Cryobiopsy in the Upper Urinary Tract: Preclinical Evaluation of a Novel Device

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OBJECTIVE To develop a novel device for cryobiopsy of the upper urinary tract (UUT) and to evaluate its feasibility in a standardized preclinical setting.

MATERIALS AND METHODS Flexible cryoprobes (diameter 0.9 mm; cooling agent CO2) were developed and used to extract biopsies in porcine UUTs. Cryosamples obtained by ureterorenoscopy were systematically compared with biopsy specimens obtained with standard of care devices in terms of physical characteristics (deflection angle and irrigation flow rates) and histologic criteria (assessability).

RESULTS Irrigation flow rates were significantly higher with introduced BIGopsy (2.8 ± 0.1) compared with standard forceps (0.94 ± 0.06; $P < .001$) and cryoprobe (1.1 ± 0.1; $P < .001$). Angular deflection was significantly reduced by the inserted cryoprobe (130.7° ± 1.2° vs 166.9° ± 1.1° [BIGopsy] or 161.4° ± 1.9° [standard forceps]; both $P < .001$). Significantly larger UUT tissue samples were obtained by the cryoprobe (mean specimen area 7.5 ± 2.5 vs 4.6 ± 2.5 mm^2 [BIGopsy] or 1.4 ± 1.4 mm^2 [standard forceps]; both $P < .001$). No crush artifacts were observed in cryosamples. Superior histologic assessability scores were achieved in samples obtained by the cryoprobe (mean 2.8 ± 0.8) and BIGopsy (2.3 ± 1.9) when compared with standard forceps (0.4 ± 0.9; $P < .001$).

CONCLUSION Cryobiopsy in the UUT is feasible and represents a viable new option to improve the diagnostic accuracy of histopathologic evaluation. Larger and more representative tissue samples can be obtained using a cryoprobe and artifacts may be avoided. Further optimization of the probe will reduce possible restrictions of ureterorenoscopy handling when the device is inserted.

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Ureterorenoscopic (URS) biopsy is the mainstay in the diagnostic evaluation of suspicious lesions in the upper urinary tract (UUT).[1] The quality of extracted tissue samples is crucial for adequate histopathologic work-up and clinical decision-making, particularly in patients harboring upper tract urothelial carcinoma (UTUC). Despite technical advantages with flexible URS and the use of modern imaging, the accuracy of UUT biopsy is poor due to significant technical limitations of the procedure and the frequent occurrence of cautery and crushing artifacts in tissue samples acquired with available devices.[2-4] Biopsy samples are frequently small and tissue compression or denudation can complicate or preclude diagnostic evaluation in a significant proportion of patients, resulting in high numbers of grading and staging errors, with both upgrading and upstaging observed in up to 38% of patients undergoing radical nephroureterectomy (RNU).[1,4-7] A recent study found that almost 50% of biopsies obtained from patients considered for pure endoscopic treatment of UTUC were nondiagnostic.[8]

Consequently, endoscopic reinterventions with repeat biopsies are frequently required. Moreover, the characterization of flat lesions in the UUT, including carcinoma in situ, remains a significant challenge in patient care, especially when tangential biopsies in the ureter are needed.[6] Moreover, a significant proportion of candidates for nephron-sparing approaches may be overtreated by RNU,[9-11] as proper selection of low-risk patients in whom renal preservation may be oncologically safe requires accurate assessment of the stage of UTUC lesions.

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Development of new forcipes has not successfully resolved issues with compression artifacts or biopsy size.[2,3] The use of cryoprobes with consecutive tissue extraction may overcome these problems. Cryobiopsy has been demonstrated to yield larger tissue specimens and higher quality samples in preclinical studies and clinical trials in lung cancer.[12-14]

The objective of the present study was to develop a minimized cryoprobe for use in URS. We assessed the feasibility of cryoextraction of tissue samples in porcine UUTs using a standardized setting. The future potential for clinical implementation to improve the diagnostic accuracy of the biopsy procedure was evaluated.

MATERIALS AND METHODS

The study was performed in an ex vivo setting. In accordance with the federal animal protection law, there was no need for an approval of the governmental review board. Specifically, the study was performed in porcine kidneys explanted from cadavers.

