [48].

A retrospective clinical study [49] collected data from eleven dogs with different types of solid tumours that received FUS treatment (VIFU2000, Alpinion Medical Systems). Clinical signs were relieved in about half of the cases, while tumour shrinkage occurred in 40%. This study demonstrated the feasibility of FUS to induce coagulation of hemorrhagic regions, thus contributing to controlling local bleeding.

The first study to evaluate the feasibility of the MRgFUS technology to accurately target canine STS was conducted in 2018 [50]. Head, truncal and spine tumours were mainly targeted in 53 canine patients using a commercial clinical system (Sonalleve V2, Profound Medical). Although the majority of tumours (81.1%) were targetable, the study suggests that treatment of the entire tumour volume with no consequential off-target damages would be feasible in only 5.7% of patients. This study underlines the significance of proper acoustic coupling with the target since ultrasonic interference with bone and air may cause thermal injuries [50].

Herein, we present the outcomes of experiments performed in cats and dogs ($n = 10$) with naturally occurring superficial sarcoma tumours using FUS. The pets were treated using an MRgFUS robotic system [51–53]. Notably, this robot is an extension of previous studies [54–58]. The study aimed to determine the system's feasibility to ablate veterinary cancer precisely. In all cases, FUS therapy was performed prior to surgical removal of the tumours. The majority of treated neoplasms were STS, mainly located at the neck, leg, face, back and belly of the animals. Due to their superficial location, a single element spherically focused transducer of 2.6 MHz was used. The dissected tumours were sent for histological analysis to examine the FUS-induced bioeffects in the exposed sites and the extent of tissue necrosis.

Materials and methods

Pet recruitment

Recruitment of pets was facilitated by directly visiting veterinarians all over the country and providing detailed information regarding the technology and experimental procedure to be followed. With this approach, we managed to enroll six dogs and four cats with naturally occurring sarcoma cancer in the study. An informed consent was signed by all pet owners.

Only fully vaccinated dogs and cats with a weight greater than 2 kg were eligible for participation. The inclusion criteria included soft tissue non-metastatic superficial tumours with dimensions greater than $20 \times 20 \times 30$ mm^3 where the malignancy had been confirmed by pathologist assessment. All recruited pets were in good condition, with no health problems other than cancer. The experiments were performed at least 1 week from the last corticosteroid administration, 2 weeks from the last chemotherapy and 6 weeks from the last radiation therapy to assess procedure-related outcomes dot affected by other therapies.

MRgFUS system

A robotic device intended for the use of the MRgFUS technology in the preclinical setting was developed and described in detail in previous studies [51–53]. The positioning mechanism features 4 PC-controlled non-magnetic motion stages actuated by piezoelectric ultrasonic motors (USR30-S3, Shinsei Kogyo Corp., Tokyo, Japan) for navigating a FUS system relative to the target site. Accurate motion control is achieved by MR-compatible linear (EM1-0–500-I, US Digital Corporation, Washington, USA) and angular (EM1-2–2500-I, US Digital Corporation, Washington, USA) optical encoders.

The FUS system included an RF amplifier (AG1016, T&C Power Conversion, Inc., Rochester, US) for applying power to a single-element spherically focused transducer (MEDSONIC, Limassol, Cyprus) operating at a central frequency of 2.6 MHz. The transducer has a diameter of 40 mm, a geometric focus of 60 mm and an efficiency of 33%. An electronic driving system provided control of the motors. The ultrasonic and motion parameters were controlled through a specially developed software. A schematic diagram of the experimental setup is shown in Fig. 1.

