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MRI-guided cryoablation - *in vivo* assessment of focal canine prostate cryolesions

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Abstract

Purpose—To analyze the appearance of acute and chronic canine prostate cryolesions on T1-weighted (T1w) and T2-weighted (T2w) MRI, compare them with contrast-enhanced (CE) MRI and histology, for a variety of freezing protocols.

Methods—Three different freezing protocols were used in canine prostate cryoablation experiments. Six acute and seven chronic (survival times ranging between 4–53 days) experiments were performed. The change in T2w signal intensity was correlated with freezing protocol parameters. The lesion area on T2w MRI was compared to CE-MRI. Histopathologic evaluation of the cryolesions was performed and visually compared to appearance on MRI.

Results—The T2w signal increased from pre-to-post freeze at the site of the cryolesion, and the enhancement was higher for smaller freeze area and duration. The T2w lesion area was between the CE non-perfused area and the hyper-enhancing CE rim. The appearance of the lesion on T1w and T2w imaging over time correlated with outcome on pathology.

Conclusion—T1w and T2w MRI can potentially be used to assess cryolesions and to monitor tissue response over time following cryoablation.

Keywords

MRI-guided; prostate; cryoablation

Introduction

Cryoablation is a promising minimally invasive therapy for localized prostate cancer. Current clinical treatment options for prostate cancer confined to the gland include whole-gland destruction through excision (radical prostatectomy), radiation (external-beam radiotherapy, brachytherapy) or cryoablation. Focal minimally invasive therapies such as cryoablation or high intensity focused ultrasound offer potential alternative treatment options with lower morbidity than the traditional whole gland therapies for patients with localized disease [1–6]. This work reports on MR imaging-based assessment of the focal cryoablation treatment area.

MRI guidance of cryoablation allows accurate monitoring of the ice ball boundary in three dimensions with excellent contrast between the frozen and unfrozen tissue. It potentially allows non-invasive temperature mapping within the iceball and the surrounding tissue during the procedure [7-8]. After completing the cryoablation, MRI can also provide the ability to evaluate tissue damage [9-14]. Accurate imaging of the ablated area with MRI immediately after treatment is clinically of value, as it could be used to evaluate if further treatment is necessary. In addition, further characterization and assessment of tissue changes *in vivo* during healing would be desirable.

It has been shown that cryolesions are visible immediately after treatment as an area of low signal on contrast-enhanced (CE) MR images and that the boundary of coagulative necrosis lies within the contrast enhancing rim [15,16]. Compared to CE, T1-weighted (T1w) and T2-weighted (T2w) imaging can be conveniently repeated acutely post-ablation. The purpose of this work was to analyze the appearance of acute and chronic cryolesions on conventional T1w and T2w images, monitor progression of the lesion appearance over time, compare that with CE-MRI and visually with histology, and investigate whether T1w and T2w imaging could provide additional information in the assessment of cryoablation lesions *in vivo*.

It is known that tissue cryoinjury is related to the freezing protocol and the thermal parameters experienced during freezing, with varying degrees of cellular damage (sub-lethal to lethal) between the outer boundary and the center of the ice-ball [17-21]. During cryotherapy of the prostate, the tissue is exposed to different cooling rates and final temperatures, as a function of distance from cryoprobe tips, variability in thermal conductance due to tissue heterogeneity, and the presence of adjacent vasculature. Therefore, in order to investigate if imaging can detect the differences in treatment outcome across a range of freezing protocols, we designed our study to include multiple freeze protocols. The goal was to identify MR imaging features that could predict the severity of cryoinjury and the subsequent tissue healing over time.

Methods

Study Design

All animal experiments were approved by the Institutional Animal Care and Use Committee. Thirteen adult intact male mixed breed dogs were preanesthetized, intubated, maintained on Isoflurane, and placed supine in the 0.5T GE Signa SP interventional MR scanner. A receive-only endorectal coil was placed in the rectum. A birdcage body coil was used for transmit. Multiple MRI-compatible cryoablation probes (1.15mm or 2mm diameter) and fiberoptic temperature sensors (Luxtron Corporation, CA) were inserted through the anterior abdominal wall into the prostate. Cryoablation was performed using an MRI-compatible cryotherapy system (SeedNet CryoHit system, Oncura Medical). The system operation is based on the Joule-Thomson effect *i.e.* the temperature change of gas during expansion. It uses argon gas for freezing, and helium for thawing. The gas pressure and duty cycle of gas flow can be controlled to achieve different freeze rates.

