SCANDINAVIAN JOURNAL OF UROLOGY
2020, VOL. 54, NO. 1, 33–39
https://doi.org/10.1080/21681805.2019.1702094

Taylor & Francis
Taylor & Francis Group

ARTICLE

Renal cryoablation – does deep endophytic ablation affect the renal collecting system?

Ahmad Makki[a], Malene B. Aastrup[a], Hanne Vinter[b], Bodil Ginnerup[c], Ole Graumann[d], Michael Borre[a] and Tommy Kjaergaard Nielsen[a]

[a]Department of Urology, Aarhus University Hospital, Aarhus, Denmark; [b]Department of Pathology, Aarhus University Hospital, Aarhus, Denmark; [c]Department of Radiology, Aarhus University Hospital, Aarhus, Denmark; [d]Department of Radiology, Odense University Hospital, Odense, Denmark

ABSTRACT

Objective: To investigate to what extent the urothelium of the renal collecting system is affected when performing deep endophytic cryoablation.

Methods: The study was conducted as an *in vivo* animal model with a total of 15 female pigs. Each animal was subjected to bilateral endophytioc renal cryoablation and randomized to a postoperative follow-up period of either one, two or four weeks. At the end of follow-up all animals had a magnetic resonance imaging (MRI) examination and bilateral nephrectomy was performed. On MRI-imaging the extent of the cryolesions, as well as signs of urinomas or fistulas, were examined. Histopathologic examinations were performed to investigate the effect on the urothelium.

Results: All animals tolerated the procedure well without any postoperative complications. MRI examinations found the renal collecting system to be involved in the cryolesions at all three stages of follow-up and revealed no signs of hematomas, urinomas or fistula formations. Epithelial edema was found at all three stages of follow-up while significant parenchymal fibrosis adjacent to the urothelium was most pronounced in the four weeks of follow-up group. The urothelium was significantly affected with luminal hemorrhage as well as hemorrhage in and underneath the urothelium and urothelial dissociation from the underlying renal parenchyma. Despite these impacts on the urothelium, this was found to be intact and vital at all three stages of follow-up, in sharp contrast to the renal parenchyma that underwent fibrotic changes.

Conclusions: In this, *in vivo* non-tumor pig model CA effectively destroyed the renal parenchyma while the impacted renal urothelium remained intact and did not undergo fibrotic changes, nor was urinomas or fistulas observed.

Abbreviations: CA: Cryoablation; MRI: Magnetic resonance imaging; TSE: Turbo spin echo; FS: Fat saturation; BGP: Bodil Ginnerup Pedersen; HE: Hematoxylin/eosin; MT: Masson Trichrome; MA: Malene Aastrup; HV: Hanne Vinter; RFA: Radiofrequency ablation.

ARTICLE HISTORY
Received 6 September 2019
Accepted 4 December 2019

KEYWORDS
Renal cryoablation; renal collecting system; treatment of renal cancer

Introduction

The increased use of cross-sectional imaging for otherwise unrelated conditions has significantly contributed to the early detection of renal masses, many of which are T1a cancers less than four cm [1,2]. According to urological guidelines partial nephrectomy remains the recommended modality for the treatment of small renal masses [3,4]. But for the past decade, ablative techniques have proven to be effective in selected patients, where the modality can offer a minimally invasive approach with the potential benefits of low complication rates, decreased morbidity and acceptable oncologic outcomes [5–7]. These techniques, including cryoablation, radio frequency ablation and microwave ablation, is considered safe but can be complicated with unintended thermal injury to the surrounding structures such as bowel loops, nerves or the urinary collecting system [8]. The efficacy of cryoablation (CA) depends on the biological characteristics of the targeted tissue, namely the rate of blood flow, density, specific heat and thermal conductivity, and therefor concerns for the possibility of persistent tumor cells following CA can be raised when treating deeper tumors located adjacent to the pelvicaliceal system [8,9]. While the management of small renal masses has been, and still is, evolving toward minimally invasive and nephron-sparing surgery, the treatment of central and endophytic tumors still remains a challenge. Treating such tumors with cryoablation can be challenging due to the tumors proximity to the renal vasculature and the collecting system, resulting in a possible disturbance in the ablation zone due to a natural heat sink primarily caused by the flow of blood and urine production [9,10].