FUS ablation

All experiments were performed at the veterinary premises. Anesthesia in dogs was induced via injectable administration of 15 mg/kg Ketamine (Nimatek, DECHRA, UK) and 1 mg/kg Xylazine (Xylapan, VETOQUINOL, UK). For cats, an amount of 22 mg/kg Ketamine and 1.1 mg/kg Xylazine was used. The animal was then placed on the robotic device in a specific position depending on the tumour location. The area of malignancy was immersed in the water above the ultrasonic transducer. With this configuration, a bottom-to-top approach of the ultrasonic

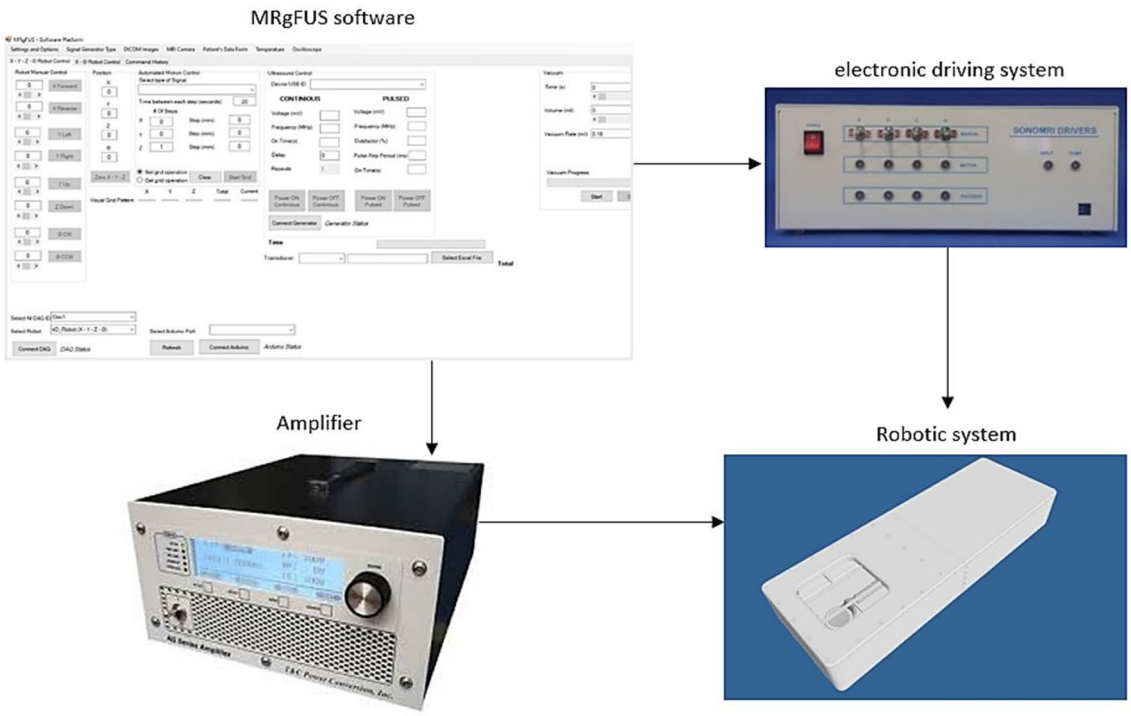


Fig. 1 Schematic diagram of the experimental set up

beam was achieved. Examples of a dog placed in a prone position and a cat placed in a supine position are shown in Fig. 2a and b, respectively.

The sonication protocol (grid, step size, sonication time, cooling time, acoustic power, focal depth) was adjusted to account for the tumour size. Small-sized tumours with a diameter up to 30 mm were managed with a single exposure. Initially, a low energy sonication of 16.5 W acoustical power (electrical power of 50 W) for 10 s was applied. If no animal was observed suffering, full exposure was applied using an increased acoustical power of 33 W (electrical power of 100 W). Notably, lower acoustical power of 24.8 W (electrical power of 75 W) was applied in the case of a small-sized face sarcoma in a dog. An exception was also made in the case of a large skin tumour in a dog that was treated applying higher acoustical power of 66 W (electrical power of 200 W) for a longer sonication time of 60 s.