Three freezing protocols were used in these experiments; as detailed in Table 1. To summarize, the key differences were:

- A. two fast, cold freeze/thaw cycles – using high freeze pressure, high duty cycle
- B. single, not very cold freeze with passive thaw – low freeze pressure, high duty cycle;

- C. single, not very cold freeze with passive thaw – high freeze pressure, low duty cycle.

The multiple freeze protocols were included to evaluate how these variable parameters affected the appearance of the cryolesion on MRI. Both protocols B and C were designed to achieve a slow freeze that was not very cold, to resemble the freezing process at the boundary of the iceball, which would be less damaging than protocol A [19,20]. This was done by varying the duty cycle and the gas pressure during the freezing. The duty cycle duration was 10 seconds. Two lesions were created in each dog's prostate. One lesion was created using protocol A, and the second with either of protocol B or C. The protocol A lesions were created using a single cryoprobe, while the protocol B and C lesions used up to 4 cryoprobes. Multiple probes were used in order to obtain a slow not very cold freeze of adequately large area. The thaw duration for protocol A lesions was 2 – 16 mins, and represents the time that the helium gas was on. For passive thaw (i.e. without active heating), the iceball was monitored till no ice was visible on the MR images, and took approximately 10 – 40 mins depending on iceball size. Seven of the dogs were used in acute studies, and were euthanized within 2-3 hrs after the cryoablation procedure. Six dogs were used in chronic studies, with survival times ranging from 4 days to 53 days. All chronic studies used only protocols A and B.

Imaging

Ice formation was monitored with T1w Fast Spin Echo (FSE) images (TE = 10.5 ms, TR = 300 ms, BW = 32 kHz, echo train length = 4, FOV = 20 cm, scan time = 20 sec). T2w-FSE images (TE = 85-92 ms, TR = 2 sec, BW = 16 kHz, echo train length = 12, FOV = 20cm, scan time = 48 sec) were acquired pre and post-ablation for 6 of the 13 dogs. Post-ablation imaging was performed when the prostate returned to body temperature after end of thawing. T1w 3D SPGR images (TE = 12.6 ms, TR = 25 ms, BW = 7.8 kHz, FOV = 13 cm, scan time = 1 min 36 sec) were acquired post-ablation for all dogs, before and after administration of gadolinium contrast agent. All of the above mentioned imaging was performed in the coronal scan plane. All acute post-ablation imaging was completed within 2 hrs following cryoablation. For the chronic studies, follow-up imaging was repeated prior to euthanasia (see Table 2).

For simplicity of terminology, the cryolesion on T1w-SPGR imaging (acquired before gadolinium contrast injection) is referred to as the T1w lesion. Correspondingly, the cryolesion on T2w-FSE images is referred to as the T2w lesion.

Histology

After euthanasia, the prostates were excised, sliced along the coronal plane at 0.5 mm intervals, fixed in 10% BNF and processed in oversized cassettes for histological analysis. Slides were stained with H&E and a Masson's Trichrome stain (to stain for collagen). Slides were evaluated by a boarded veterinary pathologist, and appropriate levels were compared with MR images.

Analysis

The boundaries of the iceball on the T1w-FSE images acquired during freezing, and of the cryolesion on CE-SPGR and T2w-FSE imaging were manually outlined using OsiriX Imaging Software. A slice from the MR images was selected approximately through the center of the frozen tissue, and the lesion area was measured in cm². Figure 1 shows the maximum iceball area achieved in the acute and chronic cases, for each of the freezing protocols. In general, smaller lesions were intentionally created in the chronic studies as compared to the acute studies, to avoid any post-ablation complications. The freeze area

achieved depended on the freeze duration and the freeze rate, as seen in Fig. 1. The slopes of the lines indicate the different freeze rates achieved for the three protocols.

Acute T2w Signal Measurements—The change in T2w signal from pre-to-post freeze was calculated as the ratio of the mean signal intensity (SI) measured in the frozen region to that in an unfrozen adjacent region within the prostate, normalized by the signal intensity ratio of the same ROI (region of interest) in the pre-freeze image.

$$\text{Acute T2w contrast} = \frac{\frac{SI \text{ in post - freeze lesion}}{SI \text{ in post freeze background}}}{\frac{SI \text{ in pre - freeze lesion}}{SI \text{ in pre - freeze background}}}$$

ROIs were manually drawn on the T2w-FSE images for all measurements. The data from 6 dogs (both acute and chronic) for which T2w imaging was performed both pre and post-ablation, was used for this analysis. One protocol B lesion in which the ice did not extend beyond the probe artifact was excluded.