CRYOBIOPSY DEVICE

New flexible cryoprobes were developed (Erbe Elektromedizin GmbH) and minimized to a diameter of 0.9 mm in order to fit into the working channel of flexible URS while maintaining angulation (Fig. 1A,B). The probe was connected to the ERBECRYO2 device (Erbe Elektromedizin GmbH), which allowed foot pedal activation of the probe. After activation, pressurized cooling agent (CO_2) is immediately applied through the central channel of the cryoprobe. When the gas reaches the tip of the probe, it expands due to a sudden decrease in pressure (Joule-Thomson effect), causing a temperature drop and subsequent freezing of the surrounding tissue. The size of the biopsy can be regulated by the activation time[12]; therefore, activation has to be maintained by keeping the foot pedal pressed until a desired cooling time has elapsed.

For the biopsy procedure, the cryoprobe was inserted into the working channel of the scope until the tip of the cryoprobe was visible. The tip of the probe was then placed either vertically or horizontally on the target tissue, depending on the position of the target, without pressure. Immediately after the predefined activation time of 10 seconds had elapsed, the biopsy sample was retrieved with a quick jerk, thereby retracting the scope together with the cryoprobe. Activation of the cryo unit was maintained until the biopsy and the scope were entirely outside the lumen to avoid loss of the specimen by defrosting of the probe and specimen.

EX VIVO MODEL

The standardized ex vivo setting using a porcine UUT model was previously described.[3] Devices were tested in a standard flexible digital URS (Storz 11278 VK/VUK (Flex-X_C). Briefly, the URS was introduced into the porcine UUT and connected to the SCB Image 1 HD Hub camera control unit (Storz Image 1 Hub 22201020). Images were displayed on a HD-Monitor. A 3-l irrigation solution bag was positioned 50 cm above the level of the table with continuous irrigation. Biopsies from the UUT were obtained from the renal pelvis under direct vision. The back loading BIGopsy device (115CM; 2.4 Fr.; IN) and a standard forceps (Richard Wolf; 829.601; 3 Fr.; Knittlingen, Germany) were used as reference standards.

INSTRUMENTS AND MEASUREMENTS

All experiments were performed by an experienced endourologist (JTK). Initially, active deflection (angle) and irrigation flow rates (mL/min) were repeatedly measured with different biopsy devices inserted into the same scope (frequently used; Flex-X_C), as described previously.[3] Measurements of active deflection with inserted cryoprobe were repeatedly performed in 3 different additional scopes with different degrees of wear and tear (new single use device; PUSEN PU3022 [Zhuhai Pusen Medical Technology Co., Ltd., Tangjiawan Town, Zhuhai, Guangdong, PRC]; Olympus URF-V [less frequently used; Olympus Europa SE & Co. KG, Hamburg, Germany] and Wolf Boa Vision

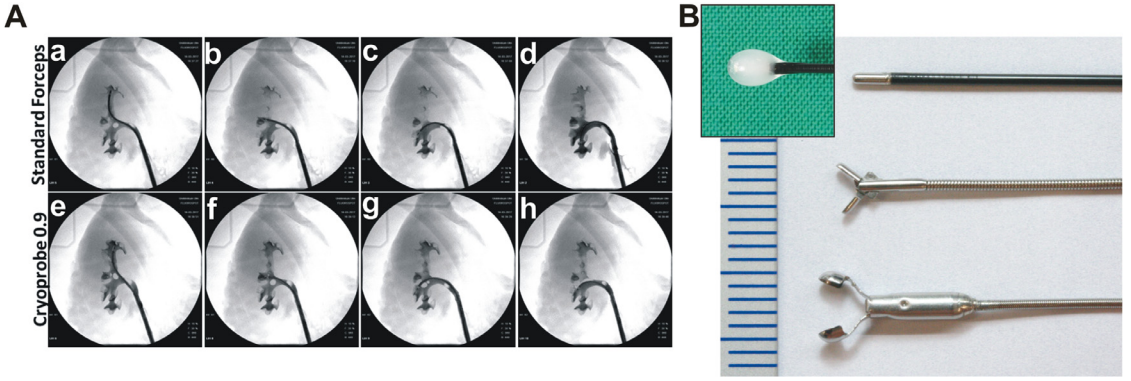

Figure 1. (A) Retrograde pyelograms in a standardized porcine UUT model, showing intrapelvic positions of the flexible digital URS (Storz 11278 VK/VUK (Flex-X_C) with different inserted devices (standard forceps **a-d**; cryoprobe **e-h**). (**B**) Overview of the new mini-cryoprobe with a tube diameter of 0.9 mm (**a**) and freezing activation (**b**), a standard forceps with a tube diameter of 0.9 mm (**c**), and the BIGopsy forceps with a tube diameter of 0.8 mm (**d**); (scaling in mm). (Color version available online.)

