ORIGINAL PAPER

UROLOGICAL ONCOLOGY

In vivo endoscopic cryobiopsy of urothelial tumors in the upper urinary tract and bladder: A feasibility pilot study in humans

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Citation: Łaszkiewicz J, Nowak Ł, Tomczak W, et al. *In vivo* endoscopic cryobiopsy of urothelial tumors in the upper urinary tract and bladder: A feasibility pilot study in humans. Cent European J Urol. 2025; doi: 10.5173/ceju.2025.0132

Article history
Submitted: Jun. 8, 2025
Accepted: Jun. 10, 2025
Published online: Aug. 24, 2025

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Introduction Biopsy of the upper tract urothelial carcinoma (UTUC) often provides low-quality or non-diagnostic material. Cryobiopsy may improve the quality of UTUC samples. Our aim was to assess the feasibility of *in vivo* endoscopic cryobiopsy of UTUC and bladder cancer (BC).

Material and methods Cryobiopsies were performed using the ERBECRYO® device and ø 1.1 mm flexible cryoprobes. Adult patients with UTUC/BC undergoing diagnostic/therapeutic endoscopic procedures were included. The cryoprobes were introduced in the proximity of the tumors and activated. The tissue samples were avulsed from the tumor, extracted and placed in a fixative.

Results Six males were included. Out of these, 4 had UTUC, while 2 had BC. The median age was 68 years. Transurethral procedures were performed in 4 patients, percutaneous in 1 and combined in 1. Cryobiopsies were conducted using cystoscopes (n = 2), rigid ureterorenoscopes (n = 2), nephroscopes (n = 1) and without endoscope, through the ureteral access sheath (n = 1). Mean obtained sample size was 6.2 × 4.7 × 3.0 mm. All the specimens allowed for a histopathologic evaluation; no crushing artifacts were reported, lamina propria was present in 4 specimens, and muscularis propria was present in 1. Bleeding from the sample bed was subjectively significantly less intense than after conventional biopsies, and the procedure was found to be less challenging than standard methods.

Conclusions Cryobiopsy represents a promising advancement in the endoscopic diagnosis of UTUC. Our pilot study demonstrates its feasibility in human *in vivo* settings. Further comparative research is warranted to establish its role in routine practice.

Key Words: cryobiopsy ‹› upper tract urothelial carcinoma ‹› UTUC ‹› bladder cancer ‹› urothelial carcinoma ‹› transitional cell cancer

INTRODUCTION

Upper tract urothelial carcinoma (UTUC) is a rare malignancy that accounts for 5–10% of all urothelial cancers. The EAU guidelines on UTUC recently introduced a new risk stratification that includes low-, weak high- and high-risk categories [1]. Hence, an endoscopic biopsy of the lesion is necessary in many patients to qualify them for the kidney sparing approach. A variety of instruments can be used to sample the lesion; however, the obtained material is often small, superficial, damaged or otherwise not representative. Adequate histopathological examination is often impossible, and it has been proven that endoscopic biopsies are associated with a significant risk of staging and grading errors [2]. That is why, the search for the optimal endoscopic biopsy method continues.

Cryobiopsy involves inserting a dedicated cryoprobe via endoscope to get it in contact with the lesion. After activation, an ice ball is formed at the cryoprobe's tip and the frozen tissue adheres to it. Pulling the probe causes the ice ball with frozen specimen to detach from the base at the phase boundary. This method is already widely used in pulmonology for transbronchial lung biopsies, because it provides large, high-quality tissue samples.

The first attempts at cryobiopsy of urothelial tumors were conducted by Klein et al., who performed *ex vivo* and porcine studies comparing flexible cryoprobes and a variety of standard endoscopic biopsy tools. Their findings demonstrated that cryobiopsy yielded larger, superior samples with fewer artifacts when compared with standard biopsy [3, 4]. Encouraged by these results, we decided to implement this technique in a clinical setting.

The aim of this human *in vivo* pilot study was to assess the feasibility of endoscopic cryobiopsy of urothelial tumors in the upper urinary tract and the bladder.

