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LABORATORY INVESTIGATION

Urinary Tract Effects After Multifocal Nonthermal Irreversible Electroporation of the Kidney: Acute and Chronic Monitoring by Magnetic Resonance Imaging, Intravenous Urography and Urinary Cytology

Johann Jakob Wendler · Maciej Pech · Markus Porsch · Andreas Janitzky · Frank Fischbach · Peter Buhtz · Klaus Vogler · Sarah Hühne · Katrin Borucki · Christof Strang · Dirk Mahnkopf · Jens Ricke · Uwe-Bernd Liehr

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Abstract
Purpose The nonthermal irreversible electroporation (NTIRE) is a novel potential ablation modality for renal masses. The aim of this study was the first evaluation of NTIRE's effects on the renal urine-collecting system using intravenous urography (IVU) and urinary cytology in addition to histology and magnetic resonance imaging (MRI).
Methods Eight percutaneous NTIRE ablations of the renal parenchyma, including the calyxes or pelvis, were performed in three male swine. MRI, IVU, histology, and urinary cytology follow-ups were performed within the first 28 days after treatment.
Results MRI and histological analysis demonstrated a localized necrosis 7 days and a localized scarification of the renal parenchyma with complete destruction 28 days after NTIRE. The urine-collecting system was preserved and showed urothelial regeneration. IVU and MRI showed an unaltered normal morphology of the renal calyxes, pelvis, and ureter. A new urinary cytology phenomenon featured a temporary degeneration by individual vacuolization of detached transitional epithelium cells within the first 3 days after NTIRE.
Conclusions This first urographical, urine-cytological, and MRI evaluation after porcine kidney NTIRE shows multifocal parenchyma destruction while protecting the involved urine-collecting system with regenerated urothelial tissue. NTIRE could be used as a targeted ablation method of centrally located renal masses.

Keywords Non-thermal irreversible electroporation · Ablation · Kidney · Animal model · MRI · Urography · Urinary cytology

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J. J. Wendler (✉) · M. Porsch · A. Janitzky · S. Hühne · U.-B. Liehr
Department of Urology, University of Magdeburg, 39120 Magdeburg, Germany
e-mail: johann.wendler@med.ovgu.de

M. Pech · F. Fischbach · J. Ricke
Department of Radiology and Nuclear Medicine, University of Magdeburg, 39120 Magdeburg, Germany

P. Buhtz · K. Vogler
Institute of Pathology, University of Magdeburg, 39120 Magdeburg, Germany

K. Borucki
Institute of Clinical Chemistry, University of Magdeburg, 39120 Magdeburg, Germany

C. Strang
Department of Anaesthesiology, University of Magdeburg, 39120 Magdeburg, Germany

D. Mahnkopf
Institute of Medical Technology and Research, Rottmersleben, Germany

Introduction

Nonthermal irreversible electroporation (NTIRE) is a new nonthermal soft-tissue ablation technology that applies high current pulses with inserted needle-like electrodes that cause irreversible membrane permeabilization of in vivo cells by way of induced high electric-field transmembrane voltage leading into cell death by loss of homeostasis [1, 2]. We focused on NTIRE as a novel potential ablation modality for centrally located renal masses in our

investigations [3–5]. Whenever technically feasible, nephron-sparing surgery by kidney tumor resection is the "gold-standard" procedure in tumors ≤7 cm [6]. The partial resection of the kidney in cases of central kidney tumors is limited by their close location to the renal hilum, resulting in the risk of organ ischemia by major vessel destruction as well as urinary leakage (by insufficient organ reconstruction) that requires nephrectomy. Cryoablation (CRA) and radiofrequency ablation (RFA) are recommended as therapeutic alternatives in inoperable patients with cortical-located kidney tumors ≤3 cm [6–12]. NTIRE promises advantages compared with the current thermal tissue ablation techniques [5]. Wendler et al. [13] demonstrated angiographically the acute protection of the renal vascular system under renal NTIRE. Tracy et al. [14] and Deodhar et al. [18] demonstrated histologically the potential of NTIRE for complete cortical renal parenchyma ablation while sparing the urine-collecting system in a porcine kidney model. The first human kidney tumor studies, by Pech et al. [4] and Thomson et al. [3], demonstrated safe NTIRE application without general side effects. Intravenous urography (IVU) and urinary cytology are routine urological examinations of the upper urinary tract [15, 16]. Dynamic contrast-enhanced and diffusion-weighted magnetic resonance imaging (MRI) provides functional information of the kidney [17]. The aim of this study was to perform the first evaluation of NTIRE's effect on the renal urine-collecting system using urography and urinary cytology in addition to histological and MRI evidence of renal tissue ablation.

