Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2016 September ; 25(9): 1361–1366. doi:
10.1158/1055-9965.EPI-16-0260.

A nomogram derived by combination of demographic and biomarker data improves the non-invasive evaluation of patients at risk for bladder cancer

Sijia Huang^{1,*}, Lei Kou^{2,*}, Hideki Furuya³, Changhong Yu², Steve Goodison^{4,5}, Michael W. Kattan², Lana Garmire¹, and Charles J. Rosser^{3,4}

¹Cancer Epidemiology Research Program, University of Hawaii Cancer Center, Honolulu, HI, USA

²Department of Biostatistics, Cleveland Clinic, Cleveland, OH, USA

³Clinical and Translational Research Program, University of Hawaii Cancer Center, Honolulu, HI, USA

⁴Nonagen BioScience Corp, Jacksonville, FL, USA

⁵Dept of Health Sciences Research, Mayo Clinic Florida, Jacksonville, FL, USA

Abstract

Background—Improvements in the non-invasive clinical evaluation of patients at risk for bladder cancer (BCa) would be of benefit both to individuals and to healthcare systems. We investigated the potential utility of a hybrid nomogram that combined key demographic features with the results of a multiplex urinary biomarker assay in hopes of identifying patients at risk of harboring BCa.

Methods—Logistic regression analysis was used to model the probability of BCa burden in a cohort of 686 subjects (394 with BCa) using key demographic features alone, biomarker data alone and the combination of demographic features and key biomarker data. We examined discrimination, calibration and decision curve analysis techniques to evaluate prediction model performance.

Results—Area under the receiver operating characteristic curve (AUROC) analyses revealed that demographic features alone predicted tumor burden with an accuracy of 0.806 [95% CI: 0.76–0.85], while biomarker data had an accuracy of 0.835 [95% CI: 0.80–0.87]. The addition of molecular data into the nomogram improved the predictive performance to 0.891 [95% CI: 0.86–0.92]. Decision curve analyses showed that the hybrid nomogram performed better than demographic or biomarker data alone.

Corresponding author: Charles J. Rosser, MD, Professor & Program Director, Clinical and Translational Research Program, University of Hawaii Cancer Center, 701 Ilalo St, Rm 327, Honolulu, HI 96813, Tel 808-564-3812, Fax 808-586-3016, crosset@cc.hawaii.edu.

*co-first authors

Disclose of potential conflict of interest

All other authors have no potential conflicts to disclose.

Conclusion—A nomogram construction strategy that combines key demographic features with biomarker data may facilitate the accurate, non-invasive evaluation of patients at risk of harboring BCa. Further research is needed to evaluate the BCa risk nomogram for potential clinical utility.

Impact—The application of such a nomogram may better inform the decision to perform invasive diagnostic procedures.

Keywords

bladder cancer; diagnostic signature; nomogram; risk; urine

INTRODUCTION

With an estimated 70,980 newly diagnosed cases of bladder cancer (BCa) and 14,330 deaths from BCa in 2015, cancer of the urinary bladder is the second most common genitourinary malignancy in the US and among the five most common malignancies worldwide [1, 2]. When detected early (*i.e.*, non-muscle invasive), the 5-year survival rate of BCa is > 90%, however at later stages (*i.e.*, muscle invasive and beyond) the 5-year survival rate is < 50%. Thus, early BCa identification, both at the initial diagnosis and at recurrence can dramatically affect outcomes [3]. Urine based assays that can noninvasively detect BCa have the potential to improve the rapid diagnosis of BCa. As such, several urine-based commercial molecular tests have been FDA-approved for BCa detection and surveillance. These tests include the measurement of soluble proteins such as bladder tumor antigen (BTA), and nuclear matrix protein 22 (NMP22), or proteins detected on fixed urothelial cells (ImmunoCyt), and chromosomal aberrations detected by fluorescent *in situ* hybridization (Urovysion) [4]. Because of their marginal detection performance, these urine-based assays have a limited role in the management of patients at risk for, or with BCa, and thus the search for non-invasive urine-based tests with clinical utility for BCa continues.