MATERIAL AND METHODS

Cryobiopsies were performed using the ERBECRYO® device and ø 1.1 mm (3.3 Fr) flexible cryoprobes (Erbe Elektromedizin GmbH, Tübingen, Germany). The device utilizes a standard CO_2 pressure cylinder and the freezing process is controlled by a dedicated foot pedal.

Patients ≥ 18 years old with UTUC (potentially low- or weak high-risk) or bladder cancer (BC) undergoing diagnostic/therapeutic endoscopic procedures (ureterorenoscopy [URS], endoscopic combined intrarenal surgery [ECIRS] or transurethral resection of the bladder tumor [TURBT]) were included in this study. The procedures were performed by a single urologist with vast experience in endoscopic procedures.

Patients were admitted with a current, reliable urine culture and received antibiotic prophylaxis accordingly. Antiplatelet and anticoagulation medications were ceased or modified according to the guidelines.

Patients were operated under spinal anesthesia.

Upper tract urinary cytology was collected in the whole cohort with UTUC.

Cryobiopsy technique used in the present study was inspired by the *ex vivo* research by Klein et al. [4]. Procedures were performed after direct visualization of the tumor using cooled 0.9% saline irrigant. The cryoprobe was introduced in the direct proximity of the tumor in such a way that the metal part of the probe was completely covered by the papillae/tumor exophytic parts. The cryoprobe was activated for approximately 10 seconds, without moving it. Then, the probe with the ice ball (including tissue sample) was avulsed from the tumor and extracted from the urinary tract by withdrawing the entire endoscope. The avulsion and extraction were conducted with the cryoprobe being intermittently activated, to avoid the tissue detachment. Finally, the specimens were visually inspected and gently placed in a fixative (10% formalin solution).

Patients with BC underwent classic TURBT or laser ablation, and those with UTUC underwent laser ablation of the tumor.

A standard histopathologic evaluation was performed in all cases. What is more, the size of the specimens was recorded before fixation in formalin.

Bioethical standards

The study was approved by the Institutional Bioethics Committee of the Wrocław Medical University (KB 28/2025). Informed consent was obtained from all the patients prior to the procedures.

RESULTS

Study cohort

In total, 6 patients who underwent cryobiopsies were included in this study. Out of these, 4 patients had tumors located in the upper urinary tract, while 2 had tumors in the bladder.

All the included patients were male. The median age was 68 years, and the most patients had a history of urothelial carcinoma (n = 5, 83%). Transurethral procedures were performed in 4 patients, percutaneous in 1 and combined in 1. Cryobiopsies were conducted using cystoscopes (n = 2), rigid URS (n = 2), nephroscopes (n = 1) and without endoscope, through the ureteral access sheath (n = 1). Mean obtained sample size was 6.2 × 4.7 × 3.0 mm. All the specimens allowed for a histopathologic evaluation; no crushing artifacts were reported, lamina propria was present in 4 specimens (67%), and muscularis propria was present in 1 (17%) (Table 1). What is more, the bleeding from the sample bed was subjectively significantly less intense than after conventional forceps/basket biopsies, and the procedure was found to be less challenging than standard methods.

Bladder tumors

A 35-year-old man with a 4th recurrence of BC (detected in a control cystoscopy) was qualified for

TURBT. The previous tumors were all assessed as pTa LG G2. A small tumor on the posterior wall of the bladder was confirmed during cystoscopy at the beginning of the procedure. A cryobiopsy of the lesion was performed using the flexible 16 Fr cystoscope (Figure 1A). After tissue sampling, the tumor was ablated using holmium laser. The size of the material from the cryobiopsy was 8 × 5 × 3 mm, and the final report assessed it as pTx LG G2. No lamina propria, nor muscularis propria were present.

The next patient was a 68-year-old actively smoking male with an extensive history of urothelial carcinoma of the bladder and upper urinary tract.

