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Features Article

Association between precystectomy epithelial tumor marker response to neoadjuvant chemotherapy and oncological outcomes in urothelial bladder cancer

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Abstract

Introduction and Objectives: We previously reported that elevated precystectomy serum levels of epithelial tumor markers predict worse oncological outcome in patients with invasive bladder cancer (BC). Herein, we evaluated the effect of neoadjuvant chemotherapy (NAC) on elevated tumor marker levels and their association with oncological outcomes.

Methods: Under IRB approval, serum levels of Carbohydrate Antigen 125 (CA-125), Carbohydrate Antigen 19-9 (CA 19-9) and Carcinoembryonic Antigen (CEA) were prospectively measured in 480 patients with invasive BC from August 2011 through December 2016. In the subgroup undergoing NAC, markers were measured prior to the first and after the last cycle of chemotherapy (prior to cystectomy).

Results: Three hundred and thirty-seven patients were eligible for the study, with a median age was 71 years (range 34–93) and 81% (272) male. Elevated precystectomy level of any tumor markers (31% of patients) was independently associated with worse recurrence-free survival (hazard ratio [HR] = 2.81; $P < 0.001$) and overall survival (HR = 3.97; $P < 0.001$). One hundred and twenty-five (37%) patients underwent NAC, of whom 59 had a complete tumor marker profile and 30 (51%) had an elevated pre-NAC tumor marker. Following completion of chemotherapy, 10/30 (33%) patients normalized their tumor markers, while 20/30 (67%) had one or more persistently elevated markers. There was no difference in clinical or pathological stage between groups ($P = 0.54$ and $P = 0.09$, respectively). Further analysis showed a significantly lower rate and longer median time to recurrence/progression in the responder group (50% in responders vs. 90% in nonresponders at a median time of 22 vs. 4.8 months, respectively; $P = 0.015$). There was also significant difference in mortality rates and median overall survival between the study groups (30% in responders vs. 70% in nonresponders at a median time of 27.3 vs. 11.6 months respectively; $P = 0.037$). Two of the three patients that died in the normalized tumor marker group had tumor marker relapse at recurrence prior to their death.

Conclusions: To our knowledge, this is the first study showing tumor marker response to NAC. Patients with persistently elevated markers following NAC have a very poor prognosis following cystectomy, which may help identifying chemotherapy-resistant tumors. A larger, controlled study with longer follow up is needed to determine their role in predicting survival.

Keywords (MeSH): Bladder cancer; Tumor markers; Neoadjuvant chemotherapy; Oncological outcomes; Prognosis

Abbreviations: TM, tumor markers; RC, radical cystectomy; UBC, urothelial bladder cancer; BC, bladder cancer; TURBT, transurethral resection of bladder tumor; NAC, neoadjuvant chemotherapy; CAMs, cellular adhesion molecules; CA 125, carbohydrate antigen 125; CA 19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen

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1. Introduction

With an incidence of 79,000 new cases per year, bladder cancer (BC) is estimated to cause over 16,000 deaths annually in the US [1]. Approximately 25% to 30% of diagnosed patients have muscle-invasive bladder cancer (MIBC), for which neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) and pelvic lymph node dissection is still considered standard of care [2,3]. Despite this aggressive treatment, nearly one-third of patients have recurrence and subsequent high likelihood of death from disease within five years [3]. This is in large part due to pathologic upstaging to extra-vesical/lymph node-positive disease in approximately 40% of patients, reflecting the current inaccuracy of clinical staging [3]. Therefore, much emphasis has been placed on studying predictors of recurrence and outcome in order to better prognosticate for our patients and direct management of this aggressive disease.

By comparing transurethral resection of bladder tumor (TURBT) specimen with RC pathology, nonresponse to NAC as well as progression on NAC have been associated with inferior survival [4,5]. Thus, just as it is important to find predictors of recurrence and survival, markers of NAC response could also inform patient management and prognostication.

Carbohydrate antigens (CAs) and cellular adhesion molecules (CAMs) (such as CEA) are commonly used serum tumor markers (TM) and have been implicated in epithelial invasion and immune system evasion during malignant transformation of epithelial cells and tumor progression [6–8]. The markers have clinical utility in several surgical subspecialties, including for the detection of early recurrence, and more recently, for evaluation of preoperative chemotherapy response [9–11]. To date, few series have been published on the prognostic role of these markers in invasive BC, with small case numbers and conflicting results [12–16]. We previously demonstrated that elevated precystectomy serum levels of carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) are associated with worse oncological outcome in patients with invasive BC [17]. No studies to date have evaluated the effect of NAC for invasive bladder cancer on these markers.

Therefore, our objectives in this study were to evaluate the effect of NAC on elevated serum tumor marker levels in patients with BC and, second, to evaluate their association with oncological outcomes in patients in the setting of NAC. In doing so, we also examined the predictive role of elevated precystectomy serum levels of epithelial tumor markers on oncological outcome in a prospective fashion.

2. Materials and methods

2.1. Study population

Between August 2011 and December 2016, in this institutional review board–approved prospective single-center study from the Institute of Urology at the University of Southern California, we enrolled all 480 consecutive patients who underwent RC, pelvic lymph node dissection and urinary diversion for invasive BC. The diagnosis of BC was based on pathology from transurethral resection of the bladder tumor.

Exclusion criteria were any history of colon, pancreatic, and ovarian pathologies or those with moderate to severe hydronephrosis (which can lead to false elevation of CA 19-9). Those with nonbladder primary, inoperable disease, distant metastatic disease at diagnosis, incomplete tumor marker profile as well as those with insufficient or no follow-up were also excluded from the study.

2.2. Tumor markers

Serum levels of Carbohydrate Antigen 125 (CA-125), Carbohydrate Antigen 19-9 (CA 19-9) and Carcinoembryonic Antigen (CEA) were measured in all patients before surgery. In the subgroup undergoing NAC, markers were measured prior to the first and after the last cycle of chemotherapy (prior to cystectomy).

Measurements of these markers were performed by electro-chemiluminescent immunoassay method (Roche Cobas 6000 analyzer, Roche Diagnostics, IN). Reference cutoff points from our laboratory were used to define elevated values of the markers (CA 19-9: 0–37 U/ml; CA 125: 0–35 U/ml; and CEA: 0–3.8 ng/ml). Elevation of any of the three markers was classified as elevated marker status, while normal values of all three markers implied a normal profile.

2.3. Data collection, outcome measures, and analysis

Patients' demographics and clinic-pathologic information were collected. Follow up data were obtained from regular clinic visits as well as outside sources where patients were followed outside our institution. Oncological outcomes including recurrence-free survival (RFS) and overall survival (OS) were used as outcome measures. We evaluated the association of tumor markers status and oncological outcomes at three different time points: precystectomy, pre-NAC and following NAC (Fig. 1).