Multiple sonications were applied in grid patterns for managing larger tumours. Initially, low power was applied similarly to the single sonication protocol. After confirming that no animal suffering occurred, a full dose was applied to create overlapping lesions. The sonication pattern consisted of 2×2 or 3×3 spots, with a spatial step of 3 mm and a time delay of 30 s between adjacent sonications. A pulse of 66 W acoustical power (200 W electrical power) and 10 s duration was applied at each spot, with one exception where acoustical/electrical power of 49.5/150 W was used.

Vital signs were monitored regularly throughout the FUS procedure to identify any treatment-related adverse events that could compromise the welfare of the animal. The

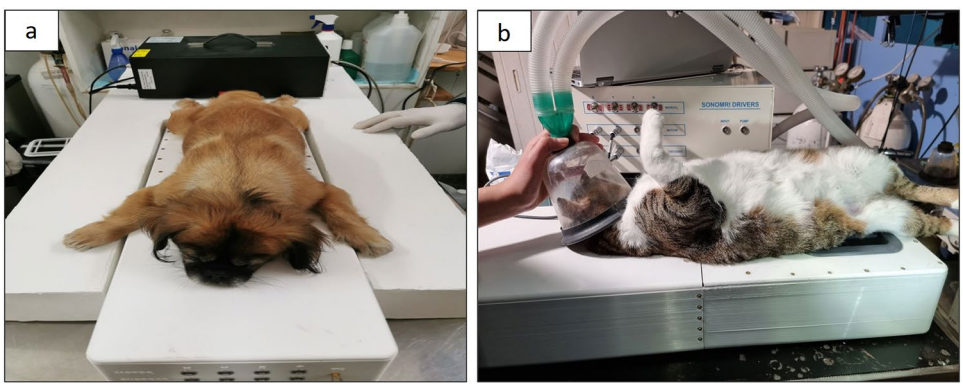


Fig. 2 Placement of a **a** dog in prone position and a **b** cat in supine position on the robotic device at the veterinary premises

characteristics of recruited pets and the sonication protocols used for managing small and large tumours are summarized in Table 1.

Histological examination

Upon execution of the FUS treatment, the tumour was surgically removed by the referring veterinarian and placed in a solution of 10% formalin for preservation purposes. The specimens were instantly sent to a specialized center for H&E histological examination to identify the FUS-induced bioeffects in the malignant cells. The histological slides were viewed with a microscope (Olympus BX51, Shinjuku City, Tokyo, Japan) and stored as high-resolution digital slides in a PC using a high-speed bright-field slider scanner (VENTANA DP 200, Roche Diagnostics International AG, Rotkreuz, Switzerland).

Table 1 Characteristics of recruited pets and sonication protocols for single and overlapping lesions

Pet characteristics	
Weight of cats (Kg)	3–5.55
Weight of dogs (Kg)	7–14
Age (years)	7–15
Tumour type	Sarcomas
Single sonication	
Electrical power (W)	75–200
Pulse duration (s)	10/60
Focal depth (cm)	0/1
Multiple sonications	
Size of tumour (mm^3)	$20 \times 20 \times 30$ – $80 \times 80 \times 60$
Grid size	$2 \times 2/3 \times 3$
Step size (mm)	3
Cooling time (s)	30
Electrical power (W)	150/200
Pulse duration (s)	10
Focal depth (cm)	1/2

Results

Pet recruitment

The anatomical sites of the treated tumours were the neck ($n=1$), leg ($n=2$), face ($n=1$), back ($n=2$) and belly ($n=4$). An example of skin tumour located close to a dog's eye is shown in Fig. 3a, whereas Fig. 3b and c show photos of sarcomas that were located in cats' back. Imaging the target anatomy for accessing proximity to sensitive structures and a proper acoustic window was unnecessary since all tumours were directly visible and accessible by ultrasound. Figure 4 shows a sarcoma on cat's leg immersed in the water container directly above the transducer.