Table 1. *Baseline patient, tumor, procedure and specimen characteristics*

Patient	Gender	Age	Prior UC	Tumor location	Access	Endoscope used for cryobiopsy	Sample size (mm)	Pathological stage and grade	Lamina propria present	Muscularis propria present
All	Male: 6 (100%)	Median (IQR): 68 (67.25–68)	Yes: 5 (83%) No: 1 (17%)	Bladder: 2 Ureter: 3 Renal pelvis: 1	Transurethral: 4 Percutaneous: 1 Combined: 1	Cystoscope: 2 Rigid URS: 2 Nephroscope: 1 None: 1	Mean: 6.2 × 4.7 × 3.0	NA	Yes: 4 (67%)	Yes: 1 (17%)
No. 1	Male	35	Yes	Bladder	Transurethral	Cystoscope	8 × 5 × 3	Tx LG G2	No	No
No. 2	Male	68	Yes	Bladder	Transurethral	Cystoscope	5 × 3 × 3	Tx HG G3	No	No
No. 3	Male	73	Yes	Renal pelvis	Combined	Nephroscope	8 × 6 × 3	Ta HG G3	Yes	No
No. 4	Male	68	Yes	Ureter	Transurethral	Rigid URS	6 × 6 × 3	Ta LG G2	Yes	No
No. 5	Male	68	No	Ureter	Transurethral	Rigid URS	5 × 5 × 4	Ta LG G2	Yes	No
No. 6	Male	67	Yes	Ureter	Percutaneous	None	5 × 3 × 2	Ta LG G2	Yes	Yes

IQR – interquartile range; HG – high-grade; LG – low-grade; UC – urothelial carcinoma; URS – ureterorenoscope