In contrast to other ablative modalities CA appears to spare the renal collecting system, but this aspect is based on very limited and contradictory evidence [9,11,12].

CONTACT Ahmad Makki A.makki88@gmail.com Department of Urology, Aarhus University Hospital, Aarhus, Denmark

In the present study, we systematically investigate the impact of CA on the urothelium of the renal collecting system in an *in vivo* pig model by utilizing magnetic resonance imaging (MRI) examinations and histopathologic examinations.

Materials and methods

The present study was conducted at the Department of Urology, Aarhus University Hospital and the Department of Clinical Medicine, Aarhus University. The study was approved by the Danish Authority on Animal Experiments (registry number: 2013-15-2934-00778). The Animal Research: Reporting of *In Vivo* Experiments guidelines on reporting animal experiments were adhered to [13]. All animals were female Danish Landrace pigs, weighing approximately 40 kg and being approximately 100 days old. A total of 15 animals were used. All animals were kept for one week at a certified research pigsty for acclamation and optimization of bowl function prior to intervention. Before treatment, each animal was randomized into one of three follow-up groups after which laparoscopic-assisted bilateral renal CA was performed. After the treatment, all animals were returned to the pigsty facilities and dependent on randomization they were kept at the pigsty for one, two or four weeks postoperatively before undergoing MRI examination prior to nephrectomy. All pigs were humanely euthanized using an overdose of pentobarbital after nephrectomy.

Anesthesia and postoperative care

Preanesthesia medication consisted of 2 mL Penovet Vet (300.000 IU/mL), 4 mL Stresnil (40 mg/mL), 4 mL Dormicum Vet (5 mg/mL), and 10 mL Hypnomidate (2 mg/mL). During surgery, animals were under general anesthesia using Propofol 15 mL/h and kept pain free with an infusion of 10 mL/h Fentanyl (50 mg/mL). At each laparoscopic port site, 2.5 mL of Bupivacain (5 mg/mL) was injected. A dose of 750 mg Cefuroxime was given both pre- and postoperatively, and an injection of 2 mL Finadyne (50 mg/mL) was administrated after surgery as well. All animals were given a catheter a demure during the procedure.

Postoperative pain management consisted of Finadyne paste (50 mg/g) for the first four days postoperatively. The well-being of the animals was systematically monitored and registered daily by means of a scoring chart for the first five days and thereafter by daily interactions with the animal caretakers.

Cryoablation procedure

Using a standard three-port laparoscopic approach, the kidneys were identified and mobilized, allowing for ultrasound-guided cryoprobe placement (BK Medical 4-Way Laparoscopic 8666-RF; Herlev, Denmark). One 17-gauge cryoprobe (IceSphere, BTG Galil Medical, Arden Hills, Minnesota, USA) was inserted to a depth of 30 mm at the lateral rim five cm from the top of the upper renal pole, ensuring close proximity to the renal collection system and confirming this by intra abdominal ultrasound. A 17-gauge multipoint Thermal Sensor (BTG Galil Medical) was placed parallel to the cryoprobe at a distance of approachable 10 mm. CA was performed and monitored using the Visual-ICE System (BTG Galil Medical) with argon gas for freezing and helium gas for thawing. The CA procedure consisted of a standard double freeze cycle with ten minutes of freezing followed by six minutes of passive thaw and then two minutes of active thaw. The same procedure was performed on the contralateral kidney.

MRI examination

The MRI examination of the renal parenchyma involved a respiration-triggered examination of the animals in the supine position on a 3-T magnet (Siemens Magnetom Skyra, Siemens, Erlangen, Germany) with an 18 channels body coil. Three sequences were performed before intravenous contrast was administered; axial 4 mm T2 turbo spin-echo (TSE), axial 5 mm T1 gradient echo sequence with fat saturation (FS), and coronal 4 mm T2 TSE. Intravenous contrast, Dotarem (gadoterate meglumine, 279.3 mg/mL; Guerbet, France), was administered at 0.2 mL/kg body weight, and a nondynamic postcontrast 4-mm coronal T1 TSE sequence with FS was undertaken (T1-FS + gadoterate meglumine contrast agent [Gd]). A board-certified senior radiologist (BG?) with 8 years of experience in body MRI evaluated all images. The radiologist was blinded to the length of the follow-up period.

