

A Urine-Based DNA Methylation Test to Detect Upper Tract Urothelial Carcinoma: Results from a Prospective Pilot Study

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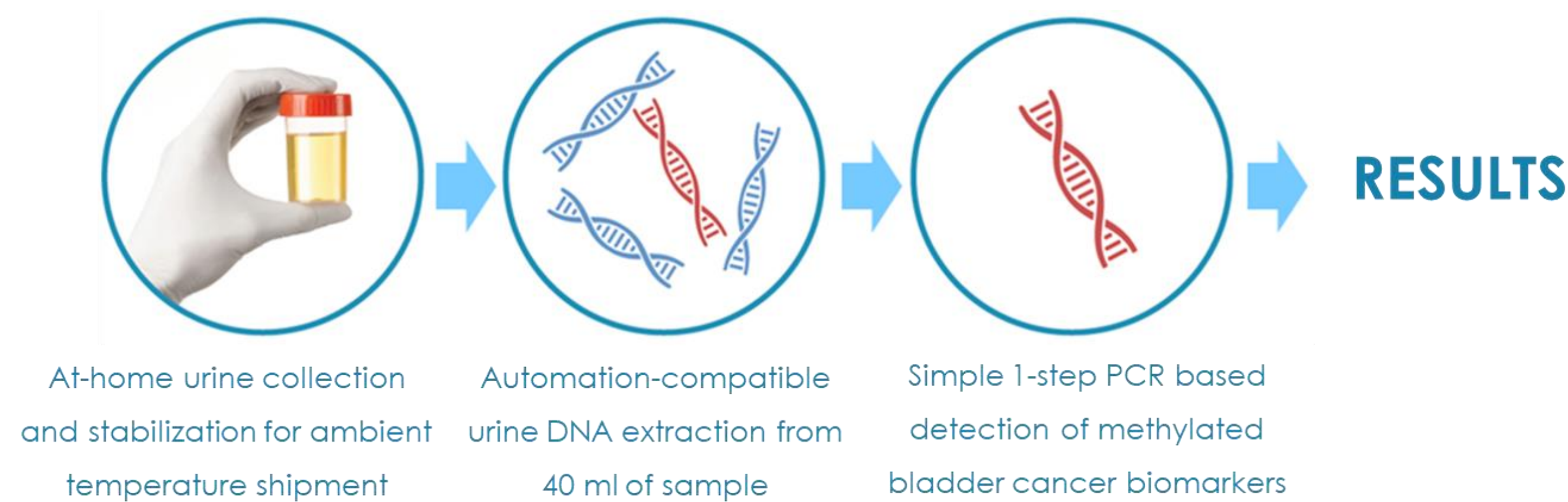
INTRODUCTION

- Upper Tract Urothelial Carcinoma (UTUC) is an uncommon, yet lethal malignancy of the urinary tract.
- Diagnosis and preoperative risk stratification of UTUC patients present distinct challenges given the limitations of currently available tools such as urine cytology, endoscopic biopsy, and cross-sectional imaging.
- Decision-making and prognosis of UTUC relies heavily on TNM stage and pathological grade, which are not accurately available until RNU is performed.

METHODS

- Type of study:** Prospective cohort.
- Study group:** Urine samples collected from UTUC patients prior to RNU, DistalU, or URS/biopsy between December 2019 and March 2022.
- Exclusion criteria:**
 - Patients with bladder cancer/positive TURBT in the past 3 months.
 - Samples with insufficient DNA concentration (n=5).
- Control group:** 1:1 sex/age-matched cancer-free healthy individuals.
- Diagnostic methods:**
 - Urine Cytology**
 - Bladder CARE™ test:** Bladder CARE™ is a urine-based qPCR cancer diagnostic test which detects the methylation level of urothelial-specific cancer biomarkers and internal control loci.

Figure 1: Bladder CARE™ test workflow



Bladder CARE™ results are represented as Bladder CARE™ Index (BCI) which is proportional to the abundance of cancer DNA in the urine sample. Patients with a BCI < 2.5 are negative for the presence of urothelial cancer, while patients with a BCI > 2.5 and > 5 are classified high-risk and positive, respectively. Both, high-risk and positive results are considered abnormal, and follow-up is recommended.

RESULTS

- A total of 50 UTUC patients and 50 healthy donors were included in the final analysis.
- Median age: 72 (64-79) years.
- Procedures: 40 RNU, 7 ureterectomy, and 3 URS. All patients who underwent URS were confirmed to have UTUC through biopsy.
- Urine cytology was available in 35 patients of which 22 were false negative.