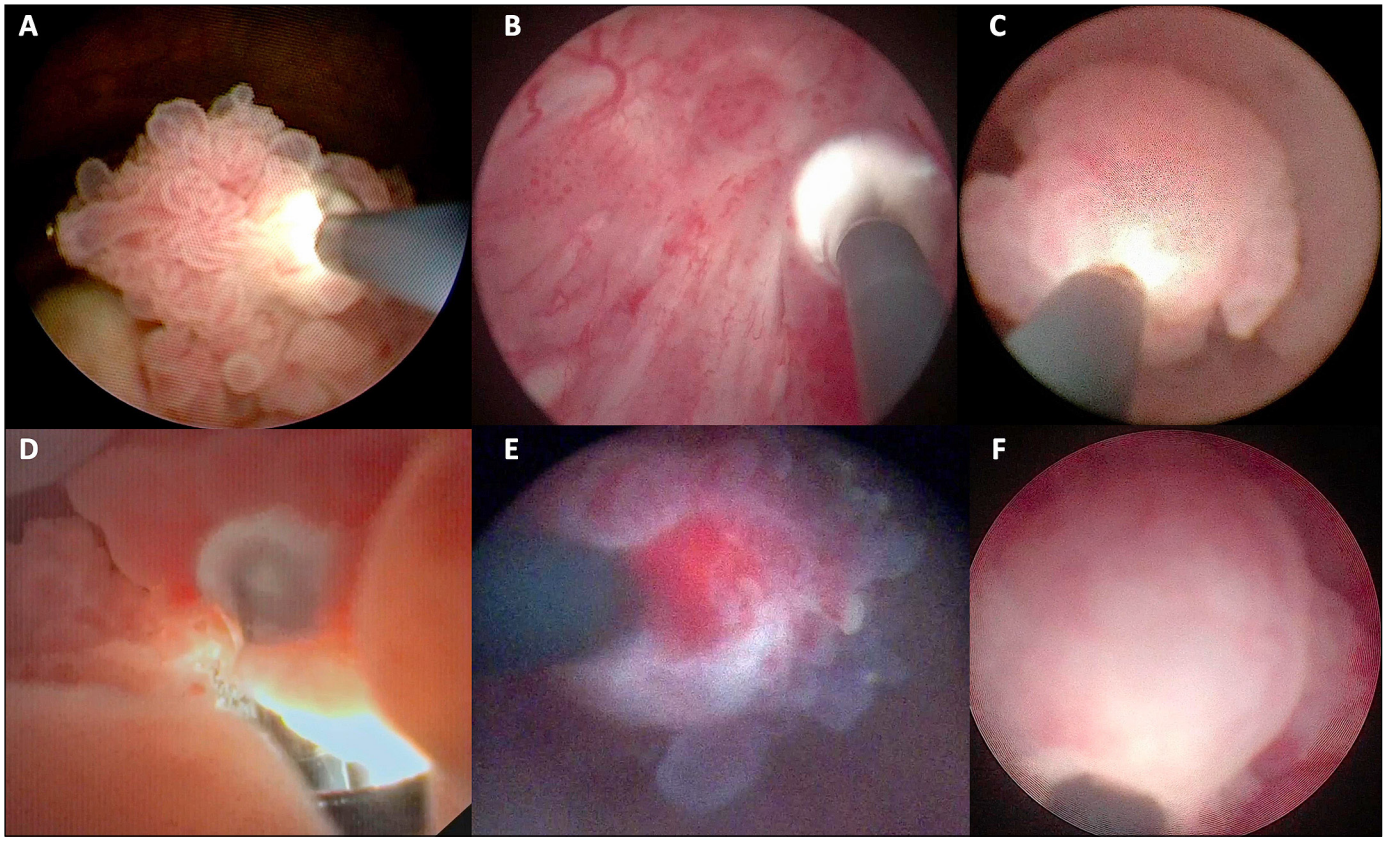

Figure 1. *Endoscopic views of cryobiopsies:* **A, B)** *cystoscopy;* **C)** *percutaneous nephroscopy;* **D)** *flexible ureterorenoscopy visualising percutaneous nephroscopy;* **E, F)** *rigid ureterorenoscopy.*

Over the course of 20 years, he has undergone over 30 TURBT, BCG therapy, left-sided radical nephroureterectomy (RNU) and 2 laser ablations of tumors in the right renal pelvis. Previous examinations revealed extensive cancerous lesions in the bladder (pTa HG G3 and pTis) and the upper urinary tract (pTa HG G3). Despite very high-risk BC and high-risk UTUC, the patient has refused the radical treatment. During cystoscopy, extensive, carpet-like lesions were observed. A cryobiopsy was taken using the rigid 17 Fr cystoscope (Figure 1B). Then, a maximal resection of the lesions was conducted. Finally, a flexible URS revealed neoplasms covering a major part of the left renal pelvis, which could be ablated only partially. The size of the cryobiopsy from the bladder was 5 × 3 × 3 mm and assessed as pTx HG G3. The urinary cytology from the renal pelvis was high-grade (Paris V). No lamina propria, nor muscularis propria were present.

Upper urinary tract tumors

The patient was a 73-year-old male who had already undergone 7 TURBTs (highest stage and grade: pT1 HG G3), BCG therapy and right RNU. After CT diagnosis of voluminous UTUC in the left kidney, retrograde intrarenal surgery with biopsy was attempted, but because of the narrow ureter, only biopsy and limited ablation were performed. A 6 Fr double-J stent was placed, and the patient was planned for the next stage. The histopathologic report described a low-volume, diagnostically inadequate material, which was assessed as "probably LG." The urinary cytology was negative. Patient was informed about the available treatment options and strongly opted for conservative approach. Therefore, ECIRS was performed using the 7,5 Fr flexible ureterorenoscope (URS) and 12 Fr nephroscope in a 15/16 Fr Amplatz sheath. The tumor was directly visualized through both accesses. Cryobiopsy was conducted through the percutaneous tract using the nephroscope. The process was also visualized by ureterorenoscope (Figure 1C, D). After the biopsy, the tumor was ablated simultaneously with two lasers through both endoscopes. The size of the obtained specimen was 8 × 6 × 3 mm. The sample was evaluated as pTa HG G2, which was additionally confirmed by selective urinary cytology – high-grade urothelial carcinoma (Paris V). The lamina propria was present in the specimen, but we did not obtain the muscularis propria. The patient was assigned into the high-risk category (according to the EAU guidelines) and qualified for RNU, which he once again refused. The follow-up endoscopy was scheduled after 6 weeks.