Histopathology

Nephrectomy was performed immediately after MRI. A section cut was placed through the middle of the cryolesion in order to obtain optimal fixation (10% buffered formalin). The tissue containing the cryolesion and the surrounding renal parenchyma was cut in four mm thick sections and dehydrated in a Tissue-Tek VIP tissue processor (Sakura, Nagano, Japan). Following dehydration, tissue was embedded in paraffin and cut at two to three μm and stained with hematoxylin/eosin (HE) and Masson Trichrome (MT). Each specimen was systematically examined by microscope to identify the affected urothelium and determine whether it was intact or urothelial dissociation had occurred (categorized as yes/no). Likewise, the presence of liponecrosis and hemorrhage in the urothelium, underneath the urothelium and luminar hemorrhage were examined and categorized as yes/no. Urothelial edema and parenchymal fibrosis were categorized as much/little/none. Inflammatory cells were categorized as many/few.

Whole slide images were captured by Nanozoomer 2.0HT (Hamamatsu Photonics K.K., Hamamatsu City, Japan) at magnification 20X. The distance from the cryoablative induced tissue necrosis to the urothelium was manually outlined and measured using NDP Viewing software U12388-01 (Hamamatsu Photonics K.K., Hamamatsu City, Japan).

Histological examinations were independently performed by MA and HV, and in case of discrepancy the case was disused and settled with a genitourinary pathologist with more

than 35 years of experience (SH). All examiners were blinded to the length of follow-up and all specimens were examined twice using the same microscope.

Results

Apart from expected perioperative gross hematuria all 15 animals tolerated the procedure very well with no postoperative complications. No animals were excluded from the study.

Macroscopic examination

In the one-week group, the cryolesions appeared edematous with blood stasis surrounding a large part of the renal collection system. In the two and four-week groups, the cryolesions gradually retracted and the edematous appearance changed toward a more sharply demarcated fibrotic appearance that extended toward the renal collection system (Figure 1).

MRI findings

At all three stages of follow-up, the cryolesions were found to extend through the renal parenchyma involving the adjacent renal collecting system (Figure 2). No urinomas or fistulas in relation to the cryolesions were observed at any of the three stages of follow-up.

Pathology

The histopathologic findings for each of the three follow-up groups are listed in Table 1. Each group consisted of five animals with a total of ten kidneys treated in each group.

The shortest distance from the urothelium to the affected parenchyma was found to be 580 μm (IQR 494), 444 μm (IQR 589) and 2411 μm (IQR 783) in the one, two and four weeks of follow-up groups, respectively (Table 2). Luminal hemorrhage within the renal collecting system was observed in 90% of the specimens in both the one and two-week follow-up groups and in 80% of the specimens with four weeks of follow-up. Urothelial dissociation from the underlying renal parenchyma was found in 63%, 33% and 50% of the specimens in the one, two and four weeks of follow-up groups, respectively. Hemorrhage underneath the urothelium and hemorrhage within the urothelium was observed in 80%, 50%, 20% and 80%, 60%, 30% of the specimens in the one, two and four weeks of follow-up groups, respectively. Significant urothelial edema was found in 30%, 60% and 20% of the specimens in the one, two and four weeks of follow-up groups, respectively. Liponecrosis was observed in 25%, 38% and 10% of the specimens in the one, two and four weeks of follow-up groups, respectively. Significant parenchymal fibrosis adjacent to the urothelium was found in 33%, 40%, 90% and a high number of inflammatory cells was observed in 50%, 40% and 20% of the specimens in the one, two and four weeks of follow-up groups, respectively. Examples of histology sections are found in Figure 3.