Materials and Methods

The study was approved by the Veterinary Ethic Commission of the State Office of Consumers and Health Protections and the Scientific Board. Three German domestic male pigs (weighing 28 to 33 kg and 3 months old) received professional care in compliance with the Principals of Good Laboratory Practice (GLP) and the German Animal Protection Act by the animal laboratory. In each pig, the right kidney was treated with two to three multifocal NTIRE ablations under general anaesthesia and muscle relaxation. After sterile preparation, applicator-needle electrodes (NanoKnife® Systems; AngioDynamics® Inc., USA) were percutaneously placed 10 to 20 mm into the right kidney in left-lateral position under computed tomography (CT) guidance (Toshiba Aquilion S16; Toshiba-MS) with the aim of multifocal ablation of the renal cortex and renal medulla, including renal calyxes and renal pelvis (Fig. 1a, b). We used a single 19G needle bipolar probe in six ablations and a triple pack of 16G needle monopolar probes in two ablations with a planned spacing of 10 mm each (see Table 1). The technical feasibility, differences in ablation efficacy, and histological results should be evaluated between these two probe techniques. Each NTIRE procedure (9 sets of 10 DC electrical pulses, pulse length 70 to 100 µs, input current 22 to 27 A, and input voltage 2300 to 2700 V) was performed under R-synchronization per continuous three-lead electrocardiogram (AccuSync72, MRC) until all pulse cycles were completed (no low or high currents were displayed) (Fig. 1c).

Sedation was accomplished using ketamine (6 to 8 mg), xylazin (4 to 7 mg), and propofol (3 to 4 mg/kg); general anaesthesia using isoflurane (1 to 2 vol%) and nitrous oxide (60 to 70%; 2 l/min); muscle relaxation using pancuronium bromide (1 to 2 mg); analgesia using meloxicam (0.4 ml/kg); and antibiosis using oxytetracycline (20 mg/kg). All animals were studied for a 28-day period and killed with an overdose (10 ml) of potassium chloride (25%) after nephrectomy. Blood and urine samples (midstream and bladder punction) were taken right before and 5 min as well as 1, 3, 7, 17, and 28 days after NTIRE.

Non-contrast-enhanced MRI, dynamic contrast-enhanced MRI, MR-diffusion-weighted imaging (MR-DWI), and T1-MR urogram scans of the kidneys (1.5 Tesla Achieva Philips, four-channel surface coil, and Gadovist; Bayer-Schering) were performed right before and 30 min as well as 7 and 28 days after NTIRE. The following MRI sequences were used: T1-survey-TFE, T2-SSH-TSE, T2-HR-SPIR-TSE, T2-HR-RT, DWI, T1-Dual-2D-GE, T1-Thrive1-4-3D-GE, and T1-Thrive5-3D-GE. Thrive2-4

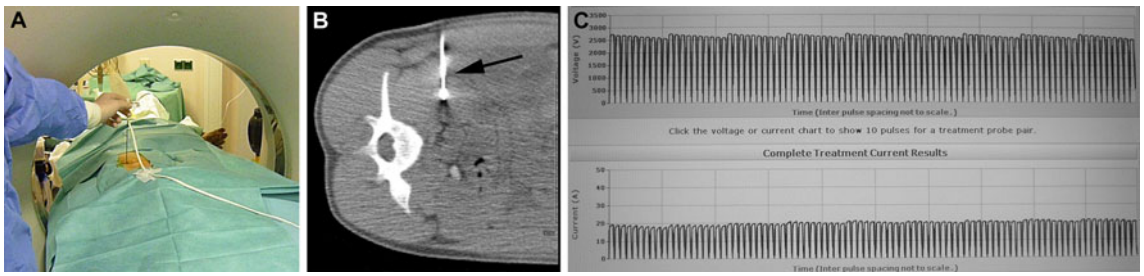

Fig. 1 CT-guided percutaneous NTIRE (**a** and **b**) of the right porcine kidney with the bipolar NTIRE probe (*arrow*). NTIRE application protocol of voltage and current amplitudes (**c**)