[frequently used; R. Wolf GmbH, Knittlingen, Germany]). To retrieve tissue samples, the different biopsy devices were individually inserted into the scope and introduced into the UUT. Up to 12 biopsies were taken from 3 different positions of the calices (upper, middle, and lower if possible) with each device. The order of instruments within the experiment and the site of biopsy per instrument were randomly assigned. The biopsies were directly processed for pathologic examination.

PATHOLOGIC EVALUATION

All biopsy specimens were fixed in 4.5% neutral buffered formalin, processed into paraffin blocks, and stained with Hematoxylin and Eosin for evaluation of total sample area (mm^2), percent of artifact-free tissue, and presence of crush artifacts. All samples were assessed by 1 reference pathologist (H.B.) using predefined histologic criteria, as previously described.[15,16] The pathologist was blinded to the biopsy method. Evaluation focused on sample size, general quality, the presence of crushing artifacts, and infiltration depth of the biopsy sample, using a standard microscope (Axio Vision LE REL 4.4; Carl Zeiss Microimaging, Göttingen, Germany). Specimens were declared representative if (1) the sample area was large enough for analysis, (2) characteristic tissue was present, (3) crushing artifacts were limited, and (4) the histologic assessability achieved a score of 2 or greater (Table 1).

STATISTICAL ANALYSES

All data were analyzed using GraphPad 6 (GraphPad Software Inc.). Continuous variables were presented as means and standard deviations or median. The chi-square test was used when comparing proportions. One-way analysis of variance or nonparametric testing (Kruskal-Wallis test with post hoc multiple comparison) was used where appropriate to evaluate differences between the groups. Pairwise comparison between groups was performed using Dunn's Test for nonparametric data. P values $< .05$ were considered significant.

RESULTS

All renal calices were reached by flexible URS with inserted forceps (Fig. 1A; a-d); however, the lower medial calyx was difficult to scope with the cryoprobe due to limited deflection (Fig. 1e-h). When the cryoprobe was bent before insertion into the scope, lower parts could be more easily reached. Irrigation flow rates were highest with BIGopsy (2.8 ± 0.1 mL) and significantly lower flow rates were found with the standard forceps (0.94 ± 0.06 mL) or cryoprobe (1.1 ± 0.1 mL) inserted (both $P < .001$), with no significant difference between the latter devices ($P = .443$; Fig. 2A). The degree of angular deflection varied between cryoprobe, BIGopsy, and standard forceps when the Flex-X_C scope was used (Fig. 2B). The cryoprobe (130.7° ± 1.2°) caused higher impairment of the deflection angle of the scope when compared with the BIGopsy (166.9° ± 1.1°; $P < .001$) and the standard forceps (161.4° ± 1.9°; $P < .001$). Angular deflection of the BIGopsy forceps was superior to the standard forceps ($P = .002$). The deflection angles of the cryoprobe inserted into the Wolf Boa Vision (158.0° ± 0.7°; $P = .029$), Olympus URF-V (172.7° ± 4.9°; $P = .016$), or PUSEN PU3022 (146.1° ± 1.1°; $P = .029$) were higher than the Flex-X_C.

Biopsy samples were obtained during each procedure. The mean specimen area obtained by the cryoprobe was 7.5 ± 2.5 mm^2, significantly larger compared with 4.6 ± 2.5 mm^2 for the BIGopsy forceps ($P < .001$) and 1.4 ± 1.4 mm^2 for the standard forceps ($P < .001$; Figs. 2C, 3A-C). There was also a significant difference between the BIGopsy and standard forceps ($P < .001$).

Histologic assessability mean score was 0.4 ± 0.9 for the standard forceps, significantly less than the 2.8 ± 0.8 for cryoprobe samples ($P = .0002$) and 2.3 ± 1.9 for BIGopsy ($P = .001$; Fig. 2D). There was no statistically significant difference between the scores of the cryoprobe and the BIGopsy ($P = .903$; Fig. 2D). Figure 3 illustrates representative and nonrepresentative (in case of forceps use) samples obtained with each instrument. No significant artifacts were observed in cryosamples, and according to further evaluation criteria (type of tissue, area, histologic assessability) all cryosamples were considered

Table 1. Histologic assessment score, defining the quality gradations of the specimens with regard to morphologic and histologic features (*according to scoring method previously described in references 14 and 15*). Scores 0 and 1 represent poor quality specimens, which are unsuitable for potential diagnosis. Scores 2-6 range from acceptable to excellent quality, allowing for "feasible" to "unrestricted" histologic evaluation. Scores 1, 3, and 5 are fine gradations between the denoted scores 0, 2, 4, and 6

