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Synergic effects of cancer stem cells markers, CD44 and embryonic stem cell transcription factor Nanog, on bladder cancer prognosis

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ABSTRACT

Background: Therapy that targets cancer stem cells has the potential to eradicate cancer and prevent tumour recurrence. Therefore, we hypothesized the combined prognostic significance of stem cell markers CD44 (prevalent in basal layer of urothelial carcinoma) and Nanog (embryonic stem cell transcription factor) in bladder cancer.

Material and Methods: CD44 and Nanog expression were determined by immunohistochemistry in 112 bladder cancer cases of which 79 were non-muscle invasive and 33 muscle invasive.

Results: A significant correlation was found between CD44 and Nanog expression ($r = 0.41$, $p < 0.001$). The bladder cancer patients with high CD44 and Nanog expression had poor recurrence-free survival and poor overall survival (all $p < 0.01$). Multivariate Cox regression analysis identified lymph node positivity (hazard ratio; HR 3.81, 95% confidence interval; CI 1.66–8.75), CD44 (HR/95%CI 7.03 [3.04–16.22]) and Nanog (HR/95%CI 2.89 [1.23–6.77]) as independent prognostic biomarkers for recurrence-free survival, whilst a combined index of CD44 and Nanog expression (high expression group; HR/95%CI 25.45 [6.71-96.50] for recurrence-free survival) and lymph node positivity (HR/95%CI 3.68 [1.63-8.33] for recurrence-free survival) were independent prognostic biomarkers for recurrence-free survival and overall survival (all $p < 0.001$).

Conclusions: A combined index of CD44 and Nanog expression is a promising prognostic predictor of recurrence-free survival and overall survival in bladder cancer. It may help identification of patients who will benefit from intensive treatment.

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CD44; Nanog; bladder cancer; cancer stem cell; recurrence; prognosis

Introduction

Bladder cancer is present in 3% of both sexes worldwide, and is characterized by a poor prognosis with a 5-year recurrence-free survival rate of 58–80% post-treatment [1,2]. The high incidence of post-treatment recurrence contributes to the economic burden as well as poor clinical outcome of the patients [3]. Therefore, prognostic biomarkers are required to predict the risk of recurrence for the development of personalized therapeutic strategies. Accumulating evidence has led to the discovery of a subset of a tumour population known as cancer stem cells, with characteristics of self-proliferation and self-renewal in addition to chemotherapy resistance, suggesting they may be candidates for the evaluation of prognosis of tumour recurrence post-treatment [4–6].

Animal data indicate that the basal layer of urothelium hosts bladder stem cells, revealed on staining with bromodeoxyuridine, where 9% of stained basal cells had longer life span and low proliferation competence [7]. Moreover, bladder stem cells at different tumour locations are linked to poor recurrence-free survival [8]. Cluster of differentiation 44 (CD44) is a cancer stem cell marker ubiquitously expressed in the basal layer of the bladder, interacting with extracellular matrix protein such as hyaluronan and promoting stem cell-like properties [9]. The stem cell properties of CD44 are enhanced by its interaction with embryonic stem cell transcription factor Nanog [6]. Nanog is one of the most important pluripotency regulating factors which is encoded by *NANOG1*, its upregulation in a number of solid tumours promotes poor prognosis [10]. The reduced expression of Nanog inhibits multidrug resistance protein 1 gene expression and causes efflux of chemotherapy drugs in ovarian (SK-OV-3) and breast (MCF-7) cancer cell lines [6]. $CD44^+$ cells have the unique tendency to express stem cell markers Oct-4, Nanog and Sox-2. The presence of these proteins acts on cancer stem cells and causes epithelial to mesenchymal transition [11].