To understand the variability in the lesion appearance on T2w imaging, and to determine the dependence of T2w signal change on the experimental variables, the Pearson's correlation coefficient of the T2w signal change was calculated for the following parameters: freeze duration, freeze area, number of freeze cycles and time from end of freeze to imaging. For protocol A (double-freeze) lesions, the above mentioned time was measured from the end of the second freeze.

Chronic T2w Signal Measurements—The change in T2w signal over time was calculated as the ratio of the mean signal intensity measured within the cryolesion to that in an adjacent unfrozen region outside the cryolesion.

$$\text{Chronic T2w contrast} = \frac{SI \text{ in lesion ROI}}{SI \text{ in background ROI}}$$

Similar slices (through centers of lesions) were selected from the imaging on different days. Even as the T2w lesion size decreased over time, the ROI was chosen to be within the T2w lesion. The measured T2w contrast was compared for the freezing protocols A and B to observe trends over time.

The T1w lesions were variable in visibility (explained further in results section), so the lesion sizes were difficult to measure and therefore were not used in area comparisons.

Results

Representative images of the iceball, T1w-SPGR, T2w-FSE and CE-SPGR acquired acutely post-ablation for 2 subjects are shown in Figs. 2 and 3. Figure 2 shows the images from an acute study with protocol A and C lesions. The cryolesions are visible on MR images, and appear differently on T1w, T2w and CE images. The macro histological section of the prostate from this subject demonstrates acute hemorrhagic necrosis comprising the majority of the lesions. Figure 3 shows the images acquired acutely post-ablation for a chronic study with protocol A and B lesions. The two cases show the variability in appearance of acute T1w and T2w lesions. For example, the T2w contrast of the lesions differs noticeably between the two subjects.

T2w Imaging

The prostatic tissue demonstrated increased T2w signal from pre-to-post freeze at the site of the cryolesion. The appearance of the T2w lesions ranged from slightly hyperintense to very hyperintense.

Figure 4 represents the scatter plots of the acute T2w contrast as a function of the freezing protocol parameters. The T2w signal change correlated well with the freeze area, shown in Fig. 4a, (Correlation coefficient $r = -0.81$, p -value = 0.003), and with freeze duration, shown in Fig. 4b ($r = -0.79$, $p = 0.003$). There was no significant correlation with number of freeze cycles, as shown in Fig. 4c ($r = 0.21$, $p = 0.48$) or with time between ablation and imaging, as shown in Fig. 4d ($r = 0.28$, $p = 0.34$).

The acute T2w contrast was greater for a smaller freeze area, and a shorter freeze duration in our experiments. However, these parameters are related, as freeze area itself was highly correlated with freeze duration ($r = 0.67$, $p = 0.01$). This is expected due to the nature of the freezing process, as a longer time is needed to freeze a larger area. The time interval from cryoablation to imaging in these acute cases was short - approximately 1 hour, therefore any dependence on time may not be measured in these cases.

For the chronic studies, the T2w lesion appearance over time differed for the two freezing protocols A and B, as shown in Fig. 5. For protocol A, the observed T2w contrast first increased, and then decreased. For protocol B lesions, T2w contrast decreased from the Day 0 value with one exception; the lesion contrast for dog 3 first increased then decreased, similar to protocol A. At day 53 the protocol B lesion was not visible, while the protocol A lesion was very slightly hyperintense.

Lesion Size Comparison—In general, the boundaries of the T2w lesion and the CE lesion corresponded well. A comparison of the acute T2w and CE lesion areas (for all three freeze protocols) is shown in the scatter plot of Fig. 6a. The T2w lesion area lies along the identity line. Acutely post-ablation, the T2w lesion area was greater than the non-enhancing area on CE images, but smaller than the non-enhancing CE lesion plus the surrounding hyper-enhancing rim. This finding was consistent over time for the chronic studies as both T2w and CE lesion sizes decreased on follow-up imaging. Figure 6b depicts an example of one such case.