The next patient was a 68-year-old man with primary tumors in the right ureter. The patient had a history of recurrent BC (pTa HG G2), managed with TURBT, as well as of contralateral UTUC (pT2 HG G2), which was managed with RNU. The current follow-up MRI showed a 14 × 13 × 11 mm mass in the uretero-pelvic junction, as well as small, suspected lesions in along the ureter. During endoscopy, a widely open ureteral orifice with tumors emerging into the bladder were observed. A cryobiopsy of the lesion located in the distal part of the ureter was performed using a rigid 8 Fr URS (Figure 1E). Complete ureteroscopy was impossible, due to the tumors obstructing the lumen of the ureter. The ascending ureteropyelography has shown multiple tumors in the ureter, which could not be managed endoscopically (Figure 2). The cryobiopsy specimen was 6 × 6 × 3 mm and assessed as pTa LG G2.

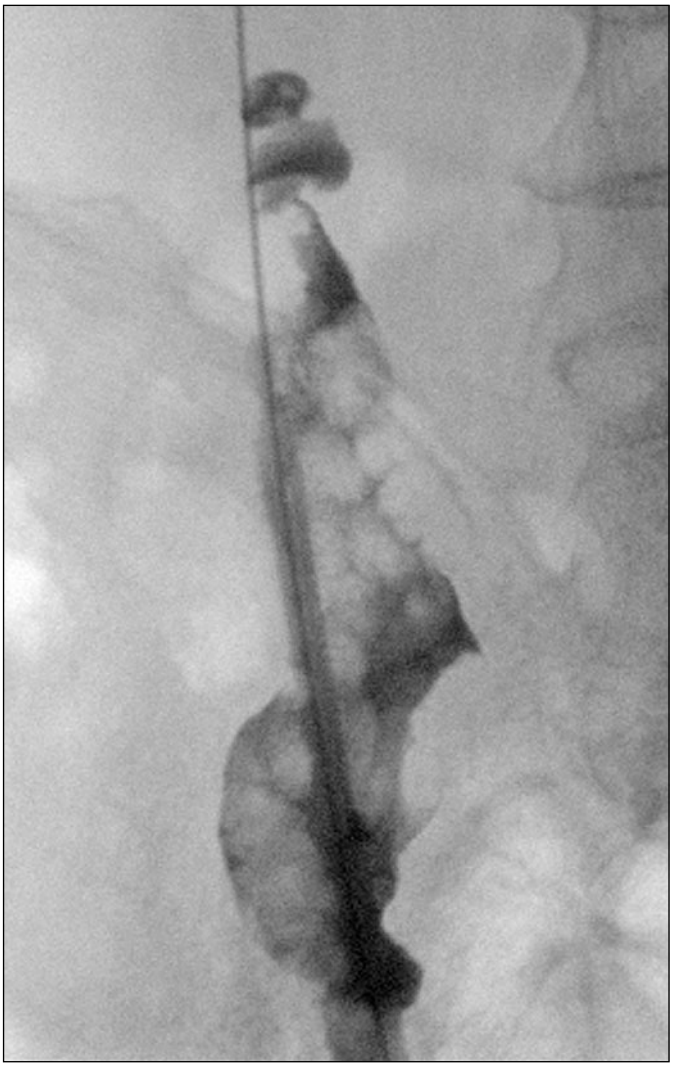

Figure 2. *Ascending ureteropyelography showing multiple tumors in the right ureter.*

The lamina propria was present in the specimen, but we did not obtain the muscularis propria. The urinary cytology was high-grade (Paris V). Due to voluminous disease, the patient was deemed eligible for RNU and radical cystectomy.

Next cryobiopsy was performed in a 68-year-old man, who presented with hematuria. In CT urography, a single lesion in the left ureter was visualized. BC was ruled out by office cystoscopy. Endoscopy confirmed the presence of a single tumor in the mid-ureter and the cryobiopsy was taken using the rigid 6 Fr URS (Figure 1F). Then, a complete ablation of the lesion was conducted. The tissue sample was 5 × 4 × 5 mm and evaluated as pTa LG G2 (Figure 3). The lamina propria was present in the specimen, but we did not obtain the muscularis propria. The urinary cytology was suspicious for high-grade urothelial carcinoma (Paris IV). The patient was therefore scheduled for a follow-up URS 3 months later.

