THE SIGNIFICANCE OF LABORATORY INDICES TO DETERMINE THE NUMBER OF LESIONS IN PATIENTS WITH NON-MUSCLE INVASIVE URINARY BLADDER CANCER

UDC 616.62.001.6–006–074
Received 13.03.2012

© **L.A. Derzhavets,** PhD, Head of Clinicodiagnostic Laboratory

Republic Scientific Practical Centre of Oncology and Medical Radiology named after N.N. Alexandrov, Lesnoy village-2, Minsky district, Minsk, Republic of Belarus, 223040

The aim of the investigation is to estimate laboratory indices to determine the number of lesions in patients with non-muscle invasive urinary bladder cancer.

Materials and methods. There were studied 552 patients with non-muscle invasive urinary bladder cancer, who had been treated in Republic Scientific Practical Centre of Oncology and Medical Radiology named after N.N. Alexandrov in 2002–2010 (Republic of Belarus). Modern laboratory techniques (immunoenzyme, immunoturbidity method, spectrofluorometry, ozonation, electron paramagnetic resonance spectrometry) were used to study tumour markers, growth factors, angiogenesis, cell-cell adhesion, endothelial damage, the products of inflammation and oxidative modifications of proteins and lipids in biological fluids (blood serum and plasma, urine). The investigation had been carried out before special treatment was started. The patients were divided into two groups depending on the number of lesions (266 patients with a single mass lesion, and 286 — with multiple mass lesions).

Results. Statistical analysis of the data obtained enabled to reveal four indices from 27 studied parameters (Urinary Bladder Cancer — UBC, Tissue Polypeptide Antigen Specific — TPS, Intercellular Adhesion Molecule — sICAM, Transmembrane HER2-neu receptor — p185) correlating maximally with the number of lesions and minimally — among themselves, and form two regression equations on their basis. The equations make it possible to determine the presence of multiple mass lesions in patients with non-muscle invasive urinary bladder cancer preoperatively, the sensitivity of equations being 81–84%. Thus, clinicians can carry out an additional thorough examination of a patient (photodynamic diagnosis) in due time in order to reveal multifocal tumour growth.

Key words: urinary bladder cancer, tumour foci, laboratory indices, biological fluids.

The significance of laboratory indices to determine the number of lesions in patients with non-muscle invasive urinary bladder cancer

Nonmuscle invasive bladder cancer (BC) is the major part among the tumours of urogenital system. The results of its treatment directly depend on early diagnosis. Currently, cystoscopy with biopsy (combined with transurethral resection is preferable) plays the leading role in the diagnostics of bladder cancer tumours, it enables to estimate the number, localization, size, and the growth character of the mass. Cystoscopy is required to be used in postoperative follow-up period as well [1]. However, this modality, along with its advantages has a number of drawbacks, and the foremost ones are discomfort for a patient, the probability of complications, e.g., infection. Moreover, in nonmuscle invasive BC (T_a, T_1, T_{is}) one of the most serious problems is failure to visualize all lesion in cystoscopy. According to literature, such diagnostic mistakes can amount to 30–40% [2]. The problem can be solved by introducing photodynamic diagnostics of BC into clinical practice [3], but the investigation is rather expensive, labour-intensive, and requiring special equipment, therefore, it is unadvisable for a patient with suspicion of bladder cancer [4]. In recent years the researchers are searching for various clinical and biochemical, molecular and biological prognostic markers showing the presence or absence of multiple lesions that would permit to abandon cystoscopy in postoperative period in favour of less invasive laboratory tests of blood und urine tests. For the past decade in Russian and foreign literature there are published the works studying the information content of some biochemical indices, tumour markers, and cytokines for BC diagnosis and monitoring [5–9].

The aim of the investigation is to estimate the significance of laboratory indices to determine the number of lesions in patients with nonmuscle invasive bladder cancer.

Materials and Methods.

There were studied the blood serum and plasma, urine of 552 patients (497 male and 55 female) with the diagnosis of nonmuscle invasive BC, who had been treated in the Department of Oncourological Pathology of Republic Scientific Practical Centre of Oncology and Medical Radiology named after N.N. Alexandrov (Republic of Belarus) in 2002–2010. The patients' age ranged from 30 to 85 years, the majority falling in the category of those over 60 yrs. BC diagnosis was made on the basis of radiologic, endoscopic, clinical methods with morphological verification required.

For contacts: Derzhavets Liliya Alexandrovna, tel.: +375(17)265-38-52, +375 29-629-81-83; e-mail: L-Dzerzhavets@mail.ru

The patients were divided into two groups depending on the number of tumours: the 1st group (n=266) — patients with a single mass lesion, and the 2nd group (n=286) — with multiple mass lesions revealed.

Before special therapy in all the examined patients there were determined the concentration of tumour markers in biological fluids: Nuclear Matrix Protein 22 (NMP-22), Bladder Tumor Antigen (BTA), Tissue Polypeptide Antigen (TPA), Tissue Polypeptide Antigen Specific (TPS), Cytokeratin Fragment 19 (Cyfra 21-1), Urinary Bladder Cancer (UBC), carcinoembryonal antigen (CEA), thymidine kinase (TK); growth gactor, angiogenesis, cell-cell adhesion, and soluble forms of their receptors: Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF), Intercellular Adhesion Molecule (sICAM), Platelet Endothelial Cell Adhesion Molecule (sPECAM), Tumor Necrosis Factor-α (TNF-α), Tumor Necrosis Factor Receptor (p55), Transmembrane HER2-neu receptor (p185); endothelium damage factors: endostatin, Willebrand factor, homocysteine; inflammation products: C-reactive protein (CRP), antitrypsin, interleukin-8 (IL-8); the products of oxidative modification of proteins and lipids: olefinic bonds of lipid blood fraction (OB), malonic dialdehyde (MDA), nitrosodimethylamines, ESR-parameter α.