T1w Imaging

Acutely post-ablation, the T1w lesion was observed in only 6 of the 13 dogs. The appearance of the acute T1w lesions (as seen in Figs. 1 and 2) ranged from isointense to slightly hypointense, and they were generally surrounded by a dark rim. The lesion centers often appeared heterogeneous. In some slices the dark rim was poorly demarcated or discontinuous, and the T1w lesion was not present in all slices that contained a CE lesion. There were also no apparent differences observed between appearance of acute T1w lesions for different protocols.

In the only dog imaged at 4 days post-ablation, the T1w lesions, which were not visible at day 0, were now apparent. Their appearance was similar to the acute T1w lesions described above. At 7 days post-ablation, all cryolesions on T1w images demonstrated T1 shortening and appeared hyperintense. At days 14 and 21, for protocol A lesions, the outer border was hypointense (longer T1), while the center remained hyperintense. There was one exception, where the lesion in dog 5 at day 21 was centrally hypointense, corresponding to a fluid filled cavity documented grossly at necropsy. All of the four protocol B lesions at days 14 and 21 were hypointense. At day 53, only the protocol A lesion was still visible, and the entire

lesion was hypointense. Thus, the T1 changes over time appear to occur from the rim of the lesion inwards.

Histology

Histologically, the acute cryolesions for all three protocols appeared similarly hemorrhagic with severe gland fragmentation and necrosis. For the chronic studies, as time progressed, both protocol A and B lesions became less hemorrhagic and demonstrated regeneration of glands and formation of scar tissue. Overall, protocol B lesions were smaller in area and caused slightly less damage, ultimately resulting in more gland regeneration.

Specifically, lesions at day 4 were predominantly hemorrhagic but around the periphery of the lesion, regeneration and healing had begun. Glands at the lesion borders, were hyperplastic and lined by irregularly piled up epithelial cells. By day 14, the cryolesion consisted of hyperplastic glands, granulation tissue (immature scar tissue and new capillary formation), hemosiderin laden macrophages and mixed inflammatory cells. By day 21, the lesions consisted of denser, slightly hyaline (glassy) scar tissue. In both dogs euthanized at this time point, the prostatic capsule adjacent to the site of cryoinjury had contracted into the body of the prostate and merged with the healing cryolesion. In one of these dogs (dog 5), a large irregular “hole” or cyst lined by attenuated prostatic epithelial cells occupied the lesion site. By day 53, the lesion consisted entirely of dense scar tissue surrounded by regenerated glands

Histological comparison with MR imaging in select subjects

The imaging and histology results from the protocol A cryolesion in two subjects (dogs 4 and 5) are presented in this section. These subjects were chosen as they developed different histopathological outcomes, though both received similar treatments and both were euthanized at 21 days post-ablation. Both subjects were imaged on days 0, 7 and 21 post-cryoablation. Figures 7 and 8 represent T1w, T2w, and CE images of the prostates of the two dogs. Photomicrographs of the corresponding histological sections (macro and microscopic images) are shown in Fig. 9. In both subjects, the protocol A cryolesion is on the left side of the prostate (*i.e.* the right side of the image). Table 3 summarizes the appearance of the lesion on the MR images and histology. The T1w and T2w lesions in Figs. 7 and 8 correspond well with the CE lesions. The appearance at day 0 and 7 was described in the above sections. Here, the lesion appearances on T1w, T2w imaging and histology at day 21 are compared.

In dog 4 at day 21, the T2w lesion was slightly hyperintense, while the T1w lesion had a hyperintense center, surrounded by a slightly hypointense outer border. Histologically, this lesion consisted of a central area of dense maturing scar tissue surrounded by regenerating glands. Also in this dog, due to the somewhat peripheral location of the cryolesion, the prostatic capsule was contracted into the healing cryolesion. At day 21 in dog 5, the cryolesion was very hyperintense on T2w images, and was hypointense on T1w images. This corresponded to a fluid filled hole or cystic structure observed grossly and histologically. This cystic space was surrounded by regenerating glands, scar tissue and inflammatory cells, which corresponded to the slightly hypointense outer border of the T1w lesion.

The different gross and microscopic appearance of the lesions in these 2 dogs helps explain the differences in imaging findings. Thus, the appearance of the lesion on T1w and T2w imaging over time correlates with outcome on pathology.