Finally, we included a 67-year-old male with a primary UTUC in the left ureter that was discovered during an endoscopic lithotripsy of a massive stone in the distal ureter. In the past, the patient underwent 10 TURBTs (highest stage and grade: pTa LG G2) and BCG therapy for BC, as well as radiotherapy for prostate cancer. At previous URS, a biopsy was taken, which had suboptimal quality, but was evaluated as pTa LG G2. Because of multiple previous procedures that resulted in the contracted bladder and stricture of the left ureteral orifice, the patient underwent bilateral percutaneous nephrostomy. During this procedure, by using the previous nephrostomy tract on the left side, a percutaneous flexible URS was performed using a 6.3 Fr instrument. The multilocular papillary tumor was visualized in the intramural ureter. Distally from the tumor, a complete stricture of the ureter was observed. A 9,5 Fr ureteral access sheath (UAS) was introduced antegradely. The working channel of 6.3 Fr flexible URS was too narrow to allow a passage of the 3.3 Fr cryoprobe. Hence, the distal end of the UAS was placed just proximal to the lesion under visual control. Then, the UAS was fixed in place, scope was removed and the cryoprobe was inserted under fluoroscopy control. The tip of the cryoprobe was advanced until it touched the tumor, which was confirmed by tactile feedback and fluoroscopy (Figure 4A, B). Cryobiopsy was taken and its site was confirmed in the URS. Finally, the tumor was ablated. The size of the specimen was 5 × 3 × 2 mm and contained a significant amount of muscularis propria, which allowed accurate staging (Figure 4C). The lesion was assessed as pTa LG G2 (Figure 4D). The urinary cytology was suspicious for high-grade urothelial carcinoma (Paris IV). The patient was scheduled for a follow-up procedure after 3 months.

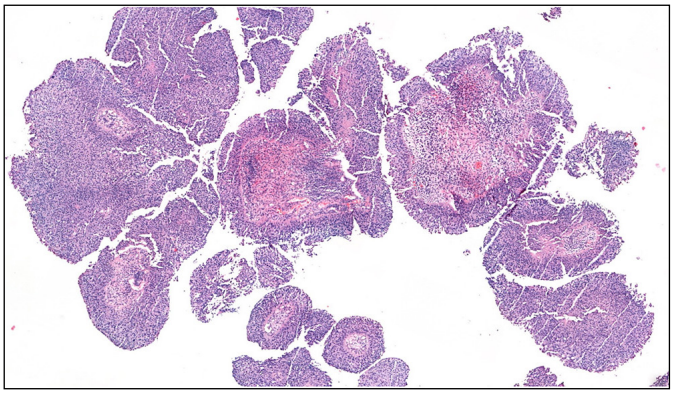

Figure 3. *Histopathological slide of the cryobiopsy specimen showing pTa LG G2 urothelial carcinoma.*

DISCUSSION

Traditional endoscopic biopsy techniques often yield small, superficial, crushed and fragmented specimens that may not provide adequate information for accurate staging and grading of UTUC. For instance, prior research indicated that 16.5% of biopsies obtained for endoscopic UTUC treatment were non-diagnostic [5]. Cryobiopsy overcomes these limitations by extracting tissue samples through a freezing process that minimizes mechanical compression and prevents crushing artifacts and fragmentation [3].

In this prospective non-comparative pilot feasibility study, we described the first human *in vivo* evaluation of cryobiopsy for the diagnosis of urothelial tumors.

The present research has been inspired by two articles by Klein et al. [3, 4]. The authors conducted studies on porcine and human *ex vivo* models that compared upper urinary tract cryobiopsy samples to those obtained through standard ureterorenoscopic biopsy techniques. Their findings indicated that cryobiopsy provided larger tissue samples with better preservation of histological architecture. Moreover, Klein papers described an excellent cryobiopsy technique that was also used in our study [3, 4].

Results of our study show that cryobiopsy is a feasible technique that allows for the extraction of relatively large, high-quality tissue samples suitable for histopathological evaluation. Additionally, what is of utmost importance, when performed properly, cryobiopsy allows for representative muscle layer sampling.