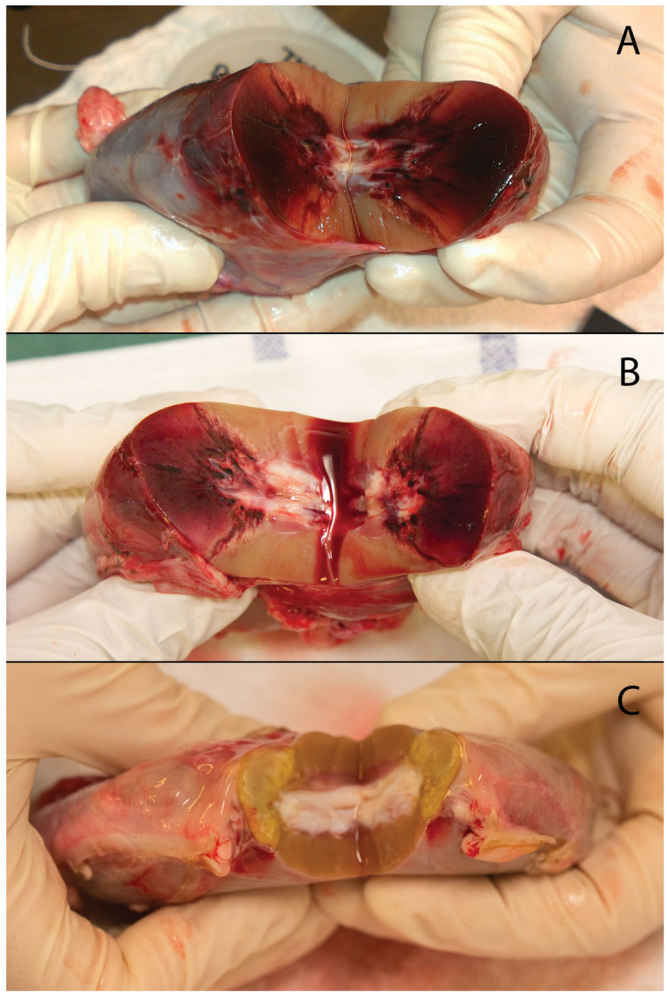

Figure 1. (Macro). Macroscopic evaluations of the cryo lesion immediately after nephrectomy. (A) The cryo lesion one week after ablation with blood stasis in the ablation zone and the involved collecting system. (B) The cryo lesion two weeks after ablation in which the ablation zone appears more well defined and with less blood stasis in the collecting system. Fibrotic transformation is starting at the edge of the cryo lesion (transformation zone). (C) The cryo lesion four weeks after ablation with almost complete fibrotic transformation and the collecting system appears completely intact as the ablation zone becomes a fibrotic scar.

Discussion

In the present study, we examined the urothelium of 30 kidneys from 15 pigs that were exposed to a renal cryoablation. Apart from the expected perioperative gross hematuria, all 15 animals tolerated the procedure very well. MRI examinations found the renal collecting system to be involved in the cryolesions at all three stages of follow-up but revealed no signs of post-operative complications. The renal collecting system was found to be significantly affected by CA but remained intact, and in contrast to the renal parenchyma the urothelium did not undergo fibrotic transformation.

In an *in vivo* pig study by Sung et al. [12] CA was intentionally performed on the renal collecting system, investigating both the short- and long-term sequelae. At one month of follow-up, regrowth of normal urothelium occurred with some scarring of the lamina propria or underlying smooth muscle and cytoplasmic vacuolization was seen in the acute

