

# Urinary Dual OncomiR Signature of miR-21 and miR-155: A Non-Invasive Approach for Bladder Cancer Detection in a Moroccan Cohort

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## INTRODUCTION & AIM

Bladder cancer is one of the most common urological malignancies worldwide. Current diagnostic methods, including cystoscopy, are invasive and may limit patient compliance. Urinary microRNAs have emerged as promising non-invasive biomarkers for cancer detection. This study aimed to evaluate the diagnostic potential of urinary miR-21 and miR-155 as a dual oncomiR signature for bladder cancer detection in a Moroccan cohort.

## RESULTS & DISCUSSION

### 1. Urinary miR-21 and miR-155 are upregulated in BCa

RT-qPCR analysis showed a significant upregulation of urinary miR-21 and miR-155 in bladder cancer (BCa) patients compared to controls ( $p < 0.0001$  for both). These results indicate strong dysregulation of both miRNAs in BCa and suggest their involvement in disease development.

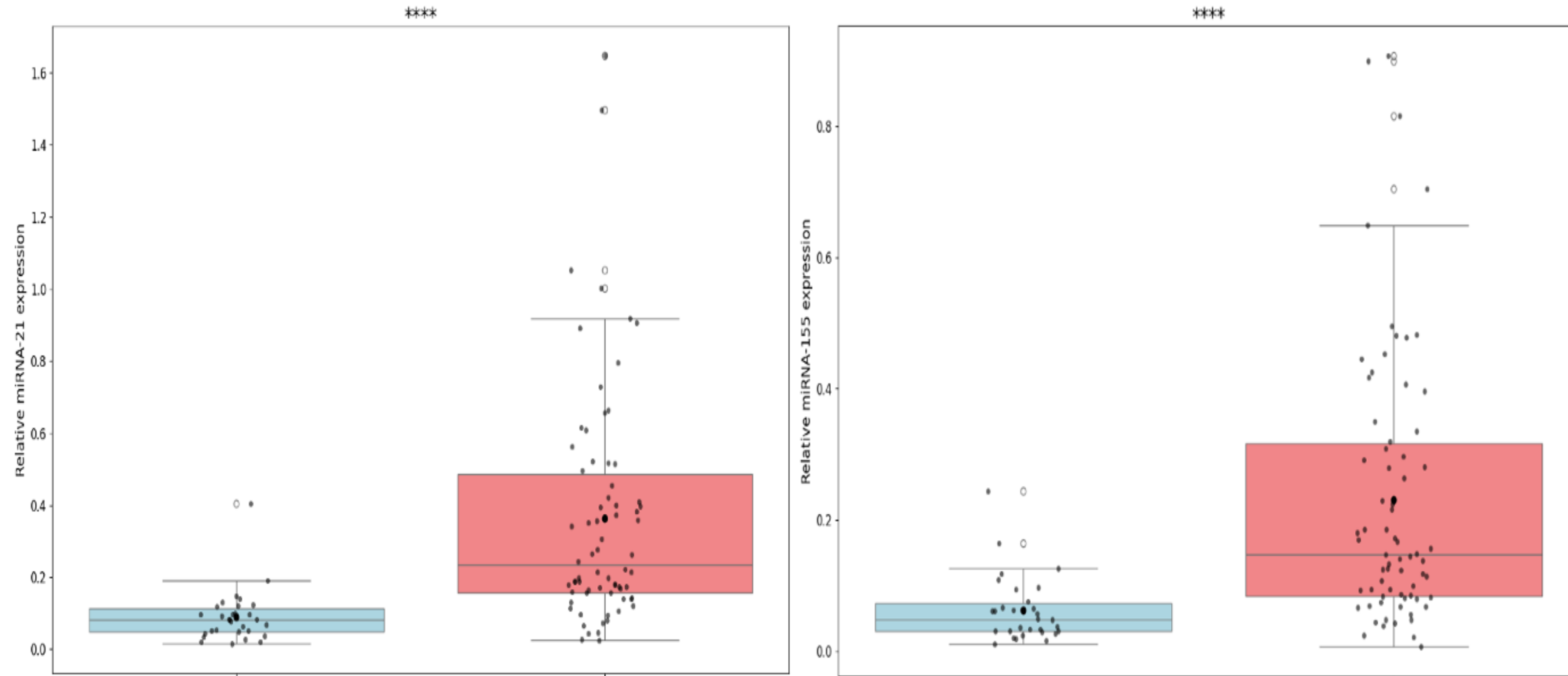


Fig. 1. Box plot representing the differential expression of miRNAs-21 and miRNA-155 in BCa patients vs controls.