Even though there is no data on cryobiopsy of urothelial tumors in humans, there is an abundance

of studies on the utilisation of this method in pulmonology. For example, a meta-analysis by Giri et al. compared transbronchial cryobiopsy and forceps biopsy in interstitial lung disease, lung tumors and peripheral pulmonary lesions. They found that cryobiopsy significantly increased diagnostic accuracy and obtained larger specimens [6]. Also, the CHEST guidelines stated that transbronchial cryobiopsy's contribution to the diagnosis of interstitial lung disease can be similar to surgical biopsy [7]. Finally, a randomised controlled trial has shown that transbronchial cryobiopsy was more accurate than needle-aspiration biopsy in mediastinal nodal staging [8]. These applications support the notion that cryobiopsy could be a valuable tool in urology for improving the accuracy of endoscopic diagnosis of UTUC.

In our initial experience, cryobiopsy did not cause any complications, including major bleeding. As this is the first study on cryobiopsy in the human urinary tract, there are no other studies available for direct comparison regarding the procedure's safety. Studies on transbronchial cryobiopsy suggest that it may be associated with a higher risk of haem-

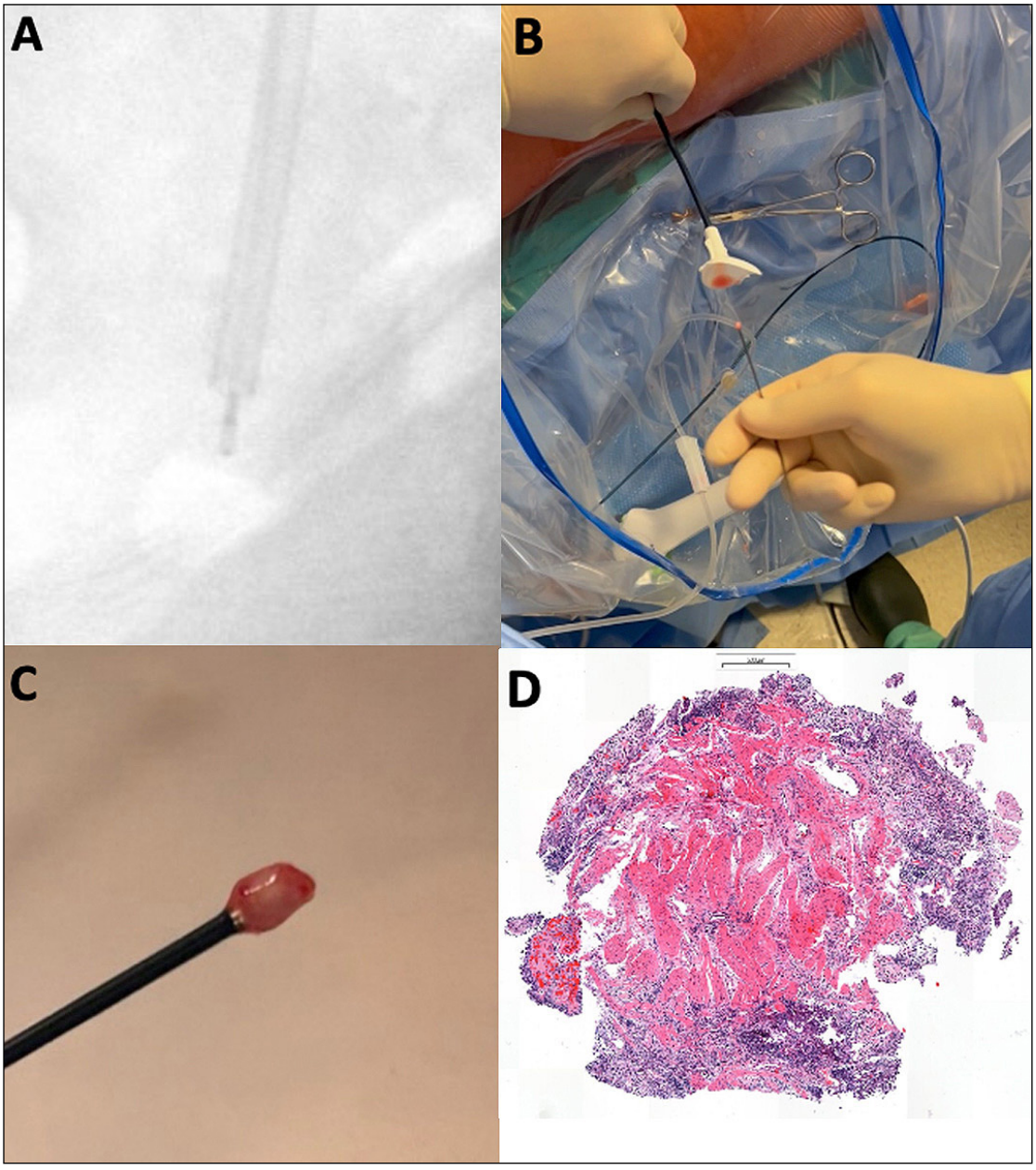

Figure 4. *Percutaneous cryobiopsy under fluoroscopic guidance:* **A)** *fluoroscopic view;* **B)** *surgeon's view;* **C)** *obtained tissue sample;* **D)** *histopathological slide showing abundant muscularis propria.*

orrhage compared to forceps biopsy [6]. However, clinically significant bleedings were rare and the procedure was generally described as safe [9–11]. Nevertheless, the anatomy and vasculature of the urinary tract and lung parenchyma differ significantly. Therefore, the safety profile of cryobiopsy in pulmonology cannot be directly extrapolated to urology. Finally, during endoscopy of the upper urinary tract, proper coagulation with a laser is usually possible when needed.

While our pilot study supports the feasibility of cryobiopsy in urothelial tumors, several technical considerations must be addressed before widespread clinical implementation. First, we were unable to perform a cryobiopsy using the standard flexible URS available on the European market. This was due to the large diameter of the probe (3.3 Fr), which does not fit into the standard working channels (3.6 Fr) of the commercially available flexible endoscopes. It has to be highlighted that the new generation of scopes with wider channels