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ORIGINAL ARTICLE

Diagnostic value of neutrophil gelatinase-associated lipocalin/matrix metalloproteinase-9 pathway in transitional cell carcinoma of the bladder

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Abstract Neutrophil gelatinase-associated lipocalin (NGAL), matrix metalloproteinase (MMP)-9, and NGAL/MMP-9 complex have been evaluated as diagnostic markers of several cancers, but results for bladder cancer are scanty. We evaluated these proteins in urine and serum of 89 patients with histologically confirmed bladder cancer and 119 cancer-free controls from a case-control study. Urinary concentrations were standardized on creatinine level. The performance of these proteins as cancer biomarkers was evaluated through the receiver operating characteristic (ROC) analysis. Urinary level of NGAL, MMP-9, and NGAL/MMP-9 complex was higher in current smokers, whereas no impact of dietary habits was observed. After adjusting for tobacco smoking, urinary concentration of MMP-9 was independently associated with cancer invasiveness, grading, and histological subtype, with elevated concentrations among T2–T4 and non-papillary bladder cancers. Conversely, NGAL and NGAL/MMP-9 complex were significantly higher in non-papillary than in papillary subtype. The pattern was less clear in serum, but correlation between urinary and serum concentration was poor, especially for Ta/is–T1 tumors. The ROC analysis confirmed that MMP-9 was the best marker (area under the ROC curve (AUC) = 0.68). Performances were much greater for muscle-invasive bladder cancers (AUC = 0.90), with elevated negative predictive values (97 %). The present study suggests that NGAL/MMP-9 pathway is associated with an aggressive phenotype of bladder cancer. The elevated negative predictive value of MMP-9 and NGAL/MMP-9 complex makes them candidate markers of exclusion test for bladder cancer. These proteins may be integrated in the surveillance of bladder cancer, thus diminishing patients' discomfort and improving compliance.

Massimo Libra and Jerry Polesel contributed equally to this work.

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Keywords Neutrophil gelatinase-associated lipocalin (NGAL) · Matrix metalloproteinase (MMP) · Bladder cancer · Diagnosis

Introduction

In developed countries, transitional cell carcinoma (TCC) of the bladder is the fourth most frequent cancer in men, with the highest incidence worldwide in southern Europe [1]. Although cytology is widely used for bladder cancer screening, definite diagnosis requires cystoscopy. This procedure, however, is expensive, is painful, and causes distress for

patients [2, 3], reducing the compliance with follow-up. Although no urinary marker can presently replace cystoscopy or lower its frequency during patients' follow-up [4, 5], validated and non-invasive biomarkers could be useful in clinical practice. Several markers have been proposed in recent years, but none is currently considered adequate to diagnose and predict the outcome of bladder cancer. Further, possible interactions between marker expression and lifestyle factors (e.g., tobacco smoking, obesity, dietary habits) have been scarcely investigated.

Neutrophil gelatinase-associated lipocalin (NGAL), also called lipocalin-2, is a secreted protein belonging to the lipocalin family proteins and actively participates into the proliferation, differentiation, and development of human tissues [6], including tumors [7]. It positively modulates the activity of the matrix metalloproteinase-9 (MMP-9) [8]—a member of the family of zinc-dependent enzymes involved in the enzymatic remodeling of the extracellular matrix. MMP-9 regulates the degradation of extracellular matrix in processes such as angiogenesis, tumor growth, and metastasis [9]. By forming the NGAL/MMP-9 complex, NGAL protects MMP-9 from proteolytic degradation, a fundamental mechanism in controlling the activity of the proteins, and enhances its enzymatic activities [10]. Notably, higher urinary MMP-9 level was found in bladder cancer cases than in cancer-free controls [11], showing positive correlations with tumor grade and invasiveness [12–16]. As a secreted protein, NGAL is detectable in many biologic fluids, including urines, where several neoplastic cells and other tumor microenvironmental factors can be directly released from bladder cancer. Strong correlations between NGAL level and severity, histological grade, and presence of metastasis were observed on samples of breast cancer tissue [17]. Significantly higher urinary concentrations of NGAL and MMP-9 were reported in women with ovarian cancer than in the control group [18]. NGAL/MMP-9 complex was expressed in breast, gastric, and esophageal cancer patients, whereas it was absent in healthy subjects [19–22].