### 2. miR-21 and miR-155 are associated with aggressive clinicopathological features

High expression of miR-21 and miR-155 was significantly associated with advanced tumor stage, high tumor grade, and muscle invasion. miR-155 was additionally associated with smoking status. These findings suggest a link between increased miRNA expression and tumor aggressiveness.

Table 1 Association Between miR-21 and miR-155 Expression Levels and Clinicopathological Parameters of BCa Patients.

Variable	N = 70	miR-21 Expression Level		P-value	miR-155 Expression Level		P-value
		High (n=48)	Low (n=22)		High (n=38)	Low (n=32)	
<b>Sex ratio</b>				0.672*			0.804*
Male	47	33	14		26	21	
Female	23	15	8		12	11	
<b>Smoking status</b>				0.182*			0.041*
Yes	46	34	12		29	17	
No	24	14	10		9	15	
<b>Age (years)</b>				0.249*			0.197*
<60	25	15	10		11	14	
≥ 60	45	33	12		27	18	
<b>T-stage</b>				0.002**			0.04*
pTa-pT1	39	21	18		17	22	
≥ pT2	31	27	4		21	10	
<b>Tumor grade</b>				0.001**			0.002*
High grade	29	26	3		22	7	
Low grade	41	22	19		16	25	
<b>Muscle Invasion</b>				0.004**			<0.001**
No	44	25	19		17	27	
Yes	26	23	3		21	5	

### 3. Both miRNAs show strong diagnostic performance

ROC analysis demonstrated strong diagnostic accuracy for miR-21 (AUC = 0.876) and miR-155 (AUC = 0.845). The combined model improved performance (AUC = 0.907), indicating enhanced diagnostic value when both markers are used together.

## CONCLUSIONS

- Urinary miR-21 and miR-155 form a promising non-invasive biomarker duo for bladder cancer detection and risk stratification, with strong diagnostic performance and association with tumor aggressiveness. This first Moroccan cohort study supports their clinical relevance and potential use in resource-limited settings. Further large-scale validation is needed before clinical application.

## METHOD

### • Study Population and Sample Collection

A case-control study was conducted including 70 histopathologically confirmed bladder cancer (BCa) patients and 30 healthy controls recruited at Mohammed V Military Hospital, Rabat, Morocco (2023–2024). Midstream urine samples were collected following informed consent and ethical approval. Urine was processed within 2 h of collection, centrifuged to remove cellular debris and extracellular vesicles, and stored at  $-80^{\circ}\text{C}$  until analysis.

### • RNA Extraction and RT-qPCR

Total RNA, including small RNAs, was isolated from urine using the mirVana™ kit. Expression levels of miR-21 and miR-155 were quantified by TaqMan® RT-qPCR, with U6 snRNA used as the endogenous control. Relative expression was calculated using the  $2^{-\Delta\Delta\text{Ct}}$  method.

### • Statistical Analysis

Differences in miRNA expression between groups were evaluated using the Mann–Whitney U test. Associations with clinicopathological parameters were assessed using Chi-square or Fisher's exact tests. Diagnostic performance was evaluated by ROC curve analysis, and combined biomarker performance was assessed using multivariate logistic regression. Statistical significance was set at  $p < 0.05$ .