The chi-square test and Student t test were used to compare categorical and continuous variables, respectively. Kaplan-Meier (KM) curves were delineated for the survival analysis, with log-rank test to detect the difference between the curves. Multivariable (MVA) Cox regression analysis was used to identify independent predictors of RFS and OS. Data were analyzed using SAS (version 9, SAS Institute Inc, Cary, NC), and 2-sided $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cohort characteristics

Of 480 patients with invasive BC, a total of 337 patients were eligible for the study. Excluded from the study were

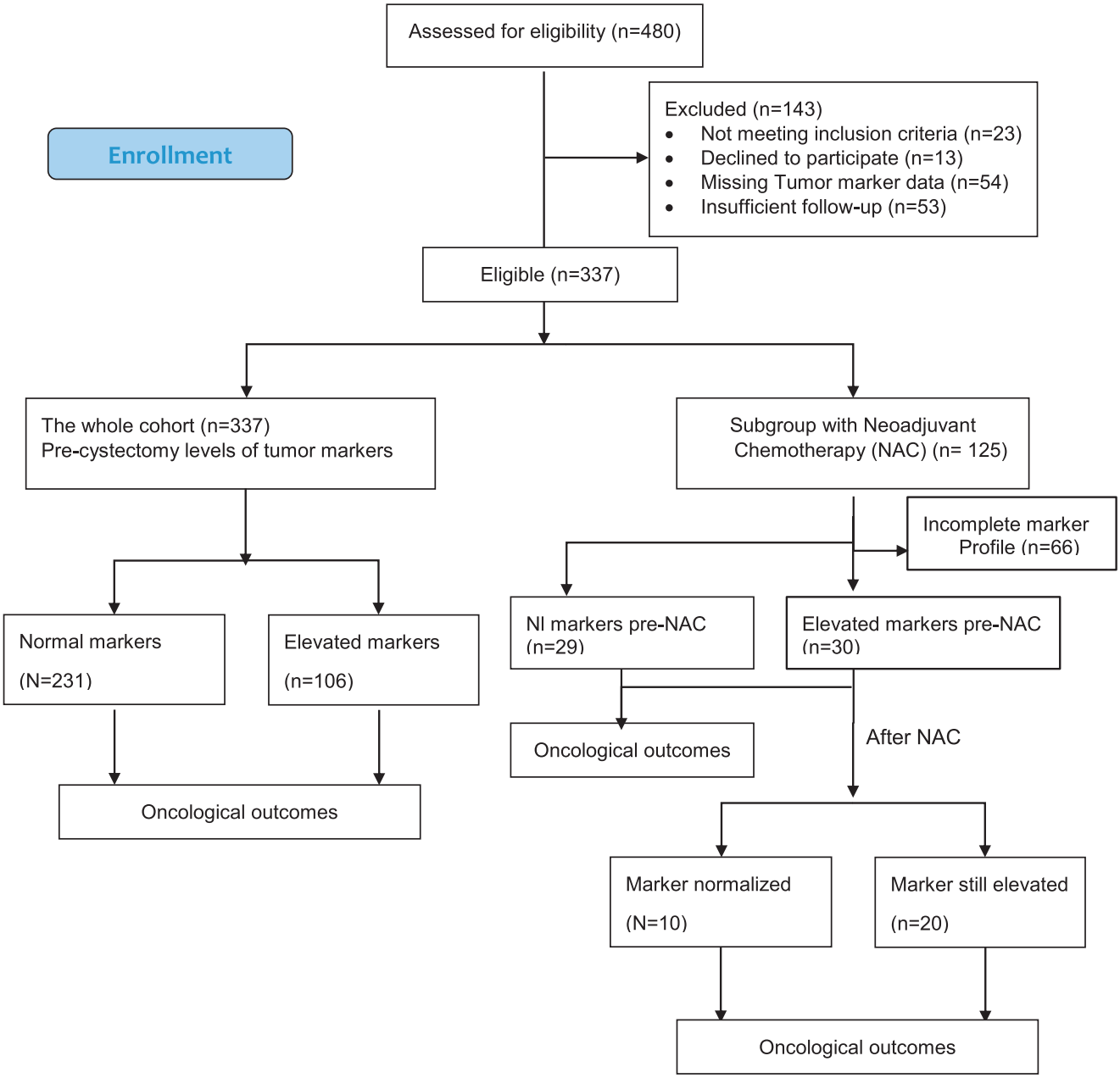

Fig. 1. Flow diagram of the tumor marker cohort of 337 invasive bladder cancer patients.

those with nonbladder primary (5), inoperable disease (5), metastatic disease (13), and those who refused to consent (13) or were missing complete tumor marker evaluation (54). Moreover, 53 patients were excluded from the study due to more recent surgery and insufficient oncologic follow-up. Four patients had concomitant malignancy, including lymphoma, adenocarcinoma of uterus, carcinoid tumor and small-cell tumor of Meckel's diverticulum; these were not excluded due to noninterference with our TM profile. (Fig. 1)

Median age of the cohort was 71 years (range 34-93) and 81% (309) were male. Two hundred and fifty-four (65%) patients had organ-confined disease while 137 (35%) had extravesical disease. Demographic and clinic-pathologic data is shown in Table 1. Table 2 also shows clinic-pathologic characteristics of the subgroup who received neoadjuvant chemotherapy.

3.2. Tumor marker results

Mean precystectomy levels of CA 125, CA 19-9 and CEA were 20 U/ml (range 1.6–563), 32.8 U/ml (range 0.1–1510) and 5.3 U/ml (range 0.2–611), respectively. One hundred and six (31.5%) patients had at least one abnormal precystectomy tumor marker level, including

Table 1
Clinical and pathological characteristics of 337 patients with invasive bladder cancer eligible for tumor marker study.

N = 337	No (%)
Median age (year-range)	71 (34–93)
< 70	144 (43)
> =70	193 (57)
Gender	
Male	272 (81)
Female	65 (19)
Mean BMI (kg/m^2)	27 ± 4.7
Median Charlson comorbidity index	1.0
Clinical stage	
T stage: T0	5 (1)
Tis	26 (8)
Ta	9 (3)
T1	73 (22)
T2	138 (41)
T3	59 (18)
T4	24 (7)
N stage: N0	304 (90)
N1	12 (4)
N2	17 (5)
N3	2 (0.5)
Nx	2 (0.5)
N stage: M0	334 (99)
M1	2 (0.5)
Mx	1 (0.5)
Neoadjuvant chemotherapy	125 (37)
Gemcitabine/Cisplatin	72 (58)
Dose-dense MVAC	25 (20)
Others	28 (22)
Diversion type	
Orthotopic	206 (61)
Continent cutaneous	19 (6)
Ileal conduit	112 (33)
Pathological stage:	
T stage: T0	52 (15)
Tis/Ta	54 (16)
T1	53 (16)
T2	58 (17)
T3	68 (20)
T4	52 (13)
N stage: N0	259 (77)
N1	26 (8)
N2	45 (13)
N3	7 (2)
M stage: M0	326 (97)
M1	11 (3)
Pathologic cell-type	
Urothelial	328 (97)
Squamous	6 (2)
Adeno	1 (< 1)
Sarcoma	3 (1)
Lymphovascular invasion	99 (29)
Adjuvant chemotherapy	38 (11)

24 (7%) for CA 125, 48 (15%) for CA 19-9 and 69 (21%) for CEA.