Therefore, both cancer stem cells markers CD44 and Nanog predict poor clinical outcomes in cancer patients. A significant association was found with the co-expression of $CD44^{++}$/$Nanog^{++}$ and poor overall survival in tongue squamous cell carcinoma [12]. Human bladder tumour initiating cells subpopulation was analysed for $CD44^+$ expression, and only 36% of the total population showed CD44 expression

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whereas, in another study, positive Nanog expression was detected in all 100 bladder cancer cases with high expression in 85% of the cases [13,14]. However, the combination of CD44 and Nanog expression profile has not been fully studied in bladder cancer patients. Therefore, we hypothesized that different or equal expression profiles of cancer stem cell markers, CD44 and Nanog, in an individual has an impact on bladder tumour recurrence post-treatment. To test this hypothesis we developed a CD44/Nanog index as a marker for evaluation of combined effect of these markers on bladder cancer prognosis.

Material and methods

The study included 112 patients with histopathologically proven bladder cancer from September 2014 till February 2015. Fresh tissue samples were collected from the Department of Surgery, Era's Lucknow Medical College and Hospital (ELMC&H), and the Department of Urology, King George's Medical University, Lucknow, India. All the tissue samples were fixed in 10% formalin for the preparation of Formalin-fixed paraffin-embedded (FFPE) blocks. The samples were obtained prior to any treatment. Tumour staging and grading were performed in accordance to the American Joint Committee on Cancer/TNM 2010 stage and the World Health Organization/International Society of Urological Pathology 2004 grading system, respectively.

A written consent was obtained prior to enrolment in the study, and the Institutional Ethics Committee, ELMC&H; Lucknow, India approved the study procedure. The study was performed in accordance with the Declaration of Helsinki.

Bladder cancer comprises two pathophysiological sub-types non-muscle invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC), and majority of patients experience recurrence despite treatment. Patients with intermediate and high risk NMIBC (having tumour size ≥3 cm and/or high grade and/or multiple tumours) were treated with Bacillus Calmette Gurein (n = 59) for 6 weeks after 3 to 4 weeks of transurethral resection of bladder tumour (TURBT) while the patients with low-risk NMIBC (having tumour size <3 cm and/or, low grade and/or single tumour) underwent TURBT alone (n = 20). Patients with MIBC were treated with radical cystectomy with adjuvant chemotherapy (gemcitabine and cisplatin, n = 16) and without adjuvant chemotherapy (n = 17).

The follow-up procedure for NMIBC patients included cystoscopic surveillance every 3 months for 2 years, and every 6 months thereafter till the designated time period of the study. For MIBC patients who underwent radical cystectomy and had T2, N0 disease renal ultrasound and computed tomography/magnetic resonance imaging (CT/MRI) were performed every 6 months for the first 2 years and annually thereafter along with an annual bone scan. While patients with ≥T3 or N + disease had CT/MRI every 3 months for the first 2 years and annually thereafter along with a bone scan at every 6 months.

Primary antibodies targeting CD44 (BioGenex, CA, USA) and Nanog (Proteintech, USA) were used. All FFPE cut sections used were 3–5 µm thick and fixed on poly-L-lysine coated glass slides which were dried at 60°C for 1 h. The sections were de-paraffinized in xylene followed by rehydration in gradient alcohol (100%, 70%, and 50%) each for 3 min. Microwave antigen retrieval procedure was performed using antigen retrieval buffer (EnVisionTM FLEX, TARGET RETRIEVAL SOLUTION, HIGH pH {50X}, DAKO) pH 9, EnVision™ FLEX, Dako). Tissues were washed with Tris buffer (pH 7.4) and hydrogen peroxide was used to block non-specific peroxidase reactions. Sections were incubated with CD44 and Nanog for 120 minutes (at room temperature) and overnight (at 4 degrees C) respectively. After three washes with Tris buffer, each slide was incubated with secondary antibody (EnVision™ FLEX/HRP, DAKO) for 30 min., followed by three washes with Tris buffer. Finally, each slide was incubated for 5 min in chromagen (3,3′-diaminobenzidine; DAB) to develop a brown colour stain followed by Tris washing. Haematoxylin was used for counterstaining.