Score	Quality Denotation
0	Due to very poor specimen quality, it is not possible to assess the relevant morphologic and histopathologic features
1	
2	Due to poor specimen quality, assessment of relevant morphologic and histopathologic structures and features is severely compromised but possible
3	
4	Despite moderate limitations in specimen quality, assessment of the relevant morphologic and histopathologic structures and features is possible
5	
6	The specimen allows for complete and unrestricted assessment of all relevant morphologic and histopathologic structures and features

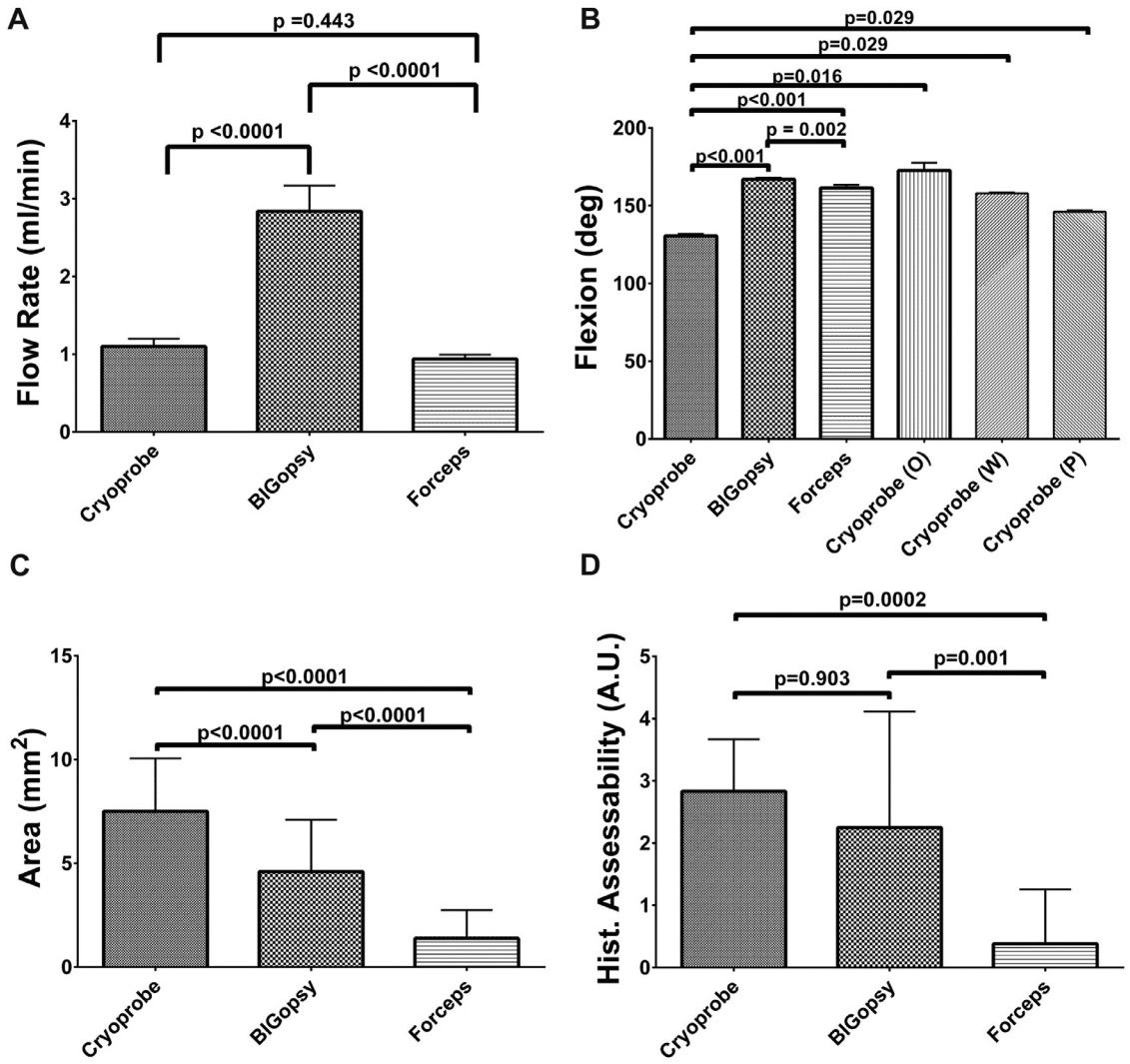

Figure 2. Irrigation flow rates (**A**) and active deflection angles (**B**) measured with inserted biopsy devices (cryoprobe, BIGopsy, and standard forceps) into the same scope (Storz). Measurements of active deflection with inserted cryoprobe were additionally performed in 3 different scopes with different degrees of wear and tear (Cryoprobe [O]: Olympus, Cryoprobe [W]: Wolf, and Cryoprobe [P]: PUSEN). Mean sample area (**C**) and histologic assessability scores (**D**) of the specimens obtained by the different biopsy devices. Specimens obtained by the cryoprobe were significantly larger and of higher quality compared with the forceps.

