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CT evaluation of patent artery after renal cell carcinoma cryoablation

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Abstract:	Rationale and Objectives: Tumor enhancement on contrast-enhanced images continues for several weeks to months after successful cryoablation; patent arteries in the ablated renal parenchyma can occasionally be observed with unknown clinical significance. We sought to determine the incidence of persistent patent artery after cryoablation and its association with early tumor progression. Materials and Methods: One hundred and fifty-nine patients underwent cryoablation (112 men [70.4%], 47 women [29.6%]; mean age, 63.6 ± 14.6 [SD] years; range, 21-91 years) for 186 renal cell carcinomas (RCCs; mean diameter, 1.9 ± 0.6 [SD] cm; range, 0.7-4.0 cm). After cryoablation, dynamic contrast-enhanced (DCE)-computed tomography (CT) with ≤2-mm slice thickness was performed within one week from the procedure, and at 1, 3, and 6 months. The time course of patent artery in the ablated renal parenchyma after cryoablation was the primary endpoint. The relationships between patent arteries 1 month after cryoablation and treatment effectiveness, tumor vascularity, tumor enhancement 1 month after cryoablation, tumor subtype, and renal function changes were evaluated as secondary endpoints. Results: DCE-CT showed patent arteries in the ablated renal parenchyma within 1 week in 166 RCCs (89.2%), at 1 month in 54 RCCs (29.0%), at 3 months in 8 RCCs (4.3%), and at 6 months in 2 RCCs (1.1%). Patent artery presence 1 month after cryoablation was significantly associated with tumor enhancement at the same time point (P = 0.015). Conclusion: Patent arteries in the ablated renal parenchyma were common on DCE-CT after cryoablation. However, they gradually disappeared and required no special treatment.

Original article

CT evaluation of patent artery after renal cell carcinoma cryoablation

Short title: Patent artery evaluation after RCC cryoablation

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Original article

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Abstract

Rationale and Objectives: Tumor enhancement on contrast-enhanced images continues for several weeks to months after successful cryoablation; patent arteries in the ablated renal parenchyma can occasionally be observed with unknown clinical significance. We sought to determine the incidence of persistent patent artery after cryoablation and its association with early tumor progression.

Materials and Methods: One hundred and fifty-nine patients underwent cryoablation (112 men [70.4%], 47 women [29.6%]; mean age, 63.6 ± 14.6 [SD] years; range, 21-91 years) for 186 renal cell carcinomas (RCCs; mean diameter, 1.9 ± 0.6 [SD] cm; range, 0.7-4.0 cm). After cryoablation, dynamic contrast-enhanced (DCE)-computed tomography (CT) with ≤2-mm slice thickness was performed within one week from the procedure, and at 1, 3, and 6 months. The time course of patent artery in the ablated renal parenchyma after cryoablation was the primary endpoint. The relationships between patent arteries 1 month after cryoablation and treatment effectiveness, tumor vascularity, tumor enhancement 1 month after cryoablation, tumor subtype, and renal function changes were evaluated as secondary endpoints.

Results: DCE-CT showed patent arteries in the ablated renal parenchyma within 1 week in 166 RCCs (89.2%), at 1 month in 54 RCCs (29.0%), at 3 months in 8 RCCs (4.3%), and at 6 months in 2 RCCs (1.1%). Patent artery presence 1 month after cryoablation was significantly associated with tumor enhancement at the same time point ($P = 0.015$).

Conclusion: Patent arteries in the ablated renal parenchyma were common on DCE-CT after

cryoablation. However, they gradually disappeared and required no special treatment.

Keywords: Tomography, X-Ray Computed; Carcinoma, Renal cell; Cryosurgery; Arteries

List of Abbreviations:

RCC	renal cell carcinoma
DCE	dynamic contrast-enhanced
CT	computed tomography
MRI	magnetic resonance imaging
eGFR	estimated glomerular filtration rate
SD	standard deviation

1. Introduction

Percutaneous thermal ablation techniques, such as cryoablation, radiofrequency ablation, and microwave ablation, are increasingly and effectively used as alternative therapy for small renal cell carcinomas (RCCs), after being histologically diagnosed by percutaneous biopsy [1, 2], especially for non-surgical candidates [3-5]. In prospective studies, the 1-, 3-, and 5-year local effectiveness of percutaneous cryoablation for RCCs was all above 94% [6, 7].

After cryoablation, dynamic contrast-enhanced (DCE)-computed tomography (CT) and DCE-magnetic resonance imaging (MRI), or both, are performed to periodically evaluate treatment effectiveness and identify complications [8]. When the target RCC is completely ablated, contrast-enhanced images (CT and MRI) eventually show the disappearance of tumor enhancement. After ablation, the unenhanced areas on CT images generally correspond to histologically proven coagulation necrosis [9]. Occasionally however, parenchymal and vascular enhancement persists in cryo-ablated RCCs in early follow-up contrast-enhanced images. Previous reports have shown that tumor enhancement continues for several weeks to months after successful cryoablation [8, 10]. However, the clinical significance of persistently patent arteries in the ablated renal parenchyma have not been sufficiently evaluated.

We hypothesized that patent arteries gradually disappear on periodic contrast-enhanced CT without clinical consequences (i.e., no association with local tumor progression). This study aimed to retrospectively evaluate the time course of patent arteries in ablated renal parenchyma

after RCC cryoablation and the relationship between patent arteries 1 month after cryoablation and early tumor progression.

2. Materials and Methods

Our institutional review board approved this retrospective study (approval number: Blinded for review). Informed consent was waived because of the retrospective use of patient data; however, all patients gave informed consent before undergoing cryoablation and periodic DCE-CT.

2.1 Patients and tumors

This study included tumors treated with cryoablation between May 2012 and August 2018 at our institution. RCC was diagnosed by percutaneous biopsy, or by imaging when a new tumor or progressively enlarging tumor was present on CT or MRI in patients with a history of treatment for histologically proven RCCs [11]. The inclusion criteria were as follows: i) tumor diameter ≤4 cm (i.e., T1a RCC), ii) cryoablation procedure completed as per our protocol, and iii) tumor assessment by DCE-CT with ≤2-mm slice thickness within 1 week and 1 month after cryoablation. The exclusion criteria were as follows: i) non-RCC histologic diagnosis (e.g., angiomyolipoma, oncocytoma, and renal metastasis), ii) transcatheter arterial embolization prior to cryoablation, or iii) lack of assessment by DCE-CT with ≤2-mm slice thickness at the previously specified time points despite the presence of patent arteries.

2.2 Endpoints

The primary endpoint was the time course of the patent artery in the ablated renal parenchyma after cryoablation. The secondary endpoints were the relationships between the patent arteries in the ablated renal parenchyma 1 month after cryoablation and treatment effectiveness, tumor vascularity, tumor enhancement 1 month after cryoablation, tumor subtype, and renal function changes.

2.3 Cryoablation procedures

Cryoablation procedures were performed for inpatients by seven experienced interventional radiologists (Blinded for review). Intraprocedural pain was addressed with local anesthesia along with conscious sedation using an intravenous infusion of fentanyl and hydroxyzine, titrated to pain tolerance and anxiety level [12]. All procedures were performed using an argon and helium gas-based cryoablation system (CryoHit, Galil Medical, Yokneam, Israel) with 17-gauge cryoprobes (Ice-Rod or Ice-seed, Galil Medical) under CT-fluoroscopy guidance (Aquilion, Canon Medical Systems, Otawara, Japan).

After local anesthesia administration, three or four cryoprobes were inserted into the tumor depending on tumor size and shape. Cryoablation was performed in two freeze cycles (10-15 min per freeze), separated by more than 5 min of passive thawing. At the end of each cycle, non-enhanced CT scans were performed to evaluate whether the ablation zone (i.e., the ice-ball) covered the entire tumor with at least a 6-mm margin [13]. If the margin was insufficient, additional

cycles were performed after repositioning the cryoprobes. If the target RCC was adjacent to a non-target organ, such as the colon, a mixture of saline and contrast medium was infused through an 18- to 21-gauge needle to separate the non-target organ from the ablation zone (i.e., hydrodissection).

2.4 Imaging follow-up

After cryoablation, abdominal DCE-CT with ≤2-mm slice thickness was performed in patients who could be intravenously administered iodinated contrast material (i.e., patients without renal dysfunction [estimated glomerular filtration rate {eGFR} <30 mL/min/1.73 m^2] and no allergy to this drug) to assess treatment effectiveness and identify complications within 1 week from the procedure (**Table 1**), at 1, 3, 6, and 12 months, and every 6 months thereafter.

DCE-CT images were obtained before and after the intravenous administration of contrast medium (300 mgI/mL at a dose of 2.0 g of iodine per kg of body weight) with a fixed injection duration of 30 s in the corticomedullary phase (36-s delay), nephrogenic phase (63-s delay), and excretory phase (240-s delay). CT images were obtained using Aquilion 16 (Canon Medical Systems), Aquilion ONE (Canon Medical Systems), Aquilion Precision (Canon Medical Systems), SOMATOM Definition (Siemens Healthineers, Erlangen, Germany), and Discovery CT750HD (GE Healthcare, Chicago, USA).

When DCE-CT could not be performed (e.g., decreased renal function during the follow-up period or history of allergy to iodinated contrast material), DCE-MRI or non-enhanced MRI was performed for evaluation of treatment effectiveness. Patent artery was not evaluated on MRI

images.

Treatment effectiveness (i.e., complete ablation or local tumor progression) was assessed by DCE-CT, DCE-MRI, or non-enhanced MRI and, when necessary, an image-guided biopsy was performed for local progression diagnosis. The appearance of a nodular focus exhibiting contrast enhancement within or adjacent to the ablation zone indicated local tumor progression [14, 15]. On a non-enhanced MRI, local tumor progression was defined as a new and enlarging focus of hyperintensity within or adjacent to low signal intensity in the ablated area on T2-weighted images [16].

2.5 Evaluation of CT images

The patent arteries in the ablated renal parenchyma on DCE-CT images (corticomedullary phase) were defined as linear enhancement with ≥1-mm length on one or more consecutive axial images. The ablated renal parenchyma area was defined as the area that showed no or poor enhancement on DCE-CT (i.e., area with increase of ≤20 HU attenuation compared to non-contrast CT) regarding the ice-ball created during cryoablation. Tumors were classified as hypervascular or non-hypervascular RCCs. Hypervascular RCCs were defined as having a peak attenuation of more than 100 HU on DCE-CT (corticomedullary phase) [17, 18].

The patent arteries in the ablated renal parenchyma, ablated tumor enhancement, and treatment effectiveness assessed by DCE-CT were retrospectively reviewed by two board-certified diagnostic and interventional radiologists with 23 (Blinded for review) and 10 years (Blinded for

review) of experience and consensus was always achieved.

2.6 Statistical analysis

The relationships between patent arteries in the ablated renal parenchyma 1 month after cryoablation and treatment effectiveness (complete ablation or local tumor progression), tumor enhancement 1 month after cryoablation (present or absent), tumor vascularity before cryoablation (hypervascular or non-hypervascular), and tumor subtype (clear cell RCC vs. non-clear cell RCC) were analyzed using Fisher's exact test.

The renal function change was measured using the following formula: (eGFR 1 month after cryoablation)/ (eGFR before cryoablation). The relationship between patent arteries 1 month after cryoablation and renal function change was evaluated using a Mann-Whitney U test.

Analyses were performed using SPSS software, version 26 (IBM, Armonk, NY). Statistical significance was set at $P < 0.05$.

3. Results

There were 386 renal tumors with diameters of ≤4 cm in 300 patients treated with cryoablation at our hospital between May 2012 and August 2018. Among these, 186 RCCs (mean diameter, 1.9 ± 0.6 [standard deviation {SD}] cm; range, 0.7-4.0 cm) in 159 patients (112 men [70.4%], 47 women [29.6%]; mean age, 63.6-± 14.6 [SD] years; range, 21-91 years) were included in the study (**Fig. 1**). One hundred and thirty-eight tumors were histologically proven RCCs (110

clear cell [79.7%], 10 chromophobe [7.2%], 8 papillary [5.8%], 1 mucinous tubular and spindle cell carcinoma [0.7%], and 9 unclassified [6.5%]). Fuhrman grading was determined in 104 (75.4%; 46 grade 1, 56 grade 2, and 2 grade 3) of 138 biopsy-proven RCCs. The remaining 48 lesions were diagnosed as RCCs based on images and a history of treatment for histologically proven RCCs.

The mean and median follow-up periods were 43.8 ± 22.1 (SD) months and 36.1 months (range, 1.0-89.0 months), respectively. Of 186 RCCs, 178 (95.7%) were completely ablated and 8 (4.3%) showed local tumor progression in a median follow up time of 19.0 months after cryoablation (mean, 20.1 ± 12.1 [SD] months; range, 5.5-44.1 months). Local tumor progression was suspected on DCE-CT (n = 6) or non-enhanced MRI (n = 1) and was histologically diagnosed by CT fluoroscopy-guided biopsy (n = 1). All RCCs with local progression were treated with cryoablation (n = 6), microwave ablation (n = 1), or molecular-targeted therapy (n = 1).

DCE-CT showed tumor enhancement in 108 RCCs (58.1%) within 1 week and in 11 (6.0%) at 1 month after cryoablation. Among these 11, DCE-CT showed disappearance of tumor enhancement at 3 months (n = 7), 6 months (n = 1), and 12 months (n = 1) after cryoablation. The remaining 2 patients were diagnosed with local tumor progression.

DCE-CT showed 392 patent arteries (mean diameter, 1.41 ± 0.33 [SD] mm; range, 1.0-3.2 mm) in the ablated renal parenchyma in 166 RCCs (89.2%) within 1 week, 81 patent arteries in 54 RCCs (29.0%) 1 month after cryoablation, 8 patent arteries in 8 RCCs (4.3%) 3 months after cryoablation, and 2 patent arteries in 2 RCCs (1.1%) 6 months after cryoablation (**Figs. 2 and 3**).

Among the two RCCs with patent arteries 6 months after cryoablation, the patent artery disappeared in one after 12 months. In the other, it still appeared 14 months after cryoablation, but subsequent DCE-CT was not performed because the patient died of hepatocellular carcinoma. The time course of patent artery by DCE-CT is shown in **Fig. 4**.

