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RESEARCH

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

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## Abstract

**Purpose** To prospectively evaluate prognostic capabilities of non-muscle invasive bladder cancer (NMIBC) biomarkers for predicting disease recurrence or progression after radical TURB (transurethral resection of bladder tumor).

**Methods** Evaluated biomarkers included blood: plasminogen activator inhibitor 1 (PAI-1), soluble urokinase plasminogen activator receptor (suPAR), interleukin 8 (IL-8) and urine: IL-8, vascular endothelial growth factor (VEGF) and apolipoprotein E (APOE). Blood and urine samples acquired before TURB for NMIBC from 223 subjects were analysed. The primary outcome was tumor recurrence or progression.

**Results** After 3 months follow-up with cystoscopy or TURB– 92 patients were tumor free (Group 1). In 131 subjects (Group 2) a recurrence of NMIBC ($n=120$) or progression to muscle invasive bladder cancer (MIBC) ($n=11$) has been observed. No major clinical differences between these two groups were spotted. The group 2 has presented with significantly higher concentrations of blood IL-8 and suPAR as well as urine VEGF and APOE. The serum IL-8 and urinary VEGF showed the highest prognostic abilities with AUROC of 0.611 (95% CI: 0.534–0.687, $p=0.0044$) and 0.632 (95% CI: 0.557–0.707, $p=0.0006$), respectively. Multivariable machine learning models which included all investigated biomarkers and European Organisation for Research and Treatment of Cancer (EORTC) risk scores have allowed to discriminate the two patient entities with AUROC of 0.84 (95% CI: 0.73–0.95, $p<0.0001$).

**Conclusions** The assessed biomarkers alone hae shown unsatisfactory prognostic capabilities to be used for prognostication of outcomes after TURB. More complex multivariable prediction models may improve their prognostic performance.

**Trial registration** The study was retrospectively registered at clinicaltrails.gov with National Clinical Trial number (NCT): NCT06235853.

**Keywords** Non-muscle invasive bladder cancer (NMIBC) · Biomarkers · Transurethral resection of bladder tumor (TURB) · Machine learning

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## Introduction

Non-muscle invasive bladder cancer (NMIBC) accounts for 75–80% cases of bladder cancer and is characterized by a high rate of recurrence and progression [1, 2]. After transurethral resection of bladder tumor (TURB) some of the patients require adjuvant intravesical treatment and all the patients require regular cystoscopic surveillance in intervals dependent on disease risk stratification. According to the current European Association of Urology (EAU) guidelines, the first cystoscopy is performed 3 months after TURB. The intensity of further surveillance should be based on the risk profile assessed with validated risk scores proposed by EAU [3].

Therefore, bladder cancers pose not only a medical challenge but also impose substantial healthcare costs and burden for the patient [4]. Hence, there is a significant need for the identification of new methods that would improve the risk assessment of the recurrence and progression of the NMIBC, limiting the frequency of cystoscopies performed.

Recent reports show, that utilization of vascular endothelial growth factor (VEGF), apolipoprotein E (APOE) and interleukin 8 (IL-8) allows to efficiently discriminate patients with bladder cancer from healthy patients [5]. Other literature reports have also shown an association between plasminogen activation system components and bladder cancer tumor histology [6]. No studies evaluating these markers in context of prediction of treatment outcome for NMIBC after a successful TURB are currently available.

The primary aim of the study was to evaluate the prognostic value of novel biomarkers associated with plasminogen activation system and inflammatory response in stratification of recurrence or progression risk in NMIBC after TURB.

## Materials and methods

### Study design

The study was designed as a prospective observational pilot study. The approval of the Bioethics Committee of the Wroclaw Medical University was obtained (approval number: KB −692/2019; Ethical Statement Date: 22nd October 2019, Wrocław). After screening and upon inclusion, every participant has signed the Informed Consent Form (ICF). The study was conducted according to the Declaration of Helsinki. The study was funded by a research grant of the Polish Ministry of Science and Higher Education (Grant number: 0073/DIA/2019/48).

### Screening and inclusion criteria

Between 01.01.2020 and 31.12.2022, 318 patients with clinical suspicion of bladder tumor visiting the Urology Outpatient Clinic of the Wroclaw Medical University Hospital were screened for eligibility. After obtaining ICF a total of 285 patients were included in the study using prespecified inclusion criteria. The flowchart of the screening and enrollment process is presented in Fig. 1.

#### Inclusion criteria

- Signed informed consent form.
- Presenting due to clinical suspicion of bladder cancer.
- ≥18 years old.

#### Exclusion criteria

- Confirmation or strong suspicion of muscle-invasive bladder cancer or metastatic disease.