representative for complete histopathologic evaluation. A significantly lower proportion of representative samples was found in biopsies obtained by BIGopsy (50%, P = .014; Fig. 3B,E) and standard forceps (8%, P < .0001; Fig. 3C and F), while no statistically significant difference was detected between BIGopsy and standard forceps (P = .069).

DISCUSSION

Obtaining representative tissue samples from the UUT by URS biopsy represents a major challenge. Biopsy samples are often insufficient in size and tissue quality, precluding valid pathologic evaluation and limiting diagnostic accuracy with regard to stage and grade.[1,4-6,8,17-19] In a series of patients who underwent RNU, pathologic upstaging occurred in up to 45% of tumors preoperatively classified as stage pTa.[20] In particular, flat UUT lesions are difficult to biopsy.[6] This may lead to a failure to diagnose UUT, including carcinoma in situ.[6,8] Some of the limitations of most currently available biopsy devices are inherent to the small working channel size of URS; however, conventional biopsy forcipes additionally induce compression artifacts of samples. In a recent comparative study, Lama et al compared the results of UUT biopsies obtained by 3 different devices (BIGopsy, standard forceps, and a nitinol basket).[7] The specimen size and quality obtained by the basket and BIGopsy device were significantly better when compared with the standard forceps. However, the overall grade concordance of UTUC biopsy with surgical specimens was still limited to 77%.

To address this clinical need and to avoid the need for repeat biopsies, we developed and tested a novel biopsy method using cryoextraction of UUT urothelial tissue in a standardized preclinical setting. We have demonstrated

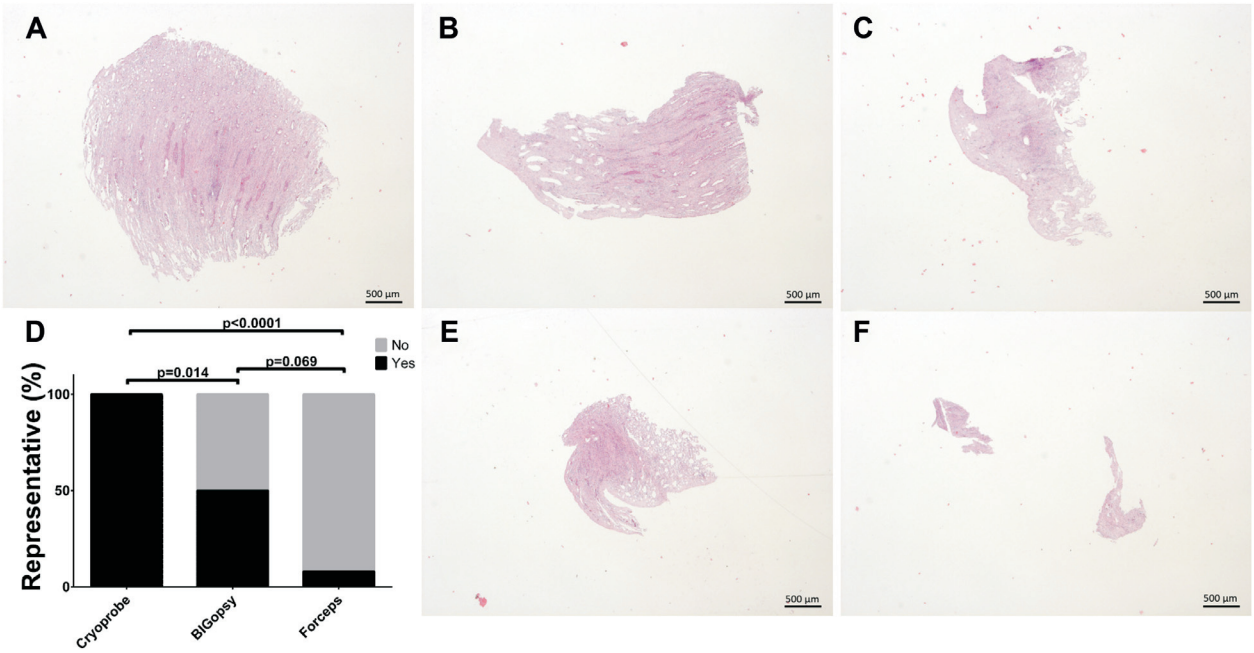

Figure 3. H&E-stained sections of representative biopsies obtained with a cryoprobe (**A**), a BIGopsy forceps (**B**), or with a standard forceps (**C**). While all specimens obtained with the cryoprobe were representative, only 50% of the BIGopsy and 8% of the specimens obtained by standard forceps were rated "representative." Biopsies taken with cryoprobes were significantly more representative (**D**) compared with the forceps biopsies. Nonrepresentative samples are illustrated for the BIGopsy (**E**) and standard forceps (**F**). (Color version available online.)