Of the 8 RCCs with local progression, patent arteries in the ablated renal parenchyma were observed in 3 RCCs (37.5%) 1 month after cryoablation. There was no association between patent arteries 1 month after cryoablation and treatment effectiveness ($P = 0.693$; **Table 2**). Tumor enhancement on DCE-CT 1 month after cryoablation was detected in 5.9% of RCCs (11/186). Patent arteries 1 month after cryoablation were significantly associated with tumor enhancement at the same time point ($P = 0.015$; **Table 2**). One hundred and fifty-three RCCs were categorized as hypervascular tumors. There was no association between patent arteries 1 month after cryoablation and tumor vascularity ($P = 0.692$; **Table 2**) or tumor subtype ($P = 0.295$; **Table 3**). There was no significant difference in renal function change between the patent artery present group (mean, 0.95 ± 0.13 [SD]; range, 0.57-1.35) and the patent artery absent group (mean, 0.96 ± 0.12 [SD]; range, 0.70-1.57; $P = 0.809$).

4. Discussion

Periodic contrast-enhanced CT and MRI are essential for accurately evaluating treatment effectiveness and complications after RCC cryoablation. In this study, DCE-CT within 1 week and 1 month after cryoablation showed patent arteries in the ablated renal parenchyma in 89.2% and

29.0% of RCCs, respectively, but they gradually disappeared and required no special treatment (i.e., there was no association with local tumor progression).

There are two recognized cell death mechanisms after cryoablation: immediate cytocidal damage related to the destruction of intracellular organelles and cell membranes and delayed cell death due to coagulative necrosis [19, 20]. A study on the effects of renal cryotherapy on renal arteries showed that arteries less than 180 μm were damaged immediately after cryoablation, but arteries larger than this size remained [21]. However, arteries with residual blood flow immediately after cryoablation gradually become thrombosed due to endothelial cell damage from freezing [21].

Lagerveld et al. showed that vascular congestion within the frozen renal parenchyma is copmleted within two weeks [22]. In our study, 20 RCCs were without patent arteries in the ablated renal parenchyma within 1 week of the procedure and were considered to have developed arterial occlusion in the interval between cryoablation and obtaining the CT acquisition. Patent arteries disappeared at 1 month in 112 of 166 RCCs with patent arteries within 1 week and were thought to have become occluded within 1 month after cryoablation, consistent with results reported by Lagerveld et al. [22]. Most of the remaining patent arteries disappeared between the 1 and 3 month follow-ups, suggesting that the arterial occlusion mechanism may take more time than previously reported [22].

Known predictive factors for local tumor progression after RCC cryoablation include large tumor size [23-25], central type tumors [24], anterior location [25], deep tumor location [26], and insufficient ice-ball margin [26, 27]. We hypothesized that patent arteries in the ablated renal

parenchyma after cryoablation might not be a risk factor for local tumor progression, and our results supported this hypothesis. Patent arteries were observed in 29.0% of RCCs at 1 month and in 4.3% of RCCs at 3 months after cryoablation, with only one RCC having a patent artery beyond one year. However, local tumor progression was observed much later (the median detection time was 19.0 months after cryoablation). There is no temporal association between the early finding of arterial patency after cryoablation and the late finding of tumor progression. Therefore, the finding, on contrast-enhanced CT, of patent arteries in the ablated renal parenchyma 1 month after cryoablation should not raise undue concern for local progression among physicians.

In a retrospective study of 23 completely ablated renal tumors, eight tumors (8/23, 34.8%) imaged within 6-36 h after cryoablation were enhanced on contrast-enhanced MRI (i.e., false-positive tumor enhancement), and no enhancement was observed in four, three, and one tumors at 3, 6, and 10 months after cryoablation, respectively [10]. In a prospective study, contrast-enhanced MRI showed false-positive tumor enhancement in 15 RCCs (15/25, 60.0%) 2-3 days, 13 RCCs (13/25, 52.0%) 5-7 days, and 1 RCC (1/25, 4.0%) 1 month after cryoablation [8]. In our study, contrast-enhanced CT 1 month after cryoablation showed tumor enhancement in 6.0% of RCCs, and patent arteries were observed significantly more frequently in the tumor enhancement group. Patent arteries may be one reason for false-positive tumor enhancement 1 month after cryoablation.

Takaki et al. reported that the rate of false-positive tumor enhancement noted 5-7 days after cryoablation for clear cell RCC (63.2%; 12/19) was significantly higher than for other RCC

subtypes (16.7%; 1/6; P <0.05) [8]. In this study, tumor vascularity and tumor subtype were unaffected by the patent arteries.

In this study, the relationships between patent arteries 1 month after cryoablation and ice-ball size and location were not evaluated. Ice-ball size is affected by various factors, such as ablation time, ablation number, type and number of cryoprobes used, location of cryoprobe insertion, and presence of close large vessels [26, 28]. Although the ice-ball covered the entire tumor with at least a 6-mm margin during cryoablation, the size of the ice-ball for RCCs (e.g., distance of ice-ball margin and volume ratio of ice-ball and RCC) was not uniform. In RCCs with centrally located ice-balls, the ablated normal parenchyma may have contained larger arteries. However, it is unclear whether ice-ball size was affected by heat sink effect and whether DCE-CT 1 month after cryoablation showed more patent arteries.

This retrospective study from a single institution has several limitations. First, although we defined patent arteries as linear enhancement with ≥1-mm length using DCE-CT with ≤2-mm slice thickness, histological correlations between the DCE-CT images and the ablated renal parenchyma were not performed; some patent arteries might not be visible on DCE-CT. Second, local tumor progression was not always histologically proven, and most lesions were diagnosed by imaging alone. Third, although the follow-up CT protocol was standardized, CT images were obtained using five types of machines. Fourth, 48 tumors were not histologically proven to be RCCs. Last, imaging evaluation was performed by consensus between two experienced radiologists and not by independent reviewers with calculation of interobserver agreement.

5. Conclusion

Patent arteries in the ablated renal parenchyma were common on DCE-CT images after cryoablation. However, they gradually disappeared and required no special treatment.

Human rights

The authors declare that the work described has been carried out in accordance with the Declaration of Helsinki of the World Medical Association revised in 2013 for experiments involving humans.

Informed consent and patient details

The authors declare that this report does not contain any personal information that could lead to the identification of the patients.

Disclosure of interest

The authors declare that they have no competing of interest.

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Author contributions

All authors attest that they meet the current International Committee of Medical Journal Editors (ICMJE) criteria for Authorship.

References

1. Iguchi T, Hiraki T, Matsui Y, Tomita K, Uka M, Tanaka T, et al. Image-guided core biopsy of 2-cm or smaller renal tumors. Diagn Interv Imaging 2020 Nov;101(11):715-720.

2. Marcelin C, Ambrosetti D, Bernhard JC, Roy C, Grenier N, Cornelis FH. Percutaneous image-guided biopsies of small renal tumors: Current practice and perspectives. Diagn Interv Imaging 2017 Sep;98(9):589-599.

3. Zhou W, Herwald SE, McCarthy C, Uppot RN, Arellano RS. Radiofrequency ablation, cryoablation and microwave ablation for T1a renal cell carcinoma: a comparative evaluation of therapeutic and renal function outcomes. J Vasc Interv Radiol 2019 Jul;30(7):1035-1042.

4. Cornelis FH, Bernhard JC. Diagnostic and interventional radiology is a milestone in the management of renal tumors in Birt-Hugg-Dubé syndrome. Diagn Interv Imaging 2019 Nov;100(11):657-658.

5. Matsui Y, Hiraki T, Gobara H, Iguchi T, Tomita K, Uka M, Araki M, Nasu Y, Furuya M, Kanazawa S. Percutaneous thermal ablation for renal cell carcinoma in patients with Birt-Hogg-Dubé syndrome. Diagn Interv Imaging 2019 Nov;100(11):671-677.

6. Georgiades CS, Rodriguez R. Efficacy and safety of percutaneous cryoablation for stage 1A/B renal cell carcinoma: results of a prospective, single-arm, 5-year study. Cardiovasc Intervent Radiol 2014 Dec;37(6):1494-1499.

7. Lim E, Kumar S, Seager M, Modi S, Mandal I, Neves JB, et al. Outcomes of renal tumors treated by image-guided percutaneous cryoablation: immediate and 3- and 5-year outcomes at a

regional center. AJR Am J Roentgenol 2020 Jul;215(1):242-247.

8. Takaki H, Nakatsuka A, Cornelis F, Yamanaka T, Hasegawa T, Sakuma H, Yamakado K. False-Positive tumor enhancement after cryoablation of renal cell carcinoma: a prospective study. AJR Am J Roentgenol 2016 Feb;206(2):332-339.

9. Kawamoto S, Permpongkosol S, Bluemke DA, Fishman EK, Solomon SB. Sequential changes after radiofrequency ablation and cryoablation of renal neoplasms: role of CT and MR imaging. Radiographics 2007 Mar-Apr;27(2):343-355.

10. Porter CA 4th, Woodrum DA, Callstrom MR, Schmit GD, Misra S, Charboneau JW, Atwell TD. MRI after technically successful renal cryoablation: early contrast enhancement as a common finding. AJR Am J Roentgenol 2010 Mar;194(3):790-793.

11. Gobara H, Nakatsuka A, Shimizu K, Yamanaka T, Matsui Y, Iguchi T, et al. Cryoablation of renal cell carcinoma for patients with stage 4 or 5 non-dialysis chronic kidney disease. Jpn J Radiol 2019 Jun;37(6):481-486.

12. Cornelis FH, Monard E, Moulin MA, Vignaud E, Laveissiere F, Ben Ammar M, et al. Sedation and analgesia in interventional radiology: Where do we stand, where are we heading and why does it matter? Diagn Interv Imaging 2019 Dec;100(12):753-762.

13. Miyazaki M, Iguchi T, Takaki H, Yamanaka T, Tamura Y, Tokue H, et al. Ablation protocols and ancillary procedures in tumor ablation therapy: consensus from Japanese experts. Jpn J Radiol 2016 Sep;34(9):647-656.

14. Ahmed M, Solbiati L, Brace CL, Breen DJ, Callstrom MR, Charboneau JW, et al. Image-

guided tumor ablation: standardization of terminology and reporting criteria–a 10-year update. J Vasc Interv Radiol 2014 Nov;25(11):1691-1705.

15. Tsivian M, Kim CY, Caso JR, Rosenberg MD, Nelson RC, Polascik TJ. Contrast enhancement on computed tomography after renal cryoablation: an evidence of treatment failure? J Endourol 2012 Apr;26(4):330-335.

16. Merkle EM, Nour SG, Lewin JS. MR imaging follow-up after percutaneous radiofrequency ablation of renal cell carcinoma: findings in 18 patients during the first 6 months. Radiology 2005 Jun;235(3):1065-1071.

17. Kim JK, Kim TK, Ahn HJ, Kim CS, Kim KR, Cho KS. Differentiation of subtypes of renal cell carcinoma on helical CT scans. AJR Am J Roentgenol 2002 Jun;178(6):1499-1506.

18. Jinzaki M, Tanimoto A, Mukai M, Ikeda E, Kobayashi S, Yuasa Y, et al. Double phase helical CT of small renal parenchymal neoplasms: correlation with pathologic findings and tumor angiogenesis. J Comput Assist Tomogr 2000 Nov-Dec;24(6):835-842.

19. Baust JG, Gage AA, Clarke D, Baust JM, Van Buskirk R. Cryosurgery--a putative approach to molecular-based optimization. Cryobiology 2004 Apr;48(2):190-204.

20. Hoffmann NE, Bischof JC. The cryobiology of cryosurgical injury. Urology 2002 Aug;60(2 Suppl 1):40-49.

21. Lagerveld BW, van Horssen P, Pes MP, van den Wijngaard JP, Streekstra GJ, de la Rosette JJ, et al. Immediate effect of kidney cryoablation on renal arterial structure in a porcine model studied by imaging cryomicrotome. J Urol 2010 Mar;183(3):1221-1226.

22. Lagerveld BW, van Horssen P, Laguna MP, van den Wijngaard JP, Siebes M, Wijkstra H, et al. Gradient changes in porcine renal arterial vascular anatomy and blood flow after cryoablation. J Urol 2011 Aug;186(2):681-686.

23. Kim EH, Tanagho YS, Bhayani SB, Saad NE, Benway BM, Figenshau RS. Percutaneous cryoablation of renal masses: Washington University experience of treating 129 tumours. BJU Int 2013 May;111(6):872-879.

24. Tsivian M, Lyne JC, Mayes JM, Mouraviev V, Kimura M, Polascik TJ. Tumor size and endophytic growth pattern affect recurrence rates after laparoscopic renal cryoablation. Urology 2010 Feb;75(2): 307-310.

25. Zargar H, Samarasekera D, Khalifeh A, Remer EM, O'Malley C, Akca O, et al. Laparoscopic vs percutaneous cryoablation for the small renal mass: 15-year experience at a single center. Urology 2015 Apr;85(4): 850-855.

26. Yamanaka T, Yamakado K, Yamada T, Fujimori M, Takaki H, Nakatsuka A, et al. CT-guided percutaneous cryoablation in renal cell carcinoma: factors affecting local tumor control. J Vasc Interv Radiol 2015 Aug;26(8):1147-1153.

27. Ge BH, Guzzo TJ, Nadolski GJ, Soulen MC, Clark TW, Malkowicz SB, et al. Percutaneous renal cryoablation: short-axis ice-ball margin as a predictor of outcome. J Vasc Interv Radiol 2016 Mar;27(3):403-409.

28. Park SY, Won JY, Oh YT, Jung DC, Kim GM, Kim MD. Assessment of cold sink effect in postulated renal cryoablation by analyzing radiographic ice ball on computed tomography. Br J

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Figure Legends

Figure 1. Study flowchart.

Figure 2. A 79-year-old woman with biopsy-proven clear cell renal cell carcinoma (RCC; 1.6-cm diameter).

A) Computed tomography (CT) image before cryoablation shows the target RCC in the right kidney (arrow).

B) CT image 2 days after cryoablation shows patent arteries (white arrows) and ablated RCC with enhancement (black arrow).

C) CT image 1 month after cryoablation shows disappearance of both the patent arteries and the enhancement of ablated RCC in ablated renal parenchyma (arrows).

Figure 3. A 70-year-old man with biopsy-proven clear cell renal cell carcinoma (RCC; 2.0-cm diameter).

A) Computed tomography (CT) image before cryoablation shows the target RCC in the right kidney (arrows).

B) Prone CT image during cryoablation with hydrodissection shows ice-ball (black arrow) and a cryoprobe (white arrow).

C) CT image (slice image 4 mm caudal to RCC) 1 month after cryoablation shows patent arteries (white arrows) and ablated renal parenchyma (black arrows).

D) CT image (slice image 4 mm caudal to RCC) 3 months after cryoablation shows disappearance of the patent arteries and shrinkage of the ablated renal parenchyma (arrows).