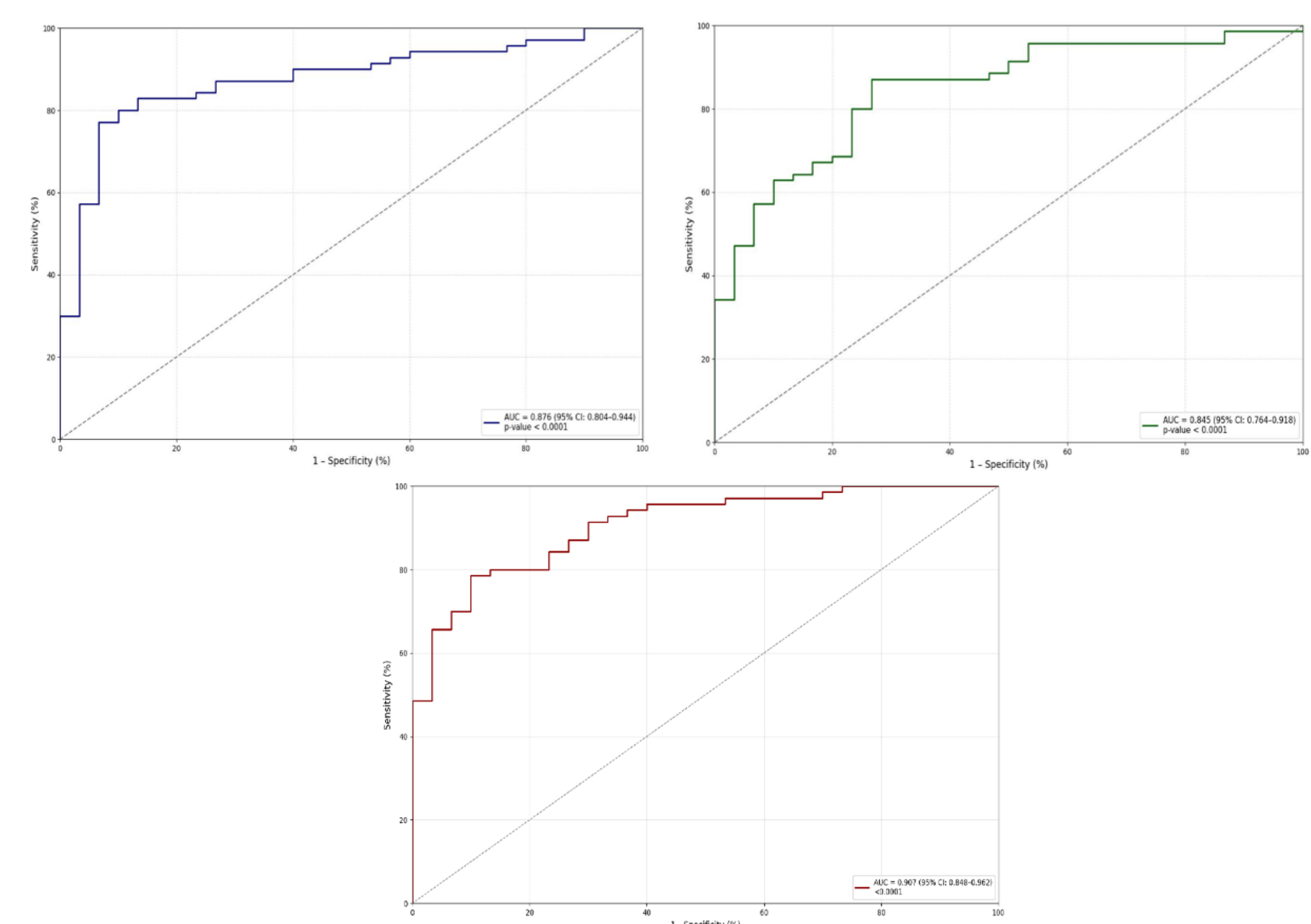


Fig. 2. ROC curve representing the diagnostic performances of miR-21 (blue), miR-155 (green) and their combined model miR-21+miR-155 (red).

### 4. miR-21 is an independent predictor of BCa

Logistic regression confirmed that miR-21 is a significant independent predictor of BCa ( $p = 0.023$ ), while miR-155 showed a borderline association ( $p = 0.057$ ), suggesting a complementary role.

Table 2 Logistic Regression Analysis of miR-21 and miR-155 Expression in BCa Patients.

Variable	Odds Ratio (OR)	95% CI	p-value
$\Delta\text{Ct}_{\text{miR21}}$	0.5747	0.3565 – 0.9265	0.0230
$\Delta\text{Ct}_{\text{miR155}}$	0.6545	0.4230 – 1.0127	0.0570

- Urinary miR-21 and miR-155 are significantly upregulated in bladder cancer and show strong diagnostic performance, enhanced when combined into a dual signature. Their association with tumor aggressiveness supports their clinical relevance as non-invasive biomarkers. This study highlights their potential utility in a Moroccan cohort.

## FUTURE WORK/ REFERENCES/ACKNOWLEDGMENT

- Future Work:** Validation in larger cohorts and evaluation of prognostic value are needed to confirm clinical utility.
- References:** Oyaert M, Van Praet C, Delrue C, Speeckaert MM. Novel urinary biomarkers for the detection of bladder cancer. *Cancers*. 2025. <https://doi.org/10.3390/cancers17081283> /Ma L, Guo H, Zhao Y, et al. Liquid biopsy in cancer: current status, challenges and future prospects. *Signal Transduct Target Ther*. 2024. <https://doi.org/10.1038/s41392-024-02021-w> /Gan L, Zheng L, Zou J, et al. MicroRNA-21 in urologic cancers: from molecular mechanisms to clinical implications. *Front Cell Dev Biol*. 2024. <https://doi.org/10.3389/fcell.2024.1437951>
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