We and others, have described the diagnostic capabilities of urine-based molecular signatures to non-invasively detect BCa [5–12]. We have refined and validated a multiplex protein biomarker panel (MMP9, MMP10, IL8, VEGFA, SERPINE1, SERPINA1, CA9, APOE, ANG and SCD1) in a series of independent cohorts [13–15]. Given the utility of key demographic features (*e.g.*, age, race, sex, tobacco history) in stratifying patients, in this study we investigated the potential utility of a hybrid nomogram that incorporates key demographic features with the results of the BCa-associated diagnostic signature in hopes of improving the evaluation of risk for harboring BCa. If proven accurate and reliable, the application of such a nomogram may guide the decision to perform invasive diagnostic procedures.

MATERIALS AND METHODS

Study Subjects

Demographic, clinical and biomarker data from 686 subjects (394 incident BCa subjects and 292 subjects with benign urologic conditions (*e.g.*, erectile dysfunction, voiding symptoms, microscopic hematuria, kidney stones) from 10 clinical sites were extracted from our series of independent cohorts previously published [13–15], Supplemental Table 1. Subjects with

urinary tract infection and gross hematuria were excluded. All molecular data were normalized to creatinine. Based on the total distribution of each biomarker's concentration, cut-points were identified deriving low/high expression status [13–15].

Primary End Point and Baseline Information

The primary end point of the study was to predict the histologic presence of urothelial carcinoma of the bladder, which was confirmed by biopsy. Tumor grade (2002 WHO classification) [16] and tumor stage (2002 TNM classification) [17] were noted for each case. No central pathology review was obtained.

Statistical Analysis

The distributions of the key demographic data as well as molecular data were examined. Multivariate logistic regression analysis was used to examine the association between these predictor variables and detection of BCa. All decisions with respect to the coding of the nomogram variables were made prior to modeling, as making these decisions afterwards can have deleterious effects on the predictive ability of the model [18]. A logistic regression model based on disease status was the basis for our nomograms, which included only key demographic data, only key biomarker data and the combination of key demographic data and biomarker data. Stepdown method was employed to obtain the reduced model with highest C-index.

Nomogram validation contained two components. The nomogram was subjected to bootstrapping as a means of calculating a relatively unbiased measure of its ability to discriminate among subjects. Briefly, we compared the predicted probability of diagnosis *vs.* actual diagnosis (*i.e.*, nomogram calibration) on the 686 subjects, using 1,000 bootstraps to reduce overfit bias, which would otherwise overstate the accuracy of the nomogram. We quantified the discrimination ability of the risk calculator by calculating the concordance index (C-index), which is a surrogate of the nonparametric area under the receiver operating characteristic curve (AUC) [19]. C-index gives the probability that, in a randomly selected pair of subjects in which one has BCa and the other does not, the subject with the BCa will be assigned the worse predicted risk [20]. C-index ranges from 0.5 (no discrimination) to 1.0 (perfect discrimination). To test the significance between the AUCs of the three nomograms (demographic data only, biomarker data only and combination of key demographic data and biomarker data), we created 1,000 C-indices for each model by using bootstrapping analysis and then calculated the differences between the paired C-indices. Lastly, nonparametric bootstrap test [21] was used to calculate the p-value for each pair of the nomograms.

The calibration of the three nomograms was compared by plotting the prediction on the X-axis and the observed outcomes on the Y-axis in the same plot [22]. In the calibration plot, the 45-degree line represents the perfect predictions. Due to binary outcomes, a smoothing technique was used to generate the observed probabilities of BCa on the X-axis. We also applied the decision curve analysis [23] on our proposed nomograms and compared the net-benefits of different examine actions. All statistical analyses were performed using S-Plus software (PC Version 3.3, Redmond, WA) and R software version 3.2.3 with additional

functions. All p values were calculated by two-sided statistical tests, unless notified otherwise.

RESULTS

Of the 686 subjects available for analysis, 394 had BCa while 292 were healthy volunteers/benign controls. Over 84% of the BCa subjects was >55 years (60% of controls), 92% of the BCa subjects were Caucasian (66% of controls), 83% of the BCa subjects were male (79% of controls). Nineteen percent of BCa subjects denied tobacco history while 37% of controls denied tobacco use (Table 1). Of the subjects with BCa, 240 of the tumors were non-invasive (Ta, Tis, T1) and 147 were muscle invasive and 7 did not have a stage reported. In addition, 134 were low-grade, 251 were high-grade and 9 did not have a grade reported.