Figure 4. The time course of patent arteries on contrast-enhanced dynamic computed tomography images with ≤2-mm slice thickness.

Response to the Reviewers' comments

We thank you very much for the opportunity to revise and resubmit this manuscript. We appreciate the detailed comments and helpful remarks from each reviewer. We have revised our paper in response to your suggestions and added a reference accordingly. Keywords were changed according to MeSH index terms. In addition, this manuscript was again edited by a native speaker. Please find below our detailed responses to your comments.

Reviewer #1: This is a retrospective study on 155 patients who underwent cryoablation (CA) for renal tumor. This study evaluated patent arteries in the ablated renal on post ablation CT scan. Several concerns should be addressed accordingly:

1. Title. Please CT on the title. (**Reviewer #1, Comment #1**)

Response: Thank you for your comment. We followed the suggestion and added "CT" in the title.

2. Discussion. Please write about Iceball size and compare to heat shrink effect. (**Reviewer #1, Comment #2**)

Response: We followed this suggestion and added one paragraph in the Discussion (Page 14, Paragraph 2).

3. Table 1: no need to differenciate tumor size <2 and >2. It would be more appropriate to differenciate < 4cm and > 4cm. (**Reviewer #1, Comment #3**)

Response: We agree that this is not a significant comparison. Table 2 has been amended accordingly.

4. Image:
2A: image does not match to the text, there is no CA area. Please correct. Please add an image of the tumor before ablation. (**Reviewer #1, Comment #4**)

Response: We followed this suggestion. The edited Figure 3A clearly shows a 2.0-cm RCC in the right kidney.

5. References

Please add the following two references and use this presentation for ALL references. (**Reviewer #1, Comment #5**)

Iguchi T, Hiraki T, Matsui Y, Tomita K, Uka M, Tanaka T, et al. Image-guided core biopsy of 2-cm or smaller renal tumors. Diagn Interv Imaging 2020 Nov;101(11):715-720.

Marcelin C, Ambrosetti D, Bernhard JC, Roy C, Grenier N, Cornelis FH. Percutaneous image-guided biopsies of small renal tumors: Current practice and perspectives. Diagn Interv Imaging 2017 Sep;98(9):589-599.

Response: We added these two references (references #1 and #2) and reformatted all references.

Reviewer #2: This is an interesting paper about evaluation of patent arteries on post-therapeutic imaging after cryoablation for RCCs. This paper may help to handle the issue represented by patent and enhanced arteries visible in the ablated renal parenchyma, that may be confusing for the diagnostic of local progression. However, this paper suffers from some problems which require clarifications:

* About your inclusion criteria, line 26:

In order to assert the external validity of your study, could you provide the reference which justifies the chosen cut-off of 4 cm for your inclusion criteria? (**Reviewer #2, Comment #1**)

Response: Thank you for your comment. With RCC, a tumor size of ≤ 4 cm is classified as T1a, as per TNM classification, and thus corresponds to the smallest detectable tumor limited to the kidney. Cryoablation has been reported to have excellent results in T1a RCCs and, in our country, only cryoablation for small renal cancers (i.e., T1a RCC) is covered by national health insurance. Hence, it is our practice to perform cryoablation for such tumors. The text has been revised for clarity (Page 5, 2.1 Patients and tumors, Line 5).

* About your inclusion criteria:

These inclusion criteria need to be clarified. Since you precise in the introduction that

both CT and MRI are available for the evaluation of cryotherapy effectiveness, why did you consider as an exclusion criterion the lack of DCE-CT? Furthermore, you say later in the manuscript, in the paragraph "Evaluation of CT images": "Treatment effectiveness (i.e., complete ablation or local tumor progression) was assessed by DCE-CT, DCE-MRI, or non-enhanced MRI". Is not that contradictory? Please clarify. (**Reviewer #2, Comment #2**)

Response: You are raising an important point which we have taken steps to clarify. The only modality we used to evaluate arterial patency was DCE-CT with ≤2-mm slice thickness. Patients who did not have this done were excluded. Other imaging modalities, such as DCE-MRI or non-enhanced MRI were used as alternatives to DCE-CT to determine treatment effectiveness but not to evaluate arterial patency. We have clarified this point in the text (Page 7, 2.4 Imaging follow-up, Paragraph 3). We have also edited the title, as per Reviewer 1's recommendation, to include the word CT.

* About cryoablation procedures: please provide references. (**Reviewer #2, Comment #3**)

Response: We added a reference on cryoablation protocol (reference #13) and a sentence about hydrodissection (Page 7, Lines 1-3).

* About evaluation of CT images:
The title of this section is about CT images but you also define MRI criteria in it. Please clarify. (**Reviewer #2, Comment #4**)

Response: As mentioned below (Response to **Reviewer #3, Comment #12)**, the sentences about MRI were deleted in the "2.5 Evaluation of CT images" section.

* About evaluation of CT images:
"DCE-MRI or non-enhanced MRI was performed when DCE-CT could not be performed". Please explain how you analyze artery patency on a non-enhanced MRI. (**Reviewer #2, Comment #5**)

Response: Please see response to **Reviewer #2, Comment #2**.When DCE-CT could not be performed, DCE-MRI or non-enhanced MRI was performed for evaluation of

treatment effectiveness. Patent artery was not evaluated by MRI. We clarify this point in the text (Page 7, 2.4 Imaging follow-up, Paragraph 3).

* About evaluation of CT images:
Please define "enhancement" and "poor enhancement". Relative to what. (**Reviewer #2, Comment #6**)

Response: On contrast-enhanced CT, a solid renal mass usually has an enhancement of greater than 20HU compared to non-contrast CT (Israel GM, Bosniak MA. Radiology 2005; 236:441–450). Therefore, "enhancement on DCE-CT" was defined by DCE-CT attenuation >20 HU compared to non-contrast CT. We revised this sentence accordingly (Page 8, 2.5 Evaluation of CT images, Lines 4-5).

* About evaluation of CT images:
You define the local progression as "The appearance of a nodular focus exhibiting contrast enhancement within or adjacent to the ablation zone indicated local tumor progression". How would you classify a persistent focal enhancement visible on the first post-cryoablation control and which does not disappear on the further imaging controls? Can you consider it as a good response? (**Reviewer #2, Comment #7**)

Response: Thank you for your important comment. In similar cases, we usually continue to follow up with periodic DCE-CT without immediate intervention (e.g., biopsy) or other imaging study (e.g., PET). If periodic follow-up DCE-CT shows an increase in size, we become concerned for possible enlargement of residual RCC and perform other imaging studies and/or biopsy. If periodic follow-up DCE-CT shows no change, the ablated RCC continues to be followed up closely.

* About evaluation of CT images:
How do you justify the evaluation of the imaging criteria with a consensus of two experienced radiologists, instead of an independent review with the calculation of the interobserver agreement? This should be acknowledged in the Discussion. (**Reviewer #2, Comment #8**)

Response: We agree that an independent review with assessment of interobserver agreement would have been preferable. We have added a comment on this as a limitation of our study in the Discussion (page 14, Paragraph 3, Lines 8-9).

* About Statistical analysis:
"Tumors were classified as hypervascular or non-hypervascular RCCs. Hypervascular RCCs were defined as a peak attenuation of more than 100 HU on DCE-CT (corticomedullary phase)": shouldn't it rather be in the paragraph "Evaluation of CT images" instead? (**Reviewer #2, Comment #9**)

Response: We followed this suggestion. These two sentences were added to the "2.5 Evaluation of CT images" section (Page 8, 2.5 Evaluation of CT images, Paragraph 1, Lines 5-7).

* About Results:
"The remaining 48 lesions were diagnosed as RCC based on images and a history of treatment for histologically proven RCCs.": please provide a reference which justifies this diagnostic reasoning. (**Reviewer #2, Comment #10**)

Response: We followed this suggestion and added a reference (Page 5, 2.1 Patients and tumors, Paragraph 1, Lines 2-4, reference 11).

* About discussion:
You treated with cryotherapy many different histological types of RCCs. These tumors do not have the same appearance and especially the same enhancement on CT and MRI. Since the artery patency is associated with tumor enhancement, how do you justify that your study population includes so many different types of tumors? Do you think the final result would be the same if you would have studied only clear cell renal carcinomas? (**Reviewer #2, Comment #11**)

Response: You are raising a valid point. We had histologic diagnosis in 138 out of 186 total RCCs (74.2%). Of these 138 cases, 110 were clear cell RCCs, corresponding to an overwhelming majority of 79.7%, with the rest being consistent with alternative histology (10 chromophobe RCCs, 8 papillary RCCs, 1 mucinous tubular and spindle cell carcinoma, and 9 unclassified). We have no reason to believe that if histology was available for all tumors, the results would be different. Previous epidemiologic studies support this assumption as clear cell RCC is the most common type of RCC. Furthermore, when we compared clear cell with non-clear cell RCC, we found no association between patent arteries 1 month after cryoablation and tumor subtype. We

have included this statistical analysis in Result section, and Table 3. We also include the Table here for your review.

		Patent arteries at 1-month after cryoablation		
		Present (n = 44)	Absent (n = 85)	*P* -value
Tumor subtype	Clear cell RCC (n = 110)	40 (36.4)	70 (63.6)	0.295
	Non-clear cell RCC (n = 19)	4 (21.1)	15 (78.9)	

Values in parentheses are percentages.

RCC: renal cell carcinoma

Ninteen non-clear cell RCCs includes 10 chromophobe RCCs, 8 papillary RCCs and 1 mucinous tubular and spindl cell carcinoma.

* About Table 1: please correct "complerte ablation." (**Reviewer #2, Comment #12**)

Response: We corrected this mistake (Table 2).

* About Figure 2A-2B:
I am not really convinced by this example. On the post-cryotherapy imaging, it seems that the treated area does not include the tumor clearly visible on the pre-treatment imaging… If the treated tumor is not the one that seems so obvious on figure 2A, please show the real treated tumor with an arrow.
Besides, the tumor visible on figure 2 does not look really enhanced (if it is, please add an image of CT before contrast injection, and ROIs with the attenuation values to prove it). However, there are patent arteries on the DCE-CT control at 1 month (figure 2B). Since you assumed that there is an association between tumor enhancement and patency of ablated parenchyma arteries 1 month after cryotherapy, maybe you should provide another example which better suits to your results.
Furthermore, even though the diagnostic was proven with histology, I suggest that you find a more representative example of clear renal cell carcinoma, since the appearance of the treated tumor is not typical for this type of RCC.
Finally, I think it would be interesting and more convincing if you add more examples of DCE-CT showing patent arteries after cryotherapy. (**Reviewer #2, Comment #13**)

Response: Thank you for this important comment. We revised Figure (Figure 3) and added new Figure (Figure 2).

Reviewer #3

As a general comment, ALL quantitative variables must be reported as means, SD and ranges. This comment applies to the whole manuscript including Abstract, Main MS and Tables. (**Reviewer #3, Comment #1**)

Response: Thank you for this suggestion, we have implemented it.

The authors should avoid using "case" Case is misleading. Use patient or RCC. (**Reviewer #3, Comment #2**)

Response: We followed this suggestion and changed “case” into “RCC.”

Specific comments

Please rewrite the Objective to be consistent with the primary endpoint and secondary endpoint. (**Reviewer #3, Comment #3**)

Response: We followed this suggestion and rewrote the Objective.

Provide a list of ALL abbreviations. (**Reviewer #3, Comment #4**)

Response: We followed this suggestion and added a list of all abbreviations.

Delete first sentence of Introduction about surgery of add relevant references. (**Reviewer #3, Comment #5**)

Response: We deleted first sentence of Introduction.

Add the following references regarding ablation of renal tumors. (**Reviewer #3, Comment #6**)

Cornelis FH, Bernhard JC. Diagnostic and interventional radiology is a milestone in the management of renal tumors in Birt-Hugg-Dubé syndrome. Diagn Interv Imaging 2019 ;100(11):657-658.
Matsui Y, Hiraki T, Gobara H, Iguchi T, Tomita K, Uka M, Araki M, Nasu Y, Furuya M, Kanazawa S. Percutaneous thermal ablation for renal cell carcinoma in patients with Birt-Hogg-Dubé syndrome. Diagn Interv Imaging 2019;100(11):671-677.

Response: We added these references (references #4 and 5).

Last sentence of the Intro should start with "The purpose of this study was to.... . and should be the same than in the Abstract. (**Reviewer #3, Comment #7**)

Response: We followed this suggestion.

M&M
Please elaborate on sedation during ablation ad add the following reference. (**Reviewer #3, Comment #8**)

Cornelis FH, Monard E, Moulin MA, Vignaud E, Laveissiere F, Ben Ammar M, Nouri-Neuville M, Barral M, Lombart B. Sedation and analgesia in interventional radiology: Where do we stand, where are we heading and why does it matter? Diagn Interv Imaging 2019;100:753-762.

Response: We followed this suggestion (Page 6, 2.3 Cryoablation procedures, Paragraph 1, Lines 2-4) and added this reference (#12).

Please provide initials of all persons involved in the study (ablation, reading). (**Reviewer #3, Comment #9**)

Response: We followed the suggestion and added the initials of the authors who performed ablation and reading. However, we hid them in this submitted revised manuscript because the manuscript is blinded for review.

Please give more details on histopathological types of RCC. (**Reviewer #3, Comment #10**)

Response: In the revised manuscript, we have described in detail the tumor subtype and Fuhrman grading (Page 9, last sentence to Page 10, Line 3). In addition, we have added a statistical comparison between clear cell RCC and non-clear cell RCC. Please see our response to Reviewer 2, comment 11 above and Table 3.

This sentence is hard to read "Most treated RCCs were initially assessed by DCE-CT

within 3 days of the procedure (1- [n = 50], 2- [n = 99], 3- [n = 34], 4- [n = 1], and 7-days later [n = 2])." Please provide this info in a Table and delete it from the text. (**Reviewer #3, Comment #11**)

Response: We followed this suggestion and added a new Table (Table 1).

MRI appears in a section entitled "Evaluation of CT images". I think that the authors should make a separate paragraph entitled Image analysis and explain how arteries were assessed, using CT in x patients and y tumors, using MRI in x patients and y tumors and a combination of CT and MRI in x patients and y tumors. More details should be given regarding MRI protocol as MRI was used for assessment. (**Reviewer #3, Comment #12**)

Response: When DCE-CT could not be performed (e.g., decreased renal function during follow-up period or history of allergy to iodinated contrast material), DCE-MRI or non-enhanced MRI was used for evaluation of treatment effectiveness. Patent artery was not evaluated on MRI images. We added one paragraph in "2.4 Imaging follow-up" section to clarify this point (Page 7, 2.4 Imaging follow-up, Paragraph 3). Because MRI evaluation was not relevant to the purpose of this study, we did not create a paragraph entitled Image analysis. Additionally, the sentences about MRI were moved to the "2.4 Imaging follow-up" section (Page 8, Paragraph 2).