CD44 staining was generally membranous. The final scoring was obtained by multiplying percentage of positive cells (0 = none, 1 = 1–9% of stained cells, 2 = 10–50% of stained cells, 3 = 51–80% of stained cells and 4 = 81–100%) with the intensity of staining (0 = none, 1 = weak, 2 = moderate and 3 = strong). CD44 expression was categorized into high and low based on the findings, i.e. 0–8 was defined as low CD44 expression while 9–12 was defined as high CD44 expression [15]. Nanog staining was nuclear and cytoplasmic. Final scoring was calculated by the same formula as CD44 expression but the score for positive percent cells was assigned 0 = none, 1 = 1–9%, 2 = 10–50% and 3 = more than 50%. The value of 0–4 was considered as low expression while 6–9 as high expression [14]. Examination of slides was done by two independent observers, who were unaware of the clinical findings. The CD44/Nanog index classification was done into three groups: low expression group (n = 38) included patients with both low CD44 and low Nanog; intermediate expression group (n = 34) comprised patients with low CD44 and high Nanog or high CD44 and low Nanog, while high expression group (n = 40) had high CD44 and high Nanog expression.

Data analysis was done by SPSS software (version 20; IBM, US). Sample size was calculated on the basis of hazard ratio (HR) of CD44 [16], giving a final sample size of 112. The Pearson's chi-square test was performed to find association between CD44 and Nanog with clinical parameters. The correlation was determined by Spearman's method. Data are presented as

mean with standard deviation [SD]. The overall survival and recurrence-free survival was determined by the Kaplan–Meier method using log-rank test. The overall survival was calculated from the date of treatment till death or the completion of follow-up while recurrence-free survival was calculated from the date of treatment till the first recurrence event or death. The significant prognostic variables in univariate analysis were built-in multivariate Cox proportional hazard regression model. In this instance, significance is present as hazard ratio (HR) with 95% confidence interval (CI). All analysis was two-sided and $p < 0.05$ was considered to indicate a statistically significant difference.

Results

The mean [SD] age of the patients was 58.6 [11.2] years. Of the 112 patients, 70.5% had NMIBC (Ta-T1), 40.5% were high-risk tumours, 34.2% were intermediate-risk tumours, and 25.3% were low-risk tumours. Fifty-eight per cent of the tumours were high grade, and 41.9% tumours were low grade. Tumour size was 3.52 [1.52] cm in the longest diameter: 36.6% had tumour size <3 cm, whereas 63.3% had ≥3 cm. Positive urine cytology was found in 53.8% of high-grade tumours. Lymph node positivity was present in 12.5% of patients.

Positive CD44 staining was observed in 90.2% of the cases of which low CD44 expression (Figure 1(b)) was present in 45.6% while high CD44 (Figure 1(c)) expression was present in 44.6%. The expression was predominantly on the cell membrane in addition to basal cells. Among 101 CD44 positively stained specimens strong intensity staining was observed in 51.5%, moderate staining in 26.7% and weak staining patterns in 21.8%. Associations of CD44 with clinical parameters are shown in Table 1. Nanog positive staining was observed in 95.5% of the cases with 38.4% low Nanog expression (Figure 1(e)) and 44.6% high Nanog expression (Figure 1(f)).

Statistical analysis between Nanog expression and clinical parameters are shown in Table 1. Nanog positive nuclear and cytoplasmic staining was observed in 50.5%, cytoplasmic localization in 43.9% and nuclear expression in 5.6%. Among 107 Nanog positive cases, 19.6% were weakly stained, 29% were moderately stained while 51.4% were strongly stained. CD44 and Nanog expression were strongly correlated ($r = 0.41$, $p < 0.001$).