Of 125 (37%) patients underwent NAC, 59 had a complete tumor marker profile before and after therapy and 30 (51%) had one or more elevated pre-NAC tumor markers. Mean levels of CA 125, CA 19-9 and CEA before NAC were 82.7, 333, 6 U/ml and the mean levels after chemotherapy were 43.3, 121, 7.6 U/ml, respectively.

Table 2
Clinical and pathological characteristics of 125 patients with invasive bladder cancer with neoadjuvant chemotherapy (NAC) and 59 patients with elevated pre-NAC markers

N (%)	NAC total N = 125	NAC TM nl N = 29	NAC TM high N = 30
Clinical T stage			
Tis/Ta	2 (2)*	1 (3)	1 (3)
T1	7 (6)**	1 (3)	0 (0)
T2	57 (46)	13 (45)	5 (17)
T3	41 (33)	9 (31)	13 (43)
T4	16 (13)	5 (18)	11 (37)
Clinical N stage			
N0	99 (79)	26 (90)	20 (66)
N1	9 (7)	1 (3)	2 (7)
N2	15 (12)	2 (7)	6 (20)
N3	2 (2)	0 (0)	2 (7)
Pathologic T stage			
T0	30 (24)	5 (17)	5 (17)
Tis/Ta	19 (15)	9 (31)	0 (0)
T1	15 (12)	3 (10)	1 (4)
T2	17 (14)	6 (21)	2 (7)
T3	20 (16)	2 (7)	10 (34)
T4	24 (19)	4 (14)	11 (38)
Pathologic N stage			
N0	87 (70)	22 (76)	15 (50)
N1	9 (7)	2 (6)	2 (6)
N2	25 (20)	4 (14)	10 (34)
N3	4 (3)	1 (4)	3 (10)
Pathologic M stage			
M0	114 (91)	26 (87)	22 (73)
M1	11 (8)	3 (13)	8 (27)

* One patient received NAC due to involvement of prostatic urethra; Other one participated in a trial of chemo-radiation for Cis.
** Three patients were node-positive; one had prostatic urethral involvement; and the other three received NAC at discussion of outside oncologist.

Following completion of chemotherapy, 10/30 (33%) patients normalized tumor markers, while 20/30 (67%) had one or more persistently elevated marker. There was no significant difference in clinical or pathological stage between groups (P = 0.54 and P = 0.09, respectively). Within the normalized marker group, 8/10 (80%) had complete or partial clinical response and 5/10 (50%) had pathological down-staging, whereas of patients with persistently elevated markers, 9/20 (45%) had some clinical response and only 4/20 (20%) had pathological down-staging. Details of clinic-pathologic characteristics, tumor marker course and oncological outcomes of this subgroup are provided in Table 3.

3.3. Oncological outcome with regard to precystectomy marker levels

Median follow up was 631 days (IQR 162–1156 days). Overall, there were 80 recurrences (40 in normal TM and 40 in elevated TM group) and 66 deaths in the

Table 3
Breakdown of subgroup undergoing neoadjuvant chemotherapy with available tumor markers, in terms of clinical and biomarker response and oncological outcome