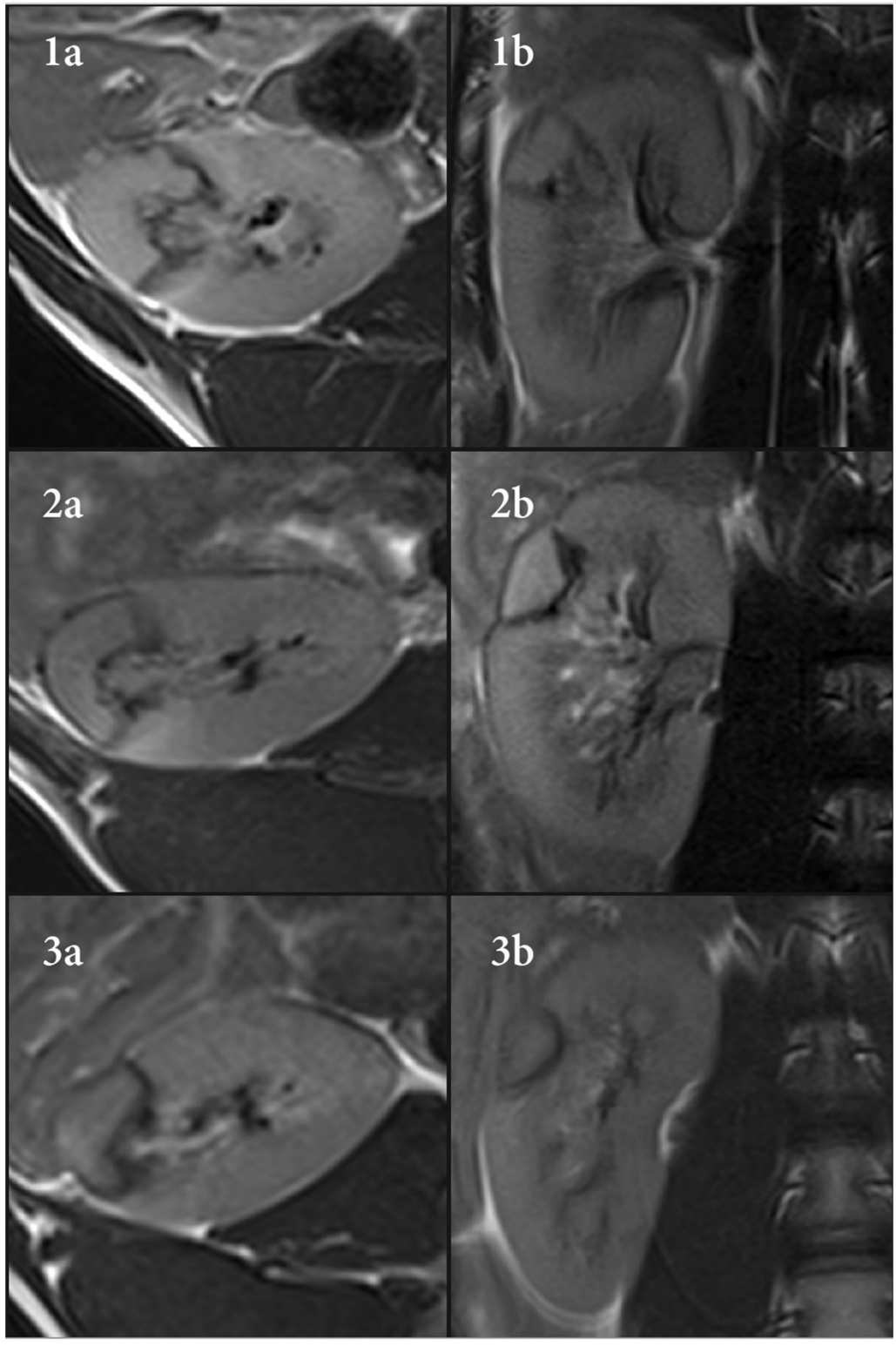

Figure 2. (MR). T2-weighted magnetic resonance imaging of the endophytic cryo lesions after one (1a/b), two (2a/b) and four weeks (3a/b) of follow-up. The cryo lesions are involving the entire renal parenchyma and extending into the renal sinus and collecting system. After four weeks (3a/b) the cryolesions decrease in size as the fibrotic transformation increases.

cryolesions, comparable with the histopathologic findings of the present study. Also, as found in the present study, the authors did not see find any signs of post-operative urinary extravasation. In this study we found the highest concentration of inflammatory cells in the one and two-weeks follow-up groups while the number of inflammatory cells was considerable decreased in the group with four-weeks of follow-up, indicating only a relative short period of

Table 1. Histologic finding.

	1 week	2 weeks	4 weeks	Total
Intact urothelium, *n* (%)[a]				
Yes	10 (100)	10 (100)	10 (100)	30 (100)
No	0	0	0	0
Epithelial edema, *n* (%)[a]				
Much	3 (30)	6 (60)	2 (20)	11 (37)
Little	7 (70)	3 (30)	6 (60)	16 (53)
None	0	1 (10)	2 (20)	3 (10)
Urothelial Dissociation, *n* (%)[b]				
Yes	5 (63)	3 (33)	5 (50)	13 (48)
No	3 (37)	6 (67)	5 (50)	14 (52)
Parenchyma fibrosis, *n* (%) [b]				
Much	2 (33)	4 (40)	9 (90)	15 (54)
Little	5 (45)	6 (60)	1 (10)	12 (39)
None	2 (22)	0	0	2 (7)
Liponecrosis, *n* (%)[b]				
Yes	2 (25)	3 (38)	1 (10)	6 (23)
No	6 (75)	5 (62)	9 (90)	20 (77)
Inflammatory cells, *n* (%)[a]				
Many	5 (50)	4 (40)	2 (20)	11 (37)
Few	5 (50)	6 (60)	8 (80)	19 (63)
Hemorrhage underneath the urothelium, *n* (%)[a]				
Yes	8 (80)	5 (50)	2 (20)	15 (50)
No	2 (20)	5 (50)	8 (80)	15 (50)
Hemorrhage in the urothelium, *n* (%)[a]				
Yes	8 (80)	6 (60)	3 (30)	17 (57)
No	2 (20)	4 (40)	7 (70)	13 (43)
Luminar hemorrhage, *n* (%)[a]				
Yes	9 (90)	9 (90)	8 (80)	26 (87)
No	1 (10)	1 (10)	2 (20)	4 (13)