Logistic regression analysis identified key demographic risk factors (*e.g.*, age, race, sex and tobacco use) and molecular biomarkers (MMP9, MMP10, IL8, VEGFA, SERPINE1, SERPINA1, CA9, APOE, ANG and SCD1) associated with BCa. The key demographic factors were used to generate a demographic only model with AUROC of 0.81 [95% CI: 0.76–0.85] and with C-index of 0.806. The key biomarker data were used to generate a biomarker only model with AUROC of 0.84 [95% CI: 0.80–0.87] and with C-index of 0.835. Under the likelihood ratio test, the biomarker model performed better than the demographic model ($p = 6.745e-4$). Subsequently, these two nomograms were combined to create a hybrid nomogram that incorporated key demographic and biomarker data (Figure 1). The AUROC of the hybrid nomogram was 0.89 [95% CI: 0.86–0.92], which based on the nonparametric bootstrap test, was significantly improved from the demographic model (0.81 [95% CI: 0.76–0.85], $p < 0.0001$) and the biomarker model (0.84 [95% CI: 0.80–0.87], $p < 0.0001$) (Figure 2). The hybrid model possessed a C-index of 0.891. Using the hybrid nomogram, we were able to calculate the sensitivity and specificity for a range of probability for BCa (Table 2).

Figure 3 illustrates how the predictions from the hybrid nomogram compare with actual outcomes for the 686 subjects. The X-axis is the prediction calculated with use of the hybrid nomogram and the Y-axis is the actual freedom from cancer for our subjects. The dashed line represents the performance of an ideal nomogram, in which predicted outcome perfectly corresponds with actual outcome. Our hybrid nomogram performance after adjusting the over-fitting bias with bootstrap is plotted as the solid line. Note that, because the solid line is relatively close to the dashed reference line, the predictions calculated with the use of our hybrid nomogram approximate the actual outcomes. In general, the performance of the hybrid nomogram appears to be within 10% of actual outcome, and possibly slightly more accurate at very high levels of predicted probability.

We also applied decision curve analysis to measure the performance of our hybrid nomogram for BCa (Figure 4). We tested the theoretical net benefits of all actions in a range of threshold probabilities for BCa. Basically, the net benefit is measuring how our action can affect the examined relative value of false positives and false negatives, which is, when our hybrid nomogram is compared to cystoscopy and biopsy. The decision curve analyses

showed that the hybrid nomogram performed better than demographic data alone above the risk threshold of 6% as well as biomarker data alone above 24% to 88%.

DISCUSSION

Predictive and prognostic nomograms in bladder cancer have been published in both nonmuscle-invasive [24] and muscle-invasive bladder cancer [25, 26]. Specifically, non-muscle-invasive nomograms of precystoscopy urinary levels of NMP22 improved the ability of age, gender and VUC to predict tumor stage and grade as well as tumor recurrence [24]. While in muscle-invasive nomograms, precystectomy clinical and pathologic factors pT and pN stages at the time of cystectomy [25] and to estimate the probabilities of recurrence and all-cause and bladder cancer-specific survival [25, 26] after cystectomy.

To the best of our knowledge, this is the first study to evaluate and internal validate a BCa diagnostic nomogram composed of pertinent demographic features and our BCa-associated diagnostic signature. Previously, we have reported and confirmed in voided urines our BCa-associated diagnostic signature comprised of 10 biomarkers in three separate studies [13–15]. The first study was a case-control study of 127 patients (64 tumor bearing subjects) in which we reported a sensitivity of 92% and specificity 97%, significantly outperforming voided urinary cytology [13]. Subsequently, in another case-control study, we tested the BCa diagnostic signature in 308 patients (102 tumor bearing subjects and 206 subjects with varying urological disorders, *e.g.*, urolithiasis, gross hematuria, urinary tract infection, moderate to severe voiding symptoms), recording a sensitivity of 74% and specificity of 90%, which outperformed voided urinary cytology and the UroVysion® cytogenetic test [14]. Recently, we published a multicenter, international case control study of 320 patients (183 tumor bearing subjects) and demonstrated continued diagnostic performance with a sensitivity of 79% and a specificity of 79% [15].