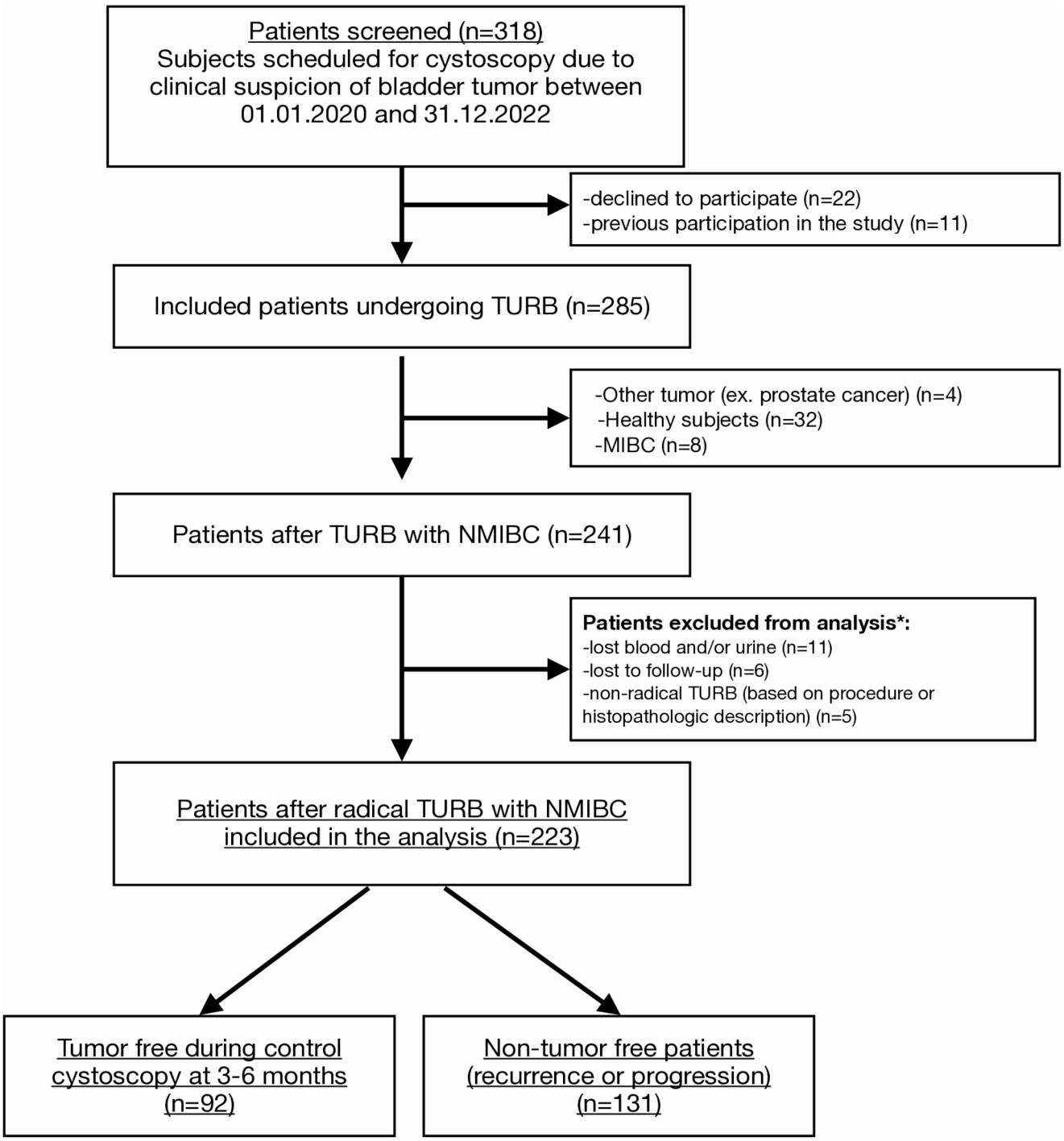
After inclusion, urine and blood samples were drawn from each patient before their visit in the Outpatient Urology Clinic. A hospital admission to perform cystoscopy (and possibly TURB) was appointed if deemed indicated by the attending physician. The blood and urine samples were secured, processed and frozen until the end of the enrollment process, according to manufacturer instructions. During hospitalization cystoscopy was carried out and where it was indicated, TURB was performed. Surgical data was gathered using procedure description. After an endoscopic procedure was carried out, medical history and data regarding tumor histology were collected, when appropriate.

### Follow-up

All patients underwent a control cystoscopy, which timing was at the discretion of the treating physician. In cases of evident or suspected disease recurrence patients were qualified for TURB. If the patients changed the treating center or did not show up for their control visits, follow-up by means of telephone contact was attempted. The decision to introduce Bacillus Calmette-Guérin (BCG) treatment or intravesical chemotherapy was made individually by the treating physician.

### Study outcomes

The main outcome of interest was the composite of the recurrence of NMIBC and the progression of NMIBC to




- Flowchart of the enrollment process.

*some patients were both lost to follow-up and had no specimens collected

MIBC - muscle invasive bladder cancer, NMIBC - non-muscle invasive bladder cancer, TURB - transurethral resection of bladder tumour

**Fig. 1** Flowchart of the enrollment process, follow-up and study workflow

muscle invasive bladder cancer (MIBC) during the first surveillance cystoscopy.