Number	NAC regimen	Clinical stage	Pathological stage	Clinical response to neoadjuvant	Preneoadjuvant*			Postneoadjuvant (30 d)			Biomarker response	Survival status	OS days	Recurrence status	RFS days
					CA 125	CA 19−9	CEA	CA 125	CA 19−9	CEA					
1	**GemCis**	**T3b**	**T4aN0M1**	**Yes**	**17.2**	**41.1**	**0.8**	**16.6**	**12.6**	**0.7**	**Y**	**Alive**	**396**	**Recur/Progress**	**379**
2	**DDMVAC**	**T3a**	**T2bN0M0**	**No**	**19.2**	**309.0**	**2.6**	**3.9**	**10.7**	**1.8**	**Y**	**Alive**	**631**	**NED¶**	**631**
3	**GemCis**	**T2a**	**T0N0M0**	**Yes**	**162.3**	**290.8**	**2.7**	**12.1**	**10.5**	**2.6**	**Y**	**Alive**	**657**	**Recurrence**	**624**
4	**GemCis**	**T3b**	**T3aN2M0**	**Yes**	**135.3**	**0.6**	**8.7**	**22.0**	**0.6**	**1.1**	**Y**	**Alive**	**407**	**NED**	**407**
5	**DDMVAC**	**T2a**	**T0N3M0**	**Yes**	**29.7**	**119.7**	**1.5**	**15.5**	**26.6**	**2.1**	**Y**	**Alive**	**680**	**NED**	**680**
6	**GemCis**	**T3b**	**T0N0M0**	**Yes**	**13.3**	**9.0**	**16.1**	**5.4**	**3.5**	**2.5**	**Y**	**Alive**	**661**	**NED**	**661**
7	**GemCis**	**T3b**	**T0N0M0**	**Yes**	**82.0**	**196.0**	**3.4**	**9.7**	**10.5**	**0.7**	**Y**	**DOD§**	**458**	**Recurrence**	**132**
8	**DDMVAC**	**T3a**	**TisN0M0**	**Yes**	**10.7**	**137.0**	**1.7**	**17.8**	**37.0**	**2.8**	**Y**	**Alive**	**1062**	**NED**	**1062**
9	**GemCis; Taxol**	**T2a**	**T3bN2M1**	**Yes: Bladder, PLNs No: Bone mets**	**8.1**	**89.2**	**0.5**	**5.3**	**16.6**	**0.9**	**Y**	**DOD**	**302**	**Recur/Progress**	**101**
10	**DDMVAC**	**T4a**	**T4aN2M1**	**No**	**84.5**	**< 0.6**	**1.9**	**15.5**	**< 0.6**	**1.3**	**Y**	**DOD**	**212**	**Recur/Progress**	**145**
11	**GemCis; MVAC**	**T4b**	**T4bNXM1**	**Yes**	**106.4**	**1238.0**	**6.6**	**59.7**	**34.7**	**0.8**	**N**	**DOD**	**152**	**Recur/Progress**	**73**
12	**GemCis; Carbo**	**T4a**	**T4aN2M0**	**No-progressed**	**138.0**	**61.1**	**3.8**	**494.2**	**587.1**	**4.0**	**N**	**DOD**	**43**	**Recur/Progress**	**43**
13	**ddMVAC**	**T3b**	**T3aNxM1**	**Yes: Bladder No: PLNs**	**3.8**	**203.1**	**4.9**	**2.0**	**184.7**	**6.0**	**N**	**DOD**	**58**	**Recur/Progress**	**58**
14	**GemCis**	**T2a**	**T3aN0M0**	**No progress**	**16.1**	**35.9**	**11.0**	**15.7**	**38.4**	**5.5**	**N**	**DOD**	**190**	**Recurrence**	**120**
15	**Cisplatin**	**T2a**	**T3aN0M0**	**Yes**	**8.5**	**108.1**	**5.7**	**14.0**	**14.8**	**5.8**	**N**	**Alive**	**529**	**Recurrence**	**222**
16	**GemCis**	**T3b**	**T3bN2M0**	**No**	**10.5**	**28.4**	**4.1**	**10.9**	**17.8**	**4.6**	**N**	**Dead**	**41**	**NED**	**41**
17	**GemCis**	**T4a**	**T1N0M0**	**No progress**	**51.7**	**1.0**	**5.2**	**41.7**	**0.6**	**3.5**	**N**	**Alive**	**834**	**Recurrence**	**631**
18	**GemCis; Carbo**	**T4a**	**T3bN1M1**	**Yes- Lung mets No: Bladder**	**23.8**	**31.9**	**4.2**	**63.8**	**33.2**	**10.0**	**N**	**DOD**	**187**	**Recur/Progress**	**36**
19	**DDMVAC**	**T3b**	**T4bNXM0**	**No**	**334.4**	**267.1**	**2.5**	**103.1**	**19.3**	**1.8**	**N**	**Alive**	**591**	**Recur/Progress**	**148**
20	**GemCis**	**T3b**	**T0N0M0**	**Yes**	**8.0**	**64.8**	**6.2**	**8.8**	**57.9**	**5.3**	**N**	**Dead**	**333**	**Recurrence**	**65**
21	**GemCis**	**T4**	**T4bN0M0**	**No**	**26.7**	**207.7**	**5.7**	**14.9**	**160.8**	**3.7**	**N**	**DOD**	**124**	**Recur/Progress**	**56**
22	**DDMVAC**	**T4**	**T4aN2M0**	**Yes**	**745.5**	**4235.0**	**31.3**	**4523.0**	**29410.0**	**958.1**	**N**	**DOD**	**145**	**Recur/Progress**	**139**
23	**GemCis**	**T4a**	**T3bN2M1**	**No**	**27.8**	**51.1**	**3.0**	**66.5**	**25.6**	**3.9**	**N**	**DOD**	**123**	**Recur/Progress**	**123**
24	**GemCis; Taxol**	**T4b**	**T4bN2M1**	**No**	**15.0**	**< 0.6**	**12.4**	**12.0**	**< 0.6**	**27.9**	**N**	**DOD**	**729**	**Recur/Progress**	**102**
25	**GemCis**	**T3b**	**T4aN2M0**	**No**	**41.0**	**177.7**	**5.0**	**45.0**	**1510.0**	**87.7**	**N**	**Dead**	**0**	**-**	**-**
26	**GemCis**	**T3b**	**T2aN3M0**	**Yes**	**12.2**	**369.0**	**2.2**	**13.2**	**39.3**	**1.5**	**N**	**DOD**	**185**	**Recurrence**	**140**
27	**GemCis**	**T3b**	**T3bN3M0**	**Yes**	**23.9**	**66.2**	**4.4**	**15.1**	**92.1**	**6.7**	**N**	**Alive**	**106**	**Recurrence**	**43**
28	**DDMVAC**	**T3b**	**T3aN0M0**	**Yes**	**12.0**	**46.4**	**2.7**	**28.0**	**65.5**	**4.6**	**N**	**Alive**	**448**	**NED**	**448**
29	**GemCis**	**T4a**	**T4aN1M0**	**No**	**80.7**	**390.2**	**12.2**	**78.8**	**239.8**	**14.2**	**N**	**Dead**	**534**	**Recurrence**	**31**
30	**GemCis;Carbo**	**T4a**	**T4aN1M0**	**No**	**11.2**	**21.6**	**6.3**	**18.6**	**37.2**	**1.4**	**N**	**Alive**	**230**	**Recur/Progress**	**126**

*Normal values are as follows: CA 125 < 35 u/mL, CA 19-9 < 37 u/mL, CEA > 3.8 u/mL.
§DOD, dead of disease; ¶NED: No Evidence of Disease; GemCis: Gemcitabin-Cisplatin; Carbo: Carboplatin.