Kaplan-Meier survival analysis found that high CD44 expression was significantly associated with a decrease in mean survival and cumulative survival at 4 years in both overall survival ($p < 0.001$) and recurrence-free survival (Figure 2(a); $p < 0.001$). Patients with low CD44 expression had a mean overall survival of 46.2 months (95%CI 44.6–47.7) and a mean recurrence-free survival of 44 months (95%CI 41.4–46.6), while those with high CD44 expression had a mean overall survival of 37.9 months (95%CI 34.5–41.3) and a mean recurrence-free survival of 22.5 months (95%CI 18.6–26.4). NMIBC patients with high CD44 expression had a higher mean recurrence-free survival of 25.8 months than in MIBC at 16.3 months ($p < 0.001$).

High Nanog expression was linked to shorter overall survival ($p = 0.004$) and recurrence-free survival (Figure 2(b); $p < 0.001$) at 4 years. The mean overall

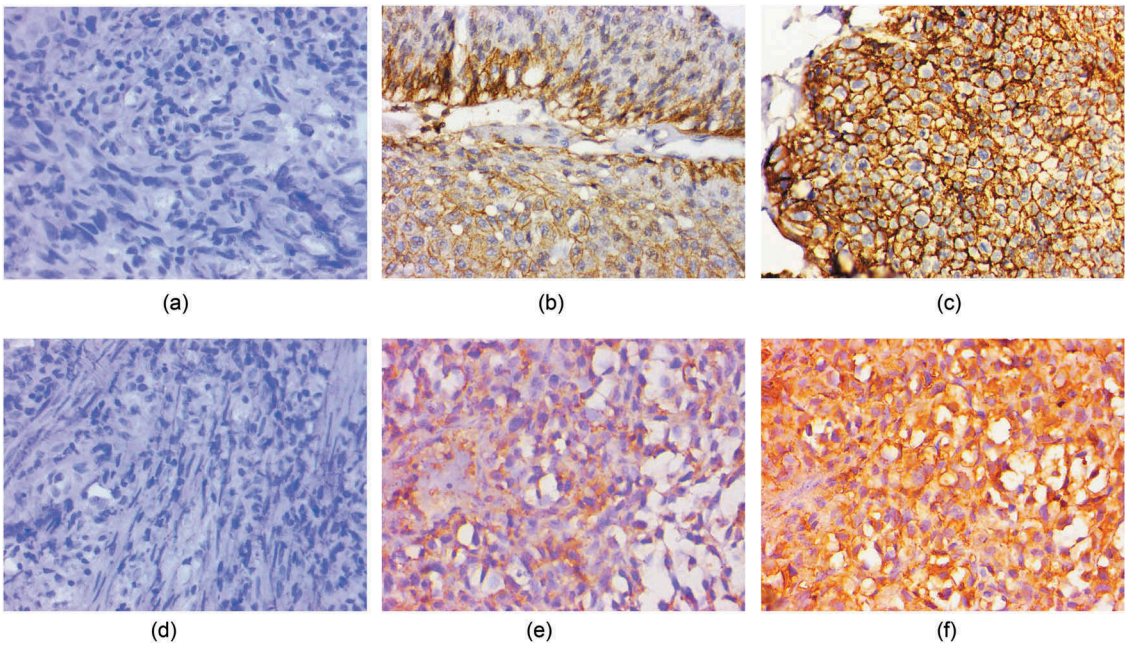

Figure 1. Evaluation of cancer stem cell markers in bladder cancer. (a) Negative control for CD44 (IHC, 400X), (b) Low expression of CD44 membranous staining (IHC, 400X) (c) High expression of CD44 membranous staining (IHC, 400X) (d) Negative control for Nanog (IHC, 400X) (e) Low expression of Nanog cytoplasmic staining (IHC, 400X) (f) High expression of Nanog cytoplasmic staining (IHC, 400X).

Table 1. Association of UBC patient's clinical parameter with the status of CD44 and Nanog expression.