Table 1 Size and shape of eight kidney ablation zones immediately, 7 and 28 days after NTIRE per MRI radiological (T2HR-SPIR/SHH sequence) and histological findings

Pig no.	NTIRE, technique, electrodes	Renal position of ablation zone	Lesion size on MRI at day 7 (mm)	Lesion size on MRI at day 28 (mm)	Macroscopic lesion shape on surface at day 28 (mm)	Macroscopic lesion size on surface at day 28 (mm)	Microscopic lesion size at day 28 (mm)
1	1 Bipolar	UP, ventrolateral	26 × 13 × 13	13 × 10 × 5	Elongate	15 × 3	15 × 8 × 4
1	1 Bipolar	MP, ventrolateral	19 × 16 × 12	5 × 6 × 6	Circular	6 × 6	8 × 6 × 4
1	3 monopolar	LP, ventrolateral	15 × 17 × 10	4 × 10 × 4	Star-shaped	6 × 6 × 5	6 × 8 × 4
2	1 Bipolar	UP, dorsolateral	15 × 14 × 13	8 × 10 × 6	Circular	9 × 9	9 × 9 × 8
2	3 Monopolar	MP, dorsolateral	17 × 14 × 13	10 × 8 × 4	Circular	12 × 11	12 × 10 × 6
3	1 Bipolar	UP, ventrolateral	21 × 9 × 9	12 × 5 × 4	Elongate	20 × 4	20 × 6 × 5
3	1 Bipolar	MP, ventrolateral	10 × 13 × 12	7 × 7 × 7	Circular	5 × 4	5 × 9 × 7
3	1 Bipolar	LP, ventrolateral	9 × 11 × 9	6 × 6 × 4	Circular	6 × 4	8 × 8 × 7

Length × width × depth. Upper pole with upper principal calyxes (UP), lower pole with lower principal calyxes (LP), and middle part with medium principal calyxes and renal pelvis (MP)

images were obtained at 20, 40, and 70 s after administrating the contrast agent. T1-MR urogram (Thrive5) was performed between 9 and 19 min (average 14) after contrast agent administration. MRI scans were evaluated using Infinitt PACS software (INFINITT PACS, INFINITT Europe GmbH, Germany).

IVU by conventional X-ray imaging (Artis zee Floor-Mounted System; Siemens) was performed 3 days after NTIRE. Images were taken right before and every 5 min (during a 90-min period) after injection of 30 ml iodine contrast agent (Ultravist-370, 86 kV) (Fig. 2a).

Urinary cytology centrifugates were stained with May–Grünwald–Giemsa stain and then fixed with Canada balm for transmitted-light microscopy with and without oil immersion. Removed kidneys were fixed in 10% formalin. Sections of 5-mm intervals in the transverse plane were made, and the sections were embedded in paraffin. Representative 5-µm microtome sliced sections were stained with haematoxylin and eosin (HE), Elastica-van-Gieson, Berlin blue, Azan, and antihuman-cytoceratin-7-antibody for histological differentiation.

Results

All of the eight renal parenchyma ablations included the renal calyxes or pelvis (two to three ablations/right kidney) (Table 1). All animals survived the experiment with no severe complications. One animal presented a temporary macrohaematuria within the first 3 days after NTIRE. Serum creatinine values showed an increase from preoperative levels of 90 µmol/l (range 67–118) to post-NTIRE levels of 136 µmol/l (range 108–182) at day 28 after the procedure, but they remained within the standard value for German domestic pigs (≤191 µmol/l).

X-Ray IVU and MRI

IVU evaluation showed regular contrast media elimination and no extravasation or gap of the treated renal calyxes and pelvis. The first radiological detection of the X-ray contrast-agent excretion was noted 5 min after injection without disparity in side. The maximal contrast-agent excretion was between 30 and 80 min and showed a consecutive decrease along with increased bladder filling. There were no qualitative or morphological differences in the contralateral untreated kidneys with regard to physiological findings (Fig. 2a, b).