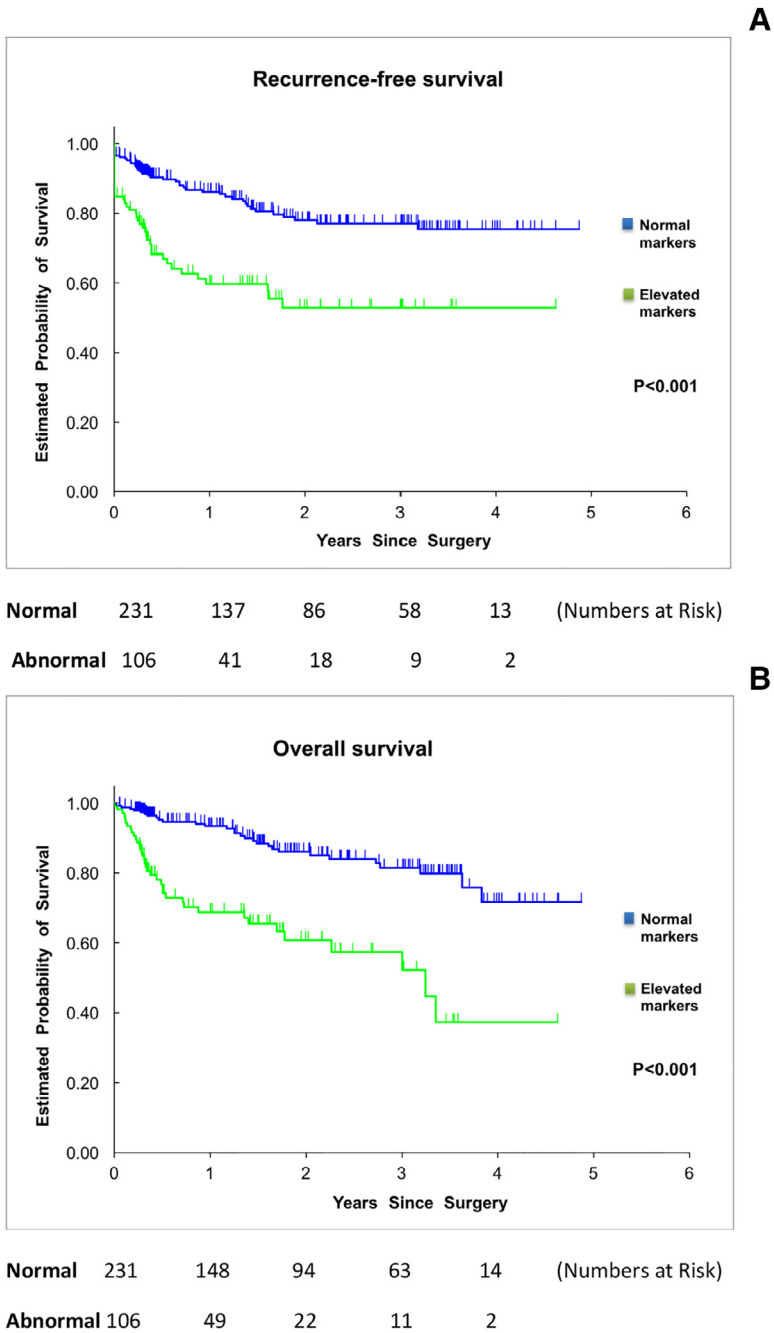

Fig. 2. Kaplan-Meier survival curves for (A) recurrence-free survival and (B) overall survival in patients with invasive bladder cancer with respect to precystectomy triple tumor marker status.

entire cohort (29 in normal TM and 37 in elevated group) during the follow up period. Survival analysis showed that elevated precystectomy level of any tumor marker (31% of patients) was associated with worse RFS (HR = 2.81; $P < 0.001$) and OS (HR = 3.97; $P < 0.001$). Kaplan-Meier curves reflecting this are shown in Fig. 2. MVA using Cox-regression modeling revealed that this effect is independent after controlling for age, gender, clinical or pathological stage, NAC (any regimen) and histologic cell type for both RFS (HR = 1.80; confidence interval [CI] 1.13-2.87; $P = 0.01$) and OS (HR = 2.40; CI 1.44-3.98; $P < 0.001$) (Table 4)

With regards to individual levels of precystectomy tumor markers, we found significant associations between each marker and the oncological outcomes. This included association between elevated precystectomy levels of CA 125 and worse RFS ($P < 0.001$) or OS ($P = 0.002$), elevated CA 19-9 and RFS ($P < 0.001$) or OS ($P < 0.001$), as well as elevated CEA with worse RFS ($P = 0.013$) or OS though ($P < 0.001$) (KM curves in supplemental material).

Table 4
Multivariable Cox regression analysis for prediction of recurrence-free survival and overall survival by elevated precystectomy epithelial tumor markers in 337 patients with invasive bladder cancer

	Recurence-free survival			Overall survival		
parameter	HR	95% CI	*P* value	HR	95% CI	*P* value
Abnormal tumor markers	**1.72**	**1.08–2.73**	**0.02**	**2.40**	**1.44–3.98**	**< 0.001**
Age	0.98	0.96–1.00	0.1	1.02	0.99–1.05	0.06
Sex	0.81	0.46–1.41	0.46	1.09	0.59–1.97	0.78
Neoadjuvant chemotherapy (any regimen)	1.27	0.79–2.05	0.31	1.43	0.86–2.39	0.16
Pathological stage (OC vs EV/LP)*	10.31	5.54–19.17	**< 0.001**	6.34	3.37–11.94	**< 0.001**
Histologic cell type	1.68	0.76–3.71	0.19	0.73	0.21–2.46	0.61

* CI, confidence interval; EV, extra-vesical; HR, hazard ratio; LP, lymph node positive; OC, organ-confined.

3.4. Oncological outcome regarding pre-NAC marker levels

Survival analysis comparing patients with normal and elevated pre-NAC tumor marker levels showed that elevated pre-NAC level of any tumor marker (51% of patients) was associated with worse 2 year RFS (68% in normal group vs. 18% in elevated; $P < 0.001$) and OS (72% in normal group vs. 27% in elevated group; $P = 0.009$) (Fig. 3).

There were also significant associations between elevated pre-NAC individual levels of CA 125 and worse RFS ($P = 0.04$) or OS ($P = 0.05$) as well as elevated CA 19-9 and RFS ($P = 0.02$) or OS ($P = 0.03$). Elevated CEA was not associated with worse RFS ($P = 0.27$) or OS though ($P = 0.14$) (KM curves not shown).

3.5. Effect of tumor marker response to NAC on oncological outcomes

There was a significantly lower rate and longer median time to recurrence/progression in the responder group (50% in responders vs. 90% in nonresponders at a median time of 22 vs. 4.8 months respectively; $P = 0.015$). There was also a significant difference in mortality rate and median overall survival between the groups (30% in responders vs. 70% in nonresponders at a median time of 27.3 vs. 11.6 months respectively; $P = 0.037$). Kaplan-Meier curves are shown in Fig. 4. Notably, two of the three patients that died in the normalized tumor marker group had tumor marker relapse at recurrence prior to their death.

4. Discussion

The potential clinical utility of CAs and CAMs has been reported across human malignancies but remains a relatively new topic in invasive BC. There is a breadth of evidence supporting the prognostic role of these tumor markers in various malignancies. For example, lower levels of CA 19-9 and CEA have been associated with better survival or response to chemotherapy in advanced pancreatic cancer and colorectal cancer, respectively [18,19]. In several pancreatic cancer cohorts, serum CA 19-9 has been prognostic for overall survival [10,18]. It has also been validated in randomized controlled trials as a postoperative prognostic marker for OS [20]. Another study showed that CEA is an efficient way to detect postoperative recurrence of colorectal surgery [21]. Serial measurement of serum levels of CA 125 is recommended for detecting early recurrence of ovarian cancer, although it is not recommended for initial screening [9]. The utility of these markers was expanded to organ-confined bladder cancer by Mergel et al. in 2007 [14]. We previously showed that elevated precystectomy serum CA 19-9 and CEA levels are associated with worse oncological outcome in invasive UBC [17]. Other, smaller studies corroborate these findings [22,23]. In this prospective study, we confirmed through multivariable analysis that elevated precystectomy serum tumor markers are independently associated with worse RFS and OS. Herein we also report the first prospective study of CA 19-9, CA 125, and CEA in patients with invasive UBC undergoing NAC.