designed for suction purposes were not available in Poland when the study was performed. Moreover, the probe's size significantly limited the flow of irrigation fluid in rigid endoscopes. Importantly, Klein et al. successfully used a prototype 2.7 Fr (0.9 mm) cryoprobe, which may help resolve these issues, yet, the probe is currently not available commercially [4]. Another technical difficulty might be a significantly reduced manoeuvrability of the flexible URS when a cryoprobe is inserted. Klein et al. noted a substantial decrease in active deflection angles when using cryoprobes, which could impact the ability to access tumors in certain anatomical locations [3]. However, further optimisation of cryoprobe design may mitigate this issue.

The extraction of the specimens after cryobiopsy poses a challenge as well. The cryoprobe requires continuous or intermittent activation during sample retrieval to prevent tissue detachment, even if the flow of irrigation fluid is turned off or if cooled irrigation is used. This theoretically raises the issue of the thermal damage risk to the ureter or urethral wall when manoeuvring the probe in their vicinity. Finally, in the first cases, we did not obtain samples containing the muscular layer. This was clearly because of the fact that the probe was positioned in the exophytic, papillary part of the tumor. However, in the last case the probe was placed at the bottom of the tumor. The detaching was much more challenging, yet, the tissue sample contained abundant volume of representative muscle.

This pilot study has other limitations that need to be disclosed. This is a non-comparative study, which included a very small and heterogeneous cohort with no statistical power.

CONCLUSIONS

Cryobiopsy represents a promising advancement in the endoscopic diagnosis of UTUC. Our pilot study demonstrates its feasibility in human *in vivo* settings, confirming the findings of prior preclinical research. By providing large, high-quality tissue specimens with minimal artifacts, cryobiopsy has the potential to improve the clinical decision-making in UTUC. Further research is warranted to compare this technique with the traditional biopsy methods and establish its role in routine urological practice.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

FUNDING

This research received no external funding.

ETHICS APPROVAL STATEMENT

The study was approved by the Institutional Bioethics Committee of the Wroclaw Medical University (KB 28/2025).

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