These two sentences have no reasons for being in Stat Analysis "Tumors were classified as hypervascular or non-hypervascular RCCs. Hypervascular RCCs were defined as a peak attenuation of more than 100 HU on DCE-CT (corticomedullary phase) [10, 11]." and should be in Image analysis. (**Reviewer #3, Comment #13**)

Response: As mentioned above (Response to Reviewer #2, Comment #9), these two sentences were moved to the "2.5 Evaluation of CT images" section.

Would be interesting to have some information about renal function and possible association between renal arteries and renal function changes. (**Reviewer #3, Comment #14**)

Response: We followed this suggestion in the revised manuscript. The renal function change was measured using the following formula: (eGFR 1 month after cryoablation)/

(eGFR before cryoablation). Next, the relationship between patent artery 1 month after cryoablation and renal function change was evaluated using a Mann-Whitney U test. There was no significant difference in renal function change between the patent artery present group (mean, 0.95 ± 0.13 [SD]; range, 0.57-1.35) and the patent artery absent group (mean, 0.96 ± 0.12 [SD]; range, 0.70-1.57; *P* = 0.809).

Results
Proportions should be added to % for qualitative or categorical variables. (**Reviewer #3, Comment #15**)

Response: We followed this suggestion and added %.

Figures
Please provide flow charts as fully editable PowerPoint documents. (**Reviewer #3, Comment #16**)

Response: We followed this suggestion (Figures 1 and 4).

Do not use "case" in the flow charts. (**Reviewer #3, Comment #17**)

Response: We followed this suggestion and changed “case” into “RCC.”

Please provide flow charts using Journal style. See a recent article of the Journal. (**Reviewer #3, Comment #18**)

Response: We followed this suggestion (Figures 1 and 4).

Add arrows on figures to show the specific points. Use white or black arrows. (**Reviewer #3, Comment #19**)

Response: We followed this suggestion (Figures 2 and 3).

Original article

CT evaluation of patent ~~Patent~~ artery ~~evaluation~~ after renal cell carcinoma cryoablation

(Reviewer #1, Comment #1)

Short title: Patent artery evaluation after RCC cryoablation

Abstract

Rationale and Objectives: Tumor enhancement on contrast-enhanced images continues for several weeks to months after successful cryoablation~~, but;~~ patent arteries in the ablated renal parenchyma can occasionally be observed with unknown clinical significance~~have not been evaluated~~. We sought to determine the incidence of persistent patent artery after cryoablation and its association with early tumor progression.~~This study retrospectively evaluated the patent artery in the ablated renal parenchyma after cryoablation.~~

Materials and Methods: One hundred and fifty-nine patients underwent cryoablation (112 men [70.4%], 47 women [29.6%]; mean age, 63.6 ± 14.6 [SD] years; range, 21-91 years) for 186 renal cell carcinomas (RCCs; mean diameter, 1.9 ± 0.6 [SD] cm; range, 0.7-4.0 cm). After cryoablation, dynamic contrast-enhanced (DCE)-computed tomography (CT) with ≤2-mm slice thickness was performed within one week from ~~of~~ the procedure ~~initial follow-up~~, and at 1-, 3-, and 6-months. The time course of patent artery ~~course~~ in the ablated renal parenchyma after cryoablation was the primary endpoint. The relationships between patent arteries 1 -month after cryoablation and treatment effectiveness ~~tumor size~~ (Reviewer #1, Comment #3), tumor vascularity, tumor

enhancement 1 -month after cryoablation, tumor subtype (Reviewer #2, Comment #11), and renal function changes and the treatment effectiveness were evaluated as secondary endpoints using Fisher's exact test.

Results: Of 186 RCCs, 178 (95.7 %) were completely ablated and 8 (4.3 %) showed local tumor progression 19.0 months (median duration) post-cryoablation. DCE-CT showed patent arteries in the ablated renal parenchyma within 1 week in 166 RCCscases (89.2%), at 1 month in 54 RCCscases (29.0%), at 3 months in 8 RCCscases (4.3%), and at 6 months in 2 RCCscases (1.1%). Patent artery presence 1 -month after cryoablation was significantly associated with tumor enhancement at the same time point 1-month after cryoablation (P = 0.015).

Conclusion: Patent arteries in the ablated renal parenchyma were common on DCE-CT after cryoablation. However, they gradually disappeared and required no special treatment.

Keywords: Tomography, X-Ray Computed; Computed tomography, Carcinoma, Renal cell; Renal cell carcinoma, Cryosurgery; Cryoablation, ; Arteries Artery

List of Abbreviations:

RCC	renal cell carcinoma
DCE	dynamic contrast-enhanced
CT	computed tomography
MRI	magnetic resonance imaging

eGFR	estimated glomerular filtration rate
SD	standard deviation

1. Introduction

~~Conventionally, surgical resection is performed for small renal cell carcinomas (RCCs).~~ (Reviewer #3, Comment #5) Percutaneous thermal ablation techniques, such as cryoablation, radiofrequency ablation, and microwave ablation, are increasingly and effectively used as alternative therapy for small renal cell carcinomas (RCCs), after being histologically diagnosed by percutaneous biopsy [1, 2], especially for non-surgical candidates [3-5]. ~~However, recently, some percutaneous thermal ablation treatments, such as cryoablation, radiofrequency ablation, and microwave ablation, have been performed for small RCCs as alternative and effective local therapies, mainly for non-surgical candidates [1]~~. In prospective studies, the 1-, 3-, and 5-year local effectiveness of percutaneous cryoablation for RCCs was~~were~~ all above 94% [6, 7~~2, 3~~].

After cryoablation, dynamic contrast-enhanced (DCE)-computed tomography (CT) and DCE-magnetic resonance imaging (MRI), or both, are performed to periodically evaluate treatment ~~the~~ effectiveness ~~of the treatment~~ and identify complications [8~~4~~]. When the target RCC is completely ablated, contrast-enhanced images (CT and MRI) eventually show the disappearance of tumor enhancement. After ablation, the unenhanced areas on CT images generally correspond to histologically proven ~~the histological areas of~~ coagulation necrosis [9~~5~~]. Occasionally however, parenchymal and vascular enhancement persists in cryo-ablated RCCs ~~However, occasionally after~~

~~cryoablation, treated RCCs and small arteries in the ablated renal parenchyma are enhanced~~ in early follow-up contrast-enhanced images. Previous reports have shown ~~showed~~ that tumor enhancement continues~~continued~~ for several weeks to months after successful cryoablation [8, 10~~4, 6~~]~~, but patent arteries in the ablated renal parenchyma have not been sufficiently evaluated~~. However, the clinical significance of persistently patent arteries in the ablated renal parenchyma have not been sufficiently evaluated.

We hypothesized that patent arteries gradually disappear on periodic contrast-enhanced CT without clinical consequences ~~with little clinical significance~~ (i.e., no association with~~influence on~~ local tumor progression). This study aimed to retrospectively evaluate the time course of patent arteries in ablated renal parenchyma after RCC cryoablation and the relationship between patent arteries 1 month after cryoablation and early tumor progression.

2. Materials and Methods

Our institutional review board approved this retrospective study (approval number: Blinded for review). Informed consent was waived because of the retrospective use of patient data; however~~, and~~ all patients gave informed consent before undergoing ~~performing~~ cryoablation and periodic DCE-CT.

2.1 Patients and tumors

This study included tumors treated with cryoablation between May 2012 and August

2018 at our institution. RCC was diagnosed by percutaneous biopsy, or by imaging when a new tumor or progressively enlarging tumor was present on CT or MRI in patients with a history of treatment for histologically proven RCCs [11]. (Reviewer #2, Comment #10) The inclusion criteria were as follows: i) tumor diameter ≤4 cm (i.e., T1a RCC) (Reviewer #2, Comment #1), ii) cryoablation procedure completed as per ~~the planned cryoablation procedure was completed according to~~ our protocol, and iii) tumor assessment ~~tumors were assessed~~ by DCE-CT with ≤2-mm slice thickness within 1 -week and 1 -month after cryoablation. The exclusion criteria were as follows: i) ~~the tumors were histologically diagnosed as~~ non-RCC histologic diagnosis (e.g., angiomyolipoma, oncocytoma, and renal metastasis), ii) ~~a~~ transcatheter arterial embolization prior to ~~was performed before~~ cryoablation, or iii) lack of assessment by DCE-CT with ≤2-mm slice thickness at the previously specified time points despite the presence of patent arteries ~~the tumors were not assessed by DCE-CT with ≤2-mm slice thickness until 1-year after cryoablation, despite observing patent arteries~~.

2.2 Endpoints

The primary endpoint was the time course of the patent artery in the ablated renal parenchyma after cryoablation. The secondary endpoints were the relationships between the patent arteries in the ablated renal parenchyma 1 -month after cryoablation and treatment effectiveness, ~~tumor size,~~ (Reviewer #1, Comment #3) tumor vascularity, tumor enhancement 1 -month after cryoablation, tumor subtype, and renal function changes ~~and treatment effectiveness~~.

2.3 Cryoablation procedures

Cryoablation procedures were performed for inpatients ~~with conscious sedation~~ by seven experienced interventional radiologists (Blinded for review). (Reviewer #3, Comment #9) Intraprocedural pain was addressed with local anesthesia along with conscious sedation using an intravenous infusion of fentanyl and hydroxyzine, titrated to pain tolerance and anxiety level [12]. (Reviewer #3, Comment #8) All procedures were performed using an argon and helium gas-based cryoablation system (CryoHit, Galil Medical, Yokneam, Israel) with 17-gauge cryoprobes (Ice-Rod or Ice-seed, Galil Medical) under CT-fluoroscopy guidance (Aquilion, Canon Medical Systems, Otawara, Japan).

After local anesthesia administration, three or four cryoprobes were inserted~~placed~~ into the tumor depending on tumor ~~according to their~~ size and shape. Cryoablation was performed in two freeze cycles (10-15 min per freeze), separated by more than 5 min of passive thawing. At the end of each cycle, non-enhanced CT scans were performed to evaluate whether the ablation zone (i.e., the ice-ball) covered the entire tumor with at least a 6-mm margin [13] (Reviewer #2, Comment #3). If the margin was insufficient, additional cycles were performed after repositioning the cryoprobes. If the target RCC was adjacent to a non-target organ, such as the colon, a mixture of saline and contrast medium was infused through an 18- to 21-gauge needle to separate the non-target organ from the ablation zone (i.e., hydrodissection). (Reviewer #2, Comment #3)

2.4 Imaging follow-up

After cryoablation, abdominal DCE-CT with ≤2-mm slice thickness was performed in patients who could be intravenously administered iodinated contrast material (i.e., patients without renal dysfunction [estimated glomerular filtration rate {eGFR} <30 mL/min/1.73 m^2] and no allergy to this drug) to assess ~~the~~ treatment effectiveness and identify complications within 1-week from the procedure (**Table 1**) ~~of the initial follow-up~~ (Reviewer #3, Comment #11), at 1-, 3-, 6-, and 12 -months, and every 6 -months thereafter. ~~Most treated RCCs were initially assessed by DCE-CT within 3 days of the procedure (1- [n = 50], 2- [n = 99], 3- [n = 34], 4- [n = 1], and 7-days later [n = 2]).~~

DCE-CT images were obtained before and after the intravenous administration of contrast medium (300 mgI/mL at a dose of 2.0 g of iodine per kg of body weight) with a fixed injection duration of 30 s in the corticomedullary phase (36-s delay), nephrogenic phase (63-s delay), and excretory phase (240-s delay). CT images were obtained using Aquilion 16 (Canon Medical Systems), Aquilion ONE (Canon Medical Systems), Aquilion Precision (Canon Medical Systems), SOMATOM Definition (Siemens Healthineers, Erlangen, Germany), and Discovery CT750HD (GE Healthcare, Chicago, USA). ~~DCE-MRI or non-enhanced MRI was performed when DCE-CT could not be performed.~~

When DCE-CT could not be performed (e.g., decreased renal function during the follow-up period or history of allergy to iodinated contrast material), DCE-MRI or non-enhanced MRI was performed for evaluation of treatment effectiveness. Patent artery was not evaluated on MRI

images. (Reviewer #2, Comment #2)

Treatment effectiveness (i.e., complete ablation or local tumor progression) was assessed by DCE-CT, DCE-MRI, or non-enhanced MRI and, when necessary, an image-guided biopsy was performed for local progression diagnosis. The appearance of a nodular focus exhibiting contrast enhancement within or adjacent to the ablation zone indicated local tumor progression [14, 15]. On a non-enhanced MRI, local tumor progression was defined as a new and enlarging focus of hyperintensity within or adjacent to low signal intensity in the ablated area on T2-weighted images [16].

2.5 Evaluation of CT images

The patent arteries in the ablated renal parenchyma on DCE-CT images (corticomedullary phase) were defined as linear enhancement with ≥1-mm length on one or more consecutive axial images. The ablated renal parenchyma area was defined as the area that showed no or poor enhancement on DCE-CT (i.e., area with increase of ≤20 HU attenuation compared to non-contrast CT) (Reviewer #2, Comment #6) regarding the ice-ball created during cryoablation.

~~Treatment effectiveness (i.e., complete ablation or local tumor progression) was assessed by DCE-CT, DCE-MRI, or non-enhanced MRI and, when necessary, an image-guided biopsy was performed for local progression diagnosis. The appearance of a nodular focus exhibiting contrast enhancement within or adjacent to the ablation zone indicated local tumor progression [7, 8]. In a non-enhanced MRI, local tumor progression was defined as a new and enlarging focus of~~

~~hyperintensity within or adjacent to low signal intensity in the ablated area on T2-weighted images [9].~~ (Reviewer #2, Comment #4) Tumors were classified as hypervascular or non-hypervascular RCCs. Hypervascular RCCs were defined as having a peak attenuation of more than 100 HU on DCE-CT (corticomedullary phase) [17, 18]. (Reviewer #2, Comment #9)

The patent arteries in the ablated renal parenchyma, ablated tumor enhancement, and treatment effectiveness assessed by DCE-CT were retrospectively reviewed by ~~a consensus of~~ two board-certified diagnostic and interventional radiologists with 23 (Blinded for review) and 10 years (Blinded for review) of experience and consensus was always achieved. (Reviewer #3, Comment #9)

2.6 Statistical analysis

The relationships between patent arteries in the ablated renal parenchyma 1 -month after cryoablation and treatment effectiveness (complete ablation or local tumor progression), tumor enhancement 1 -month after cryoablation (present or absent), ~~tumor size (≤2 cm or >2 cm)~~ (Reviewer #1, Comment #3)~~, and~~ tumor vascularity before cryoablation (hypervascular or non-hypervascular), and tumor subtype (clear cell RCC vs. non-clear cell RCC) (Reviewer #2, Comment #11) were analyzed using Fisher's exact test. ~~Tumors were classified as hypervascular or non-hypervascular RCCs. Hypervascular RCCs were defined as a peak attenuation of more than 100 HU on DCE-CT (corticomedullary phase) [10, 11].~~

The renal function change was measured using the following formula: (eGFR 1 month

after cryoablation)/ (eGFR before cryoablation). The relationship between patent arteries 1 month after cryoablation and renal function change was evaluated using a Mann-Whitney U test.