The gold standard for initial clinical diagnosis and staging of BCa involves cystoscopic examination of the bladder together with cytologic examination for malignant cells in the urine. Cystoscopy is an unpleasant invasive procedure, which may involve anesthetizing the patient and resection of biopsies for histopathological diagnosis and staging. Cystoscopy may also have certain side effects such as urinary tract infection, voiding symptoms and stenosis of the urethra. Voided urine cytology (VUC) remains the method of choice for the noninvasive detection of bladder cancer, with its main use being to recognize the presence of recurrence and early progression in stage and grade. VUC can be used to diagnose new malignancy, yet while it has a specificity of >93%, its sensitivity is only 25–40%, especially for low-grade and low-stage tumors [4, 27, 28]. Thus, current methods to non-invasively detect BCa leave much to be desired. The inadequate power of these single markers must partly explain this. The concept that the presence or absence of one molecular marker will aid diagnostic or prognostic evaluation has not proved to be the case. A number of molecular signatures have been derived and are being made commercially available as clinical assays, especially in the breast cancer field [29, 30]. We have employed a range of genomic [12, 31] and proteomic [11, 32] profiling approaches to study voided urine samples in hopes of identifying a unique, yet accurate, molecular signature associated with BCa.

In biomarker research, though a variable maybe statistically significant in a multivariate model, it does not necessarily equate to the biomarker improving the model's predictive accuracy. For example, a biomarker with an odds ratio of 3 may be a poor classifier and thus an odds ratio of 10 or more may be required. In addition, a single measure of association such as an odds ratio may not meaningfully describe a biomarker's ability to risk classify patients [33]. Thus, it is critical to determine if the addition of biomarker(s) to an existing clinical and pathologic model possesses the ability to improve the predictive accuracy of this model. The accuracy of the hybrid nomogram improved to 0.89 [95% CI: 0.86–0.92] compared to key demographic model (0.81 [95% CI: 0.76–0.85], $p = 5.886\text{e-}8$) and biomarker model (0.84 [95% CI: 0.80–0.87], $p < 7.707\text{e-}5$) (Fig. 2). In general, the performance of the hybrid nomogram appears to be within 10% of actual outcome, and possibly slightly more accurate at very high levels of predicted probability. We also applied decision curve analysis to measure the performance of our hybrid nomogram for BCa. The decision curve analyses showed that the hybrid nomogram performed better than demographic data alone above the risk threshold of 6% as well as biomarker data alone above 24% to 88%.

The main clinical utility of a hybrid nomogram in the described setting is to facilitate the decision on whether a patient requires cystoscopy with subsequent bladder biopsy. The hybrid nomogram would provide a probability of harboring BCa. For example if the probability of harboring BCa is $< 10\%$ perhaps the patient and physician would forego an invasive procedure. However if the risk was substantial (*i.e.*, $> 70\%$) then mostly likely the patient would be compelled to undergo confirmative diagnostic procedure. In the absence of definitive risk thresholds, it would be important to provide a range of threshold probabilities (Table 2).

We acknowledge that this study is limited due to its retrospective design, to the analysis of banked urine samples collected from high volume centers and so may not be representative of the general population at risk for BCa and to limitations of available data (*e.g.*, tobacco history). Nevertheless, this cohort reflects a contemporary cohort of BCa patients, which enabled the derivation of a hybrid nomogram for testing in larger, more diverse prospective studies.

In this study, we developed a hybrid nomogram that facilitates the accurate prediction of the probability of a patient harboring BCa. The hybrid nomogram has been constructed by combining readily available key demographic factors with key biomarker data. If such a nomogram is proven to be reliable, adoption may assist the physician and patient in deciding whether or not further evaluation is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

None

Financial Support

This work was supported by research grants from Weinman Foundation Fund (C.J. Rosser), R01 CA198887 (C.J. Rosser), 5P30CA0717890-6071 (C.J. Rosser), R44 CA173921 (S. Goodison) and RO1 CA116161 (S. Goodison), K01 ES025434 (L.X. Garmire) and P20 GM103457 (L.X. Garmire).

Drs. Rosser and Goodison are officers at Nonagen BioScience Corp and hold a patent related to this work (US patent # 9249467).