### Investigated biomarkers

Six biomarkers were tested from blood and urine specimens collected from each patient prior to their first cystoscopy. These included blood: plasminogen activator inhibitor 1 (PAI-1), soluble urokinase plasminogen activator receptor (suPAR), interleukin 8 (IL-8) and urine: IL-8, vascular endothelial growth factor (VEGF) and apolipoprotein E (APOE).

### Study registration

The study was retrospectively registered in the Clinicaltrials.gov, with National Clinical Trial number (NCT): NCT06235853.

### Statistical analysis

No preliminary sample size calculation was performed due to lack of previous data on the examined biomarkers.

Non-binary (continuous) data was presented as mean standard deviation (SD) or median and interquartile ranges (IQR), as appropriate. The distribution of the data was assessed utilizing the Shapiro-Wilk test. Categorical data were presented as numbers and percentages. To compare categorical data, we performed Chi2 tests or Fisher exact as appropriate. The statistical significance of differences in continuous data were tested using the Student's t or Mann-Whitney U test according to the data distribution. To determine the prognostic utility of the investigated biomarkers receiver operating characteristic (ROC) curves were generated. For each ROC curve area under the curve (AUC) with 95% confidence intervals (CI), specificity, sensitivity, Youden-point with the best cut-off value were calculated. Correlations between continuous data were tested using Spearman rank correlation (due to skewed data distribution). A two-tailed P value of $<0.05$ was statistically significant. All analyses were performed using Statistica Software version 13.2 (StatSoft, Tulsa, Oklahoma, United States).

### Statistical considerations for machine learning models

To investigate more complex relationships between biomarker levels and follow-up events, machine learning (ML) hyper-parameter tuning was conducted for the: Random Forest, Support Vector Machine (SVM), Naïve Bayes, K-nearest Neighbours (KNN) and MLP, AdaBoost, XGBoost and LightGBM models. The results of our computations highlighted the Random Forrest and LightGBM as the architectures which achieved superior results. For each model the relative feature importance was evaluated. The code and data are available on GitHub repository: https://github.com/FilipKubackiSoton/Biomarkers-of-Recurrence-and-Progression-in-Non-muscle-Invasive-Bladder-Cancer/blob/main/Analyses.ipynb.

## Results

A total of 318 patients were screened for eligibility and 241 were included in the study. After excluding cases of screening failure, lost laboratory specimens and patients who were lost to follow-up, a final group of 223 patients with complete dataset was analysed. The process of enrollment as well as study workflow is presented on flowchart in Fig. 1.

The patients were divided into two groups according to whether they were tumor-free after the initial follow-up ($n=92$)– Group 1 (classified by either cystoscopy with cytology or histopathology result from subsequent TURB), and those in whom tumor recurrence occurred or cancer progression to MIBC was documented during the follow-up period ($n=131$)– Group 2. The median time to first surveillance cystoscopy after primary TURB was 97 days. The characteristics of both groups and levels of investigated biomarkers are presented in Table 1.

The patients from Group 2 were more moribund with a significantly higher Charlson Comorbidity Index (CCI): 4 (IQR: 2.5–5) vs. 5 (IQR: 4–6), $p<0.0001$. Restaging TURB (re-TURB) was performed after initial TURB in 5 cases in the tumor free group and in 11 cases in the non-tumor free group. No further major differences regarding the group characteristics (including the histology of the bladder tumor) were observed. Precise description of the clinicopathologic features of NMIBC tumors is included in Online Resource 6.

The Group 2 has presented with significantly higher concentrations of serum IL-8 (24.4; IQR 13.9–84.6 vs. 19.0; IQR 10.1-35.71, $p=0.0065$) and serum suPAR (2.86; IQR 2.06–3.75 vs. 2.4; IQR 1.89–3.08, $p=0.0033$). Group 2 has also shown significantly higher concentrations of urine VEGF (153; IQR 78.84–329.3 vs. 96; IQR 72.45–149.1, $p=0.0011$) and APOE (9.91; IQR 5.58–24.53 vs. 7.9; IQR 4.33–14.69, $p=0.0434$). Both groups presented similar concentrations of serum PAI-1 and urine IL-8.

To assess the ability of the investigated biomarkers to predict outcomes after TURB due to NMIBC, ROC curves for each marker have been created and presented in Fig. 2. The parameters of each ROC curve are presented in Table 2.