HE stained[a], MT stained[b].

Table 2. Urothelium measurements.

	1 week	2 weeks	4 weeks
Urothelial thickness			
Meanvalues, µm	23.4	21.3	26.0
Distance to necrotic tissue*			
Meanvalues, µm (IQR)	579.8 (493.8)	443.7 (589.3)	2411.3 (783.3)

*shortest distance measured from urothel to necrotic tissue.

time where the urothelium is affected by an acute inflammatory response following CA. Furthermore, hemorrhage within the urothelium and underneath the urothelium was seen in 80% of the specimens with one week of follow-up, compared to only 20% and 30% of the specimens with two and four-weeks of follow-up, respectively. In general, we found the changes of urothelium to be more similar to each other in the one and two weeks-groups, compared to the four weeks-group where the acute inflammatory response appeared to be in regression. These findings imply only a short-term, and non-lethal impact, to the urothelium following CA. These findings were supported in a study by Janzen et al. [14], where the authors found that CA resulted in preservation of the urothelial architecture in 62% of cases, compared to only 8% in radio frequency ablation (RFA) lesions. In a study by Brashears et al. [15] the authors found a high risk of urinary fistulas in RFA procedures. RFA procedures are heat ablation and therefore not completely comparable to CA. In the present study, we did also not find signs of urinomas or fistulas at any of the three stages of follow-up, supporting the findings by Sung et al. [12] and Janzen et al. [14].

Gross hematuria was seen perioperatively and up to 24 h after the procedure in all animals, confirming the involvement of the renal collecting system. This was confirmed by both the postoperative MRI findings and the macroscopic examination of the specimens after nephrectomy. In the present study, MRI was not performed immediately after, which might have provided a good opportunity to evaluate the *in vivo* extend of the cryolesion. An acute inflammatory response in the urothelium is generally characterized by edema, presence of inflammatory cells and hemorrhage. In the present study, the inflammatory response was found to peak two weeks after CA. Furthermore, to evaluate the consolidation of the cryolesions we systematically measured the distance from the urothelial lining to the presence of the fibrotic change in the parenchyma. We observed how there was no significant difference in the distance between the one and two weeks of follow-up groups, confirming that the fibrotic transformation of the cryolesion is limited during this period. In the four weeks, follow-up group this distance was significantly higher, supporting the macroscopic findings where the cryolesions were found to consolidate and retract from the urothelial lining without permanent damage to the urothelium.

Advanced surgical technologies and novel techniques are often evaluated in animal models. However, the pig model may be a less adequate model for evaluating interventional impacts in relation to the renal collecting system, especially with respect to complications. In a study by Souza et al. [16], it was demonstrated that the pig collecting system regenerated after partial nephrectomy without any kind of closure of the collecting system or internal drainage. The authors suggest that this might be due to the small and more muscular collecting system found in pigs. Ames et al. [17] support these findings as no urinomas occurred after partial nephrectomy without suturing the collecting system, in eight pigs.

Finally, other studies have suggested that the renal urothelium in pig may exhibit a more rapid regeneration with fewer tendencies to develop known complication, compared to human kidneys [16–18].

The present animal model is a non-tumor model and thus limiting the direct transferability to clinical settings. Also, only perioperative laparoscopic ultrasound was used to guide the cryoprobe placement and the monitoring of the extent of the ice ball, making it difficult to monitor the deep endophytic margins of the ice ball. The follow-up time being limited to only one month excludes this study from assessing any potential long-term sequelae, e.g. strictures, ureteropelvic junction obstructions and arteriovenous malformations.