Table 1: Pathologic information of the UTUC patients

Variables	Value
Tumor Laterality, n (%)	
Right	27 (54)
Left	23 (46)
Tumor Location, n (%)	
Pelvis	28 (56)
Ureter	15 (30)
Pelvis and Ureter	7 (14)
Tumor Size, median, cm	3 (1.5-4.8)
Tumor Histology, n (%)	
Pure Urothelial Carcinoma	43 (86)
UC with Variant Histology	7 (14)
Lymphovascular Invasion, n (%)	4 (8)
T Stage, n (%)	
Ta	22 (44)
T1	7 (14)
T2	7 (14)
T3	11 (22)
T4	3 (6)
Node +, n (%)	4 (8)
Grade, n (%)	
Low	9 (18)
High	41 (82)

Figure 2: UTUC patients have significantly higher BCI values compared to the controls (189.3 vs. 1.6; p < 0.001)

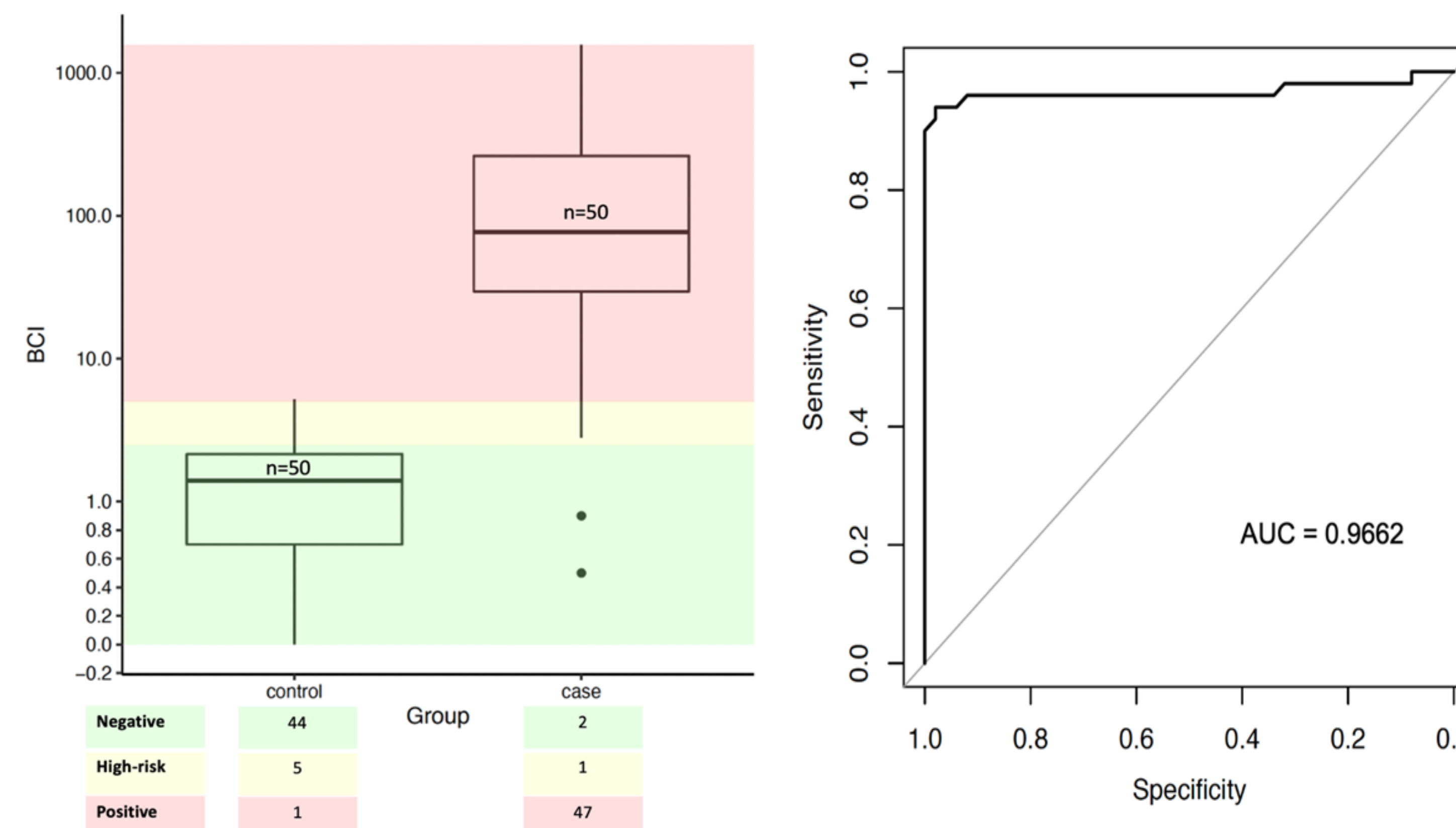


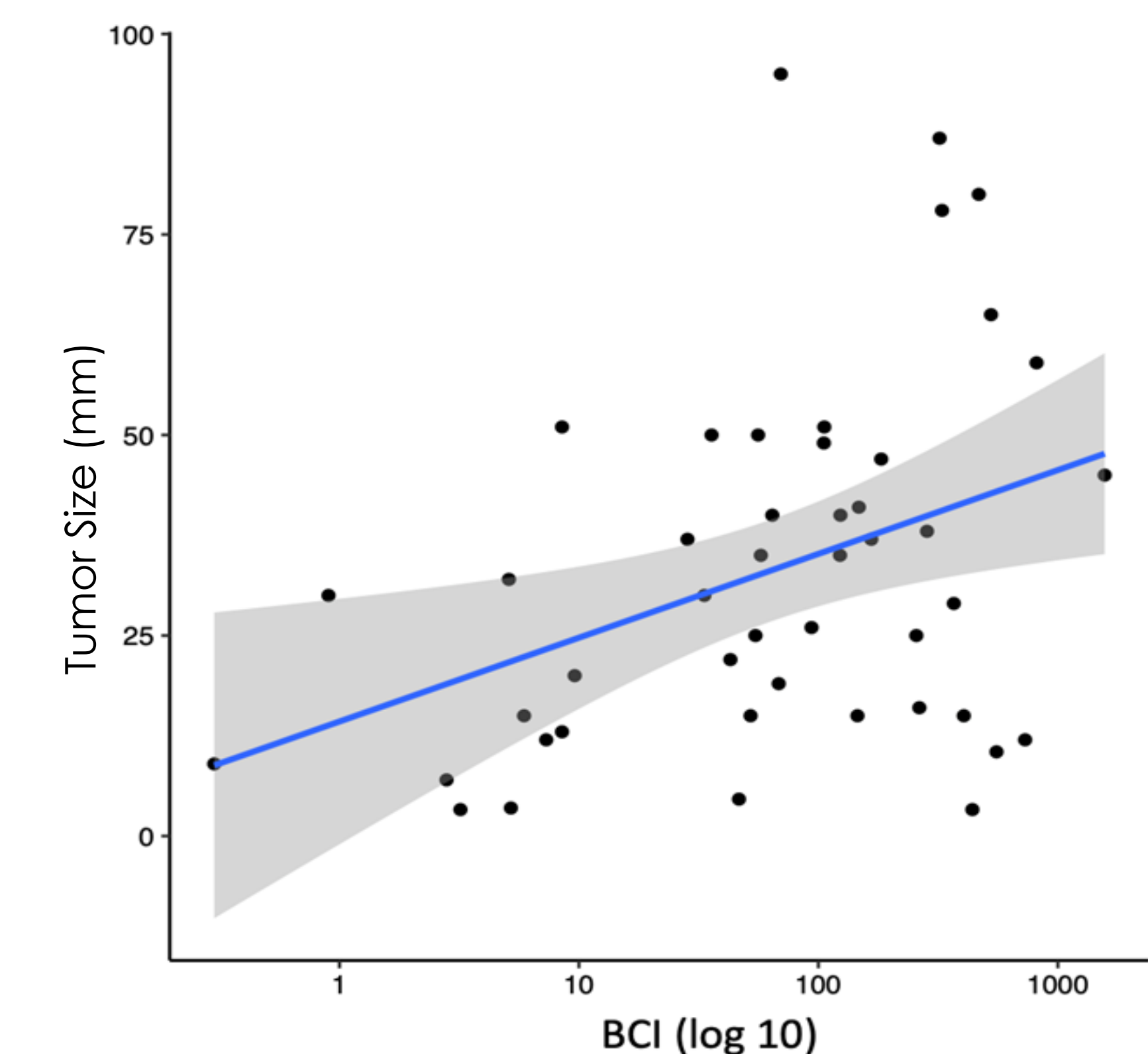
Table 2: Comparison of Bladder CARE™ and urine cytology in UTUC patients

		Urine Cytology			Bladder CARE™		
		Neg	Pos	NA	Neg	HR	Pos
UTUC	Low-grade (n=9)	5	0	4	1	0	8
	High-grade (n=41)	17	13	11	1	1	39

Table 3: Performance of Bladder CARE™ and urine cytology

	Bladder CARE™	Urine Cytology
Sensitivity	96%	37%
Specificity	88%	NA
PPV	89%	NA
NPV	96%	NA

Figure 4: A significant correlation was found between BCI values and tumor size (p = 0.009)



CONCLUSIONS

- In this prospective pilot study, the proposed urine-based epigenetic assay (Bladder CARE™) showed high sensitivity and negative predictive value for the detection of UTUC.
- In addition, the sensitivity of Bladder CARE™ was exceedingly higher than the standard urine cytology.
- A significant correlation was found between BCI values and tumor size; this may help with preoperative risk stratification of UTUC patients.
- A larger sample size study to validate the accuracy of this test is the next step.

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