All the investigated markers have shown poor discriminatory properties to select patients who are at risk of disease

**Table 1** Characteristics of the whole study population and study subgroups with investigated biomarker levels

| | Whole study population ($n=223$) | Tumor free post-TURB ($n=92$) | Non-tumor free ($n=131$) | $p$-value between groups |
|---|---|---|---|---|
| Age [years] | 70; IQR (65–76) | 69; IQR (64–74) | 70; IQR (65–77) | 0.1969 |
| Sex [males / females] | 162 / 61 | 63 / 29 | 99 / 32 | 0.3629 |
| BCG therapy prior to enrollment | 107 (45.3%) | 51 (55.4%) | 56 (42.7%) | 0.0767 |
| Intravesical chemotherapy prior to inclusion | 42 (18.8%) | 22 (23.9%) | 20 (15.3%) | 0.1189 |
| CCI | 5; IQR (4–6) | 4; IQR (2.5–5) | 5; IQR (4–6) | <0.0001 |
| Obesity | 13 (5.8%) | 8 (8.7%) | 5 (3.8%) | 0.1517 |
| Cardiovascular disease | 44 (19.7%) | 15 (16.3%) | 29 (22.13%) | 0.3092 |
| Chronic kidney disease | 7 (3.1%) | 1 (1%) | 6 (4.6%) | 0.2970 |
| Diabetes | 53 (23.8%) | 22 (23.9%) | 31 (23.7%) | 0.9999 |
| Smoker | 32 (14.3%) | 11 (11.9%) | 21 (16.0%) | 0.4422 |
| EORTC recurrence points | 2; IQR (1–3) | 1; IQR (0–2) | 3; IQR (2–4) | <0.0001 |
| EORTC progression points | 2; IQR (0–6) | 2; IQR (0–4) | 4; IQR (2–6) | 0.0001 |
| **Baseline tumor histology** | | | | |
| Primary tumor | 83 (37.2%) | 36 (39.1%) | 47 (35.9%) | 0.6737 |
| Secondary tumor | 140 (62.8%) | 56 (60.9%) | 84 (64.1%) | |
| pTis | 11 (4.9%) | 2 (2.2%) | 9 (6.9%) | 0.1294 |
| pTa | 112 (50.2%) | 45 (48.9%) | 67 (51.1%) | 0.9999 |
| pT1 | 83 (37.2%) | 39 (42.4%) | 44 (33.6%) | 0.2062 |
| Unknown* | 17 (7.6%) | 6 (6.5%) | 11 (8.4%) | |
| Low Grade (LG) | 92 (41.3%) | 36 (39.1%) | 56 (42.7%) | 0.2941 |
| High Grade (HG) | 114 (51.1%) | 50 (54.4%) | 64 (48.9%) | 0.2510 |
| Grade unknown | 17 (7.6%) | 6 (6.5%) | 11 (8.4%) | |
| Recurrence | 120 (50.8%) | n/a | 120 (91.6%) | |
| Progression | 11 (4.7%) | n/a | 11 (8.4%) | |
| **Investigated biomarkers** | | | | |
| Serum PAI-1 | 3.65; IQR (2.17–6.2) | 3.51; IQR (2.16–6.51) | 3.76; IQR (2.16–5.44) | 0.8057 |
| Serum suPAR | 2.69; IQR (1.97–3.58) | 2.4; IQR (1.89–3.08) | 2.86; IQR (2.06–3.75) | 0.0332 |
| Serum IL-8 | 21.6; IQR (12.43–66.4) | 19.0; IQR (10.12–35.71) | 24.4; IQR (13.93–83.57) | 0.0065 |
| Urine IL-8 | 55.9; IQR (16.9–67.2) | 58.7; IQR (18.1–67.2) | 53.96; IQR (16.06–67.15) | 0.9716 |
| Urine VEGF | 122.5; IQR (74.9–250.8) | 96.0; IQR (72.45–149.1) | 153.4; IQR (78.84–329.3) | 0.0011 |
| Urine APOE | 9.54; IQR (5.19–21.46) | 7.9; IQR (4.33–14.69) | 9.91; IQR (5.58–24.53) | 0.0434 |

BCG– Bacillus Calmette-Guérin, CCI– Charlson Comorbidity Index, EORTC - European Organisation for Research and Treatment of Cancer

recurrence or progression in univariable analysis. The serum IL-8 and urinary VEGF showed the highest prognostic abilities with AUROC of 0.611 (95% CI: 0.534–0.687, $p=0.0044$) and 0.632 (95% CI: 0.557–0.707, $p=0.0006$), respectively. The serum PAI-1 and urine IL-8 showed no clinical utility in this context.

To evaluate whether investigated biomarkers may add additional prognostic value to preexisting European Organisation for Research and Treatment of Cancer (EORTC) risk score, a multivariable logistic regression analysis with retrograde stepwise elimination was performed. The number of EORTC progression points, urine VEGF and serum IL-8 remained independent predictors of bad outcome with model AUROC 0.806 and SE 0.031. Detailed results of analysis are presented in Table 3.

To further explore multivariable interactions and relationships between the investigated biomarkers, a ML approach was implemented and several ML-models were tested. The diagnostic performance of models including multiple variables (age, BCG therapy, EORTC recurrence and progression points and all the investigated markers) was presented in Fig. 3. The peak prognostic precision was achieved using Light GMB model, which yielded AUROC of 0.84 (95% CI: 0.73–0.95, $p<0.0001$). To tackle the issue of unbalanced dataset, we used the state-of-the-art methodology recommended and tested in a recent article [7]. For four of the best performing models relative feature importance was evaluated and presented in Fig. 4.