NAC has become standard of care in the management of invasive UBC, and adoption of NAC protocols has led to an increase in its utilization, and improved survival [2,5,24,25]. Furthermore, among nonresponders, those with stable disease have better OS and RFS than those who progress on NAC [4]. Thus, given the range and lack of reliable clinical criteria of NAC response, there is a clear role for markers such as serum CAs and CAMs to predict response (or lack thereof) and oncological outcomes.

There is a paucity of literature on the prognostic value of epithelial tumor marker kinetics during chemotherapy, and most data come from nonbladder malignancies like cholangiocarcinoma, ovarian and colorectal cancer [10,11,26,27]. Lee et al. showed in 2016 that among various prognostic factors for patients with advanced epithelial ovarian cancer undergoing chemotherapy, serum levels of CA 125 following the first chemotherapy cycle and time to normalization were the most important for both OS and PFS [28]. Rodriguez et al. showed that epithelial ovarian cancer patients

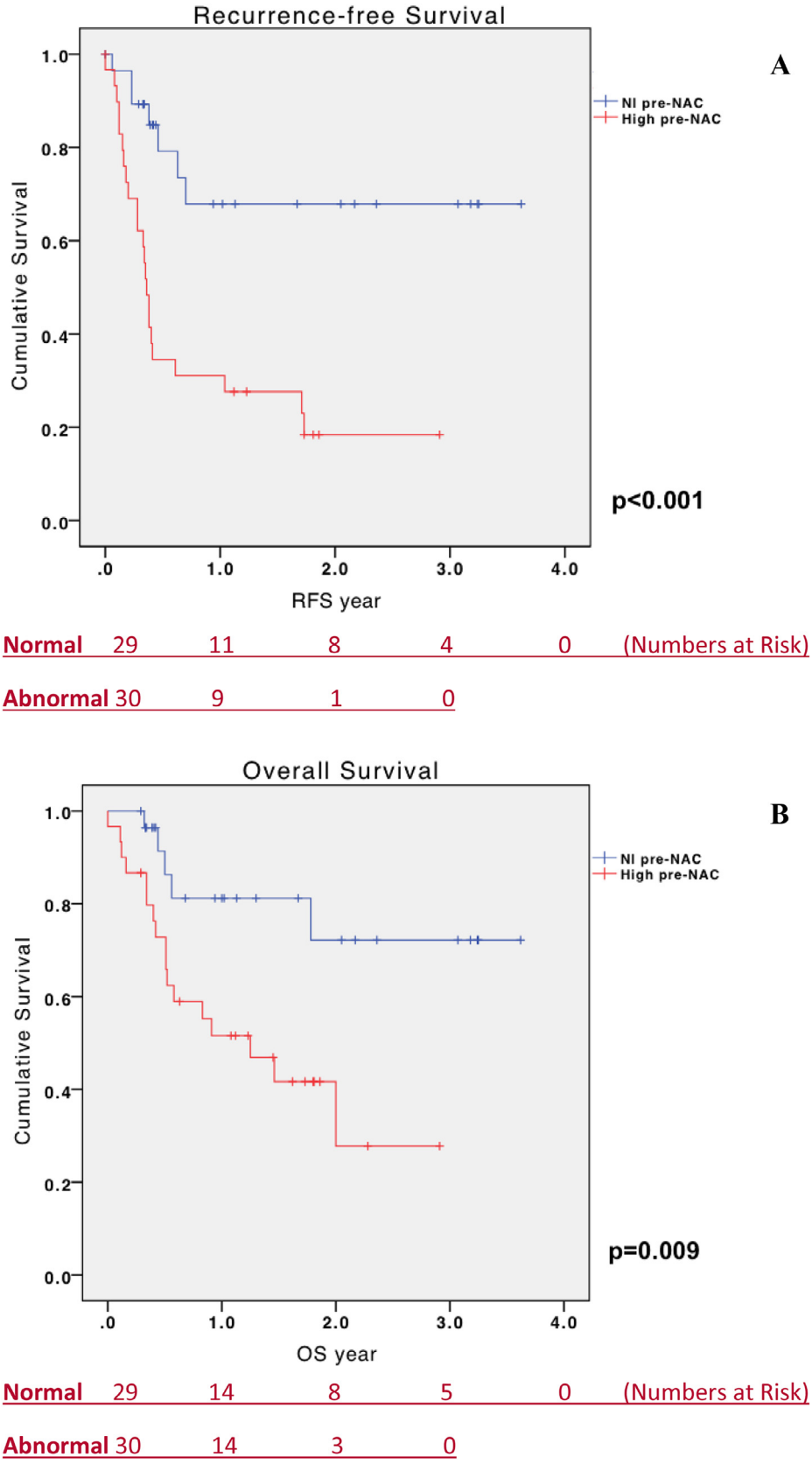

Fig. 3. Survival curves for (A) recurrence-free survival and (B) overall survival comparing normal vs. elevated pre-neoadjuvant tumor marker groups.