Cancer development and invasion depend on different interactions taking place between tumor cells and non-neoplastic cells [23]. Such interactions may be modulated by several factors, including dietary habits. High fat diet and obesity were associated with both tumor growth and molecular changes that in turn may determine alteration of several molecules contributing to the tumor microenvironment [24]. Among these molecules, the role of NGAL has been recently explored in cancer [25]. Our recent in silico analysis suggested an active role of NGAL in tumor development of several cancer types, including that of the bladder [26]. However, validation of these findings is still lacking.

On these grounds, we thought to compare NGAL release in urine and serum samples from bladder cancer patients with that of cancer-free controls. Further investigations, aimed to emphasize the role of NGAL in cancer, were performed by analyzing MMP-9 and NGAL/MMP-9 complex levels in the same subset of patients. NGAL and MMP-9 levels were also evaluated according to different lifestyle and diet habits to understand if these may play a role in the perturbation of cancer microenvironment.

Materials and methods

Human subjects

The data were derived from a case-control study conducted from 2004 to 2009 on TCCs within an established Italian network of collaborating centers. This analysis included the earliest cases enrolled up to August 2007 in the province of Pordenone whose urine and blood samples were available [27]. Cases were 89 Caucasian patients aged 18 years or older (median age 66 years) with incident histologically or cytologically confirmed TCC admitted to major general hospitals. Using the TNM classification, cases were classified in non-muscle-invasive (i.e., Ta/is–T1) and muscle-invasive (i.e., T2–T4) tumors following the guidelines of European Association of Urology [4].

The control group included 119 Caucasian patients (median age 66 years) admitted to the same network of hospitals for a wide spectrum of acute, non-neoplastic conditions unrelated to tobacco and alcohol consumption, to known risk factors for bladder cancer, or to conditions associated with long-term diet modification. All study subjects signed an informed consent, according to the recommendations of the Board of Ethics of the study hospitals.

Trained nurses administered a validated, structured questionnaire [28] to cases and controls during their hospital stay, thus keeping refusal below 5 % for both cases and controls. The questionnaire collected information on sociodemographic factors and lifestyle factors, including smoking and alcohol drinking habits. Patient dietary habits in the 2-year preceding study enrolment was investigated through a validated food-frequency questionnaire, including seven sections: (i) milk, hot beverages, and sweeteners; (ii) bread, cereals, and first courses; (iii) second courses (e.g., meat and other main dishes); (iv) side dishes (i.e., vegetables); (v) fruits; (vi) sweets, desserts, and soft drinks; and (vii) alcoholic beverages. For vegetables and fruit, seasonal variation was considered in the analysis.

Marker analysis

Each patient enrolled in the study provided peripheral blood and urine samples on the day that they were interviewed. Samples were collected before patients had undergone any

treatment. Standard clean-catch procedure for urine collection (50-mL sample of first voided for each patient) was performed to prevent sample contamination. Half of the sample (25 mL) was immediately frozen at −80 °C, and the remaining half was stored in CytoLyt solution at 4 °C. Blood samples were centrifuged at 1500*g* for 10 min obtaining serum, buffy coat, and red blood cells and then stored at −80 °C. Serum, plasma, and urine samples were stored at −80 °C until analyses.