Features with the most significant input to the models' performance were relatively consistent across the spectrum of utilized methods and included: EORTC punctation, urine VEGF and serum IL-8, but also serum suPAR.

Secondary analyses, including diagnostic value of the discussed markers for distinguishing MIBC from NMIBC

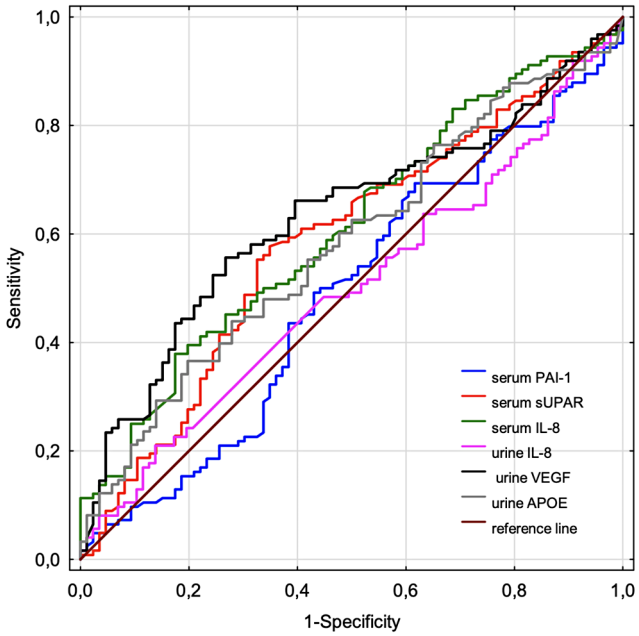



**Fig. 2** Cumulated ROC curves of the investigated biomarkers

**Table 2** Characteristics of ROC curves for predicting the study outcome

| | AUC | AUC 95% CI | SE | *p*-value |
|---|---|---|---|---|
| Serum PAI-1 | 0.49 | 0.41–0.57 | 0.041 | 0.9062 |
| Serum suPAR | 0.587 | 0.508–0.665 | 0.040 | 0.0305 |
| Serum IL-8 | 0.611 | 0.534–0.687 | 0.039 | 0.0044 |
| Urine IL-8 | 0.499 | 0.42–0.577 | 0.040 | 0.9704 |
| Urine VEGF | 0.632 | 0.557–0.707 | 0.038 | 0.0006 |
| Urine APOE | 0.582 | 0.505–0.660 | 0.040 | 0.0375 |
| EORTC recurrence points | 0.778 | 0.748–0.804 | 0.030 | <0.0001 |
| EORTC progression points | 0.654 | 0.580–0.728 | 0.038 | <0.0001 |
| Best multivariable model[a] | 0.806 | 0.775–0.837 | 0.031 | <0.0001 |

EORTC - European Organisation for Research and Treatment of Cancer

[a] Model described in Table 3

(Online Resource 1–3), and NMIBC from healthy subjects have been performed and presented in the Supplementary Information (SI) in Online Resource 4. Based on the limited number of patients with MIBC in a univariable analysis, serum suPAR and urine: IL-8, VEGF and APOE diagnosed NMIBC from MIBC (at the moment of control TURB after 3 months) with AUC exceeding 0.7. The markers however, did not allow to discriminate healthy subjects from patients with bladder cancer (due to predominance of NMIBC). In Online Resource 5 biomarker concentrations were compared in subgroups, based on tumor type, histology and previous BCG therapy, however no major differences were spotted.

## Discussion

The results of our study have shown that none of the investigated biomarkers alone is sufficient for predicting recurrence or progression after TURB for NMIBC. However, multivariable predictive models which have utilized the investigated biomarkers along with clinical features and EORTC risk score have achieved satisfactory diagnostic precision with model AUC reaching 0.84 for machine learning models.

According to current EAU Guidelines on Non-muscle invasive Bladder Cancer [3] it is recommended to stratify patients into risk groups to predict both progression and recurrence. Depending on the predicted outcome and planned BCG treatment different risk scores have been put forward by the EAU. The EORTC risk score proposed in 2021 to predict progression post-TURB has shown good predictive value with Harell's bias corrected *c* index of c = 0.80 [8]. However, patients receiving BCG installations after TURB have been excluded from the cited study cohort and therefore its generalizability is limited.