	CD44 expression			NANOG expression		
	Low	High		Low	High	
Clinical parameters	n = 62	n = 50	p-value	n = 48	n = 64	p-value
Age						
<50 years	15	9	0.268	12	12	0.102
50–70 years	41	31		33	39	
>70 years	6	10		3	13	
Gender						
Male	49	40	0.9	39	50	0.685
Female	13	10		9	14	
Tumour grade						
Low	39	8	<0.001	30	17	<0.001
High	23	42		18	47	
Tumour stage						
Ta-T1	47	32	0.173	35	44	0.632
T2-T4	15	18		13	20	
Tumour Size						
<3 cm	25	16	0.363	20	21	0.336
≥3 cm	37	34		28	43	
Type of tumour growth						
Solitary	42	23	0.02	30	35	0.407
Multiple	20	27		18	29	
Tumour location						
Posterior wall	13	13	0.019	9	17	0.002
Trigone	13	23		8	28	
Left wall	12	7		12	7	
Right wall	9	4		10	3	
Anterior wall	5	2		2	5	
Dome	10	1		7	4	
Lymph node status						
N–	59	39	0.006	46	52	0.021
N+	3	11		2	12	
Urine cytology						
Negative	41	22	0.019	35	28	0.002
Positive	21	28		13	36	
Treatment Modality						
Non-BCG	18	2	0.005	13	7	0.057
BCG	29	30		22	37	
RC with adjuvant therapy	6	10		4	12	
RC without adjuvant therapy	9	8		9	8	
Tumour recurrence						
No	51	9	<0.001	40	20	<0.001
Yes	11	41		8	44	

BCG: Bacillus Calmette Gurein, RC: Radical cystectomy.

survival was 45.2 months (95%CI 43.0–47.4) and a mean recurrence-free survival was 44.3 months (95%CI 41.4–47.1) for patients with low Nanog expression in contrast to high Nanog expression with a mean overall survival of 40.4 months (95% CI 37.6–43.2) and a mean recurrence-free survival of 27.2 months (95% CI 23.3–31.1). A shorter mean recurrence-free survival was found in MIBC patients with high Nanog expression (20.5 months) than NMIBC (30.4 months) ($p < 0.001$).

The multivariate Cox regression models (Table 2(a)) showed no significant association of overall survival with tumour grade, CD44 and Nanog expression except for lymph node positivity ($p < 0.001$). Therefore, only lymph node status appears to an independent prognostic factor for overall survival. On the contrary, the multivariate Cox regression models (Table 2(a)) for recurrence-free survival showed lymph node status ($p = 0.002$), CD44 ($p < 0.001$) and Nanog ($p = 0.014$) expression were independent prognostic factors. Both the cancer stem cell markers CD44 and Nanog are thus associated with increased risk of bladder tumour recurrence.

The survival analysis in regard of the CD44/Nanog index and overall survival and recurrence-free survival expectancy is as follows. The mean overall survival was 46.7 months (95% CI 44.9–48.4), 43.7 months (95% CI 40.7–46.7) and 37.4 months (95% CI 33.6–41.3) in low expression, intermediate expression and high expression group, respectively ($p < 0.001$). In recurrence-free survival (Figure 2(c)) low expression group had a mean survival of 46.5 months (95%CI 44.0–48.9); intermediate expression group had mean survival of 38.5 months (95%CI 34.0–43.0) while the high expression group had mean survival of 19.5 months (95%CI 15.7–23.4)($p < 0.001$). Furthermore, a multivariate Cox regression analysis (Table 2(b)) showed the CD44/Nanog index to be an independent prognostic factor for both overall survival and recurrence-free survival along with lymph node positivity in bladder cancer patients.