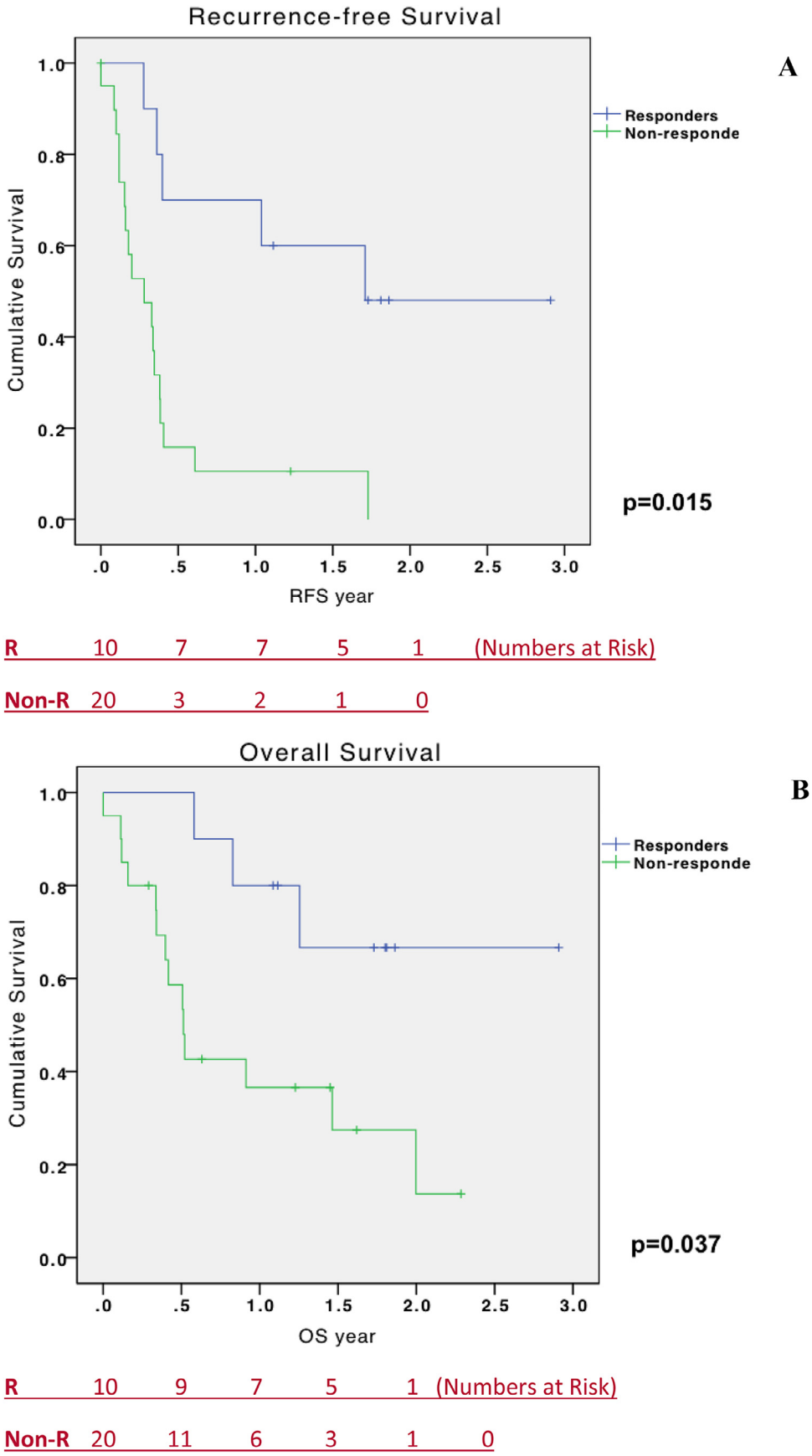

Fig. 4. Survival curves for (A) recurrence-free survival and (B) overall survival for tumor marker-response groups after neoadjuvant chemotherapy.

whose CA 125 levels decreased following NAC were very likely to be cyto-reduced by surgery [29]. Our study is unique in that we are the first to examine this relationship between tumor markers and NAC in bladder cancer patients.

We demonstrated that CA 19-9, CA 125, and CEA normalized in approximately one third of patients following NAC. Patients whose markers normalized had a significantly lower rate and longer median time to recurrence/progression. Furthermore, mortality and median overall survival was significantly better in patients with marker normalization. Notably, there was no significant difference in clinical or pathological stage between responders versus nonresponders. We integrated all NAC regimens (GemCis, ddMVAC, others) into our MVA model to determine whether it affects the prognostic role of precystectomy tumor markers on oncological outcomes. The effect was independent from NAC itself and different NAC regimens (data not shown). We also included all histologic types and variants in the multivariable model, which showed no significant differences. This may imply that epithelial tumor markers can be used across histologic subtypes.

We showed that patients with elevated markers prior to NAC have worse oncological outcome. This significant difference may imply that those with elevated markers were less likely to respond to chemotherapy, which also shows another useful aspect of these markers.

We also compared clinical response to NAC with the biomarker response. A study comparing WHO criteria, RECIST criteria, and a computerized 3D-modeling failed to show any significant difference in treatment response assessment, with most area-under-curves between 0.60s and 0.70s, which is not remarkable [30]. Table 3 shows multiple patients (for example #11, 20, 22, 26) who were clinical responders to NAC, but whose tumor markers failed to respond. All four patients recurred shortly after surgery and died. In practice, patients are not restaged clinically following NAC as imaging is not a very reliable source of staging. Many investigations are currently underway to predict response to neoadjuvant chemotherapy using genomics, however these tumor markers are currently a simple tool that represent a phenotype of this disease. However, due to small case number of the NAC cohort, we cannot reliably rule out collinearity between clinical and marker responses. For example, five out of seven patients who down-staged to $<$T2 after NAC in the same table, were among TM responders.

Our results suggest that the ability of serum tumor markers to predict oncological outcome is applicable to patients in the setting of NAC as suggested by their association with recurrence/progression, mortality rate, and median overall survival. Furthermore, serum tumor markers are easily obtainable and relatively inexpensive. Thus, pending further study, markers such as CA 19-9, CA 125, and CEA could easily be adopted into clinical practice.

Our study is not without limitations. First, besides having one of the largest prospectively collected precystectomy tumor marker cohorts, we are limited by a relatively small NAC sample size and by the fact that not all patients had serum tumor markers drawn both before and after receiving NAC. We acknowledge the possible selection bias in reporting on only those patients that had tumor marker response results. In addition, markers obtained during NAC course were rarely available. Such interim data would be valuable for assessing chemoresponsiveness, i.e. whether the markers can be predictive of NAC response early in the course of neoadjuvant treatment. In summary, a larger, multi-institutional effort with controls should be undertaken in order to confirm the findings of our study.

5. Conclusion

To our knowledge, this is the first study showing tumor marker response to NAC. Patients with persistently elevated markers following NAC have a very poor prognosis following cystectomy, which may help identifying chemotherapy-resistant tumors. A larger, controlled study with longer follow up is needed to determine their role in predicting survival.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.urolonc.2018.09.008.

References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin 2017;67(1):7–30.
[2] Meeks JJ, Bellmunt J, Bochner BH, Clarke NW, Daneshmand S, Galsky MD, et al. A systematic review of neoadjuvant and adjuvant chemotherapy for muscle-invasive bladder cancer. Eur Urol 2012;62(3):523–33.
[3] Stein JP, Lieskovsky G, Cote R, Groshen S, Feng AC, Boyd S, et al. Radical cystectomy in the treatment of invasive bladder cancer: long-term results in 1,054 patients. J Clin Oncol 2001;19(3):666–75.
[4] Chappidi MR, Kates M, Brant A, Baras AS, Netto GJ, Pierorazio PM, et al. Assessing cancer progression and stable disease after neoadjuvant chemotherapy for organ-confined muscle-invasive bladder cancer. Urology 2017;102:148–58.
[5] Gandhi NM, Baras A, Munari E, Faraj S, Reis LO, Liu JJ, et al. Gemcitabine and cisplatin neoadjuvant chemotherapy for muscle-invasive urothelial carcinoma: predicting response and assessing outcomes. Urol Oncol 2015;33(5):204.e1–7.
[6] Cao Y, Stosiek P, Springer GF, Karsten U. Thomsen-Friedenreich-related carbohydrate antigens in normal adult human tissues: a systematic and comparative study. Histochem Cell Biol 1996;106(2):197–207.
[7] Okegawa T, Pong RC, Li Y, Hsieh JT. The role of cell adhesion molecule in cancer progression and its application in cancer therapy. Acta Biochim Pol 2004;51(2):445–57.