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin.* 2012; 62:10–29. [PubMed: 22237781]
2. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011; 61:69–90. [PubMed: 21296855]
3. Hall MC, Chang SS, Dalbagni G, Pruthi RS, Seigne JD, Skinner EC, et al. Guideline for the management of nonmuscle invasive bladder cancer (stages Ta, T1, and Tis): 2007 update. *J Urol.* 2007; 178:2314–2330. [PubMed: 17993339]
4. vanRhijn BW, van der Poel HG, van der Kwast TH. Urine markers for bladder cancer surveillance: a systematic review. *Eur Urol.* 2005; 47:736–748. [PubMed: 15925067]
5. Chen CL, Lin TS, Tsai CH, Wu CC, Chung T, Chien KY, et al. Identification of potential bladder cancer markers in urine by abundant-protein depletion coupled with quantitative proteomics. *J Proteomics.* 2013 Jun 24;85:28–43. [PubMed: 23631828]
6. Aaboe M, Marcussen N, Jensen KM, Thykjaer T, Dyrskjøt L, Orntoft TF. Gene expression profiling of noninvasive primary urothelial tumours using microarrays. *Br J Cancer.* 2005; 14:1182–1190.
7. Holyoake A, O'Sullivan P, Pollock R, Best T, Watanabe J, Kajita Y, et al. Development of a multiplex RNA urine test for the detection and stratification of transitional cell carcinoma of the bladder. *Clin Cancer Res.* 2008; 14:742–749. [PubMed: 18245534]
8. Hanke M, Kausch I, Dahmen G, Jocham D, Warnecke JM. Detailed technical analysis of urine RNA-based tumor diagnostics reveals ETS2/urokinase plasminogen activator to be a novel marker for bladder cancer. *Clin Chem.* 2007; 53:2070–2077. [PubMed: 17921261]
9. Mengual L, Burset M, Ribal MJ, Ars E, Marin-Aguilera M, Fernandez M, et al. Gene expression signature in urine for diagnosing and assessing aggressiveness of bladder urothelial carcinoma. *Clin Cancer Res.* 2010; 16:2624–2633. [PubMed: 20406841]
10. Bartoletti R, Cai T, Dal Canto M, Boddi V, Nesi G, Piazzini M. Multiplex polymerase chain reaction for microsatellite analysis of urine sediment cells: a rapid and inexpensive method for diagnosing and monitoring superficial transitional bladder cell carcinoma. *J Urol.* 2006; 175:2032–2037. [PubMed: 16697794]
11. Yang N, Feng S, Shedden K, Xie X, Liu Y, Rosser CJ, et al. Urinary Glycoprotein Biomarker Discovery for Bladder Cancer Detection Using LC/MS-MS and Label-Free Quantification. *Clin Cancer Res.* 2011; 17:3349–3359. [PubMed: 21459797]
12. Urquidi V, Goodison S, Cai Y, Sun Y, Rosser CJ. A Candidate Molecular Biomarker Panel for the Detection of Bladder Cancer. *Cancer Epidemiol Biomarkers Prev.* 2012; 21:2149–2158. [PubMed: 23097579]
13. Goodison S, Chang M, Dai Y, Urquidi V, Rosser CJ. A multi-analyte assay for the non-invasive detection of bladder cancer. *PLoS One.* 2012; 7(10)
14. Rosser CJ, Ross S, Chang M, Dai Y, Mengual L, Zhang G, et al. Multiplex Protein Signature for the Detection of Bladder Cancer in Voided Urine Samples. *J Urol.* 2013; 190:2257–2262. [PubMed: 23764080]
15. Chen LM, Chang M, Dai Y, Chai KX, Dyrskjøt L, Sanchez-Carbayo M, et al. External validation of a multiplex urinary protein panel for the detection of bladder cancer in a multicenter cohort. *Cancer Epidemiol Biomarkers Prev.* 2014; 23:1804–1812. [PubMed: 24920641]
16. Montironi R, Lopez-Beltran A. The 2004 WHO classification of bladder tumors: a summary and commentary. *Int J Surg Pathol.* 2005; 13:143–153. [PubMed: 15864376]