Serum and urine concentrations of MMP-9, NGAL, and MMP-9/NGAL complex were assayed, according to the manufacturer's protocols, by specific, commercially available, through enzyme-linked assay (ELISA) kits (Quantikine, R&D Systems Inc., USA) in accordance with the manufacturer's instructions and analyzed with an ELISA reader (Tecan Systems) at 450 nm. Urinary concentrations were standardized according to creatinine (Cr) level and expressed as nanogram per milligram Cr. Urinary Cr was assayed using ABX Pentra Enzymatic Creatinine CP kit (HORIBA ABX INC, USA) according to the manufacturer's instructions. Colorimetric intensity was assayed at 545 nm using ABX Pentra 400 analyzer (HORIBA ABX INC, USA). All analyses were carried out at the Department of Biomedical and Biotechnological Sciences, University of Catania.

Statistics

The effect of sociodemographic characteristics, lifestyle factors, and dietary habits on markers' level was evaluated in controls through multivariable regression models. All markers' concentrations were log-transformed, except for urinary NGAL/MMP-9 complex where the log transformation was not applicable due to the elevated number of undetectable concentrations. Marker concentration was entered in the model as dependent variable, and each factor was entered as predictor: corresponding β coefficient was tested through t test.

Differences of urinary and serum levels of NGAL, MMP-9, and NGAL/MMP-9 complex according to tumor characteristics were evaluated using the nonparametric Kruskal-Wallis test, followed by Dunn's multiple comparison post-test. To evaluate the independent effect of each tumor feature, an adjusted Kruskal-Wallis test was further adopted [29]. For each molecule, the agreement between urinary and serum concentrations was measured through Spearman's rank correlation coefficient. The performance of these proteins as cancer biomarkers was evaluated in terms of sensitivity and specificity, overall and according to tumor characteristics. Receiver operating characteristic (ROC) analysis was performed to determine the optimal cutoff for diagnostic purpose, and sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) were calculated. Discrimination was quantified by the area under the ROC curve (AUC) [30].

Results

The majority of cases with TCC were by far men and aged ≥65 years (Table 1). Ever smoking was reported by 85.4 % of cases and 66.4 % of controls, whereas no difference was observed for education and drinking habit. Non-muscle-invasive tumors (i.e., Ta/is–T1) represented 78.4 % of cases, whereas papillary feature was reported in 79.8 % of TCCs.

Table 2 shows the effect of sociodemographic characteristics, lifestyle factors, and dietary habits on marker concentrations. Age and current tobacco smoking were positively correlated ($\beta > 0$) to increased level of all urinary markers, but only to serum NGAL. Red meat and cereal consumptions were directly associated to urinary NGAL and serum NGAL, respectively. However, these associations may be compatible with casual association. Considering these results, differences between cases and controls in Table 3 were tested adjusting for age and smoking habits.

Table 1 Distribution of 89 cases of transitional cell carcinoma (TCC) of the bladder and 119 hospital controls according to sociodemographic characteristics, tobacco smoking, alcohol drinking, and clinical pathological factors

Variables	TCCs ($n=89$)		Controls ($n=119$)		χ^2 test
	n	(%)	*n*	(%)	
Sex					
Men	75	(84.3)	99	(83.2)	0.04; $p=0.84$
Women	14	(15.7)	20	(16.8)	
Age (years)					
<65	34	(38.2)	47	(39.5)	0.15; $p=0.93$
65–74	39	(43.8)	49	(41.2)	
≥75	16	(18.0)	23	(19.3)	
Education					
<7	42	(47.2)	53	(44.5)	0.15; $p=0.93$
7–11	31	(34.8)	43	(36.1)	
≥12	16	(18.0)	23	(19.3)	
Smoking status					
Never	13	(14.6)	40	(33.6)	27.76; $p<0.01$
Former	37	(41.6)	64	(53.8)	
Current	39	(43.8)	15	(12.6)	
Alcohol drinking status					
Never/former	7	(7.9)	17	(14.3)	2.05; $p=0.15$
Current	82	(92.1)	102	(85.7)	
Invasiveness[a]					
Ta/is	50	(56.8)			
T1	19	(21.6)			
T2–T4	19	(21.6)			
Grading[a]					
Well/moderately differentiated	40	(45.5)			
Poorly diff./undifferentiated	48	(54.6)			
Histological subtype					
Papillary	71	(79.8)			
Non-papillary	18	(20.2)			