When it comes to recurrence prognostication EAU recommends using two different risk scores after TURB depending on whether BCG treatment has been introduced. The recommended 2006 EORTC score (for patients not treated with BCG) presents with only moderate discriminatory capabilities with c score 0.74–0.75 [8]. What is more, the Club Urologico Español de Tratamiento Oncologico (CUETO) risk score designed for patients treated with BCG

**Table 3** Multivariable logistic regression model with stepwise elimination of variables for predicting study outcome

| Variable | Univariable analysis | | Multivariable analysis | |
|---|---|---|---|---|
| | Unadjusted OR (95% CI) | *p*-value | Adjusted OR (95% CI) | *p*-value |
| Serum PAI-1 | 1.014 (0.969–1.060) | 0.5535 | - | - |
| Serum suPAR | 1.271 (1.012–1.598) | 0.0396 | - | - |
| Serum IL-8 | 1.005 (1.002–1.009) | 0.0051 | 1.006 (1.001–1.010) | 0.0091 |
| Urine IL-8 | 1.001 (0.999–1.003) | 0.3278 | - | - |
| Urine VEGF | 1.002 (1.001–1.003) | 0.0258 | 1.002 (1.001–1.003) | 0.0478 |
| Urine APOE | 1.006 (1.000–1.013) | 0.0642 | - | - |
| EORTC recurrence points | 2.531 (1.927–3.323) | <0.0001 | 2.466 (1.866–3.259) | <0.0001 |
| EORTC progression points | 1.183 (1.080–1.297) | 0.0003 | - | - |

Model AUC = 0.806 (95% CI: 0.775–0.837, $p = 0.0001$)

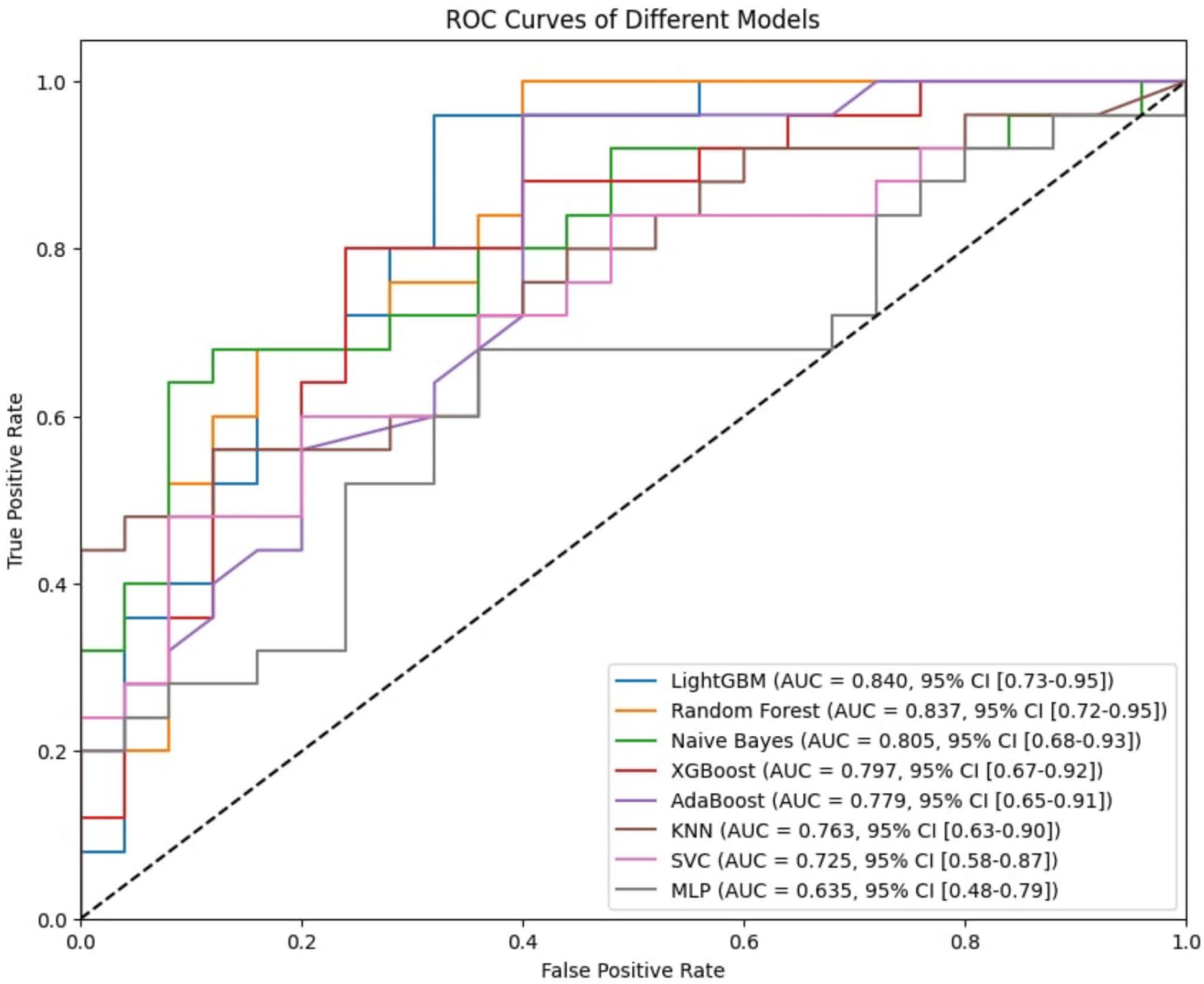



**Fig. 3** The performance of created machine learning models

showed even worse performance with c score of 0.69–0.70 [9]. Therefore, the prognostication of outcomes after TURB is multifactorial and relies mostly on patient- and tumor clinical features rather than a singular risk score. That is why we have developed a more pragmatic, short term observation model than can be applied to the whole population of NMIBC patients without distinguishing for BCG treated and non-treated groups. Although our model and the EAU recommended models cannot be directly compared (due to methodological reasons) it seems that their accuracy remains moderate.