17. Greene, FL. American Joint Committee on Cancer, American Cancer Society. AJCC Cancer Staging Manual. New York: Springer-Verlag; 2002.
18. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Stat Med*. 1996; 15:361–387. [PubMed: 8668867]
19. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology*. 1982; 143:29–36. [PubMed: 7063747]
20. Harrell FE, Califf RM, Pryor DB, Lee KL, Rosati RA. Evaluating the yield of medical tests. *JAMA: The Journal of the American Medical Association*. 1982; 247:2543–2546. [PubMed: 7069920]
21. Davison, AC.; Hinkley, DV. Bootstrap Methods and Their Application. Cambridge University Press; 1997.
22. Harrell, FE, Jr. Regression Modeling Strategies with Applications to Linear Models, Logistic Regression, and Survival Analysis. New York: Springer Verlag; 2001.
23. Vickers AJ, Elkin EB. Decision curve analysis: A novel method for evaluating prediction models. *Med Decis Making*. 2006; 26:565–574. [PubMed: 17099194]
24. Shariat SF, Zippe C, Ludecke G, Boman H, Sanchez-Carbayo M, Casella R, et al. Nomograms including nuclear matrix protein 22 for prediction of disease recurrence and progression in patients with Ta, T1 or CIS transitional cell carcinoma of the bladder. *J Urol*. 2005; 173:1518–1525. [PubMed: 15821471]
25. International Bladder Cancer Nomogram Consortium. Postoperative nomogram predicting risk of recurrence after radical cystectomy for bladder cancer. *J Clin Oncol*. 2006; 24:3967–3972. [PubMed: 16864855]
26. Shariat SF, Karakiewicz PI, Palapattu GS, Amiel GE, Lotan Y, Rogers CG, et al. Nomograms provide improved accuracy for predicting survival after radical cystectomy. *Clin Cancer Res*. 2006; 12:6663–6667. [PubMed: 17121885]
27. Wiener HG, Vooijs GP, van't Hof-Grootenboer B. Accuracy of urinary cytology in the diagnosis of primary and recurrent bladder cancer. *ActaCytol*. 1993; 37:163–169.
28. Rife CC, Farrow GM, Utz DC. Urine cytology of transitional cell neoplasms. *UrolClin North Am*. 1979; 6:599–612.
29. Nguyen B, Cusumano PG, Deck K, Kerlin D, Garcia AA, Barone JL, et al. Comparison of Molecular Subtyping with BluePrint, MammaPrint, and TargetPrint to Local Clinical Subtyping in Breast Cancer Patients. *Ann SurgOncol*. 2012; 19:3257–3263.
30. Malo TL, Lipkus I, Wilson T, Han HS, ACS G, Vadaparampil ST. Treatment Choices Based on OncotypeDx in the Breast Oncology Care Setting. *J Cancer Epidemiol*. 2012;941495. [PubMed: 22927848]
31. Rosser CJ, Liu L, Sun Y, Villicana P, McCullers M, Porvasnik S, et al. Bladder Cancer-Associated Gene Expression Signatures Identified by Profiling of Exfoliated Urothelia. *Cancer Epidemiol Biomarkers Prev*. 2009; 18:4444–4453.
32. Kreunin P, Zhao J, Rosser C, Urquidi V, Lubman DM, Goodison S. Bladder cancer associated glycoprotein signatures revealed by urinary proteomic profiling. *Proteome Res*. 2007; 6:2631–2639.
33. Pepe MS, Janes H, Longton G, Leisenring W, Newcomb P. Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. *Am J Epidemiol*. 2004; 159:882–890. [PubMed: 15105181]

Figure 1. Diagnostic nomogram for predicting bladder cancer

Locate the patient's age on the age axis. Draw a straight line up to the point's axis to determine how many points toward predicting bladder cancer the patient should receive. Repeat this process for each of the remaining axes, drawing a straight line each time to the point's axis. Sum the points received for each predictive variable and locate this number on the total point's axis. Then draw a straight line down from the total points to the predicted risk score, which depicts the risk the patient has of harboring bladder cancer.

Figure 2.
Receiver operating characteristic (ROC) curves for key demographic data, key biomarker data, and the combination of both for predicting the presence of bladder cancer.