[a] The sum does not add up to the total because of missing values

Table 2 Effect estimates (β coefficients) of lifestyle factors and dietary habits on urinary and serum NGAL, MMP-9, and NGAL/MMP-9 complex concentrations (CMPLX) in 119 hospital controls

	Urinary concentrations (ng/mg Cr)						Serum concentrations (ng/mL)					
	Log NGAL		Log MMP-9		CMPLX		Log NGAL		Log MMP-9		Log CMPLX	
Lifestyle factors												
Gender (female)	0.345		0.354		1.354		0.067		0.045		0.091	
Age (5 years)	0.121	**	0.188	**	2.153	*	0.023	**	0.012		0.015	
Former tobacco smoking	0.414	*	0.383		−1.018		0.018		0.030		0.033	
Current tobacco smoking	0.534	*	0.535	*	4.485	*	0.067		0.110		0.113	
Cigarettes/day	−0.047		−0.055		−0.706		0.009		−0.008		0.014	
Current alcohol drinking	0.019		0.103		2.505		−0.074		−0.101		−0.130	
Alcohol intake (100 g/day)	−0.131		−0.063		−0.029		0.038		0.107		0.213	
Body mass index (kg/m^2)	−0.039		−0.040		−0.645		−0.010	*	−0.013		−0.011	
Diabetes mellitus	−0.286		0.440		−3.722		−0.050		0.048		0.000	
Food intake (100 g/day)												
Cereals	−0.002		−0.026		0.021		0.034	*	0.026		0.035	
Raw vegetables	0.086		0.055		−0.554		0.007		0.024		0.017	
Cooked vegetables	−0.038		−0.014		0.092		0.000		0.032		0.045	
Citrus fruits	0.062		0.051		−1.246		0.011		0.004		−0.011	
Non-citrus fruits	0.058		0.024		−0.044		−0.004		−0.020		−0.019	
White meat	−0.091		0.020		−1.324		−0.001		0.021		0.111	
Red meat	0.304	*	0.390		9.517		0.011		0.121		0.126	
Fish	−0.540		−0.204		1.552		−0.105		−0.034		−0.138	
Milk	0.013		0.007		0.723		−0.013		0.006		−0.013	

Estimated from multivariable regression model including terms for smoking habits

*$p < 0.05$; **$p < 0.01$

Urinary NGAL concentrations were significantly higher in cases than in controls (median 18.35 vs. 7.75 ng/mg Cr; $p < 0.01$); likewise, higher urinary levels of MMP-9 (median 6.54 vs 1.18 ng/mg Cr; $p < 0.01$) and NGAL/MMP-9 complex (median 1.11 vs 0.00 ng/mg Cr; $p < 0.01$) were observed in cases compared to controls (Table 3). Figure 1a shows increasing urinary concentrations of NGAL, MMP-9, and NGAL/MMP-9 according to invasiveness, with markers' concentration significantly higher in T2–T4 cases ($p < 0.01$). Interestingly, NGAL/MMP-9 complex was undetectable in 53.8 % of controls, but only in 30.3 % of all TCCs (5.3 % of T2–T4 cases). In the univariate analysis, the expression of the three molecules was higher in poorly differentiated/undifferentiated than in well/moderately differentiated TCCs and in non-papillary than in papillary subtype (Table 3). However, after mutual adjustment for tumor characteristics, age, and smoking habits, only MMP-9 was still associated to invasiveness, grading, and histological subtype; conversely, NGAL and NGAL/MMP-9 complex remained associated only to histological subtype.

No significant differences between TCC cases and controls emerged in serum (Table 3). Nonetheless, higher levels of NGAL and NGAL/MMP-9 complex were observed in patients with muscle-invasive tumors (Fig. 1b). These differences were statistically different after taking into account the other tumor characteristics, age, and smoking habits (Table 3).