for the first time that cryobiopsy via URS is feasible and offers high potential for use in the UUT. **Histologic** evaluation revealed that significantly larger tissue samples were obtained by the cryoprobe, with all samples considered representative for adequate histopathologic evaluation without crushing artifacts. The device allows for frontloading, with inherent advantages for use in standard flexible URS procedures. Moreover, the method is suitable for obtaining tangential biopsies of suspicious flat lesions in the ureter.

Different biopsy forcipes are commercially available and have been systematically evaluated in terms of irrigation flow, deflection, and field of view.[2,3,7,21-23] The Cook BIGopsy best retained flow rates and deflection but needed backloading and restricted the field of view.[3] Another study found that the BIGopsy was more likely to obtain adequate tissue for diagnostic evaluation than other devices.[21] Kramer et al recently presented a newer generation biopsy forceps, "EF-120-00-3F," which is reusable and has a rotating tip activated when grasping tissue.[2] Functional properties and usability for histopathologic evaluation in humans showed improved tissue quality and better tumor staging. No significant tissue artifacts were observed; however, the ability to evaluate deep layer involvement and invasive growth was still limited to 80% and 75%, respectively.

Cryobiopsy offers the potential for improved staging and evaluation of tumor invasion depth. The depth of freezing can be controlled by freezing time and allows for acquisition of sufficient tissue in different layers and even from suspicious flat lesions.[12] The tissue architecture and different layers generally can be preserved during the freezing procedure with no compression artifacts. However, cryoartifacts may occur and the pathologist must know how to distinguish cryoartifacts from malignant lesions when evaluating the specimens. Casoni et al reported characteristic cryoartifacts in tissue samples obtained with cryoprobes from patients with fibrotic interstitial lung diseases, but the artifacts did not significantly interfere with pathologists' interpretations.[24] In agreement with previous studies, no significant tissue damage induced by the freezing process was observed in the present study.

Whereas flow rates with cryoprobe were comparable to conventional forceps, 1 potential limitation of the cryoprobe is a reduction of the deflection angle of the URS. Although the cryoprobe prototype was optimized toward more flexion than other cryoprobes available on the market, further optimization of the probe may be necessary to achieve deflection angles similar to standard equipment. Theoretically, further minimization of the outer diameter of the probe may reduce restrictions of URS handling when the device is inserted into the scope. However, since significant forces are applied to the probe during extraction, further miniaturization is challenging. Also, the lumen for gas flow decreases with smaller diameters, reducing the cooling capacity, and smaller probes would lead to smaller specimens.[12]

The strong dependency of deflection on the wear and tear of the URS device represents further challenges for clinical application. The specimens in our study were extracted with a frequently used flexible scope (Flex-X_C),

which was probably used more often than would be required for routine URS needs and therefore must be considered as the worst-case scenario. As hypothesized, higher deflection angles were achieved with scopes that were newer or had lower degrees of wear and tear. Although this was not systematically evaluated, there seem to be differences in the deflection depending on the system used (fiber optic vs chip on the tip) and the manufacturer. Multiple cryobiopsy samples were taken with the different scopes in the present study and we did not observe any damage or significant signs of wear and tear during the procedure. Since it cannot be stipulated what URS to use in conjunction with cryobiopsy, the use of a more flexible probe may be advantageous and should be considered for further investigation.