To our knowledge there are very few scientific papers regarding the biomarkers included in our study. The choice of biomarkers, which were investigated in our paper, was due to several reports of increased protein production in bladder cancer tissues [10–13]. Several authors have already tested extensive biomarker panels for their ability to diagnose bladder cancer at the moment of primary TURB. In the study by Goodison et al. [14], the combination of three urinary biomarkers: VEGF, APOE, IL-8 has allowed to discriminate patients with NMIBC from healthy individuals with an AUC of 0.968 (95% CI: 0.942–0.992), a sensitivity of 90% and specificity of 97%. In our dataset, this diagnostic result was not observed, however an analogic set of biomarkers has allowed to diagnose MIBC from NMIBC patients in the primary TURB with AUC of 0.892 and SE equal to 0.041– as presented in Online Resource 3. To our knowledge, very few authors investigated the prognostic utility of these biomarkers. Gogalic and colleagues have attempted to correlate the levels of urinary VEGF and IL-8 (along with other proteins) with the frequency of recurrence of NMIBC. VEGF and IL-8 alone have predicted this outcome with AUC of 0.67 ($p=0.038$) and 0.69 ($p=0.066$), respectively, which is comparable to our results [15].

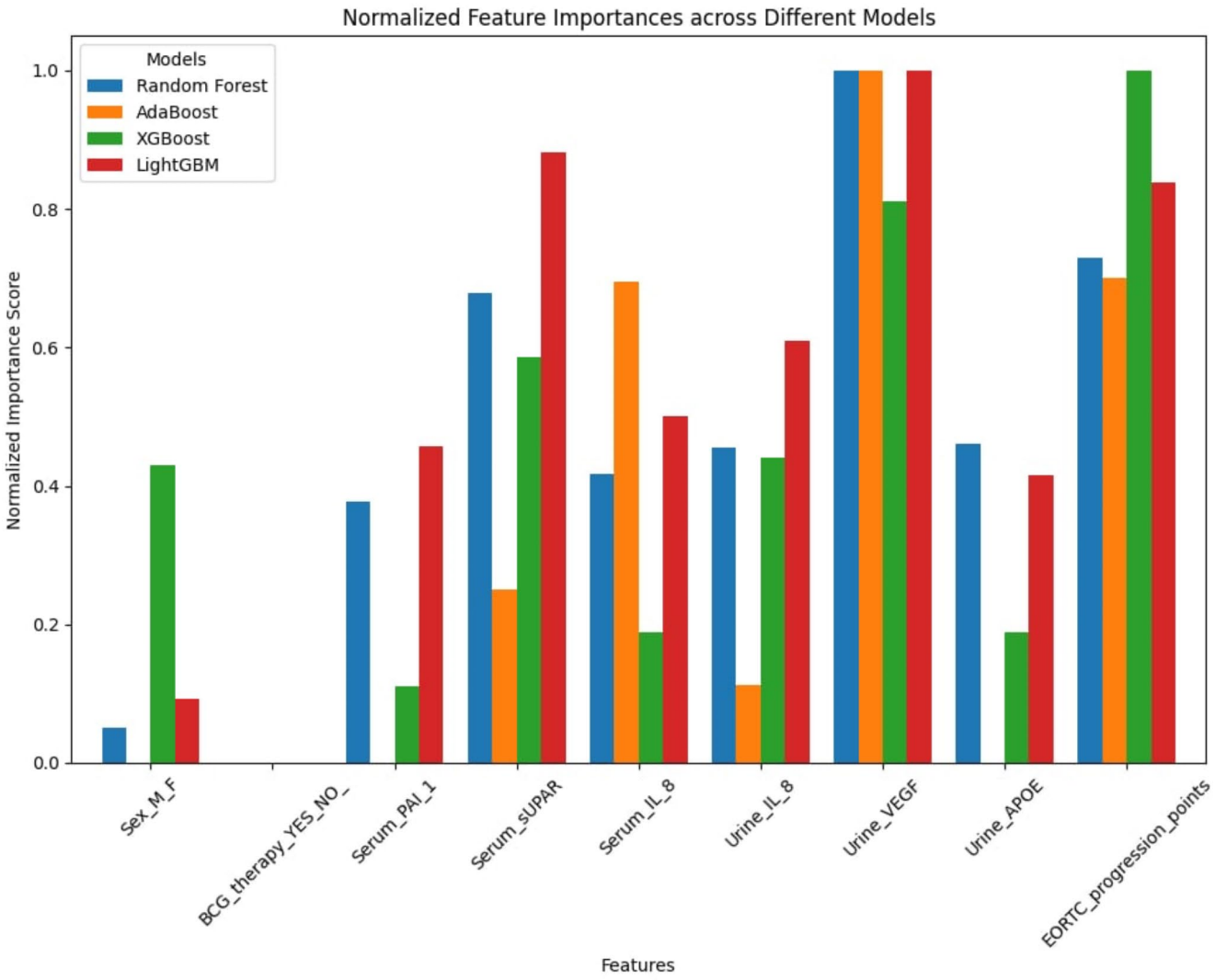



**Fig. 4** Relative feature importance for four most robust models

However, the correlation with other clinical features (past recurrences, BCG therapy and creatinine) has allowed them to achieve the model AUC 0.96. These findings have not yet been validated in larger prospective studies.

Other reports have shown an association of elevated VEGF concentrations with recurrence of bladder cancer and baseline tumor grade [16, 17]. Similar observations have been made regarding the APOE, interleukin-8 and plasminogen-system-related proteins (PAI-1 and suPAR). In several studies it has been reported that both high peripheral blood and urinary IL-8 levels were associated with tumor detection and/or recurrence [5, 18–20]. The studies on serum PAI-1 and suPAR have produced similar results and confirmed their association with oncological outcomes. Furthermore, Iwata et al. have also reported an association between suPAR and PAI-1 with the baseline grade of the bladder tumor [6]. The combination of both markers demonstrated an independent association with recurrence- and progression free survival (RFS and PFS).

All the investigated markers have been previously shown to be accurate for diagnosing bladder cancer, however their value for recurrence or progression prognostication has not been yet well established. On one hand, the number of studies investigating this problem is low, and on the other their design is heterogenous enough to preclude their synthesis. Therefore larger, prospective studies designed specifically for addressing this problem are needed to draw any meaningful conclusions on this topic. Our data suggest that serum IL-8 and urine VEGF may potentially increase the accuracy of clinical risk scores such as EORTC bladder cancer risk scale.

Although our study has produced negative results, the literature and presented MLmodels indicate that there may still be room for simple protein biomarkers for predicting outcome after TURB, as adjuncts to clinical risk scoring

