

Evaluation of six novel biomarkers for predicting recurrence of non-muscle
invasive bladder cancer after endoscopic resection – a prospective observational
study

SUPPLEMENTARY INFORMATION (SI)

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Online Resources

Online Resource 1 Comparison of biomarker concentrations in patients with NMIBC and MIBC (at the moment of study entry - diagnostic value)

Investigated biomarkers:			
Biomarker	Patients with NMIBC (n=223)	Patients with MIBC (n=8)	p-value
Serum PAI-1	3.65 (2.17 - 6.2)	4.89 (2.49 – 5.45)	0.5114
Serum sUPAR	2.69 (1.97 - 3.58)	3.70 (2.87 – 4.99)	0.0399
Serum IL-8	21.6 (12.43 - 66.4)	30.76 (25.34 – 85.43)	0.1360
Urine IL-8	55.9 (16.9 - 67.2)	280 (67.15 – 524.9)	0.0065
Urine VEGF	122.5 (74.9 - 250.8)	460.4 (214.7 - 1049)	0.0069
Urine APOE	9.54 (5.19 - 21.46)	62.37 (16.98 – 259.2)	0.0089

MIBC – muscle invasive bladder cancer, NMIBC – non-muscle invasive bladder cancer.

Online Resource 2 ROC curve analysis for investigated biomarkers to discriminate between MIBC and NMIBC (at the moment of study entry – diagnostic value)

	AUC	AUC 95% CI	SE	p-value
Serum PAI-1	0.569	0.407 - 0.732	0.083	0.4025
Serum sUPAR	0.712	0.527 - 0.898	0.095	0.0250
Serum IL-8	0.656	0.528 - 0.785	0.065	0.0170
Urine IL-8	0.773	0.580 - 0.967	0.099	0.0056
Urine VEGF	0.775	0.594 - 0.956	0.093	0.0029
Urine APOE	0.765	0.560 - 0.970	0.105	0.0112
EORTC recurrence points^a	n/a	n/a	n/a	n/a
EORTC progression points^a	n/a	n/a	n/a	n/a

MIBC – muscle invasive bladder cancer, NMIBC – non-muscle invasive bladder cancer

^a In 5 out of 8 MIBC cases the tumors were diagnosed *de-novo*, therefore EORTC score could not be calculated. Due to that fact ROC curve analysis was not performed for these parameters.

Online Resource 3 Multivariable logistic regression to diagnose between NMIBC and MIBC at the moment of study entry

The analysis was performed to replicate investigation by Goodison et. al.

Variable	Univariable analysis		Multivariable analysis	
	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Urine VEGF	1.001 (1.001 – 1.006)	0.0041	1.001 (1.000 – 1.002)	0.0330
Urine IL-8	1.003 (1.001 – 1.005)	0.0290	1.003 (1.000 – 1.005)	0.0791
Urine APOE	1.000 (0.999 – 1.001)	0.5711	1.000 (0.998 – 1.001)	0.4735

Effect shown for MIBC as modelled variable. Model's AUROC = 0.892 (SE = 0.041).

Online Resource 4 Comparison of investigated biomarkers concentration between healthy subjects and patients with any type of bladder cancer at the moment of study entry (diagnostic value)

Biomarker (median; IQR)	Healthy subjects (n=32)	Patients with any type of bladder cancer (n=223)	p-value
Serum PAI-1	3.45 (2.15 – 5.44)	3.65 (2.17 - 6.2)	0.7696
Serum suPAR	2.43 (1.80 – 3.39)	2.69 (1.97 - 3.58)	0.3824
Serum IL-8	14.75 (10.52 – 39.69)	21.6 (12.43 - 66.4)	0.1224
Urine IL-8	67.15 (33.28 – 67.15)	55.9 (16.9 - 67.2)	0.5704
Urine VEGF	95.42 (52.64 – 221.76)	122.5 (74.9 - 250.8)	0.1170
Urine APOE	9.16 (4.38 – 20.28)	9.54 (5.19 - 21.46)	0.5709