[8] Patankar MS, Jing Y, Morrison JC, Belisle JA, Lattanzio FA, Deng Y, et al. Potent suppression of natural killer cell response mediated by the ovarian tumor marker CA125. Gynecol Oncol 2005;99(3): 704–13.
[9] Duffy MJ. Tumor markers in clinical practice: a review focusing on common solid cancers. Med Princ Pract 2013;22(1):4–11.
[10] Lee BS, Lee SH, Son JH, Jang DK, Chung KH, Paik WH, et al. Prognostic value of CA 19-9 kinetics during gemcitabine-based chemotherapy in patients with advanced cholangiocarcinoma. J Gastroenterol Hepatol 2016;31(2):493–500.
[11] Sakamoto Y, Miyamoto Y, Beppu T, Nitta H, Imai K, Hayashi H, et al. Post-chemotherapeutic CEA and CA19-9 are prognostic factors in patients with colorectal liver metastases treated with hepatic resection after oxaliplatin-based chemotherapy. Anticancer Res 2015;35 (4):2359–68.
[12] Chang A, Cai J, Miranda G, Groshen S, Skinner D, Stein JP. Usefulness of CA 125 as a preoperative prognostic marker for transitional cell carcinoma of the bladder. J Urol 2004;172(6 Pt 1):2182–6.
[13] Manvar AM, Wallen EM, Pruthi RS, Nielsen ME. Prognostic value of CA 125 in transitional cell carcinoma of the bladder. Expert Rev Anticancer Ther 2010;10(12):1877–81.
[14] Margel D, Tal R, Baniel J. Serum tumor markers may predict overall and disease specific survival in patients with clinically organ confined invasive bladder cancer. J Urol 2007;178(6):2297–300; discussion 2300-1.
[15] Washino S, Hirai M, Matsuzaki A, Kobayashi Y. Clinical usefulness of CEA, CA19-9, and CYFRA 21-1 as tumor markers for urothelial bladder carcinoma. Urol Int 2011;87(4):420–8.
[16] Margel D, Tal R, Neuman A, Konichezky M, Sella A, Baniel J. Prediction of extravesical disease by preoperative serum markers in patients with clinically organ confined invasive bladder cancer. J Urol 2006;175(4):1253–7; discussion 1257.
[17] Ahmadi H, Djaladat H, Cai J, Miranda G, Daneshmand S. Precystectomy serum levels of carbohydrate antigen 19-9, carbohydrate antigen 125, and carcinoembryonic antigen: prognostic value in invasive urothelial carcinoma of the bladder. Urol Oncol 2014;32(5):648–56.
[18] Haas M, Heinemann V, Kullmann F, Laubender RP, Klose C, Bruns CJ, et al. Prognostic value of CA 19-9, CEA, CRP, LDH and bilirubin levels in locally advanced and metastatic pancreatic cancer: results from a multicenter, pooled analysis of patients receiving palliative chemotherapy. J Cancer Res Clin Oncol 2013;139(4):681–9.
[19] Vainshtein JM, Schipper M, Zalupski MM, Lawrence TS, Abrams R, Francis IR, et al. Prognostic significance of carbohydrate antigen 19-9 in unresectable locally advanced pancreatic cancer treated with dose-escalated intensity modulated radiation therapy and concurrent full-dose gemcitabine: analysis of a prospective phase 1/2 dose escalation study. Int J Radiat Oncol Biol Phys 2013;86(1):96–101.
[20] Berger AC, Garcia M Jr., Hoffman JP, Regine WF, Abrams RA, Safran H, et al. Postresection CA 19-9 predicts overall survival in patients with pancreatic cancer treated with adjuvant chemoradiation: a prospective validation by RTOG 9704. J Clin Oncol 2008;26 (36):5918–22.
[21] Yakabe T, Nakafusa Y, Sumi K, Miyoshi A, Kitajima Y, Sato S, et al. Clinical significance of CEA and CA19-9 in postoperative follow-up of colorectal cancer. Ann Surg Oncol 2010;17(9):2349–56.
[22] Margel D, Bostrom P, Baniel J, Yossepowitch O, Zlotta A, Fleshner N. External validation of a biomarker based pre-cystectomy algorithm to predict nonorgan confined urothelial cancers. J Urol 2012;187(3):840–4.
[23] Margel D, Harel A, Yossepowitch O, Baniel J. A novel algorithm to improve pathologic stage prediction of clinically organ-confined muscle-invasive bladder cancer. Cancer 2009;115(7):1459–64.
[24] Reardon ZD, Patel SG, Zaid HB, Stimson CJ, Resnick MJ, Keegan KA, et al. Trends in the use of perioperative chemotherapy for localized and locally advanced muscle-invasive bladder cancer: a sign of changing tides. Eur Urol 2015;67(1):165–70.
[25] Witjes JA, Comperat E, Cowan NC, De Santis M, Gakis G, Lebret T, et al. EAU guidelines on muscle-invasive and metastatic bladder cancer: summary of the 2013 guidelines. Eur Urol 2014;65(4):778–92.
[26] Iwanicki-Caron I, Di Fiore F, Roque I, Astruc E, Stetiu M, Duclos A, et al. Usefulness of the serum carcinoembryonic antigen kinetic for chemotherapy monitoring in patients with unresectable metastasis of colorectal cancer. J Clin Oncol 2008;26(22):3681–6.
[27] Ko AH, Hwang J, Venook AP, Abbruzzese JL, Bergsland EK, Tempero MA. Serum CA19-9 response as a surrogate for clinical outcome in patients receiving fixed-dose rate gemcitabine for advanced pancreatic cancer. Br J Cancer 2005;93(2):195–9.
[28] Lee M, Chang MY, Yoo H, Lee KE, Chay DB, Cho H, et al. Clinical Significance of CA125 Level after the First Cycle of Chemotherapy on Survival of Patients with Advanced Ovarian Cancer. Yonsei Med J 2016;57(3):580–7.
[29] Rodriguez N, Rauh-Hain JA, Shoni M, Berkowitz RS, Muto MG, Feltmate C, et al. Changes in serum CA-125 can predict optimal cytoreduction to no gross residual disease in patients with advanced stage ovarian cancer treated with neoadjuvant chemotherapy. Gynecol Oncol 2012;125(2):362–6.
[30] Hadjiiski L, Weizer AZ, Alva A, Caoili EM, Cohan RH, Cha K, et al. Treatment response assessment for bladder cancer on CT based on computerized volume analysis, World Health Organization Criteria, and RECIST. AJR Am J Roentgenol 2015;205(2):348–52.