systems. Although more advanced molecular methods are available nowadays (as UroVision or ImmunoCyt), the relatively low assay costs of protein biomarkers may increase the odds of being cost-effective for oncological risk stratification.

### Limitations of this study

The study has several limitations. In our opinion, the most important one is short observation time, which may lead to recurrence or progression underestimation. Despite short follow-up period a sufficient number of outcome events was registered to perform robust analyzes. Secondly, due to the COVID-19 pandemic, the study has recruited lower than planned number of patients. A longer 12 month observation period was not feasible due to frequent change of treating center and significant loss to follow-up. Lastly, the proposed ML models may be overfitted due to low sample size of the study group and unbalanced distribution of the outcome event. On the other hand, the proposed model was trained on a cohort which included patients who were treated with BCG installation and classifies patients according to a composite poor outcome, which makes it a more pragmatic tool rather than scores designed to prognosticate for each recurrence and progression (with or without BCG treatment).

## Conclusions

A panel of blood PAI-1, suPAR, IL-8 and urine IL-8, VEGF and APOE combined with EORTC risk scale can predict recurrence and progression at 3 months after primary TURB for NMIBC with of AUC of 0.84 (95% CI: 0.73–0.95, $p<0.0001$). Singular protein biomarkers alone are insufficient for long term prognostication in patients with NMIBC who undergo TURB.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00345-025-05485-9.

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**Author contributions** KB– study conceptualisation, study design, study coordination, gathering clinical data, registration of the study, manuscript preparation, manuscript drafting WK– study conceptualisation, study design, study coordination, manuscript drafting, gathering clinical data AK– gathering clinical data KK-K– study conceptualisation, study design, coordination of laboratory procedures, gathering clinical data DB - laboratory procedures, gathering clinical data MŻ - laboratory procedures, gathering clinical data JCh– gathering clinical data FK– statistical analysis, development of machine learning models TK– statistical analysis, manuscript drafting MK - study conceptualisation, study design, manuscript drafting TSz– study conceptualisation, study design, manuscript drafting DK– study conceptualisation, study design, study coordination, manuscript drafting. All authors have approved the submitted version of the manuscript and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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**Data availability** The whole study database is available in an open-source repository under https://doi.org/10.17632/65p8fnnf8d.1.

## Declarations

**Ethics approval and consent to participate** The approval of the Bioethics Committee of the Wroclaw Medical University was obtained (approval number: KB −692/2019; Ethical Statement Date: 22nd October 2019, Wrocław). Every study participant has signed the informed consent form (ICF).

**Competing interests** The authors declare no competing interests.



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