Discussion

The concept of cancer stem cells has been a major breakthrough in the field of oncology, with evidence of their ability to resist chemotherapy, differentiate and self-renew [4]. Thus, we require good cancer stem cells prognostic biomarkers for bladder cancer surveillance and prediction of patient's outcome. For this, we investigated CD44 and Nanog expression profile in bladder cancer and also evaluated their combined significance on bladder cancer recurrence and overall survival. CD44 is an important cell surface molecule which plays an important role in bladder cancer recurrence and progression [17,18]. CD44, upon activation by the IL6/STAT3 signalling pathway, promotes tumour invasion and epithelial to mesenchymal transition, whilst IL-6 urinary levels were significantly elevated in $CD44^+$ patients [18]. Their study also reported that 52.3% of MIBC expressed CD44 + compared to 32.6% found in our study.

The non-muscle invasive papillary upper tract urothelial carcinoma exhibits 46.9% of negative CD44 expression in high-grade tumours and was significantly associated with poor progression-free survival [19]. Szarvas et al. analysed expression of *CD44* with tumour stage, grade, muscle invasion, lymph node positivity and recurrence, but found no association, whilst Cox univariate analysis of both *CD44* and CD44 protein expression failed to link to overall survival, disease-specific survival and metastasis-free survival [20]. Others [21] found a highly significant correlation between CD44 expression and pathological features of low-grade tumours, high-grade tumours, carcinoma in situ and invasion [21]. Our present data confirm these findings, with a significant association of high CD44 expression with tumour grade, lymph node

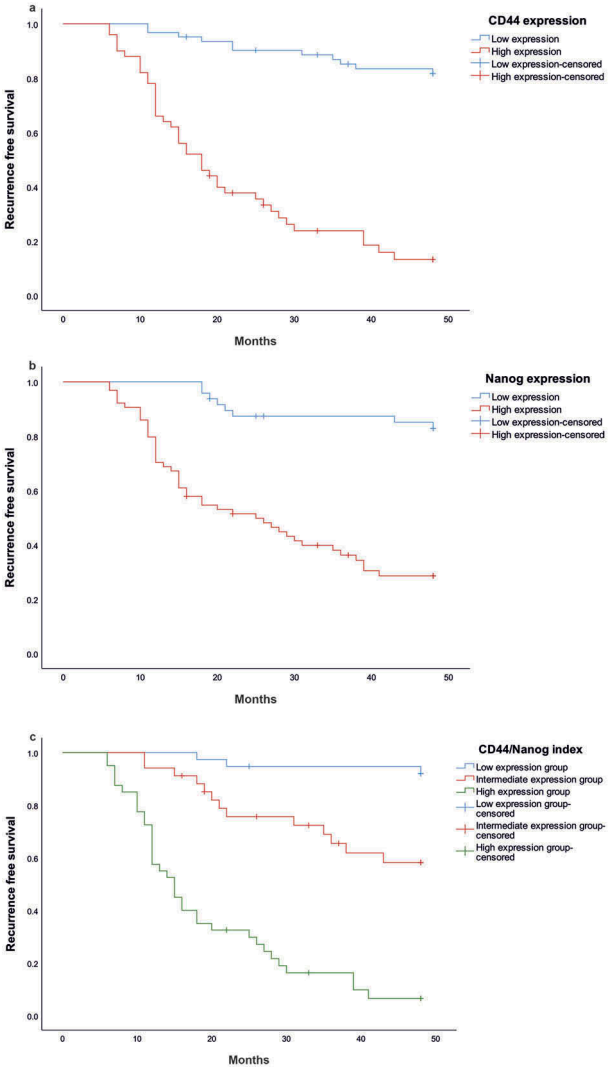

Figure 2. Survival curves of overall cohort panel, n = 112. Kaplan–Meier curves representing recurrence-free survival (RFS) for (a) CD44 (median RFS = 18 months for high CD44 expression), (b) Nanog (median RFS = 25 months for high Nanog expression), and (c) CD44/Nanog index (median RFS = 15 for high expression group).