Analyses were performed using SPSS software, version 26 (IBM, Armonk, NY). Statistical significance was set at P <0.05.

3. Results

There were 386 renal tumors with diameters of ≤4 cm in 300 patients treated with cryoablation at our hospital between May 2012 and August 2018. AmongOf these, 186 RCCs (mean [± standard deviation] diameter, 1.9 cm ± 0.6 [standard deviation {SD}] cm; range, 0.7-4.0 cm) in 159 patients (112 men [70.4%], 47 women [29.6%]; mean age, 63.6 years ± 14.6 [SD] years; range, 21-91 years) were included in the study (**Fig. 1**). One hundred and thirty-eight tumors were histologically proven RCCs (110 clear cell [79.7%], 10 chromophobe [7.2%], 8 papillary [5.8%], 1 mucinous tubular and spindle cell carcinoma [0.7%], and 9 unclassified [6.5%]). Fuhrman grading was determined in 104 (75.4%; 46 grade 1, 56 grade 2, and 2 grade 3) of 138 biopsy-proven RCCs. (Reviewer #3, Comment #10) The remaining 48 lesions were diagnosed as RCCs based on images and a history of treatment for histologically proven RCCs.

The mean and median follow-up periods were 43.8 ± 22.1 (SD) months and 36.1 months (range, 1.0-89.0 months), respectively. Of 186 RCCs, 178 (95.7%) were completely ablated and 8 (4.3%) showed local tumor progression in a median follow up time ofat the median duration of 19.0 months after cryoablation (mean, 20.1 ± 12.1 [SD] months; range, 5.5-44.1 months). Local

tumor progression was suspected on DCE-CT (n = 6) or non-enhanced MRI (n = 1) and was histologically diagnosed by CT fluoroscopy-guided biopsy (n = 1). All RCCs with local progression were treated with cryoablation (n = 6), microwave ablation (n = 1), or molecular-targeted therapy (n = 1).

DCE-CT showed tumor enhancement in 108 RCCs (58.1%) within 1 -week and in 11 (6.0%) at 1 -month after cryoablation. AmongHowever, of these 11, DCE-CT showed disappearance of tumor enhancement at 3 -months (n = 7), 6 -months (n = 1), and 12 -months (n = 1) after cryoablation. The remaining 2 patients were diagnosed with local tumor progression.

DCE-CT showed 392 patent arteries (mean diameter, 1.41mm ± 0.33 [SD] mm; range, 1.0-3.2 mm) in the ablated renal parenchyma in 166 RCCsceases (89.2%) within 1 -week, 81 patent arteries in 54 RCCsceases (29.0%) 1 -month after cryoablation, 8 patent arteries in 8 RCCsceases (4.3%) 3 -months after cryoablation, and 2 patent arteries in 2 RCCsceases (1.1%) 6 -months after cryoablation (**Figs. 2 and 3Fig. 2**). AmongIn the two RCCsceases with patent arteries 6 -months after cryoablation, the1 patent artery disappeared in one after 12 -months. In theThe other, it still appeared 14-months after cryoablation, but subsequent DCE-CT was not performed because the patient died of hepatocellular carcinoma. The time course of patent artery by DCE-CT course is shown in **Fig. 43**.

Of the 8 RCCs with local progression, patent arteries in the ablated renal parenchyma were observed in 3 RCCsceases (37.5%) 1 -month after cryoablation. There was no association between patent arteries 1 -month after cryoablation and treatment effectiveness (*P* = 0.693; **Table**

21). Tumor enhancement on DCE-CT 1 -month after cryoablation was detected in 5.9% of RCCs (11/186 RCCs). Patent arteries 1 -month after cryoablation werewas significantly associated with tumor enhancement at the same time point1 month after cryoablation (P = 0.015; **Table 21**). Sixty-seven RCCs were larger than 2 cm, and One hundred and fifty-three 153 RCCs were categorized as hypervascular tumors. There waswere no associations between patent arteries 1 -month after cryoablation and tumor size (P = 0.405) (Reviewer #1, Comment #3) or tumor vascularity (P = 0.692; **Table 21**) or tumor subtype (P = 0.295; **Table 3**) (Reviewer #2, Comment #11). There was no significant difference in renal function change between the patent artery present group (mean, 0.95 ± 0.13 [SD]; range, 0.57-1.35) and the patent artery absent group (mean, 0.96 ± 0.12 [SD]; range, 0.70-1.57; P = 0.809).

4. Discussion

Periodic contrast-enhanced CT and MRI are essential for accurately evaluating treatment effectiveness and complications after RCC cryoablation. In this study, DCE-CT within 1 -week and 1 -month after cryoablation showed patent arteries in the ablated renal parenchyma in 89.2% and 29.0% of RCCsceases, respectively, but they gradually disappeared and required no special treatment (i.e., there was no association withinfluence on local tumor progression).

There are two recognized cell death mechanisms after cryoablation: immediate cytocidal damage related to the destruction of intracellular organelles and cell membranes and delayed cell death due to coagulative necrosis [19, 2012, 13]. A study on the effects of renal cryotherapy on

renal arteries showed that arteries less than 180 μm were damaged immediately after cryoablation, but arteries larger than this size remained [2114]. However, arteries with residual blood flow immediately after cryoablation gradually become thrombosed due to endothelial cell damage from freezing [2114].

Lagerveld et al. showed that vascular congestion within the frozen renal parenchyma is copmleted completes within two weeks [2215]. In our study, 20 RCCscases were without patent arteries in the ablated renal parenchyma within 1 -week of the procedure and were considered to have developed arterialartery occlusion in the interval days between cryoablation and obtaining the CT acquisition images. Patent arteries disappeared at 1 -month in 112 of 166 RCCscases with patent arteries within 1 -week and were thought to have become be occluded within for at least 1 -month after cryoablation, consistent with results reported by Lagerveld et al. [2215]. Most of the remaining patent arteries also disappeared between the 1 -and 3 -month follow-ups, suggesting that the arterial artery occlusion mechanism may take takes more time than previously reported their results [2215].

KnownSome investigators reported predictive factors for local tumor progression after RCC cryoablation include, such as large tumor size [23-2516-18], central type tumors [2417], anterior location [2518], deep tumor location [2619], and insufficient ice-ball margin [26, 2719, 20]. We hypothesized that patent arteries in the ablated renal parenchyma after cryoablation might not be a risk factor for local tumor progression, and our results supported this hypothesis assumption. Patent arteries were observed in 29.0% of RCCscases at 1 -month and in 4.3% of

RCCscases at 3 -months after cryoablation, with and only one RCCcase having showed a patent artery beyond one year. However, local tumor progression was observed much later after a longer period (the median detection time duration was 19.0 months after cryoablation). There is no temporal association between the early finding of arterial patency after cryoablation and the late finding of tumor progression. Therefore, the finding, on contrast-enhanced CT, of patent arteries in the ablated renal parenchyma 1 month after cryoablation should not raise undue concern for local progression among physicians. It is unlikely that the patent arteries after cryoablation (early findings) were associated with local tumor progression (late findings). Therefore, even if contrast-enhanced CT reveals patent arteries in the ablated renal parenchyma 1-month after cryoablation, physicians should continue to follow the patient and treated RCC without undue fear of local progression.

In a retrospective study of with 23 completely ablated renal tumors masses, eight tumors masses (8/23, 34.8%) imaged within 6-36 h after cryoablation were enhanced on contrast-enhanced MRI (i.e., false-positive tumor enhancement), and no enhancement was observed in four, three, and one tumors masses at 3-, 6-, and 10 -months after cryoablation, respectively [106]. In a prospective study, contrast-enhanced MRI showed false-positive tumor enhancement in 15 RCCs (15/25, 60.0%) 2-3 days, 13 RCCs (13/25, 52.0%) 5-7 days, and 1 RCC (1/25, 4.0%) 1 -month after cryoablation [84]. In our study results, contrast-enhanced CT 1 -month after cryoablation showed tumor enhancement in 6.0% of RCCs cases, and patent arteries were observed significantly more frequently in the tumor enhancement group. Patent arteries may be one reason for false-positive

tumor enhancement 1 -month after cryoablation.

Takaki et al. reported that the rate of false-positive tumor enhancement noted 5-7 days after cryoablation for clear cell RCC (63.2%; 12/19) was significantly higher than for other RCC subtypes (16.7%; 1/6; *P* <0.05) [84]. In this study, tumor vascularity and tumor subtype ~~size~~ (Reviewer #1, Comment #3) were unaffected by the patent arteries.

In this study, the relationships between patent arteries 1 month after cryoablation and ice-ball size and location were not evaluated. Ice-ball size is affected by various factors, such as ablation time, ablation number, type and number of cryoprobes used, location of cryoprobe insertion, and presence of close large vessels [26, 28]. Although the ice-ball covered the entire tumor with at least a 6-mm margin during cryoablation, the size of the ice-ball for RCCs (e.g., distance of ice-ball margin and volume ratio of ice-ball and RCC) was not uniform. In RCCs with centrally located ice-balls, the ablated normal parenchyma may have contained larger arteries. However, it is unclear whether ice-ball size was affected by heat sink effect and whether DCE-CT 1-month after cryoablation showed more patent arteries. (Reviewer #1, Comment #2)

This retrospective study from a single institution has several limitations. First, although we defined patent arteries as linear enhancement with ≥1-mm length using DCE-CT with ≤2-mm slice thickness, histological correlations between the DCE-CT images and the ablated renal parenchyma were not performed; some patent arteries might not be visible on DCE-CT. Second, local tumor progression was not always histologically proven, and most lesions were diagnosed by imaging alone. Third, although the follow-up CT protocol was standardized, CT images were

obtained using five types of machines. Fourth ~~Finally~~, 48 tumors were not histologically proven to be RCCs. Last, imaging evaluation was performed by consensus between two experienced radiologists and not by independent reviewers with calculation of interobserver agreement. (Reviewer #2, Comment #8)

5. Conclusion

Patent arteries in the ablated renal parenchyma were common on DCE-CT images after cryoablation. However, they gradually disappeared and required no special treatment.

Human rights

The authors declare that the work described has been carried out in accordance with the Declaration of Helsinki of the World Medical Association revised in 2013 for experiments involving humans.

Informed consent and patient details

The authors declare that this report does not contain any personal information that could lead to the identification of the patients.

Disclosure of interest

The authors declare that they have no competing of interest.

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Author contributions

All authors attest that they meet the current International Committee of Medical Journal Editors (ICMJE) criteria for Authorship.

References

1. Iguchi T, Hiraki T, Matsui Y, Tomita K, Uka M, Tanaka T, et al. Image-guided core biopsy of 2-cm or smaller renal tumors. Diagn Interv Imaging 2020 Nov;101(11):715-720.

2. Marcelin C, Ambrosetti D, Bernhard JC, Roy C, Grenier N, Cornelis FH. Percutaneous image-guided biopsies of small renal tumors: Current practice and perspectives. Diagn Interv Imaging 2017 Sep;98(9):589-599.

3. Zhou W, Herwald SE, McCarthy C, Uppot RN, Arellano RS. Radiofrequency ablation, cryoablation and microwave ablation for T1a renal cell carcinoma: a comparative evaluation of therapeutic and renal function outcomes. J Vasc Interv Radiol. 2019 Jul;30(7):1035-1042.

4. Cornelis FH, Bernhard JC. Diagnostic and interventional radiology is a milestone in the management of renal tumors in Birt-Hugg-Dubé syndrome. Diagn Interv Imaging 2019 Nov;100(11):657-658.

5. Matsui Y, Hiraki T, Gobara H, Iguchi T, Tomita K, Uka M, Araki M, Nasu Y, Furuya M,

Kanazawa S. Percutaneous thermal ablation for renal cell carcinoma in patients with Birt-Hogg-Dubé syndrome. Diagn Interv Imaging 2019 Nov;100(11):671-677.

62. Georgiades CS, Rodriguez R. Efficacy and safety of percutaneous cryoablation for stage 1A/B renal cell carcinoma: results of a prospective, single-arm, 5-year study. Cardiovasc Intervent Radiol. 2014 Dec;37(6):1494-1499.

73. Lim E, Kumar S, Seager M, Modi S, Mandal I, Neves JB, et al. Outcomes of renal tumors treated by image-guided percutaneous cryoablation: immediate and 3- and 5-year outcomes at a regional center. AJR Am J Roentgenol. 2020 Jul;215(1):242-247.

84. Takaki H, Nakatsuka A, Cornelis F, Yamanaka T, Hasegawa T, Sakuma H, Yamakado K. False-Positive tumor enhancement after cryoablation of renal cell carcinoma: a prospective study. AJR Am J Roentgenol. 2016 Feb;206(2):332-339.

95. Kawamoto S, Permpongkosol S, Bluemke DA, Fishman EK, Solomon SB. Sequential changes after radiofrequency ablation and cryoablation of renal neoplasms: role of CT and MR imaging. Radiographics 2007 Mar-Apr;27(2):343-355.

106. Porter CA 4th, Woodrum DA, Callstrom MR, Schmit GD, Misra S, Charboneau JW, Atwell TD. MRI after technically successful renal cryoablation: early contrast enhancement as a common finding. AJR Am J Roentgenol. 2010 Mar;194(3):790-793.

11. Gobara H, Nakatsuka A, Shimizu K, Yamanaka T, Matsui Y, Iguchi T, et al. Cryoablation of renal cell carcinoma for patients with stage 4 or 5 non-dialysis chronic kidney disease. Jpn J Radiol 2019 Jun;37(6):481-486.

12. Cornelis FH, Monard E, Moulin MA, Vignaud E, Laveissiere F, Ben Ammar M, et al. Sedation and analgesia in interventional radiology: Where do we stand, where are we heading and why does it matter? Diagn Interv Imaging 2019 Dec;100(12):753-762.