Discussion

In this study we report on assessment of cryoablation tissue damage in the *in vivo* canine prostate with T1w and T2w MRI. Cryolesions created using different freezing protocols were analyzed at various post-ablation time intervals with MRI and histopathology to understand the MR appearance of the acute and chronic lesions and tissue regeneration. Lesions created in the prostate gland by cryoablation appear differently on the various MR imaging techniques as they heal. Compared to CE imaging, T1w and T2w imaging can be conveniently repeated acutely post-ablation, and can also be done between multiple freeze-thaw cycles. T1w and T2w imaging can provide additional evaluation of scar tissue formation and gland regeneration, along with the vascular information that CE provides.

The acute T2w lesion area lies between the CE-non perfused and the CE-hyperemic rim. For all lesions combined, the mean area of the CE non-perfused lesion was 69.6 mm², of the T2w lesion was 89.2 mm², and of the CE non-perfused lesion plus hyperemic rim was 114.5 mm². Similarly, a previous study showed that the necrosis area on histology also lies between the CE-non perfused and the CE-hyperemic rim in acute studies [15]. From their results for all lesions combined, the mean area of the CE non-perfused lesion was 167 mm², of tissue necrosis was 187 mm², and of the CE non-perfused lesion plus hyperemic rim was 328 mm². This comparison of the T2w lesion area with the CE lesion area in conjunction with the CE comparison to histology in [15], provides an indirect approach to understanding how the T2w lesion compares to histology. It adds to our knowledge of how the vascular injury depicted by CE MRI relates to the coagulation necrosis in tissue. Based on the combined results of these studies, T2w imaging can potentially also be a useful indicator of the area of damage. A direct comparison of the acute T2w lesion area with necrosis could not be performed in this study, and would be valuable to validate how accurately T2w imaging can predict the cryo treatment margins. Unfortunately the few cases for which both the acute T2w imaging and histology data was available, and the difficulty in matching the MR images with the histology slides limits this comparison. Future studies will include a comparison of lesion area on T2w imaging with necrosis on histology.

The T2w imaging visualized the lesion for a variety of freezing protocol parameters, though the signal intensity varied depending on these parameters. The T2w signal enhanced more for a smaller freeze area. We hypothesize that the T2w signal change is caused by fluid accumulation within the cryolesion from injured microvessels within and surrounding the lesion, and that in larger lesions the fluid is distributed over a larger volume, resulting in less T2w signal increase. The acute T2w signal analysis is complicated by the co-dependence of the freeze protocol parameters. More data is needed to separate out the effect of all these variables, to enable a multi-parametric analysis

The central hemorrhagic region of the cryolesion appears isointense or hypointense on acute T1w images, while the dark rim could be a combination of hemorrhage and edema. The T1w lesion was visible only in 6 dogs on imaging done immediately after cryoablation, though there was significant hemorrhage observed on pathology in all acute dogs. This could potentially be due to a delayed appearance of the T1w lesion on MRI. This delay in appearance was seen in 2 of our chronic studies, where the T1w lesion was not observed on day 0 but was visible on day 4 (dog 1) and day 14 (dog 2). Over time as erythrocytes break down and the hemorrhage is resolved, the lesion appearance evolves on T1w imaging. At day 7, the T1w lesion was hyperintense, indicating T1 shortening. Between day 7 and 21, the T1 lengthened. At day 53, the site of the lesion showed dense scar tissue, which was seen as a hypointense T1w lesion.

Acutely post-ablation (day 0), no differences were seen on T1w or T2w imaging between the various freeze protocols. The T1w lesion appeared hyperintense for both protocols at day 7. T2w imaging performed on day 7 however showed a difference in lesion appearance between the different protocols. A limited number of time points were imaged in this study, and it would be valuable to perform a longitudinal study with more imaging conducted between day 0-7 to resolve better the timeline of how the freezing protocols differ on T2w imaging.

The secretory epithelium that lines the prostatic glands is capable of undergoing regeneration as long as some basal cells and the structural basement membranes remain intact. At the edges of the cryolesions, where temperatures are not as cold, sub lethally damaged glands regenerate. Functionality of the glands, however, remains unclear. In addition it is unclear how closely the regenerative capability of canine prostate glands compares to the regenerative potential of human prostate tissue and prostate cancer.