The highest contrast between NTIRE lesions and renal parenchyma was obtained by T2-SPIR-weighted MRI with the following findings: (1) Postinterventional localized hyperintense edema of the renal parenchyma with no substantial lesion immediately after NTIRE; (2) hypointense necrosis-like lesion ≤26-mm diameter with perifocal hyperintense edema 7 days after NTIRE; (3) nonintense scar-like lesion with cortical shrinkage ≤13-mm diameter 28 days after NTIRE (Fig. 3a–c, Table 1). The urographical late venous MRI phase (T1-Thrive5-3D-GE urogram scans) demonstrated normal morphological appearances and normal timing of contrast media excretion with no

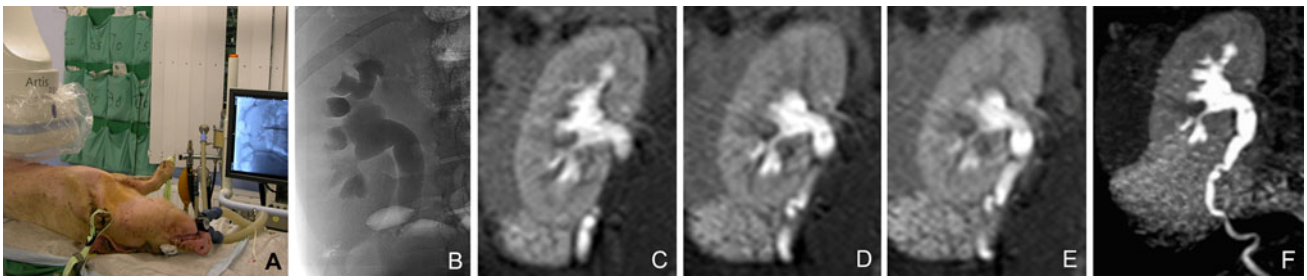

Fig. 2 X-ray IVU (60 minutes after injection) with the pig in supine position 3 days after NTIRE (**a** and **b**). Contrast-enhanced late venous MRI (Thrive5) with urographic phase of the right kidney 7 days after NTIRE (**c**, **d**, and **e**). 2D reconstruction of the superimposed coronary T1-MR urogram planes (**f**)

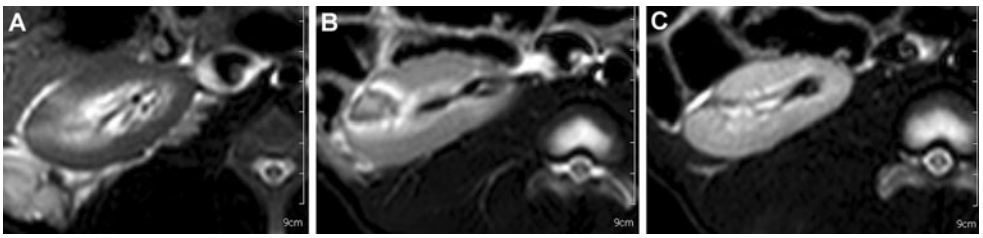

Fig. 3 Center part of the right kidney (pig no. 2) visualized with T2-SPIR MRI at 30 min (**a**), 7 days (**b**), and 28 days after NTIRE (**c**)

urine leakage or urinary obstruction. There were no qualitative or morphological differences in the contralateral untreated kidneys with regard to physiological findings of the renal calyxes and pelvis (Fig. 2c–f).

Histological Analysis and Urinary Cytology

A total of eight sharp circumscribed scars with concave retractions of the right renal surface were detected 28 days after NTIRE ablation (Table 1, Fig. 4b). The central area showed a fibrotic scar partly with fibrinoid necrosis of complete destructed tubules and glomeruli surrounded by granulomas of calcium containing foreign-body giant cells, siderin- and hematoidin-containing histiocytes, fibrotic atrophied glomeruli and tubules, follicular lymphocyte infiltrations, as well as arterioles and venules with circular calcium deposits and a thickened wall (Fig. 4c). All NTIRE ablation zones reached the renal calyxes or pelvis, which showed normal urothelium (Fig. 4d). No cicatrisation, shrinkage, or necrotic ulceration of the renal pelvis and calyxes were seen. There were no qualitative differences of histological results between the bipolar- and monopolar-probe techniques.

Urinary cytology had fundamentally changed by day 1 after NTIRE application. There were an increased number of isolated and partly aggregated transitional urothelium cells with massive degeneration: pale and partly cloudy cytoplasm with ongoing dissolution of the cell membrane as well as swollen cell nuclei with anisocaryosis, hypochromasia, and a dissoluted structure by differently sized pale vacuoles (Fig. 5a, b). From day 3 onward, normal urinary cytology of isolated transitional epithelium cells (regular relation between homogenous cytoplasm and cell nucleus) was seen (Fig. 5c).