13. Miyazaki M, Iguchi T, Takaki H, Yamanaka T, Tamura Y, Tokue H, et al. Ablation protocols and ancillary procedures in tumor ablation therapy: consensus from Japanese experts. Jpn J Radiol 2016 Sep;34(9):647-656.

147. Ahmed M, Solbiati L, Brace CL, Breen DJ, Callstrom MR, Charboneau JW, et al. Image-guided tumor ablation: standardization of terminology and reporting criteria–a 10-year update. J Vasc Interv Radiol. 2014 Nov;25(11):1691-1705.

158. Tsivian M, Kim CY, Caso JR, Rosenberg MD, Nelson RC, Polascik TJ. Contrast enhancement on computed tomography after renal cryoablation: an evidence of treatment failure? J Endourol. 2012 Apr;26(4):330-335.

169. Merkle EM, Nour SG, Lewin JS. MR imaging follow-up after percutaneous radiofrequency ablation of renal cell carcinoma: findings in 18 patients during the first 6 months. Radiology. 2005 Jun;235(3):1065-1071.

1710. Kim JK, Kim TK, Ahn HJ, Kim CS, Kim KR, Cho KS. Differentiation of subtypes of renal cell carcinoma on helical CT scans. AJR Am J Roentgenol. 2002 Jun;178(6):1499-1506.

1811. Jinzaki M, Tanimoto A, Mukai M, Ikeda E, Kobayashi S, Yuasa Y, et al. Double phase helical CT of small renal parenchymal neoplasms: correlation with pathologic findings and tumor angiogenesis. J Comput Assist Tomogr. 2000 Nov-Dec;24(6):835-842.

1912. Baust JG, Gage AA, Clarke D, Baust JM, Van Buskirk R. Cryosurgery--a putative approach to molecular-based optimization. Cryobiology. 2004 Apr;48(2):190-204.

2013. Hoffmann NE, Bischof JC. The cryobiology of cryosurgical injury. Urology. 2002 Aug;60(2 Suppl 1):40-49.

2114. Lagerveld BW, van Horssen P, Pes MP, van den Wijngaard JP, Streekstra GJ, de la Rosette JJ, et al. Immediate effect of kidney cryoablation on renal arterial structure in a porcine model studied by imaging cryomicrotome. J Urol. 2010 Mar;183(3):1221-1226.

2215. Lagerveld BW, van Horssen P, Laguna MP, van den Wijngaard JP, Siebes M, Wijkstra H, et al. Gradient changes in porcine renal arterial vascular anatomy and blood flow after cryoablation. J Urol. 2011 Aug;186(2):681-686.

2316. Kim EH, Tanagho YS, Bhayani SB, Saad NE, Benway BM, Figenshau RS. Percutaneous cryoablation of renal masses: Washington University experience of treating 129 tumours. BJU Int. 2013 May;111(6):872-879.

2417. Tsivian M, Lyne JC, Mayes JM, Mouraviev V, Kimura M, Polascik TJ. Tumor size and endophytic growth pattern affect recurrence rates after laparoscopic renal cryoablation. Urology. 2010 Feb;75(2): 307-310.

2518. Zargar H, Samarasekera D, Khalifeh A, Remer EM, O'Malley C, Akca O, et al. Laparoscopic vs percutaneous cryoablation for the small renal mass: 15-year experience at a single center. Urology. 2015 Apr;85(4): 850-855.

2619. Yamanaka T, Yamakado K, Yamada T, Fujimori M, Takaki H, Nakatsuka A, et al. CT-

guided percutaneous cryoablation in renal cell carcinoma: factors affecting local tumor control. J Vasc Interv Radiol. 2015 Aug;26(8):1147-1153.

2720. Ge BH, Guzzo TJ, Nadolski GJ, Soulen MC, Clark TW, Malkowicz SB, et al. Percutaneous renal cryoablation: short-axis ice-ball margin as a predictor of outcome. J Vasc Interv Radiol. 2016 Mar;27(3):403-409.

28. Park SY, Won JY, Oh YT, Jung DC, Kim GM, Kim MD. Assessment of cold sink effect in postulated renal cryoablation by analyzing radiographic ice ball on computed tomography. Br J Radiol 2019 Jul; 92(1093):20170951.

Figure Legends

Figure 1. Study flowchart.

Figure 2. A 79-year-old woman with biopsy-proven clear cell renal cell carcinoma (RCC; 1.6-cm diameter).

A) Computed tomography (CT) image before cryoablation shows the target RCC in the right kidney (arrow).

B) CT image 2 days after cryoablation shows patent arteries (white arrows) and ablated RCC with enhancement (black arrow).

C) CT image 1 month after cryoablation shows disappearance of both the patent arteries and the enhancement of ablated RCC in ablated renal parenchyma (arrows).

Figure 32. A 70-year-old man with biopsy-proven clear cell renal cell carcinoma (RCC; 2.0-cm diameter) ~~in the right kidney~~.

A) Computed tomography (CT) image before cryoablation shows the target RCC in the right kidney (arrows).

B) Prone CT image during cryoablation with hydrodissection shows ice-ball (black arrow) and a cryoprobe (white arrow).

~~A) A computed tomography (CT) image before cryoablation shows no and poor contrast areas in the right renal parenchyma.~~

C~~B~~) ~~A~~ CT image (slice image 4 mm caudal to RCC) 1 -month after cryoablation shows patent arteries (white arrows) and ablated renal parenchyma (black arrows).

D~~C~~) ~~A~~ CT image (slice image 4 mm caudal to RCC) 3 -months after cryoablation shows disappearance of the patent arteries ~~disappearing~~ and shrinkage of the ablated renal parenchyma ~~shrinking~~ (arrows).

Figure 43. The time course of patent arteries on contrast-enhanced dynamic computed tomography images with ≤2-mm slice thickness.

Author responsibilities, integrity, ethics

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Article title : CT evaluation of patent artery after renal cell carcinoma cryoablation

Human and animal rights

- [x] The authors declare that the work described has been carried out in accordance with the Declaration of Helsinki of the World Medical Association revised in 2013 for experiments involving humans as well as in accordance with the EU Directive 2010/63/EU for animal experiments.
- [ ] The authors declare that the work described has not involved experimentation on humans or animals.

Informed consent and patient details

- [x] The authors declare that this report does not contain any personal information that could lead to the identification of the patient(s) and/or volunteers.
- [ ] The authors declare that they obtained a written informed consent from the patients and/or volunteers included in the article and that this report does not contain any personal information that could lead to their identification.
- [ ] The authors declare that the work described does not involve patients or volunteers.

Disclosure of interest

- [x] The authors declare that they have no known competing financial or personal relationships that could be viewed as influencing the work reported in this paper.
- [ ] The authors declare the following financial or personal relationships that could be viewed as influencing the work reported in this paper:

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Author contributions

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CRediT author statement

Soichiro Kajita: Conceptualization, Methodology, Investigation, Data curation, Writing - original draft

Toshihiro Iguchi: Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing, Visualization, Project administration.

Takao Hiraki: Methodology, Writing - review & editing.

Yusuke Matsui: Conceptualization, Methodology, Investigation, Validation, Writing - review & editing.

Koji Tomita: Writing - review & editing.

Mayu Uka: Writing - review & editing.

Takashi Tanaka: Formal analysis, Writing - review & editing.

Motoo Araki: Writing - review & editing.

Hideo Gobara: Writing - review & editing.

Susumu Kanazawa: Writing - review & editing, Supervision.

Highlights

- Patent arteries in ablated renal parenchyma after cryoablation were common.
- Patent arteries in ablated renal parenchyma after cryoablation gradually disappeared.
- Patent arteries in ablated renal parenchyma after cryoablation required no special treatment.
- There was no association between patent arteries 1 month after cryoablation and treatment effectiveness.

Table 1. Date of initial follow-up CT

Date after cryoabletion (day)	Number
1	50 (26.9)
2	99 (53.2)
3	34 (18.3)
4	1 (0.5)
5	
6	
7	2 (1.1)

Values in parentheses are percentages.

Table 2. Relationships between patent arteries 1 month after cryoablation and treatment effectiveness, tumor enhancement 1 month after cryoablation, and tumor vascularity

		Patent arteries at 1 month after cryoablation		
		Present (n = 54)	Absent (n = 132)	*P* value
Treatment effectiveness	Complete ablation (n = 178)	51 (28.7)	127 (71.3)	0.693
	Local progression (n = 8)	3 (37.5)	5 (62.5)	
Tumor enhancement at 1 month after cryoablation	Absent (n = 175)	47 (26.9)	128 (73.1)	0.015
	Present (n = 11)	7 (63.6)	4 (36.4)	
Tumor vascularity	Hypervascular (n = 153)	47 (30.7)	106 (69.3)	0.398
	Non-hypervascular (n = 33)	7 (21.2)	26 (78.8)	

Values in parentheses are percentages.

Table 3. Relationship between patent arteries 1 month after cryoablation and tumor subtype

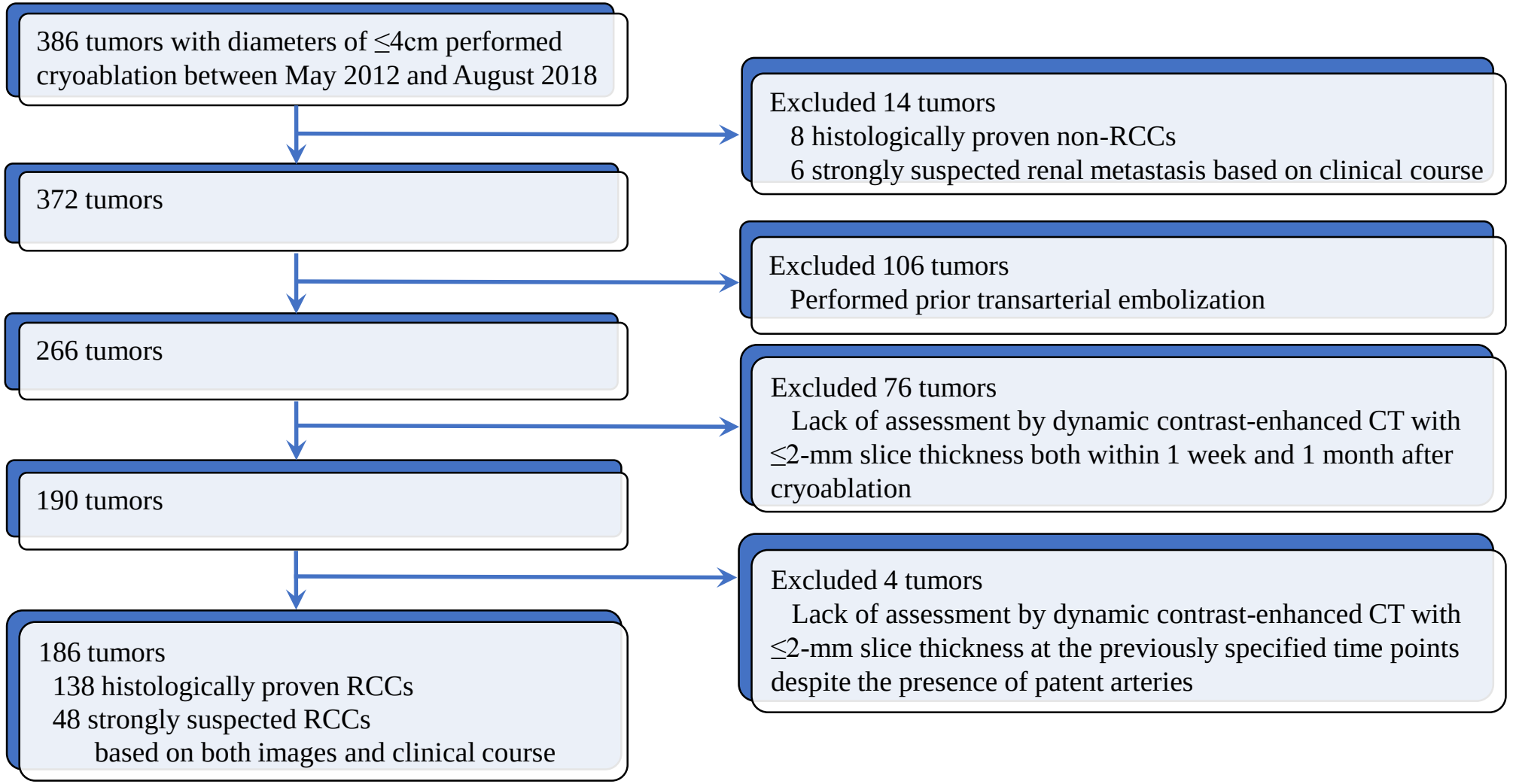
		Patent arteries at 1 month after cryoablation		
		Present (n = 44)	Absent (n = 85)	*P* value
Tumor subtype	Clear cell RCC (n = 110)	40 (36.4)	70 (63.6)	0.295
	Non-clear cell RCC (n = 19)	4 (21.1)	15 (78.9)	

Values in parentheses are percentages.

RCC: renal cell carcinoma

Ninteen non-clear cell RCCs includes 10 chromophobe RCCs, 8 papillary RCCs and 1 mucinous tubular and spindl cell carcinoma.