Stronger correlations were observed between urinary and serum levels of NGAL and MMP-9 in TCC patients. These correlations were higher in patients with muscle-invasive TCCs ($r = 0.72$ and $r = 0.54$, respectively) than in those with non-muscle-invasive TCCs ($r = 0.22$ and $r = 0.27$, respectively). The correlation was less marked for NGAL/MMP-9 complex. Among controls, urinary concentrations of NGAL, MMP-9, and NGAL/MMP-9 complex did not correlate with those in serum (Supplementary Fig. 1).

ROC curves were used to determine the optimal cutoff for the three molecules (Table 4). Low sensitivity was reported for all the three urinary markers in all TCCs, which were able to correctly classify approximately 65 % of cases. For MMP-9 and NGAL/MMP-9 complex, the sensitivities and the NPVs greatly increased among muscle-invasive cancers (Se = 84 % and NPV = 97 %). According to ROC analysis (Fig. 2), MMP-9 and NGAL/MMP-9 complex were the best markers among all TCCs (AUC = 0.68). For T2–T4 TCCs, MMP-9 and NGAL/MMP-9 complex were still the best markers showing similar diagnostic performances (AUC = 0.90 and 0.88,

Table 3 Median urinary and serum NGAL, MMP-9, and NGAL/MMP-9 complex concentrations in 119 hospital controls and in 89 cases of transitional cell carcinoma (TCC) of the bladder according to clinical pathological features

	n	Median urinary concentrations (ng/mg Cr)			Median serum concentrations (ng/mL)		
		NGAL	MMP-9	NGAL/MMP-9 complex	NGAL	MMP-9	NGAL/MMP-9 complex
Controls	119	7.75	1.18	0.00	90.49	717.94	51.86
All TCCs	89	18.35	6.54	1.11	83.70	738.51	53.44
KW test		$p<0.01$	$p<0.01$	$p<0.01$	$p=0.50$	$p=0.32$	$p=0.58$
Invasiveness[a]							
Ta/is	50	16.18	3.30	0.55	78.78	610.01	38.27
T1	19	15.24	5.98	0.29	84.09	822.82	57.24
T2–T4	19	68.55	24.29	6.64	139.32	943.55	113.85
KW test		$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p=0.01$	$p<0.01$
Adjusted KW test[b]		$p=0.56$	$p=0.02$	$p=0.08$	$p=0.02$	$p=0.21$	$p=0.03$
Grading[a]							
Well/moderately differentiated	40	13.49	4.33	0.51	78.78	615.97	38.48
Poorly diff./undifferentiated	48	20.25	10.71	1.30	100.26	825.47	68.49
KW test		$p<0.01$	$p<0.01$	$p<0.01$	$p=0.04$	$p=0.06$	$p=0.03$
Adjusted KW test[b]		$p=0.47$	$p=0.04$	$p=0.31$	$p=0.76$	$p=0.50$	$p=0.71$
Histological subtype							
Papillary	71	14.84	3.97	0.52	78.91	611.63	43.90
Non-papillary	18	49.88	44.03	10.67	113.66	937.75	75.36
KW test		$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p<0.01$	$p=0.04$
Adjusted KW test[b]		$p=0.01$	$p=0.02$	$p<0.01$	$p=0.04$	$p<0.01$	$p=0.14$

Urinary concentrations were standardized on creatinine (Cr) level and expressed in nanogram per milligram Cr