During the freezing process of the tissue on the tip of the cryoprobe, the chip or the optical fibers of the scope are at risk of damage, which can be avoided if the probe is activated only if the isolation covering of the probe is visible. Given the relatively large sample size of cryobiopsies, a retraction of the cryoprobe into the working channel of the URS would cause loss of the specimen, so retraction of the complete instrument together with the cryoprobe as 1 unit is necessary. To guarantee the adherence of the specimen to the tip of the cryoprobe, the probe is activated during the whole extraction process. An access sheath allows the surgeon to retract the specimen without damaging the mucosa of the ureter, and may facilitate tissue acquisition in some patients.[25,26]

Any new technology potentially results in additional costs. Several reports on the cost of cryobiopsy for lung diseases suggested that this procedure may be cost-effective compared with open or video-assisted surgical biopsy and would become self-financing within 6 months of introduction.[27-29] It is too early to speculate about the costs of cryobiopsy in the UUT at this stage of development. The costs of biopsies in the UUT depend on the devices (reusable vs single-use) and the technique used as well as on pricing policies.[30] However, the authors expect that cryobiopsy in the UUT will be cost-effective, given that re-interventions are frequently required after conventional biopsy because of diagnostic failure. Moreover, it is increasingly recommended to combine forceps biopsy with nitinol baskets for UUT biopsy[7], further increasing the costs of conventional biopsy.

Our study is limited by its ex vivo setting and use of porcine kidneys. The results can therefore not directly be translated into clinical practice. Potential bleeding complications induced by the cryoprobe will need to be assessed, potentially with ex vivo perfusion. The kidney model was not pathologic and therefore we were unable to evaluate a "diagnostic yield" of cryobiopsies. However, we focused on the quality of cryospecimens using standardized semi-quantitative scores that have been previously applied,[15,16] and porcine renal tissue provided well-defined **histologic** landmarks such as glomeruli, collection tubes, and renal vasculature, allowing us to assess level of preservation of structures. In summary, we focused on the feasibility of obtaining cryobiopsies from UUT tissue via URS, and aimed to provide preliminary proof-of principle data on this method.

CONCLUSION

To our knowledge, this is the first systematic report on cryobiopsy during ureterorenoscopy. Cryobiopsy in the UUT is feasible and represents a viable new option to improve the diagnostic accuracy of histopathologic evaluation. Larger and more representative tissue samples can be obtained by using a cryoprobe, while concurrently avoiding crush artifacts. Further optimization of the probe will reduce possible restrictions of URS handling when the device is inserted. Clinical trials should further shape the applicability of cryobiopsy techniques for UUT lesions.

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References

1. Roupret M, Babjuk M, Comperat E, et al. European association of urology guidelines on upper urinary tract urothelial carcinoma: 2017 update. *Eur Urol*. 2018 Jan;73:111–122.
2. Kramer MW, Abbas M, Kabbani M, et al. Superiority of the EF-120-00-3F biopsy forceps in the histopathological evaluation of upper urinary tract specimens. *World J Urol*. 2014;32:931–938.
3. Ritter M, Bolenz C, Bach T, Strobel P, Hacker A. Standardized ex vivo comparison of different upper urinary tract biopsy devices: impact on ureterorenoscopes and tissue quality. *World J Urol*. 2013;31:907–912.
4. Smith AK, Stephenson AJ, Lane BR, et al. Inadequacy of biopsy for diagnosis of upper tract urothelial carcinoma: implications for conservative management. *Urology*. 2011;78:82–86.
5. Raman JD. Kidney sparing surgery for upper-tract urothelial carcinoma. *Minerva Urol Nefrol*. 2016;68:359–371.
6. Redrow GP, Guo CC, Brausi MA, et al. Upper urinary tract carcinoma in situ: current knowledge, future direction. *J Urol*. 2017;197:287–295.
7. Lama DJ, Safiullah S, Patel RM, et al. Multi-institutional evaluation of upper urinary tract biopsy using backloaded cup biopsy forceps, a nitinol basket, and standard cup biopsy forceps. *Urology* 2018. http://dx.doi.org/10.1016/j.urology.2018.03.040. pii: S0090-4295(18)30297-8[Epub ahead of print].
8. Villa L, Haddad M, Capitanio U, et al. Which patients with upper tract urothelial carcinoma can be safely treated with flexible ureteroscopy with holmium:YAG laser photoablation? Long-term results from a high volume institution. *J Urol*. 2018;199:66–73.
9. Clements T, Messer JC, Terrell JD, et al. High-grade ureteroscopic biopsy is associated with advanced pathology of upper-tract urothelial carcinoma tumors at definitive surgical resection. *J Endourol*. 2012;26:398–402.
10. Brien JC, Shariat SF, Herman MP, et al. Preoperative hydronephrosis, ureteroscopic biopsy grade and urinary cytology can improve prediction of advanced upper tract urothelial carcinoma. *J Urol*. 2010;184:69–73.
11. A. Breda, A. Territo, A. Guttilla, et al., Correlation between confocal laser endomicroscopy (Cellvizio(R)) and histological grading of upper tract urothelial carcinoma: a step forward for a better selection of patients suitable for conservative management, Eur Urol Focus. 2017 Jun 4. pii: S2405-4569(17)30127-X. http://dx.doi.org/10.1016/j.euf.2017.05.008. [Epub ahead of print].