Conclusion

As expected CA effectively destroys the renal parenchyma while the impacted renal urothelium remains intact and does not undergo fibrotic changes, indicating that CA is suitable for treatment of deep endophytic tumors in close relation to the renal collecting system.

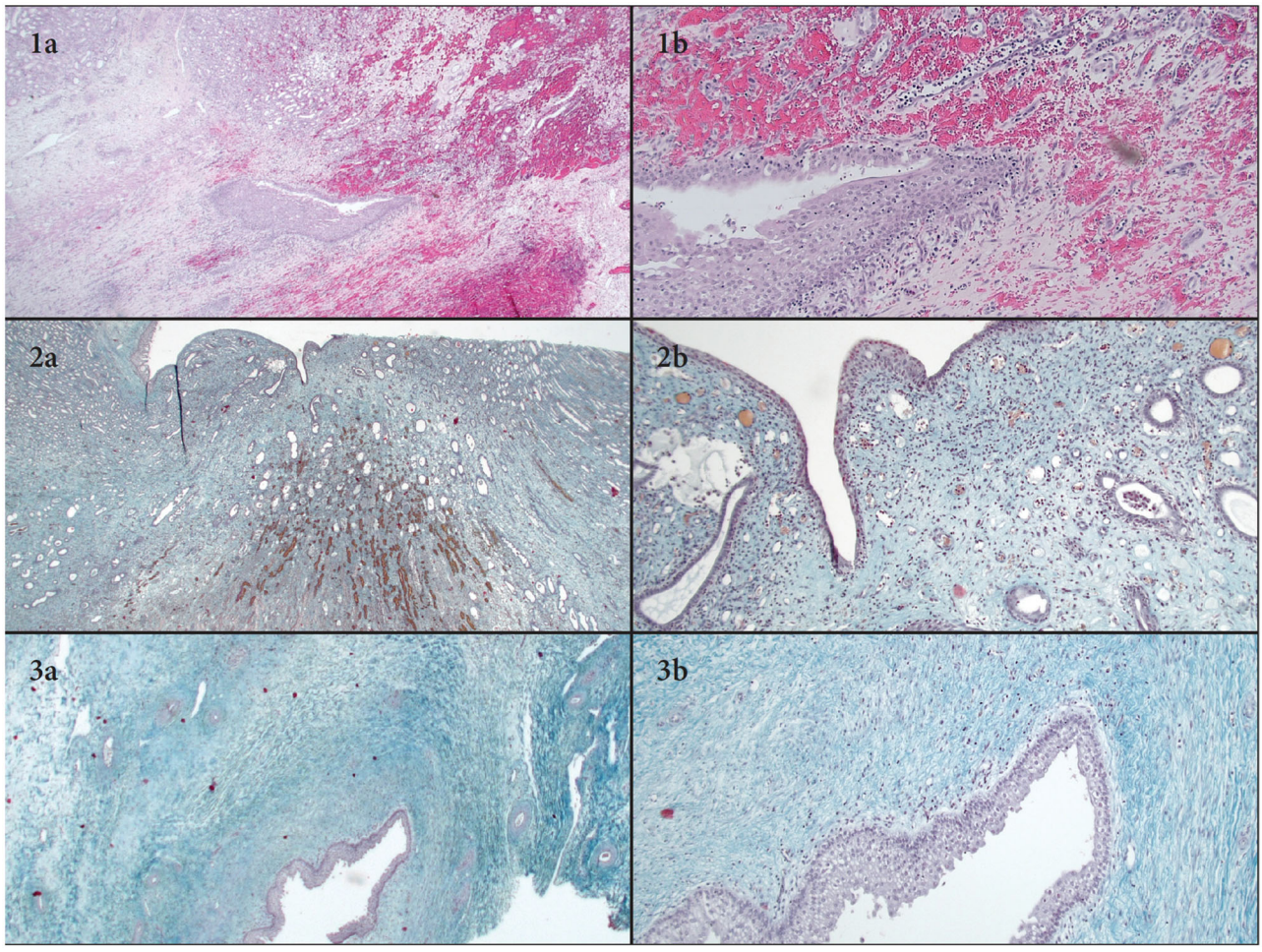

Figure 3. (Histo). Histology sections from the cryolesions. One week follow-up (1a/b, x2/x10, Hematoxylin & eosin); demonstrating hemorrhage underneath and within the cryo lesion and with a sharp demarcation to the normal renal parenchyma and the preserved urothelium. Two weeks follow-up (2a/b, x2/x10, Masson trichrome); demonstrating the cryo lesion with many inflammatory cells and preserved urothelium between the normal renal parenchyma. Four weeks follow-up (3a/b, x2/x10, Masson trichrome); demonstrating the fibrotic transformation of the renal parenchyma in the cryo lesion with a sharp demarcation in relation to the preserved urothelium.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Mager R, Frees S, Haferkamp A. Watchful waiting and active surveillance of small renal masses. Urologe. 2018;57(3): 295–299.
[2] Hollingsworth JM, Miller DC, Daignault S, et al. Rising incidence of small renal masses: a need to reassess treatment effect. J Natl Cancer Inst. 2006;20(98):1331–1334.
[3] Alameddine M, Koru-Sengul T, Moore KJ, et al. Trends in utilazation of robotic and open partial nephrectomy for management of cT1 Renal Masses. Eur Urol Focus. 2019;5(3):482–487.
[4] Zini L, Perrotte P, Capitanio U, et al. Radical versus partial nephrectomy: effect on overall and noncancer mortality. Cancer. 2009; 115(7):1465–1471.
[5] Zaegar H, Samarasekera D, Khalifeh A, et al. Laparoscopic vs percutaneous cryoablation for the small renal mass: 15-year experience at a single center. Urology. 2015;85(4):850–855.
[6] Breen DJ, Bryant TJ, Abbas A, et al. Percutaneous cryoablation of renal tumours: outcomes from 171 tumours in 147 patients. BJU Int. 2013;112(6):758–765.
[7] Tsivian M, Chen VH, Kim CY, et al. Complications of laparoscopic and percutaneous renal cryoablation in a single tertiary. Eur Urol. 2010;58(1):142–148.