KW Kruskall-Wallis test

[a] The sum does not add up to the total because of missing values

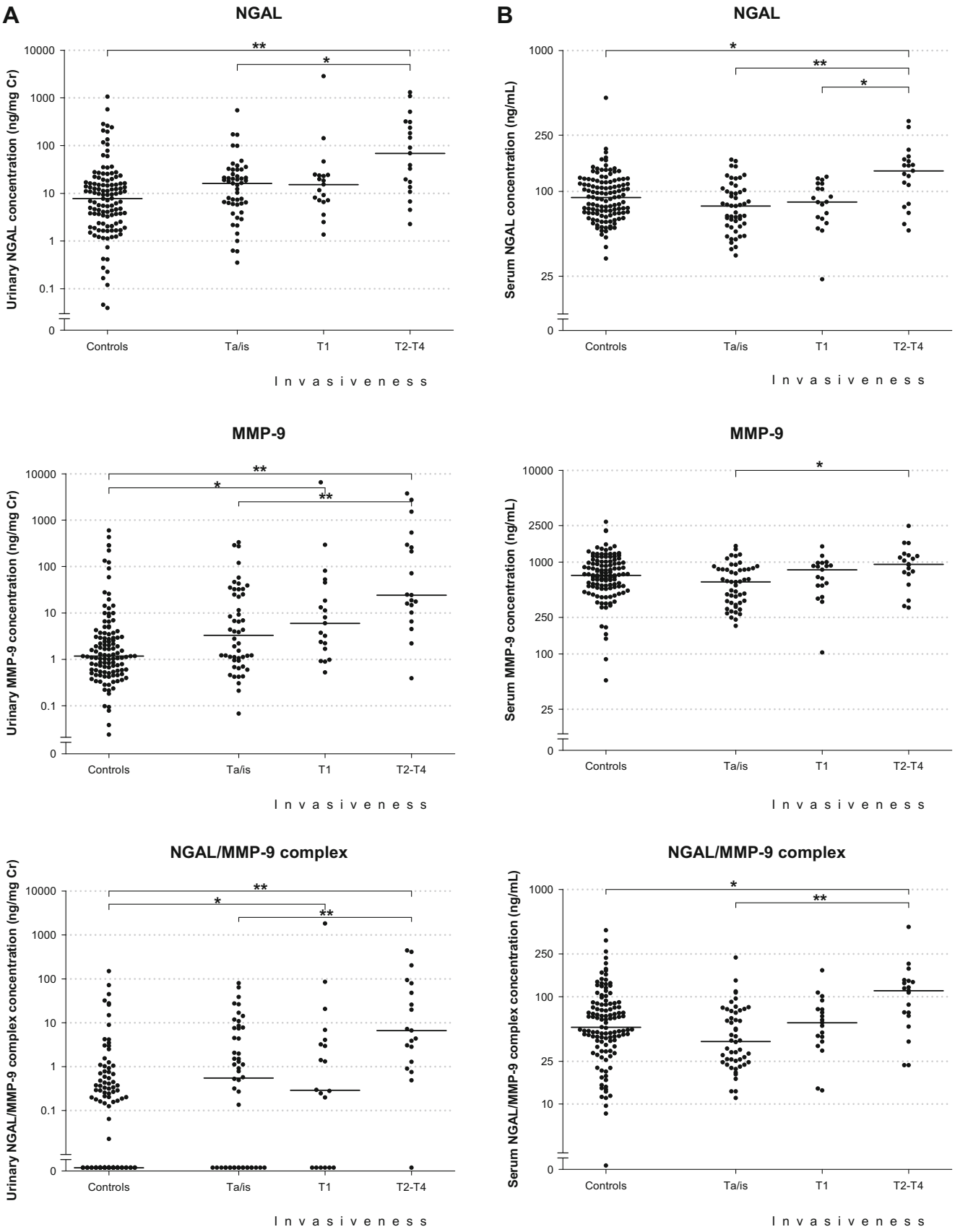
[b] Mutually adjusted for tumor invasiveness, grading, and histological subtype plus age and smoking habits

respectively, Table 4). Diagnostic performances in serum were generally lower than in urine (data not shown).

Discussion

The results from the present study showed an association between NGAL, MMP-9, and NGAL/MMP-9 complex and TCC, and the associations were consistent with respect to possible perturbation due to lifestyle factors. As expected, these three biomarkers showed higher diagnostic properties in urine than in serum; indeed, TCC is localized in the inner layer of the bladder where it can excrete these proteins directly in the urine. These findings are also supported by our previous observation in which the immunostaining of lipocalin-2 reveals its localization in bladder cancer cells [26].