Figure 3. Calibration of the hybrid nomogram for bladder cancer

Dashed line is reference line where an ideal nomogram would lie. Dotted line is the performance of hybrid nomogram, while the solid line corrects for any bias in hybrid nomogram.

Figure 4. Decision curve analysis of hybrid nomogram

The Y-axis represents the net benefit, which is calculated by summing the benefits (gaining true positives) and subtracting weighted harms (deleting false positives). A model is of clinical value if it has the highest net benefit.

Table 1
Multivariate Logistic Regression Analysis of Factors Associated with Bladder Cancer

Factor	Frequency Distribution						OR	95% CI	p
	Case		Controls						
	No.	%	No.	%					
Age, years									
55	59	15%	114	39%	0.27	(0.19,0.39)	1.26e-12		
56-74	209	53%	126	43.2%	1.49	(1.10,2.02)	0.01319		
75	125	31.7%	51	17.5%	2.20	(1.52,3.18)	9.441e-11		
NA	1	0.3%	1	0.3%					
Ethnicity									
Caucasian	365	92.64%	193	66.1%	6.46	(4.12,10.12)	2.676e-18		
African-American /Other	29	7.36%	99	33.9%	0.15	(0.10, 0.24)			
Sex									
Male	326	82.7%	232	79.4%	1.24	(0.84, 1.82)	0.3201		
Female	68	17.3%	60	20.6%	0.81	(0.55, 1.19)			
Tobacco history									
Absent	75	19%	108	37%	3.86	(2.56, 5.81)	8.777e-11		
Present	174	44.2%	65	22.3%	0.26	(0.17, 0.39)			
NA	145	36.8%	119	40.7%					
Biomarkers IL-8									
Low	133	33.8%	210	71.9%	0.20	(0.14, 0.28)	1.053e-22		
High	261	66.2%	82	28.1%	5.03	(3.61, 6.99)			
MMP9									
Low	163	41.4%	180	61.6%	0.44	(0.32, 0.60)	2.296e-07		
High	231	58.6%	112	38.4%	2.28	(1.67, 3.10)			
MMP10									
Low	194	49.2%	177	60.6%	0.63	(0.46, 0.86)	3.985e-03		
High	200	50.8%	115	39.4%	1.59	(1.17, 2.16)			

Factor	Frequency Distribution						OR	95% CI	p
	Case		Controls						
	No.	%	No.	%					
VEGF									
Low	148	37.6%	195	66.8%	0.30	(0.22, 0.41)	6.876e-14		
High	246	62.4%	97	33.2%	3.34	(2.43, 4.59)			
CA9									
Low	180	45.7%	163	55.8%	0.67	(0.49, 0.90)	0.01083		
High	214	54.3%	129	44.2%	1.50	(1.11, 2.04)			
APOE									
Low	168	42.6%	175	59.9%	0.50	(0.37, 0.68)	1.075e-05		
High	226	57.4%	117	40.1%	2.01	(1.48, 2.74)			
AIAT									
Low	141	35.8%	202	69.2%	0.25	(0.18, 0.34)	1.023e-17		
High	253	64.2%	90	30.8%	4.03	(2.92, 5.56)			
ANG									
Low	165	41.9%	178	61%	0.46	(0.34, 0.63)	1.146e-06		
High	229	58.1%	114	39%	2.17	(1.59, 2.95)			
Syndecan									
Low	168	42.6%	175	59.9%	0.50	(0.37, 0.68)	1.075e-05		
High	226	57.4%	117	40.1%	2.01	(1.48, 2.74)			
PAII									
Low	174	44.2%	169	57.9%	0.58	(0.42, 0.78)	5.112e-04		
High	220	55.8%	123	42.1%	1.74	(1.28, 2.36)			

Table 2

Sensitivity, specificity, PPV, NPV for a range of probability for detecting bladder cancer

Nomogram Probability (%)	Sensitivity (%)	Specificity	PPV (%)	NPV (%)
Test Characteristics for Predicting Any Bladder Cancer				
10	0.283	1.000	1.000	0.668
15	0.376	0.988	0.956	0.695
25	0.503	0.952	0.879	0.734
40	0.659	0.912	0.838	0.794
50	0.775	0.863	0.798	0.846
75	0.902	0.639	0.634	0.903