FUS ablation

Single exposure was performed for tumours with a diameter up to 30 mm. Figure 5a shows a typical lesion on dissected skin tumour of a dog. This lesion had a relatively large size, and thus, it was treated by applying an acoustical power of 66 W (electrical power of 200 W) for 60 s. An example of a lesion formed by multiple sonications in a grid pattern can be seen in Fig. 5b. This Figure shows a dissected skin tumour of a dog with overlapping lesions inflicted after a 2×2 ablation (spatial step of 3 mm, time delay of 30 s and focal depth of 1 cm). At each spot, an acoustical power of 66 W was applied for 10 s. Accordingly, Fig. 5c shows overlapping lesions inflicted on a skin tumour of a cat using similar acoustical parameters in a 3×3 grid.

The duration of the FUS treatment was approximately 15 min, including the placement of the animal on the system. The patients remained under general anesthesia throughout

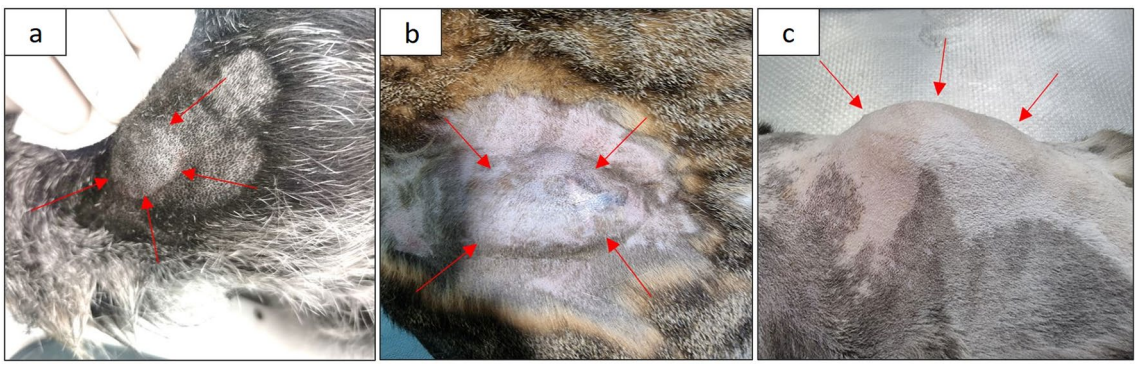


Fig. 3 **a** Skin tumour located close to the eye of a dog and **b–c** Sarcoma in the back of a cat

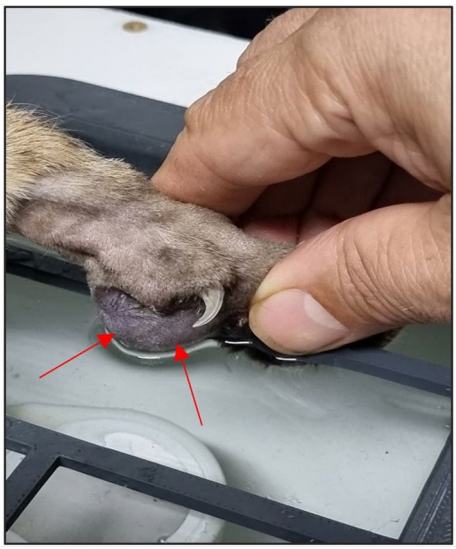


Fig. 4 Placement of a cat leg sarcoma above the ultrasonic transducer

the procedure, with no indications of suffering. Lesion creation was restricted within the tumours.

Histological examination

In 8 out of the 10 treatments, coagulative necrosis was evident in the histological slides. Although FUS caused necrosis of the exposed malignant tissue, some cancer cells were not destroyed. Figure 6 shows typical histological results with a clearly marked area of coagulative necrosis. In this case, successive sonications were performed in a 3×3 grid pattern on a cat sarcoma (Fig. 3c). An acoustical power of 49.5 W (electrical power of 150 W) for 10 s was sufficient to induce coagulative necrosis of the exposed tissue. Figure 6a and b show the necrotized area with magnifications of 10 X and 20 X, respectively. With the higher magnification, blood coagulation was visible in the borderline of the thermal lesion (Fig. 6b). Notably, this lesion was not visible on the skin following the tumour removal. Figure 7 presents histological slides of a skin tumour of a dog, which was treated using a 3×3 ablation pattern (with acoustical/electrical power of 66/200 W for 10 s and focal depth of 1 cm). Notably, almost all cancerous cells seemed to be destroyed in