Figure 1



RCC: renal cell carcinoma

Figure 2A

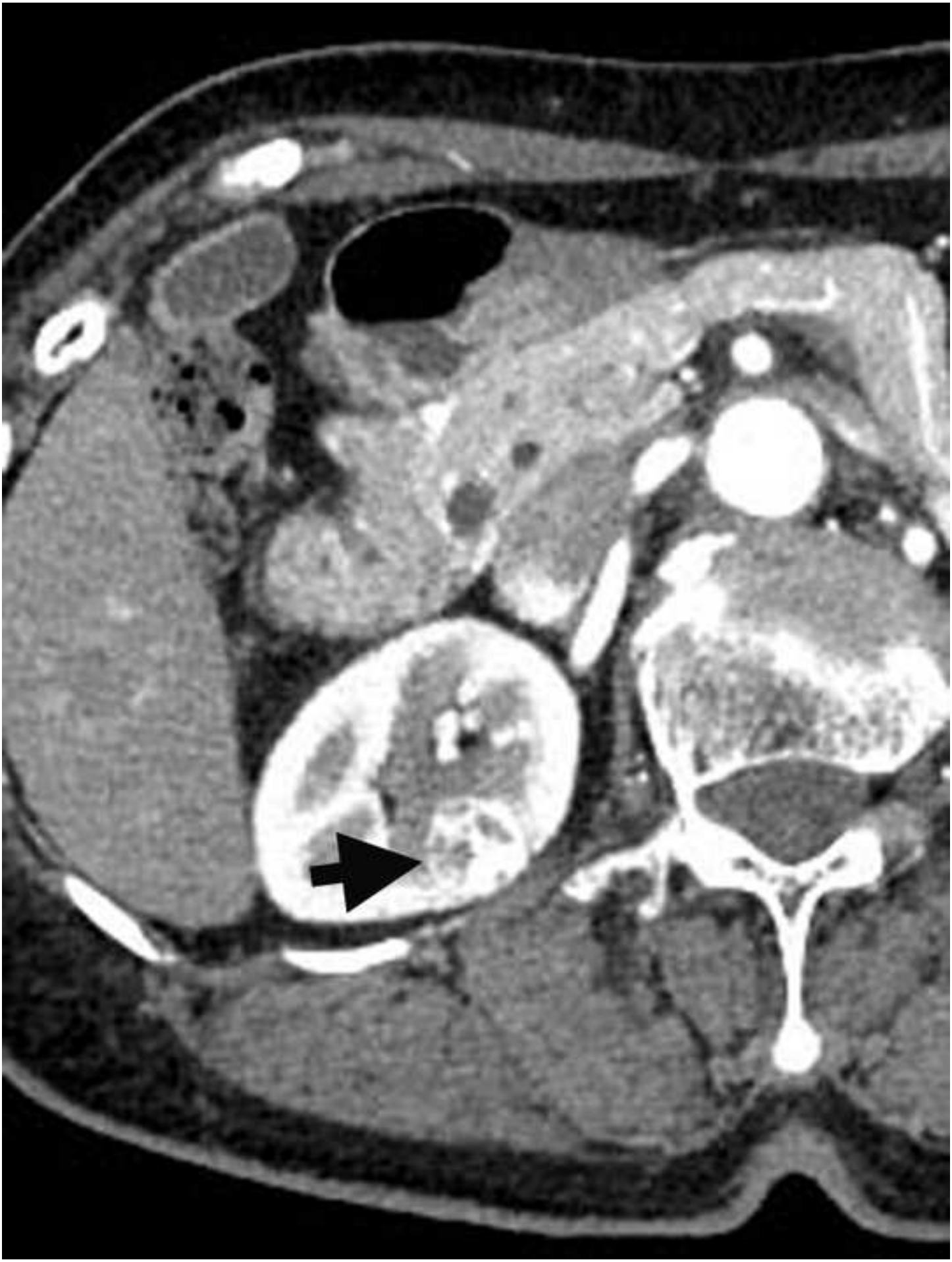


Figure 2B

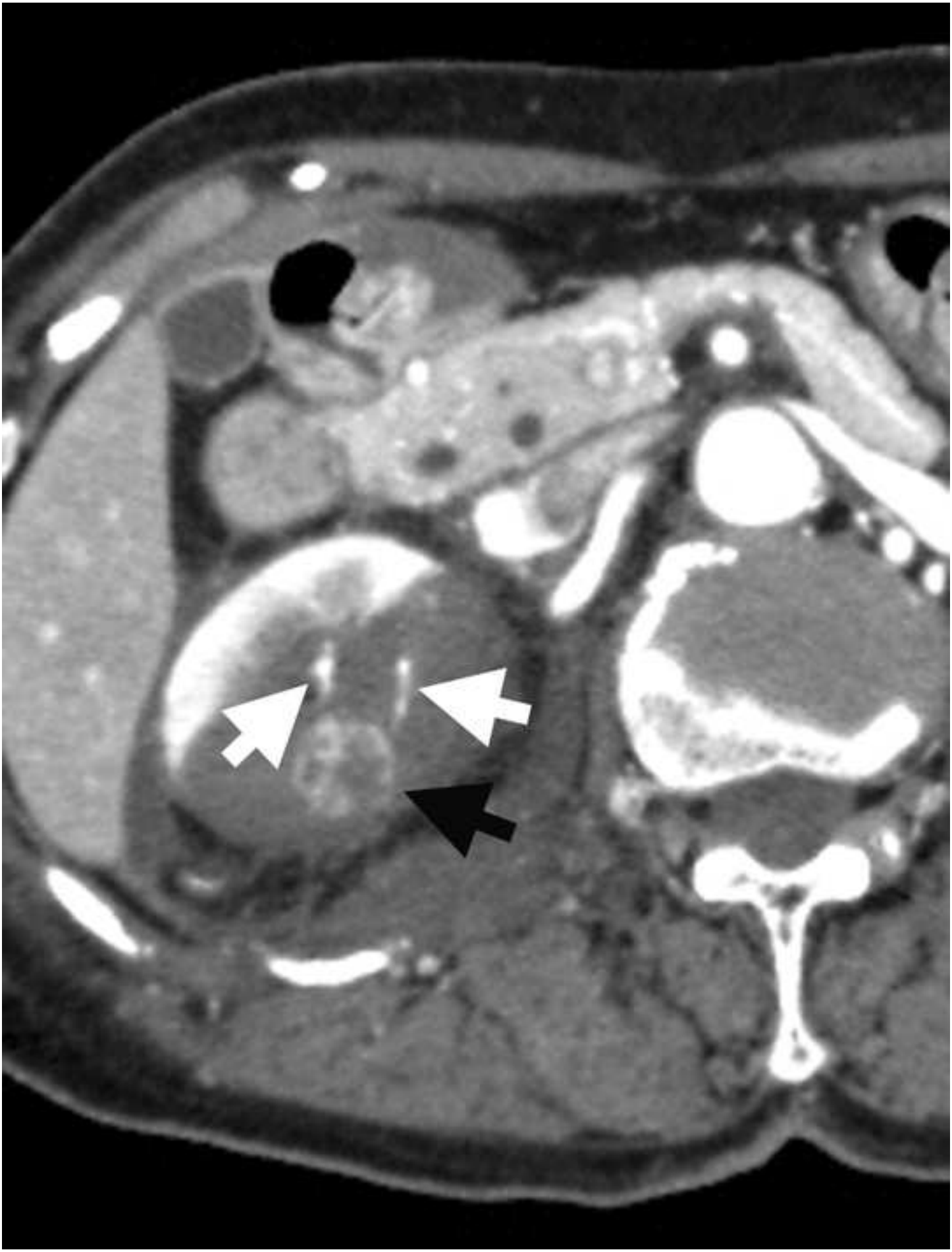


Figure 2C

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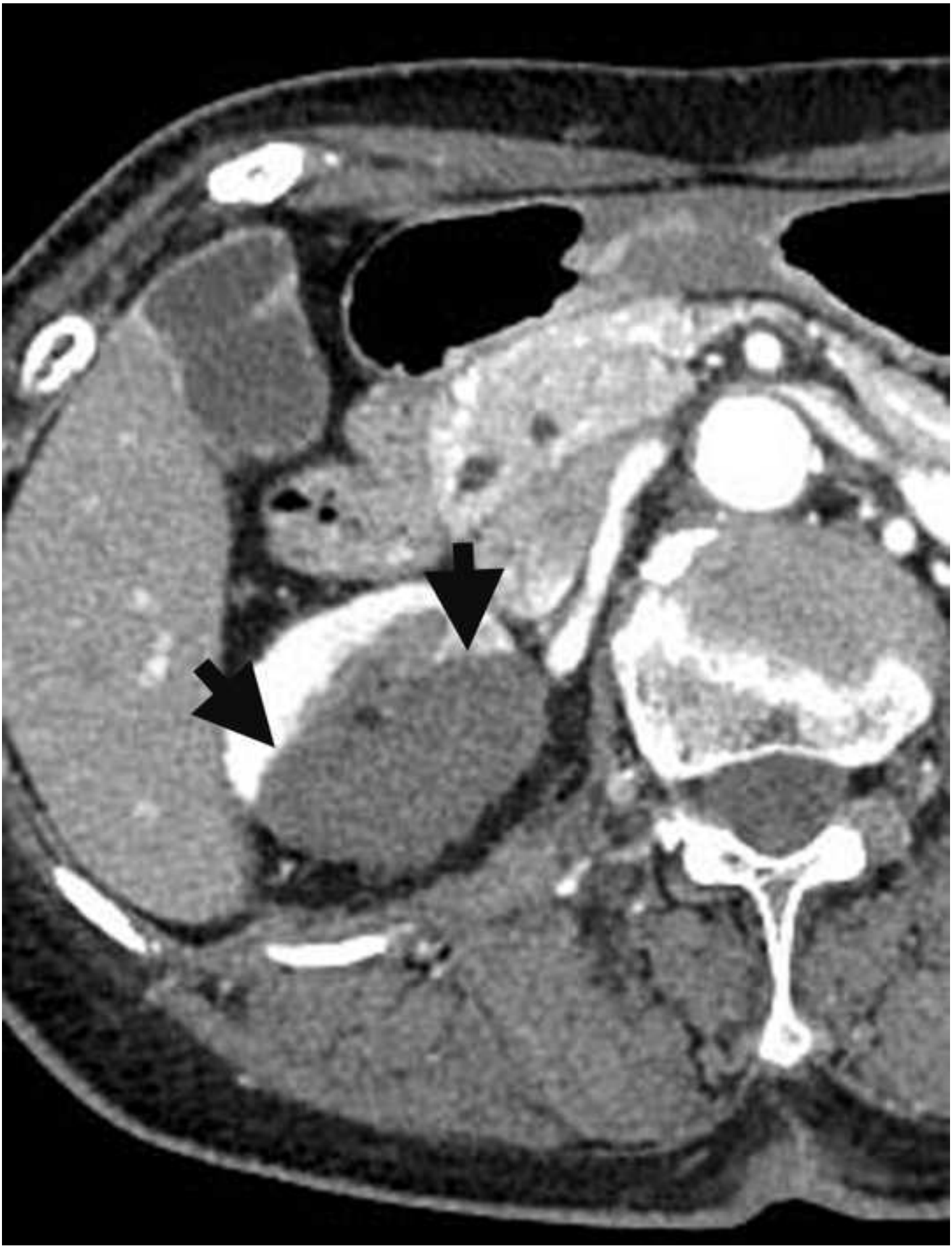