12. Franke KJ, Szyrach M, Nilius G, et al. Experimental study on biopsy sampling using new flexible cryoprobes: influence of activation time, probe size, tissue consistency, and contact pressure of the probe on the size of the biopsy specimen. *Lung*. 2009;187:253–259.
13. Hetzel J, Eberhardt R, Herth FJ, et al. Cryobiopsy increases the diagnostic yield of endobronchial biopsy: a multicentre trial. *Eur Respir J*. 2012;39:685–690.
14. Pourabdollah M, Shamaei M, Karimi S, Karimi M, Kiani A, Jabbari HR. Transbronchial lung biopsy: the pathologist's point of view. *Clin Respir J*. 2016;10:211–216.
15. Franke KJ, Linzenbold W, Nuessle D, et al. A new tool for transbronchial cryobiopsies in the lung: an experimental feasibility ex vivo study. *Respiration*. 2016;91:228–234.
16. Yarmus LB, Semaan RW, Arias SA, et al. A Randomized controlled trial of a novel sheath cryoprobe for bronchoscopic lung biopsy in a porcine model. *Chest*. 2016;150:329–336.
17. Williams SK, Denton KJ, Minervini A, et al. Correlation of upper-tract cytology, retrograde pyelography, ureteroscopic appearance, and ureteroscopic biopsy with histologic examination of upper-tract transitional cell carcinoma. *J Endourol*. 2008;22:71–76.
18. Tavora F, Fajardo DA, Lee TK, et al. Small endoscopic biopsies of the ureter and renal pelvis: pathologic pitfalls. *Am J Surg Pathol*. 2009;33:1540–1546.
19. Jeon SS, Sung HH, Jeon HG, et al. Endoscopic management of upper tract urothelial carcinoma: improved prediction of invasive cancer using a ureteroscopic scoring model. *Surg Oncol*. 2017;26:252–256.
20. Guarnizo E, Pavlovich CP, Seiba M, Carlson DL, Vaughan ED, Sosa RE. Ureteroscopic biopsy of upper tract urothelial carcinoma: improved diagnostic accuracy and histopathological considerations using a multi-biopsy approach. *J Urol*. 2000;163:52–55.
21. Al-Qahtani SM, Legraverend D, Gil-Diez de Medina S, Sibony M, Traxer O. Can we improve the biopsy quality of upper urinary tract urothelial tumors? Single-center preliminary results of a new biopsy forceps. *Urol Int*. 2014;93:34–37.
22. Carrafiello G, Fontana F, Mangini M, et al. Upper urinary tract biopsy: an old device for a new approach. *Radiol Med*. 2012;117:1152–1160.
23. Kleinmann N, Healy KA, Hubosky SG, Margel D, Bibbo M, Bagley DH. Ureteroscopic biopsy of upper tract urothelial carcinoma: comparison of basket and forceps. *J Endourol*. 2013;27:1450–1454.
24. Casoni GL, Tomassetti S, Cavazza A, et al. Transbronchial lung cryobiopsy in the diagnosis of fibrotic interstitial lung diseases. *PLoS One*. 2014;9:e86716.
25. Gorin MA, Santos Cortes JA, Kyle CC, Carey RI, Bird VG. Initial clinical experience with use of ureteral access sheaths in the diagnosis and treatment of upper tract urothelial carcinoma. *Urology*. 2011;78:523–527.
26. Breda A, Territo A, Lopez-Martinez JM. Benefits and risks of ureteral access sheaths for retrograde renal access. *Curr Opin Urol*. 2016;26:70–75.
27. Hernandez-Gonzalez F, Lucena CM, Ramirez J, et al. Cryobiopsy in the diagnosis of diffuse interstitial lung disease: yield and cost-effectiveness analysis. *Arch Bronconeumol*. 2015;51:261–267.
28. Sharp C, McCabe M, Adamali H, Medford AR. Use of transbronchial cryobiopsy in the diagnosis of interstitial lung disease-a systematic review and cost analysis. *QJM*. 2017;110:207–214.
29. Medford AR. Theoretical cost benefits of cryobiopsy. *J Thorac Cardiovasc Surg*. 2010;140:487–488. author reply 488.
30. Hennessey DB, Fojecki GL, Papa NP, Lawrentschuk N, Bolton D. Single-use disposable digital flexible ureteroscopes: an ex vivo assessment and cost analysis. *BJU Int*. 2018;121(Suppl 3):55–61.