[8] Mauri G, Nicosia L, Varano GM, et al. Tips and tricks for a safe and effective image-guided percutaneous renal tumour ablation. Insights Imaging. 2017;8(3):357–363.
[9] Park SH, Kang SH, Ko YH, et al. Cryoablation for endophytic renal cell carcinoma: intermediate-term oncologic efficacy and safety. Korean J Urol. 2010;51(8):518–524.
[10] Nielsen TK, Lagerveld BW, Østraat Ø, et al. Preoperative aspects and dimensions used for anatomical score predicts treatment failures in laparoscopic cryoablation of small renal masses. J Endourol. 2016;30(5):537–543.
[11] Nielsen TK, Østraat Ø, Graumann O, et al. Computed tomography perfusion, magnetic resonance imaging, and histopathological findings after laparoscopic renal cryoablation: an in vivo pig model. Technol Cancer Res Treat. 2017;16(4):406–413.
[12] Sung GT, Gill IS, Hsu TH, et al. Effect of intentional cryo-injury to the renal collecting system. J Urol. 2003;170(2):619–622.
[13] Kilkenny C, Browne W, Cuthill IC, et al. Animal research: reporting in vivo experiments – the ARRIVE guidelines. J Cereb Blood Flow Metab. 2011;31(4):991–993.
[14] Janzen NK, Perry KT, Han KR, et al. The effects of intentional cryoablation and radio frequency ablation of renal tissue involving

the collecting system in a porcine model. J Urol. 2005;173(4): 1368–1374.

[15] Brashears JH, 3rd, Raj GV, Crisci A, et al. Renal cryoablation and radio frequency ablation: an evaluation of worst case scenarios in a porcine model. J Urol. 2005;173(6):2160–2165.

[16] De Souza DB, Abilio EJ, Costa WS, et al. Kidney healing after laparoscopic partial nephrectomy without collecting system closure in pigs. Urology. 2011;77(2):508.e5.

[17] Ames CD, Vanlangendonck R, Morrissey K, et al. Evaluation of surgical models for renal collecting system closure during laparoscopic partial nephrectomy. Urology. 2005;66(2): 451–454.

[18] Shikanov S, Wille M, Large M, et al. Knotless closure of the collecting system and renal parenchyma with a novel barbed suture during laparoscoping porcine partial nephrectomy. J Endourol. 2009;23(7):1157–1160.