positivity and recurrence. On analysing tumour recurrence at biopsy, CD44 was linked to an hazard ratio of 2.51 (95% CI: 1.1–5.85) [21]. A meta-analysis of five studies with 417 bladder cancer patients reported high CD44 expression to significantly worsen overall survival (HR/95% CI 2.03 [1.08–3.79]), whilst three studies with 317 bladder cancer patients found the CD44 expression was not linked to overall survival at ≥5 years (HR = 2.34, 95% CI 0.89–2.50) [22]. Others have also failed to link CD44 expression to overall survival [23] or recurrence-free survival [22]. These are in contrast to the significant association between high CD44 expression and poor overall survival along with recurrence-free survival in the existing study. Low CD44 methylation frequency has significantly been linked with recurring NMIBC [24]. CD44 proves to be a predictor of poor disease outcome after treatment in MIBC [25]. The MIBC patients with high CD44 expression in the current study also showed poor survival outcomes post-treatment. The presence of high CD44 splice variant 6, CD47 and integrin beta-4 expression in tumours located at posterior wall had significant poor recurrence-free survival. Moreover, multivariate Cox regression analysis identified high ΔNp63, CD47 and integrin beta-4 expression as independent prognostic biomarkers for tumours located at trigone or posterior wall of bladder [8]. Both high CD44 and Nanog expression were significant with tumours located at trigone of the bladder. High Nanog expression was present in 91.3% high staged bladder tumours (MIBC) in contrast to 71.8% present in low staged bladder tumours (NMIBC) [14]. Higher tumour stages exhibited 60.6% of high Nanog expression while 55.6% were present in low tumour stages in our study. But we found no significant association between high Nanog as well as high CD44 expression with tumour stage. Nanog positive expression was observed in 80% of prostate cancer cases, 90% of bladder cancer cases and 100% of colon cancer cases. On comparing the tumour grades of all the three cancers Amini et al. found 100% Nanog expression in grade 3 [26]. Few studies have reported high Nanog expression to be non-significant with lymph node positivity in bladder cancer but similar to our findings it was observed significantly in tongue squamous cell carcinoma cases [12]. A random model (I^2 = 37%) depicted that high Nanog expression had an unfavourable overall survival (HR 2.19, 95%CI 1.87–2.58) in solid cancers [10]. CD44 and Nanog expression were high in tumour spheres from adenocarcinoma patients at cell membranes and nuclear, respectively, but neither was significantly linked with overall survival [27]. In contrast, high Nanog expression was significantly associated with overall survival in the present study.

We conclude that lymph node status and the intermediate/high CD44/Nanog combined score index are linked to overall survival with roughly similar hazard ratios (4.81, 4.79, 5.62, respectively). However, in predicting recurrence-free survival, the hazard ratio of lymph node involvement (3.68) was inferior to that of the intermediate score (6.12), which was, in turn, inferior to that of the high expression score (25.45). If confirmed, these data may have a role in medical/surgical management of bladder cancer in a different treatment modality to that practised to date and therefore may help facilitate the survival of patients.

This work represents an advance in biomedical science because it shows the combination of CD44 and Nanog staining may be of value for risk stratification and bladder cancer prognosis.

Table 2. (a) Univariate and multivariate Cox regression analysis of associated factors with overall and recurrence-free survival. (b) Univariate and multivariate Cox regression analysis of CD44/Nanog index with overall and recurrence-free survival.