Discussion

This preclinical study is the first examination of NTIRE's effect on the renal urine-collecting system using MRI, IVU, and urinary cytology in addition to histological evidence of renal tissue ablation. MRI of the ablation zones at day 28 correlated well with histological analysis at day 28 after NTIRE. Tracy's [14], Deadhar's [18] results, as well as ours, demonstrated histologically the potential of sparing the urine-collecting system by NTIRE of renal parenchyma in a normal porcine kidney. None of these previous studies observed the effect of the NTIRE on the urine-collecting system using urography and urinary cytology, which are routine urological examinations. Referring to a warranted tumor model for renal NTIRE, Thomson et al. [3] investigated seven patients with percutaneous NTIRE of renal masses verified by CT at 3-month follow-up. They observed complete tumor ablation in two renal cell carcinoma (RCC) patients, whereas five patients (one with bifocal RCC, one with singular RCC, one with a transitional cell carcinoma of the renal pelvis, and two with a kidney metastasis from colorectal carcinoma) did not respond to NTIRE treatment.

MRI and urography are favorable methods with which to monitor the success of renal NTIRE ablation as well as renal function and urine-collecting system morphology [15, 17]. This study demonstrated preservation and regeneration

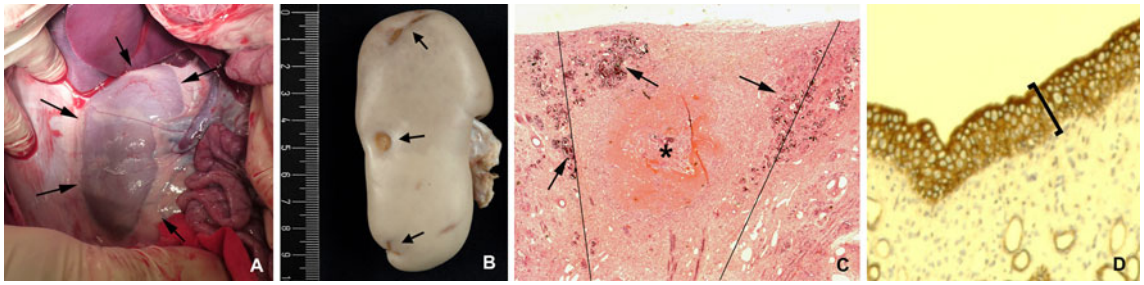

Fig. 4 Intraoperative transperitoneal view of the right kidney (**a**). Macroscopic view of the right kidney after fixing in formaldehyde and removing the connective tissue. *Arrows* show ablation zones as cortical scarred depressions (**b**). Histological analysis of the NTIRE ablation area shows a sharp delineated NTIRE ablation zone with central scar (*borderlines*) partly with a central fibrinoid necrosis (*asterisks*) and peripheral calcium deposits (*arrows*) stained with HE (**c**). The scar adjoining renal calyxes with normal urothelium (*bracket*) stained with cytoceratin-7 (**d**)

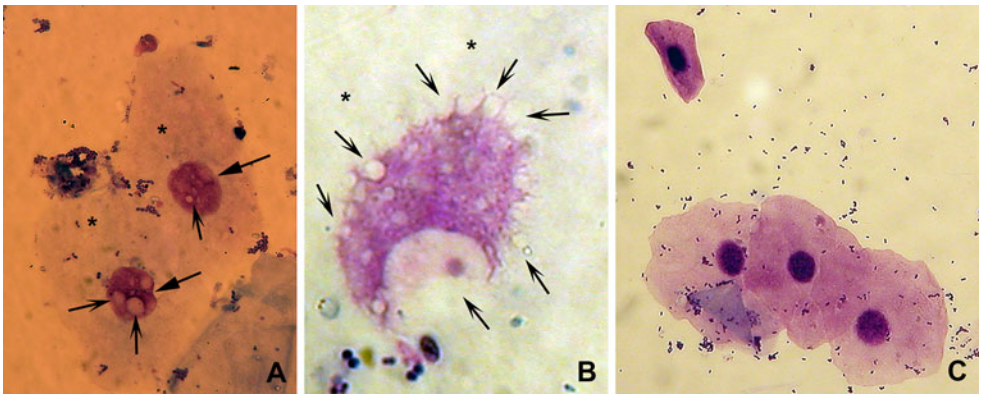

Fig. 5 Urinary cytology one day after NTIRE treatment (**a** and **b**) shows degenerated transitional urothelium cell nuclei (*closed arrows*) by vacuolization (*open arrows*) with pale cytoplasm and ongoing dissolution of the cell membrane (*asterisks*). Normal urinary cytology 3 days after NTIRE (**c**)