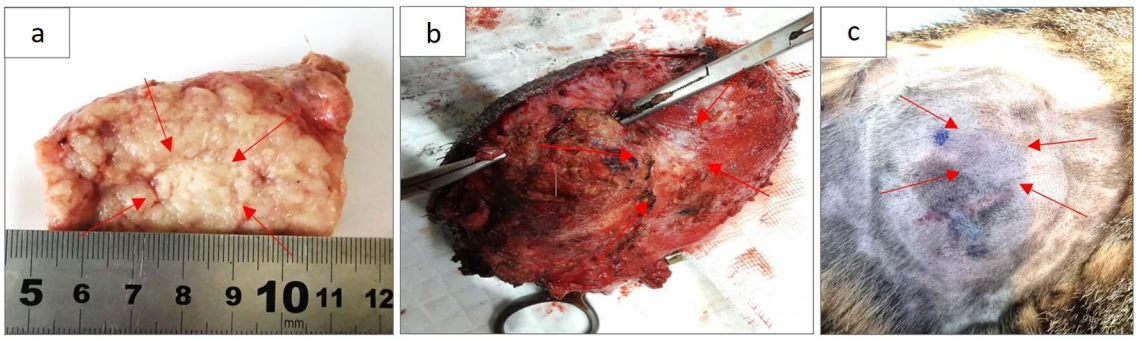


Fig. 5 **a** Lesion inflicted on skin tumour of a dog after single exposure (66 W acoustical power and 60 s pulse duration), **b** Overlapping lesions inflicted on skin tumour of a dog after ablation in a 2×2 grid pattern (3 mm step, 10 s pulse duration, 30 s delay, 66 W acoustical power, and 1 cm focal depth), and **c** Overlapping lesions inflicted on skin tumour of a cat after ablation in a 3×3 grid pattern (3 mm step, 10 s pulse duration, 30 s delay, 66 W acoustical power, and 1 cm focal depth) using the 2.6 MHz transducer (with 40 mm diameter and 60 mm radius of curvature)

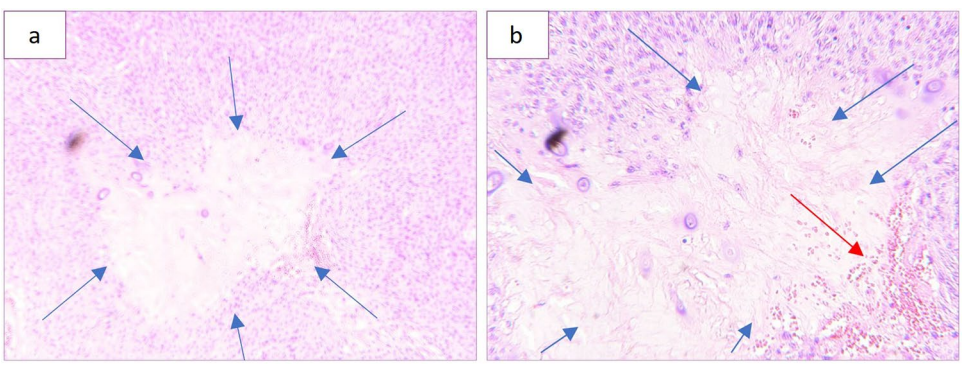


Fig. 6 Histological slides of a cat sarcoma after ablation in 3×3 grid (3 mm step, 10 s pulse duration, 30 s delay, 49.5 W acoustical power, and 2 cm focal depth) using **a** 10X and **b** 20X magnification. Blue arrows indicate a marked area of coagulative necrosis and red arrows indicates blood coagulation at the lesion borderline