Multiple proteins have been measured in bladder cancer patients showing the specificity of the analysis in biological fluid such as urine. Among these proteins, in agreement with our findings, the authors revealed a strong association of higher MMP urine levels with invasiveness and grading [11–15, 31]. A recent study on renal cell carcinomas [32] compared NGAL, MMP-9, and NGAL/MMP-9 complex in urine and serum. Expression levels of NGAL were strongly correlated in both urine and serum from these patients. However, the authors failed to demonstrate such correlation for MMP-9 and NGAL/MMP-9 complex levels. Conversely, the present study showed a strong correlation between serum and urine levels of NGAL, MMP-9, and NGAL/MMP-9 complex only in TCCs with an aggressive phenotype showing their role in invasiveness (Supplementary Fig. 1). Accordingly, MMP-9 and NGAL/MMP-9 complex showed sensitivity and specificity higher than 80 % for muscle-invasive TCCs. The particularly elevated NPV means that the probability of having the disease, given a negative test, is very low (3 % for MMP-9 and 4 % for NGAL/MMP-9 complex). On the other hand, PPVs were between 55 and 73 %, suggesting a moderate capacity to identify cases. These results suggested that these molecules could be used as exclusion test. Several investigations have previously reported similar results for MMP-9 in bladder cancer [11–15, 31]. However, these studies were heterogeneous according to tumor characteristics (e.g., histological type, stage, grade), and none of them has reported the prognostic properties according to tumor stage.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◀ **Fig. 1** Distribution of NGAL, MMP-9, and NGAL/MMP-9 complex concentrations in urine (**a**) and serum (**b**) in hospital controls and in cases of transitional cell carcinoma of the bladder (TCC) according to tumor invasiveness. Median values are represented by *horizontal lines*. *p* values computed by non-parametric Kruskal-Wallis and Mann-Whitney *U* tests. **p* value <0.05; ***p* value <0.01

Only a recent study on 41 bladder cancer cases [16] investigated the capability of urinary and serum biomarkers to discriminate muscle-invasive bladder cancers from non-muscle-invasive ones. However, its results were in contrast with those from the present study, reporting inverse association between cancer invasiveness and serum concentration of MMP-9, NGAL, and NGAL/MMP-9 complex [16]. Diverse distribution in histological subtypes in cases series may partly account for these differences, since markers' level in both urine and serum varied according to TCC type. The faculty to detect a disease at an early stage is a particularly interesting aspect in the evaluation of a new biomarker. Indeed, we found poor sensitivity and specificity for non-muscle-invasive TCCs, suggesting that the overall diagnostic properties of the three molecules were driven by T2–T4 cancers. Similar results were reported by Gerhards and colleagues [13] for urinary MMP-2.

An efficient tumor biomarker is expected to be cost-effective in the detection of cancer at an early stage and in the discrimination between low-risk and high-risk cancers [33]. According to this point of view, our finding may help to better define the diagnostic properties of NGAL, MMP-9, and NGAL/MMP-9 complex. Firstly, the correlation was stronger in urine rather than in serum, suggesting that urine is the most adequate among body fluids for these biomarkers.

Table 4 Sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV) for urinary NGAL, MMP-9, and NGAL/MMP-9 complex as biomarkers of transitional cell carcinoma of the bladder (TCC)

	Optimal cutoff (ng/mg Cr)	Se (%)	Sp (%)	PPV (%)	NPV (%)
All TCCs					
NGAL	11.59	61	61	53	67
MMP-9	2.20	65	66	59	72
NGAL/MMP-9 complex	0.265	65	67	60	72
Non muscle-invasive TCCs					
NGAL	11.59	57	61	45	71
MMP-9	1.66	62	58	46	73
NGAL/MMP-9 complex	0.19	61	61	48	73
Muscle-invasive TCCs					
NGAL	17.1	74	76	33	95
MMP-9	6.5	84	82	43	97
NGAL/MMP-9 complex	0.89	84	83	44	97