Online Resource 5 Subgroup analysis comparing biomarker concentrations

Subgroups	Biomarker	Subgroup counts (Group 1 / Group 2)	Group medians (IQR)	Test p-value
Low-grade (LG) <i>versus</i> High-grade (HG)	Serum PAI-1	Low-grade (LG): n=92	-	0.1182
	Serum suPAR		-	0.0885
	Serum IL-8		-	0.9512
	Urine IL-8	High-grade (HG): n=114	34.36 (11.23-65.02) vs 67.15 (26.21-109.51)	0.0002
	Urine VEGF		-	0.8054
	Urine APOE		-	0.9937
Primary tumour <i>versus</i> secondary tumor	Serum PAI-1	Primary tumour (n=83) secondary tumor (n=140)	-	0.1117
	Serum suPAR		-	0.5056
	Serum IL-8		-	0.8194
	Urine IL-8		-	0.0505
	Urine VEGF		145.5 (85.2-301.5) vs 107.3 (66.4-201.1)	0.0085
	Urine APOE		-	0.0618
[secondary tumors]: No previous BCG <i>versus</i> Previous BCG	Serum PAI-1	No previous BCG (n=33)	-	0.7711
	Serum suPAR		-	0.1663
	Serum IL-8		-	0.0893
	Urine IL-8	Previous BCG (n=107)	31.18 (9.87-67.15) vs 63.89 (18.22-82.09)	0.0114
	Urine VEGF		-	0.2216
	Urine APOE		-	0.4052

Online Resource 6 Clinicopathologic characteristics of NMIBC within the patient study groups

Study group / Tumor parameter	Whole study population* (n=206)	Tumor free post-TURBT* (n=86)	Non-tumor free* (n=120)
Number of tumors: class: [single / 2-7 / >7]	191 / 13 / 2	81 / 4 / 1	110 / 9 / 1
Tumor size [< 3 cm / ≥ 3 cm]	196 / 10	85 / 1	111 / 9
Primary recurrence rate [primary / ≤ 1 per year / > 1 per year]	83 / 115 / 8	36 / 49 / 1	47 / 66 / 7
T category** [pTa / pT1]	112 / 83	45 / 39	67 / 44
Cis Class: [YES / NO]***	185 / 21	83 / 3	102 / 18
Tumor grade Class: [G1 / G2 / G3]	66 / 115 / 25	24 / 54 / 8	42 / 61 / 17

*The data applies to patients with complete dataset (total n=206).

** In this category a lower number of patients is presented, as in 11 cases, the primary tumor histology was Cis.

*** In this category higher numbers of Cis histology rates are reported than in Table 1, since in some cases concomitant Cis in the bladder specimen was found (which was not classified as the main tumor histologic type).

Online Resource 7 Association between study biomarker levels and clinicopathologic characteristics of NMIBC in the whole study group

Biomarker / Tumor parameter	Serum PAI-1	Serum sUPAR	Serum IL- 8	Urine IL- 8	Urine VEGF	Urine APOE
Number of tumors: class: [single / 2-7 / >7]	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Tumor size [< 3 cm / ≥ 3 cm]	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
Primary recurrence rate [primary / ≤ 1 per year / > 1 per year]	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
T category [pTa / pT1]	<i>ns</i>	<i>ns</i>	<i>ns</i>	***	<i>ns</i>	<i>ns</i>
Cis Class: [YES / NO]	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*
Tumor grade Class: [G1 / G2 / G3]	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Comparison of baseline tumor characteristics and biomarker concentrations. The data applies to patients with complete dataset (n=206) – as presented in Online Resource 6.

ns – p-value >0.05, * p-value 0.01-0.05, ** p-value 0.001-0.01, ***p-value <0.001

The only difference found in this analysis was the difference between urine IL-8 levels between subjects with T1 and Ta tumors: 66.15 (31.18 – 134.19) vs 41.49 (13.87 – 67.01), $p < 0.0001$.