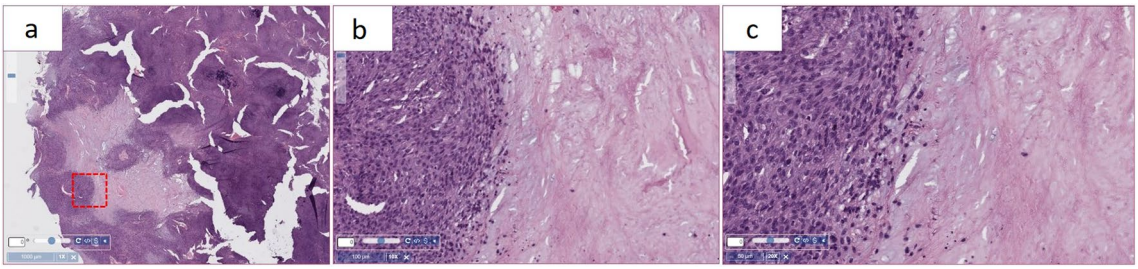


Fig. 7 Histological slides of a dog skin tumour revealing necrotic area resulted from ablation in 3×3 grid (3 mm step, 10 s pulse duration, 30 s delay, 66 W acoustical power, 1 cm focal depth) using **a** no magnification, **b** 10X, and **c** 20X magnification

the non-magnified histological image (Fig. 7a). Intact malignant cells became apparent using magnifications of 10 X and 20 X (Fig. 7b and c, respectively).

The histological images reveal the FUS-induced changes in the cellular structure of the tumours. The cancerous cells appear with irregular size and shape having multiple large, purple-stained nuclei. In contrast, necrotic cells appear anucleate and more eosinophilic, and in places swollen and discohesive. Cytoplasmic hyper-eosinophilia on H&E staining can be attributed to both loss of cytoplasmic RNA and denaturation of cytoplasmic proteins [59]. It should be emphasized that the denaturation of cytoplasmic proteins is directly related to the coagulative necrosis of cells [59].

Discussion

This study was undertaken to evaluate the efficacy of FUS in treating canine and feline superficial sarcoma cancer. The study enrolled dogs and cats with well-defined malignancies without a history of metastasis through the recruitment campaign. A total of ten pets met the inclusion criteria.

The tumours were treated with an MRgFUS robotic system described previously in the literature [51–53]. This system was previously proven efficient in ablating healthy thigh tissue of rabbit models [53]. In this study, the in-vivo performance of the system was demonstrated by treating dogs and cats with naturally occurring sarcomas. Both the hardware and software were proven efficient in a real situation. Notably, few software bugs were identified and corrected without compromising animal safety.

All the lesions were visibly targetable due to their superficial location, and thus, pre-treatment imaging for assessing an appropriate entrance for the FUS beam was not needed. The patient was placed in a proper position defined by the tumour location. In all the cases, the sonication was applied to the tumour laterally and not towards the body. Thus, penetration of the beam in the body was avoided. In line with previous studies [50], the success of ablation relied on the accurate placement of the animal to achieve proper acoustic coupling with the target and sufficient transmission of ultrasound through degassed water.

FUS was applied following the administration of anaesthesia. The ablation protocol was varied mainly depending on the tumour size. A trial sonication of low power was initially applied, thus identifying any signs of animal suffering or other procedure-related complications. The full dose was then deposited in tumours to induce necrosis of cancerous cells. After FUS, the animals were released to continue with surgery from the veterinarians. In most cases, a lesion was clearly visible on the dissected tumour. Overlapping lesions had the expected size based on the grid dimensions.

Previous work on FUS ablation of malignant canine and feline STS is quite limited. The promising results demonstrated here align with a previous retrospective clinical study. In this study, FUS resulted in symptoms alleviation and tumour size reduction of solid tumours in canine patients [49]. However, this study lacks information on the histopathological assessment of the FUS-induced bioeffects [49].