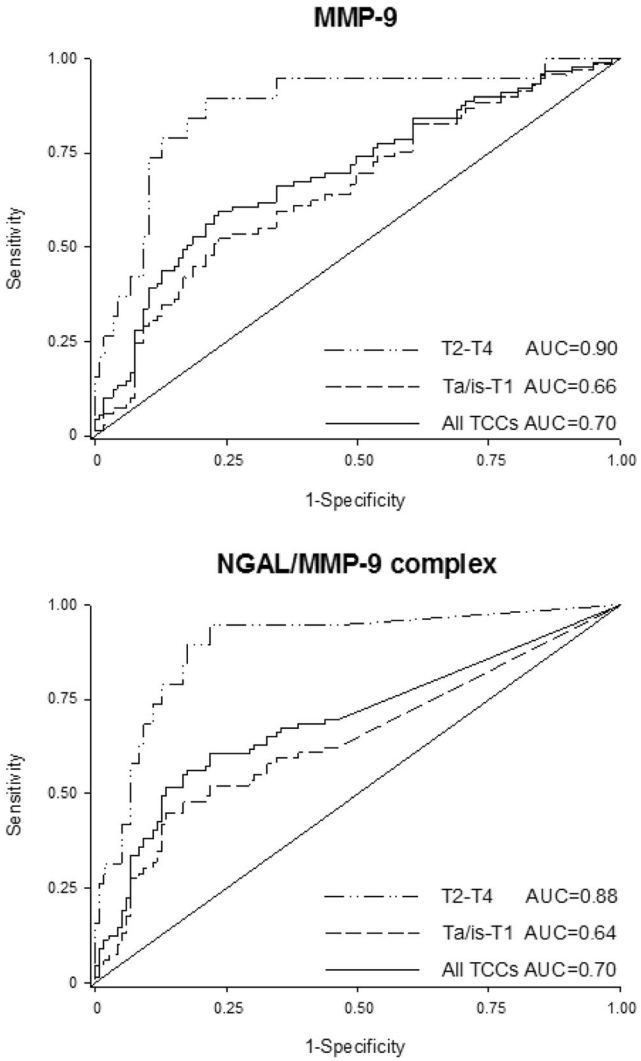

Fig. 2 Receiver operating characteristic (ROC) curves for urinary MMP-9 and NGAL/MMP-9 complex concentrations, according to tumor invasiveness

Secondly, the three proteins correlated with tumor invasiveness, being therefore able to discriminate low-risk from high-risk cancers. However, sensitivities and PPVs were very low in non-muscle-invasive TCCs, bringing to light the limits of these markers to detect early-stage cancers.

A strength of this study was the use of hospital controls, since, following a pragmatic approach, it could give more reliable information on the actual diagnostic properties of these biomarkers. Further, the availability of information on several lifestyle factors, including dietary habits, was an additional strength. Indeed, possible alteration of markers concentration due to lifestyle factors was evaluated and marker performances were tested taking into account this possible source of bias. Several previous investigations considered volunteers or healthy people as control group [12, 31], but this choice is prone to selection bias [34] and may artificially increase the marker specificity. Indeed, volunteers are

known to be generally healthier than the general population, thus reducing the number of false positives. Moreover, other advantages derive from the case-control study design [27]. First, the use of matched controls may have prevented differences in proteins' concentration due to dissimilarities between cases and controls in relation to age and/or gender (i.e., matching characteristics). Then, patients were approached during their hospital stay, limiting selection bias and ensuring that urine and blood samples were collected by trained nurses prior to any cancer treatment, adhering to standard clean-catch procedure.

In conclusion, the results from the present study suggested that NGAL/MMP-9 pathway is associated to an aggressive phenotype of TCC. Although further confirmations are needed, our findings suggest that these proteins may be integrated in the surveillance of bladder cancer, thus diminishing patients' discomfort and improving compliance.

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Compliance with ethical standards

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Conflicts of interest None

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