of the urine-collecting system after renal NTIRE. No urine extravasation or fistula, no urinary obstruction, no cicatrization or shrinkage, no necrotic ulceration, and no renal failure were observed. These observations are in direct contrast to the acute effects and early complications in the urine-collecting system seen using RFA and CRA [19]. Clinical RCC studies with sufficient outcome data after NTIRE are still warranted. In contrast, short and intermediate oncological outcome data after RFA and CRA of small renal masses (SRMs) have been extensively published, whereas the first long-term results are still awaited [8, 10, 11]. Kunkle and Uzzo [10] analysed CRA or RFA of 1375 localized SRMs (mean tumor size 26.4 mm) in 47 studies of 45 institutions: They reported local tumor progression in 5.2% of patients after CRA and in 12.9% of patients after RFA at mean 18.7-month follow-up, whereas 24.5% of patients had unknown or indeterminate primary pathology, and 33.5% had benign lesions. Rodriguez et al. [11] observed 81 percutaneous CRAs of biopsy-proven pT1-staged RCC (13 to 51 mm) with a complete ablation rate efficacy of 98%. Similarly, Zagoria et al. [12] performed percutaneous RFA of 125 biopsy-proven pT1-staged RCCs (6 to 88 mm) with a complete ablation rate efficacy of 93%.

The preserved and intact matrix, such as submucosa and the basement membrane of the urine-collecting system, as well as the regeneration capability of the urothelium may stimulate healing of the renal calyxes and pelvis after NTIRE. This study observation of urinary cytology after NTIRE represents a qualitative result of a new urinary cytological phenomenon. The current literature provides no similar observations of transitional cell alteration. We interpret this vacuolization of the detached transitional cells as an NTIRE-caused degeneration that seems to be specific in the sense of ongoing apoptosis and necrosis for cells after NTIRE. However, urinary cytology after NTIRE is unable to monitor the NTIRE ablation success, but it reflects NTIRE's individual effect on transitional cells of the urinary tract. Therefore, this new phenomenon must be taken into account when evaluating urinary cytology during the first 7 days after NTIRE. In summary, this study is an additional module by which to demonstrate preservation

and regeneration of the urine-collecting system and urothelium after NTIRE (Figs. 4, 5).

Limitations

This preclinical study of renal NTIRE ablation is limited by the missing swine kidney tumor model, the qualitative description of urinary cytology and urography only, and the small number of cases. Long-term complications, such as renal calyx obstruction, ureteropelvic junction obstruction, or ureter structures, were not assessed.

In general, percutaneous ablation of renal masses is limited by the tumor location because of the percutaneous lumbar access, close proximity to other organs, respiratory kidney motion compared with ribs having small intercostal access, and the difficult distinction between kidney tumor and renal parenchyma during the ablation procedure under non-contrast-enhanced imaging guidance. NTIRE ablation is limited by the requirement of general anaesthesia and muscle relaxation. In contrast, the NTIRE technique is limited by the need for combined multiple monopolar electrodes that are dependent on tumor size (ablation volume $\geq$3.5 cm^3), a maximum number of six probes (ablation volume $\leq$60 cm^3), precise electrode positioning (parallel distance range 5–15 mm), missing coagulation of the probe channel, and dependence on electrical impedance homogeneity of the target tissue [2].

Compared with current thermal ablative techniques, NTIRE plays a potential role in the ablation of centrally located kidney tumors by preserving the urine-collecting system and main vasculature without loss of targeted parenchyma ablation effectiveness [19].

Conclusion

This first urographical and urine-cytological evaluation after porcine kidney NTIRE shows multifocal parenchyma destruction with protection of the involved urine-collecting system. NTIRE could be used as a targeted method of ablating centrally located tumors. New histological phenomena should be taken into account by pathologists when evaluating histology and urinary cytology after NTIRE. Further human NTIRE studies of primary bioptic verified kidney tumors followed by tumor resection approximately 4 weeks after NTIRE are warranted to determine ablation efficacy of this novel technology in different kidney cancers.

Conflict of interests The authors declare that they have no conflict of interest. This study was performed independently of the manufacturer of the devices used.

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