Figure 3A

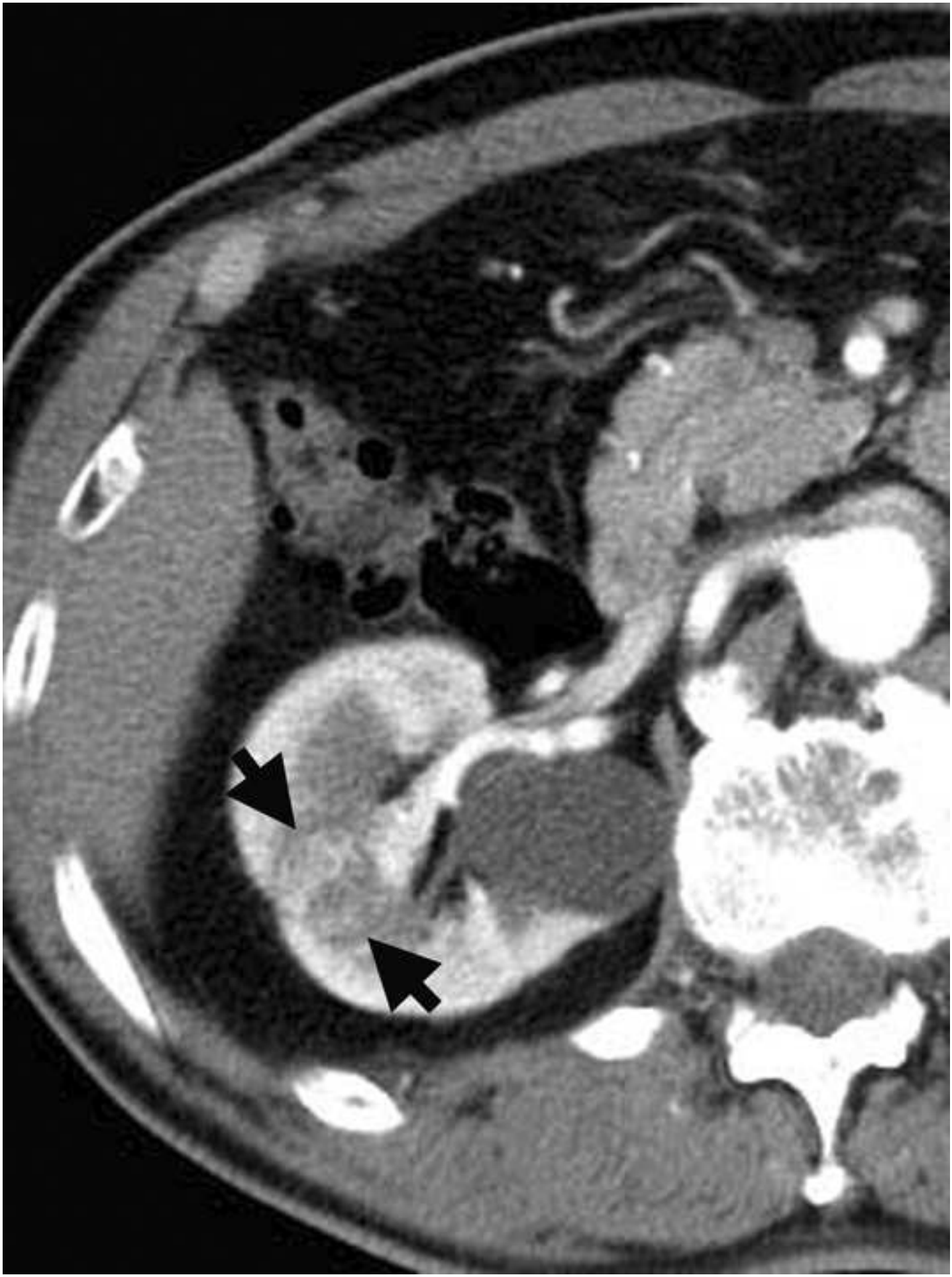

Figure 3B

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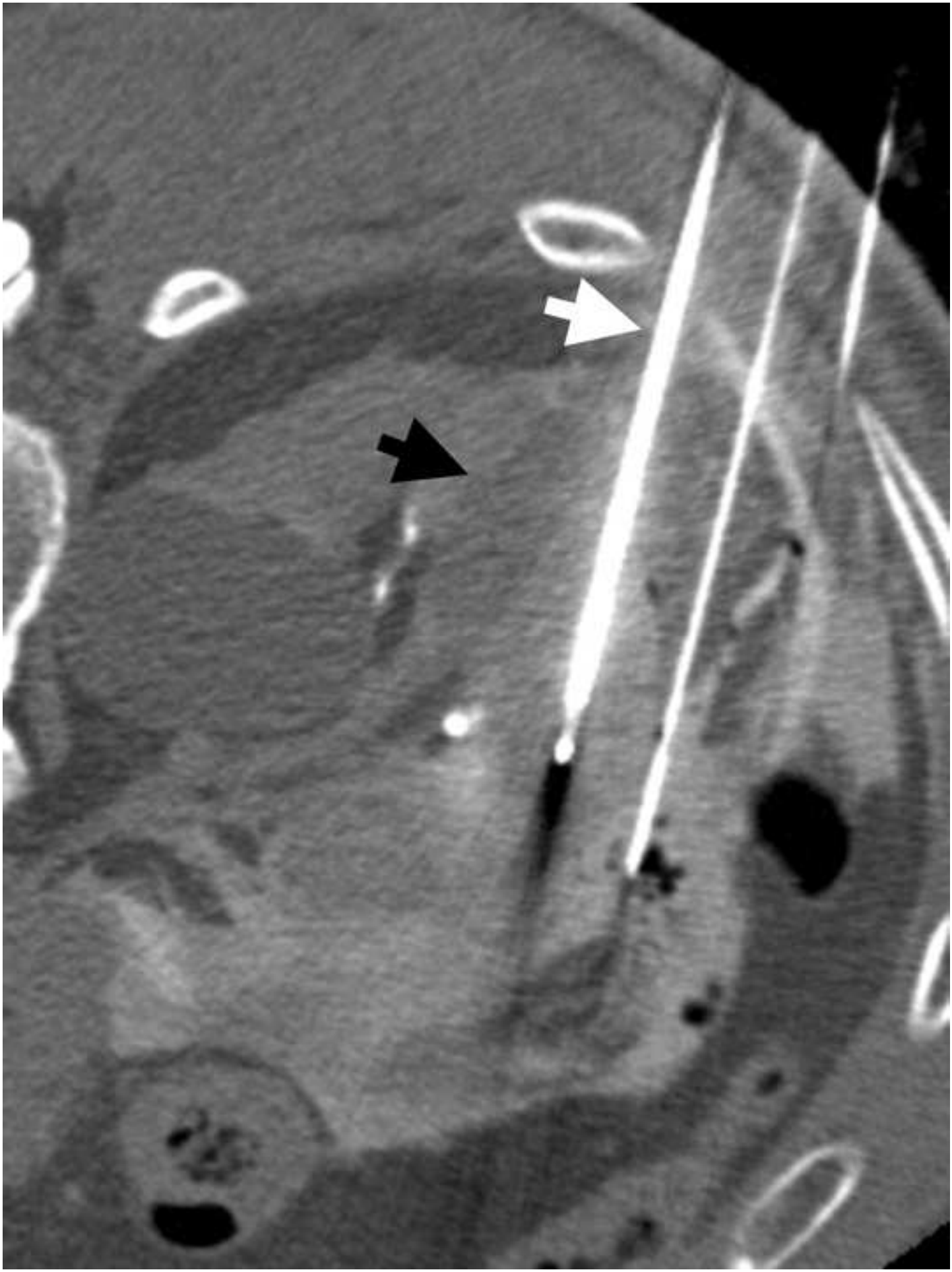

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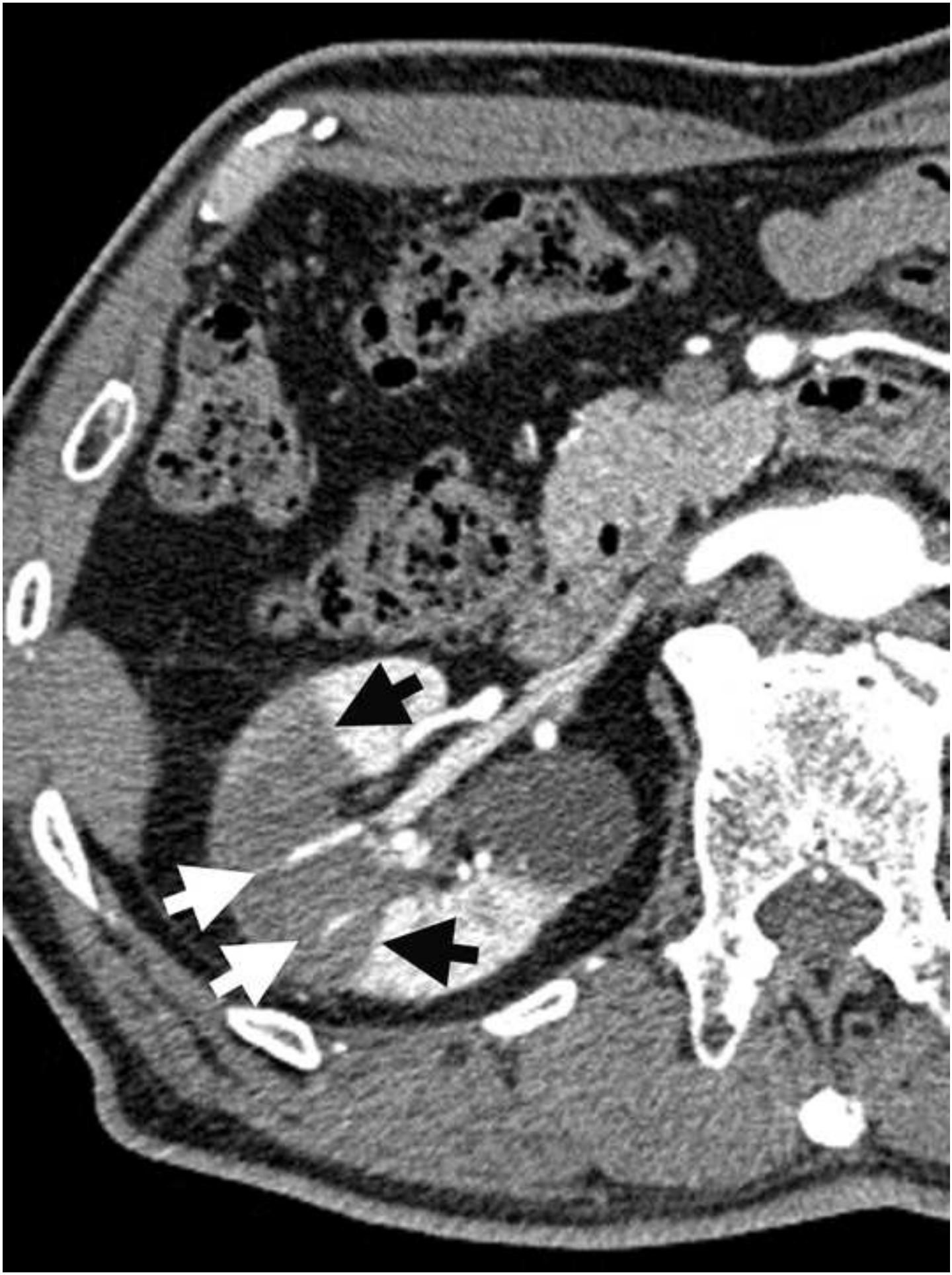

Figure 3D

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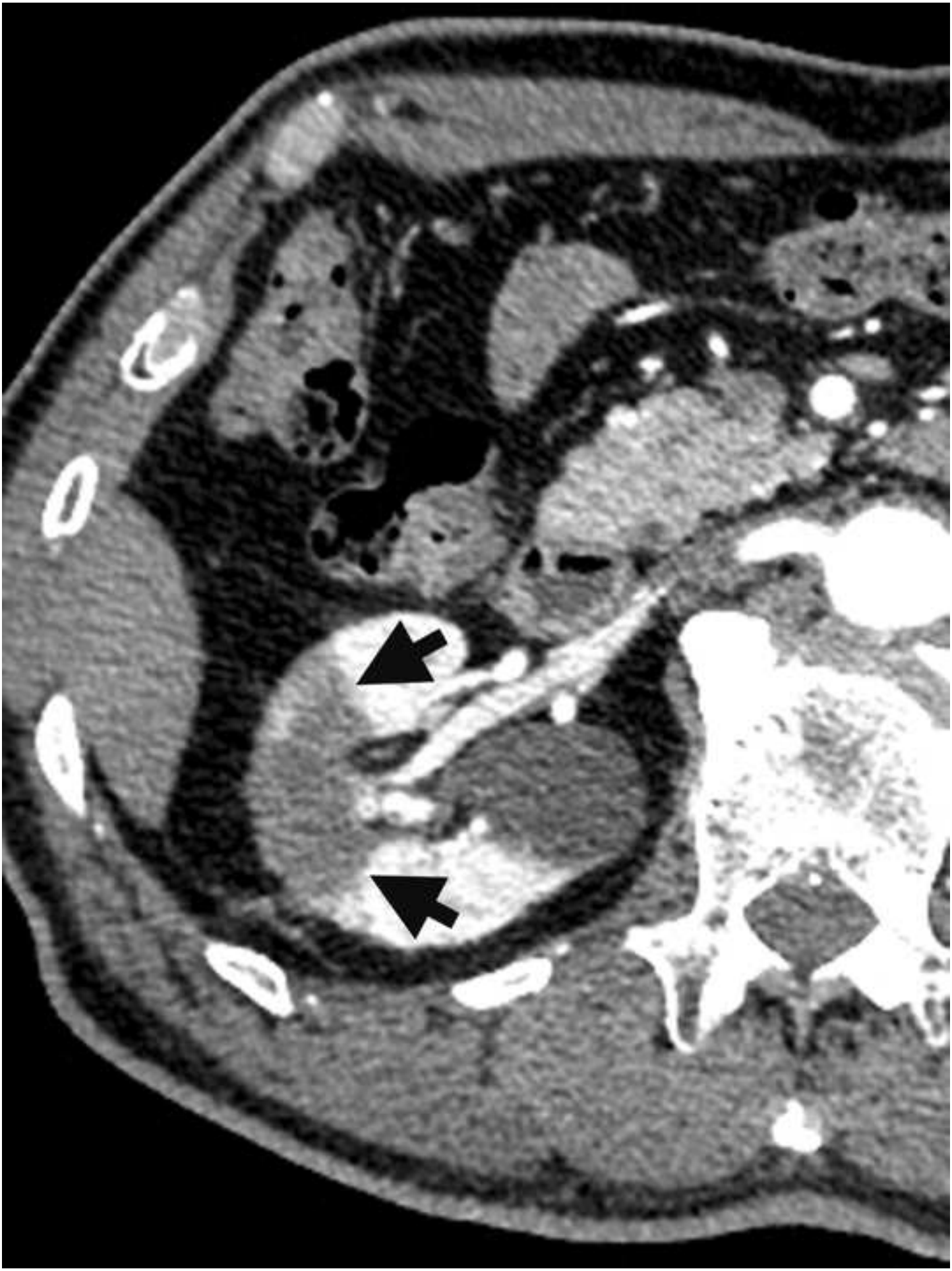

Figure 4

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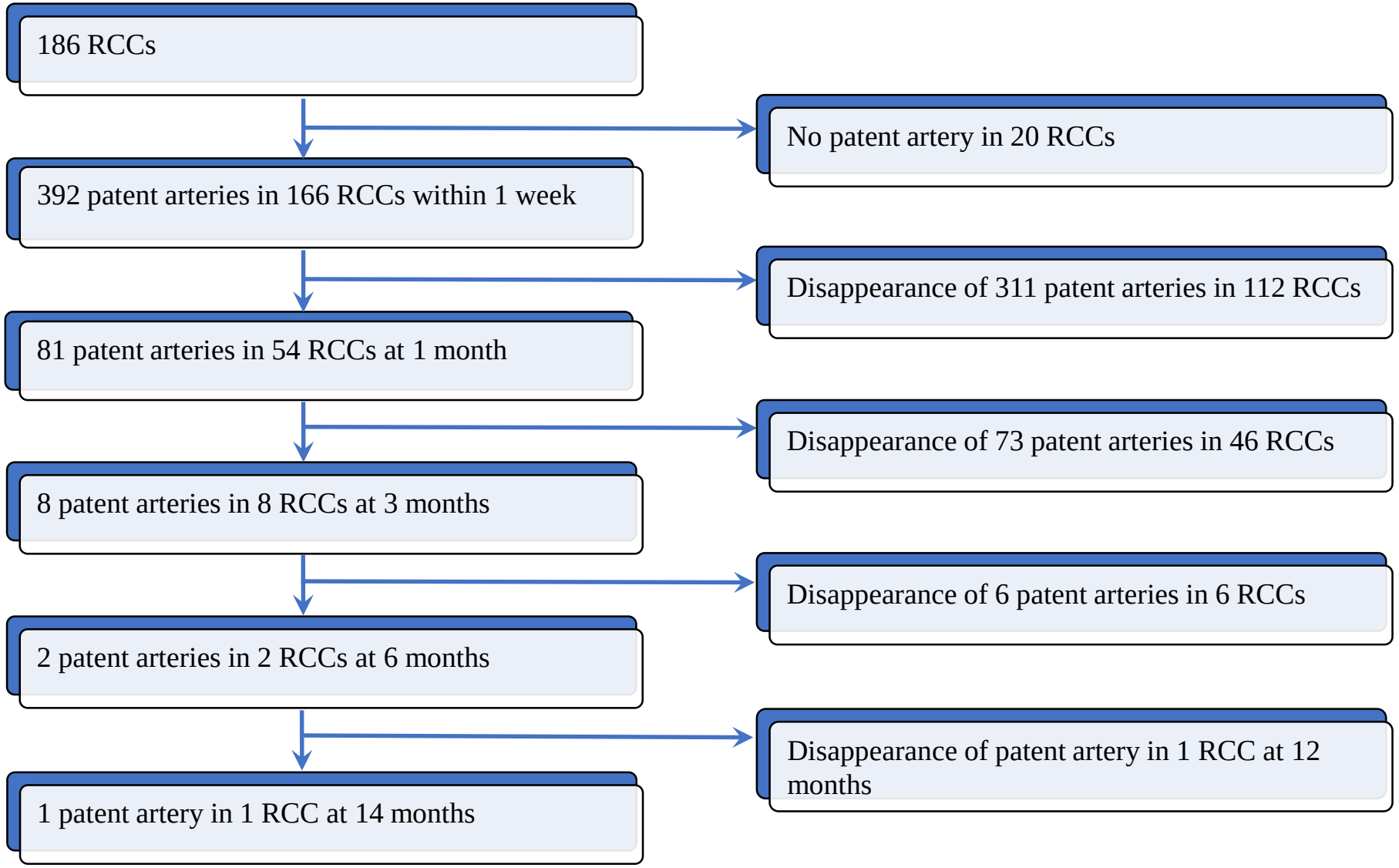

RCC: renal cell carcinoma