In the skin model of wound repair, healing begins within approximately 24 hrs as the associated hemorrhage clots and inflammatory cells migrate to the site. Within 3-7 days, a bed of granulation tissue is established, while the formation of mature scar tissue may take several weeks [24]. We have documented a similar time frame for healing in the cryolesioned prostate tissues.

In conclusion, T1-weighted and T2-weighted MRI can potentially be used to assess cryolesions and to monitor tissue response over time following cryoablation. Further refinement and interpretation of subtle differences noted between the various freezing protocols on the different imaging modalities is desirable.

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Figure 1.

Ice-ball size as a function of freeze duration for each of the freeze protocols. The different freeze protocols are represented by the characters 'A', 'B' and 'C'. The data from the acute studies are in black, and from the chronic studies are in gray text color. The different freeze protocols had different freeze rates, represented by the slope of the lines. The chronic studies generally had smaller freeze areas to avoid post-operative complications. Freeze area is correlated with freeze duration, as a longer time is required to freeze a larger area.

Figure 2.

Representative images of maximum freeze T1-FSE, T1-3DSPGR, T2-FSE, CE-SPGR, and histopathology sections for an acute study with protocol A and C lesions. The protocol A lesion is on the left side of the prostate (*i.e.* right side of image), as marked on the iceball image.

Figure 3.

Acute images taken immediately post-ablation from a chronic study with protocol A and B lesion show the maximum iceball on T1-FSE, and the cryolesion after ablation on T1-3DSPGR, T2-FSE, and CE-SPGR. The protocol A lesion is on the left side of the prostate (*i.e.* right side of image), as marked on the iceball image.

Figure 4.

Acute T2w signal change as a function of freeze protocol parameters. The different freeze protocols are represented by the characters 'A', 'B' and 'C'. T2w contrast correlated well with freeze area (a) and freeze duration (b). The plots in (c) and (d) show the correlation of T2w contrast with number of freeze cycles or with time after freeze is not significant.

Figure 5.

Evolution of T2w contrast over time for the two freeze protocols in chronic experiments. The T2w contrast for protocol A lesions first increased (day 4,7), and then decreased. The T2w contrast for protocol B lesions decreased after day 0 with one exception – dog 3 protocol B lesion contrast increased & then decreased, similar to protocol A lesion.

Figure 6.

(a) Comparison of acute T2 lesion area vs. CE lesion area. The T2 lesion area lies along the identity line. The T2 lesion area is greater than the CE non-perfused area but smaller than the CE with bright rim area. (b) Similar to the acute case, the T2 lesion size stays greater than CE non-perfused area, but less than CE with bright rim area, even as lesion decreases in size over time.

Figure 7.

T1w, T2w and CE images acquired at different days post-ablation for Dog 4 show the evolution of the cryolesion over time. The appearance of the lesion at day 21 correlates with the histology in Fig. 9.

Figure 8.

T1w, T2w and CE images acquired at different days post-ablation for Dog 5 show the evolution of the cryolesion over time. The protocol A lesion at day 21 was seen as hyperintense on T2w and hypointense on T1w images, and was found to be a cyst on pathology (shown in Fig. 9).

Figure 9.

Top row shows the H&E and trichrome stain macro sections for dogs 4 & 5, corresponding to the MR images shown in Figs. 7 and 8. The H&E microscopic sections for the protocol A lesion are shown in the bottom row.

Table 1

Details of the freezing protocol parameters used in this study.

Protocol	A	B	C
Freeze cycles	2	1	1
Thaw	Active	Passive	Passive
Freeze duty cycle	100 %	100 %	20 - 40 %
Gas pressure	3500 - 4000 psi	1000 - 1500 pi	3000 - 3500 pi
Target end temperature	< -40° C	> -10° C	> -10° C
Freeze duration	2 - 25 mins	14 - 30 mins	30 – 40 mins
Iceball area	0.886 – 5.049 cm2	0.495 – 1.869 cm2	2.85 – 4.29 cm2
No. of cryoprobes	1	2 - 4	1

Table 2

Follow-up imaging time points for the chronic experiments. The dogs were euthanized immediately after last imaging session.

Subject	1	2*	3	4	5	6
Follow-up imaging (days post-ablation)	D0, D4	D0, D14	D0, D7, D14	D0, D7, D21	D0, D7, D21	D0, D53

* Dog 2 did not have T2w imaging acutely post-ablation, only on Day 14. T1w imaging was performed on both days.