Herein, the dissected tumours were sent for H and E staining to examine the formed lesions. Coagulative necrosis became apparent through the histological slides in 80% of cases. Histological examination suggests that well-circumscribed areas can be precisely necrotized by overlapping lesions. Necrotic regions appeared in H&E staining as well-defined eosinophilic areas with loss of cytoplasmic and nuclear details. Occasionally, blood coagulation was visible on the images. The histopathology results are in agreement with a study by Ranjan et al. [42], in which an oral tumour of a canine patient was successfully ablated using FUS.

Although FUS was able to induce necrosis of malignant tissue, a few cancerous cells in the treated areas remained unaffected. In many cases, magnifications of the histological images up to 10X falsely suggested that all cancer cells were destroyed. Magnifications of 20X and 40X were sufficient to reveal that some cancer cells were still intact. This observation suggests that FUS should be supplemented by radiotherapy or chemotherapy to eliminate the remaining cancer cells.

Efficient system performance was also evident in terms of animal safety. All lesions were restricted in the targeted tumour tissue, and no other procedure-related adverse events were recorded. Thereby, when FUS is applied under proper anesthesia, there is no threat to animal wellbeing. However, risks and discomforts of animals should be further investigated for more challenging tumour locations.

Non-superficial lesions would be far more challenging to be treated. An imaging modality such as MRI is required to define the exact location and the boundaries of deep tumours and establish a safe acoustic path for the beam. Moreover, targeting organs located behind the diaphragm requires maneuvering the ultrasonic beam close to sensitive and bonny structures. In such cases, Magnetic Resonance Imaging (MRI) would be of great importance in accurately targeting the malignancy and monitoring tumour necrosis through MR thermometry [31]. Since the robotic system used in this study can safely operate in an MRI environment, large veterinary clinics with MRI scanners could benefit from real-time MRI monitoring of the procedure. Veterinary MRI scanners are now available for animal trials (Vet-MR Grande, Esaote, Genoa, Italy).

A recent study [50] evaluated the feasibility of targeting canine STS in several anatomic locations with MRI using a clinical commercial MRgFUS system with promising results. However, the capability of FUS to destroy the targeted malignancies was not investigated. Thereby, FUS ablation of challenging tumours is an important issue to be considered in a future study.

As only superficial neoplasms were managed in this preliminary experience, the robotic system was accompanied by a 2.6 MHz transducer. However, it is suitable for targeting any tumour upon minimal modification, such as replacing the transducer with one of lower frequency so that ultrasonic waves can easily penetrate deep tissue.

Another limitation of the study is that lesion creation was restricted within the tumour, while complete destruction of its entire volume was not attempted. Complete tumour removal will require a tumour-specific sonication grid of bigger size and motion of the FUS transducer in all four degrees of freedom. This is also left to be addressed by future research.

Conclusions

Well-delineated thermal lesions were inflicted in superficial canine and feline sarcomas using an MRgFUS system. As a result, coagulative necrosis was confirmed by histopathology assessment. The findings are promising for translating the system to the clinic in the future. Further research is required to optimize the therapeutic protocols to provide efficient treatment of veterinary cancer that meets the clinical requirement and could eventually lead to human applications.

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Author contributions AA mainly contributed to the drafting of manuscript. NE contributed to the implementation of animal experiments. SP contributed to the histological assessment of tumours. KS contributed to the implementation of animal experiments. CD supervised the implementation of the experiments and drafting of the manuscript.

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Data availability All data generated or analysed in the present study are available from the corresponding author on reasonable request.

Code availability Not applicable.

Declarations

Conflict of interests All authors declare no conflict of interest.

Consent to participate Not applicable. The study does not include data on patients.

Consent for publication Not applicable. The study does not include data on patients.

Ethical approval All animal experiments have been approved by the authorities of Veterinary Services, Ministry of Agriculture, Cyprus under the study license (CY/EXP/PR.L6/2019). Six dogs and four cats with naturally occurring sarcoma cancer were enrolled in the study. An informed consent was signed by all pet owners. All animals were treated in a responsible way that did not compromise their welfare.

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