	Overall Survival				Recurrence free survival			
	Univariate Cox		Multivariate Cox		Univariate Cox		Multivariate Cox	
	HR (95% CI)	p-value	HR (95% CI)	p-Value	HR (95% CI)	p-value	HR (95% CI)	p-value
(a)								
Tumour Grade	5.65 (1.97–16.22)	0.001	2.19 (0.67–7.18)	0.195	3.96 (2.03–7.76)	<0.001	0.81 (0.31–2.08)	0.67
Type of tumour growth	1.43 (0.70–2.94)	0.321			1.35 (0.78–2.33)	0.276		
Tumour Location (trigone versus remaining tumour location)	0.93 (0.42–2.03)	0.863			2.69 (1.55–4.65)	<0.001	1.47 (0.79–2.72)	0.215
Lymph node status	7.96 (3.74–16.92)	<0.001	4.38 (1.94–9.85)	<0.001	4.51 (2.27–8.97)	<0.001	3.81 (1.66–8.75)	0.002
Urine cytology	1.86 (0.90–3.84)	0.09			2.49 (1.43–4.34)	0.001	1.81 (0.97–3.37)	0.061
Treatment modality								
Non-BCG	Reference							
BCG	3.18 (0.73–13.79)	0.121			3.02 (1.05–8.62)	0.039	0.94 (0.25–3.43)	0.926
RC with adjuvant therapy	3.64 (0.70–18.78)	0.122			7.02 (2.27–21.67)	0.001	1.63 (0.37–7.02)	0.512
RC without adjuvant therapy	4.23 (0.85–21.00)	0.077			2.62 (0.76–8.96)	0.125	0.63 (0.15–2.66)	0.536
CD44 expression	5.14 (2.20–12.02)	<0.001	2.63 (0.97–7.09)	0.056	9.91 (4.99–19.68)	<0.001	7.03 (3.04–16.22)	<0.001
Nanog expression	3.37 (1.37–8.24)	0.008	1.32 (0.47–3.67)	0.593	6.83 (3.20–14.61)	<0.001	2.89 (1.23–6.77)	0.014
(b)								
Tumour Grade	5.65 (1.97–16.22)	0.001	2.22 (0.70–7.08)	0.175	3.96 (2.03–7.76)	<0.001	0.86 (0.34–2.19)	0.763
Type of tumour growth	1.43 (0.70–2.94)	0.321			1.35 (0.78–2.33)	0.276		
Tumour Location (trigone versus remaining tumour location)	0.93 (0.42–2.03)	0.863			2.69 (1.55–4.65)	<0.001	1.46 (0.79–2.70)	0.223
Lymph node status	7.96 (3.74–16.92)	<0.001	4.81 (2.09–11.05)	<0.001	4.51 (2.27–8.97)	<0.001	3.68 (1.63–8.33)	0.002
Urine cytology	1.86 (0.90–3.84)	0.09			2.49 (1.43–4.34)	0.001	1.78 (0.95–3.31)	0.068
Treatment modality								
Non-BCG	Reference							
BCG	3.18 (0.73–13.79)	0.121			3.02 (1.05–8.62)	0.039	1.03 (0.28–3.73)	0.955
RC with adjuvant therapy	3.64 (0.70–18.78)	0.122			7.02 (2.27–21.67)	0.001	1.74 (0.40–7.46)	0.455
RC without adjuvant therapy	4.23 (0.85–21.00)	0.077			2.62 (0.76–8.96)	0.125	0.73 (0.178–3.06)	0.676
CD44/Nanog index								
Low expression group	Reference							
Intermediate expression group	5.38 (1.16–24.91)	0.031	4.79 (1.01–22.6)	0.048	6.36 (1.81–22.39)	0.004	6.12 (1.68–22.2)	0.006
High expression group	11.59 (2.69–49.84)	0.001	5.62 (1.22–25.91)	0.027	32.52 (9.79–107.99)	<0.001	25.45 (6.71–96.50)	<0.001

BCG: Bacillus Calmette Gurein, RC: Radical cystectomy.

Summary table

What is known about this subject:
- Increased tissue expression of CD44 and Nanog are linked to the prognosis of bladder cancer, although some have failed to confirm this finding.

What this paper adds:
- High CD44 and Nanog expression is significantly associated with poorer bladder cancer prognosis.
  A score combining expression of CD44 and Nanog predicts both bladder cancer survival and recurrence.

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Disclosure statement

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