Table 1

The content of the studied laboratory indices in biological fluids of patients with nonmuscle invasive BC

Index	Compared groups	Statistic parameters			
		n	Me (25%; 75%)	min–max	p
UBC, mcg/ml	I	266	18.5 [15.3; 23.5]	4.4–190.9	<0.0001
	II	286	23.7 [18.8; 27.9]	3.7–196.8	
TPS, IU/ml	I	264	97.45 [89.5; 101.4]	64.0–126.0	<0.0001
	II	286	101.0 [95.5; 105.0]	11.8–130.0	
TPA, IU/ml	I	264	131.0 [127.0; 140.0]	93.0–162.0	<0.0001
	II	286	134.0 [129.4; 142.0]	99.0–162.0	
Cyfra 21-1, ng/ml	I	266	2.10 [1.68; 2.40]	0.42–4.90	<0.0001
	II	285	2.30 [1.90; 2.70]	0.52–8.20	
BTA, U/ml	I	266	13.7 [8.70; 19.60]	2.4–500.0	<0.0001
	II	286	16.4 [10.60; 28.00]	0.5–500.0	
NMP-22, U/ml	I	262	22.25 [16.7; 33.4]	9.1–112.0	<0.0006
	II	283	29.40 [19.00; 36.60]	9.2–112.0	
VEGF, pg/ml	I	265	258.30 [214.00; 315.80]	54.2–975.1	<0.0001
	II	285	296.50 [235.80; 376.80]	34.8–1265.6	
sICAM, ng/ml	I	264	122.80 [116.00; 138.00]	76.8–456.0	<0.0001
	II	282	131.35 [120.00; 146.00]	15.0–410.0	
sPECAM, ng/ml	I	264	102.80 [95.45; 113.85]	65.0–220.0	0.0006
	II	282	109.00 [98.50; 116.80]	47.3–210.0	
TNF-α, pg/ml	I	263	9.78 [5.92; 13.93]	1.41–64.80	<0.0001
	II	281	12.40 [8.64; 24.54]	1.02–45.32	
p55, ng/ml	I	261	3.30 [2.40; 4.70]	1.0–18.0	<0.0001
	II	272	4.15 [2.80; 5.40]	1.5–11.8	
EGF, ng/ml	I	257	21.60 [18.60; 27.40]	5.5–200.5	0.0005
	II	270	24.70 [19.50; 31.70]	9.8–152.8	
p185, ng/ml	I	255	4.43 [3.70; 5.90]	2.8–12.5	<0.0001
	II	263	5.70 [4.30; 7.00]	0.5–11.6	
FGF, pg/ml	I	262	132.00 [112.40; 155.60]	19.10–348.14	<0.0001
	II	277	143.60 [122.70; 198.00]	16.68–423.5	
Interleukin-8, pg/ml	I	165	23.90 [18.40; 33.90]	11.2–112.3	0.0076
	II	176	29.40 [920.10; 45; 65]	11.7–98.4	
Antitrypsin, g/l	I	258	1.20 [1.00; 1.40]	0.8–2.4	<0.0001
	II	281	1.30 [1.10; 1.50]	0.7–2.8	
C-reactive protein, mg/dL	I	263	0.55 [0.45; 0.70]	0.1–7.0	<0.0001
	II	285	0.65 [0.52; 0.80]	0.1–4.2	
	II	237	25.80 [21.80; 29.66]	10.26–45.70	

VEGF, FGF, sICAM, sPECAM, endostatin, homocysteine, TPA, TPS, TK in blood serum, UBC, NMP-22, BTA in urine were determined using immunoenzymoassay on automatic analyzers of Alisei (Seac, Italy) and Elecsys 2010 (Roche, Switzerland) using reagent kit made by BioSource (Belgium), R&DSystems (USA), BenderMedSystems (Austria), DRG (USA), Roche (Switzerland). Using immunoturbidimetric method, there were measured the level of Willebrand factor in blood plasma on hemostasis analyzer, STA Compact (Diagnostica Stago, France), and determined the concentration of CRP and antitrypsin in blood serum on automatic analyzer Hitachi-912 Roche (Switzerland). By spectrofluorometric method using fluorescence spectrophotometer Hitachi-MPF-4 Roche (Switzerland) there was studied the content of MDA in blood; OB were determined by ozonation method using "AMMO" analyzer (Russia), ESR-parameter α — by the method of spin ESR-spectrometry on EPRA-9300 analyzer (ADANI, Belarus).

Statistical processing of data was performed using Statistica 6.0 "StatSoft" and included the testing of normalcy of quantitative characteristics distribution in the sampling using Shapiro–Wilk test. Quantitative values of the characteristics not following the normal probability law were described as median-quartile characteristics: median, 25 and 75 percentiles (Me [25; 75]), minimum and maximum values (min-max). Statistical differences were studied using Mann–Whitney test. To analyze the relations between the indices studied there was used Spearman nonparametric correlation analysis, $p<0.05$ was taken as the criterion of statistical significance. Logistic regression and ROC-analysis were used to estimate the significance of laboratory values to determine the number of tumours.

Results and Discussion. Statistical processing of the obtained results of the study in the groups of patients with single and multiple tumour foci revealed statistically significant differences of 17 from 27 of laboratory parameters studied (Table 1).

Spearman nonparametric correlation analysis showed that the following indices moderately correlate ($R=0.35–0.55$; $p<0.05$) with the number of tumour foci: UBC, TPS, p185, TPA, ICAM, CRP, p55, FGF, TNF-α. The sensitivity and specificity analysis of the above listed indices was performed relying on characteristic ROC-curves tracing and AUC (Area Under Curve) determination. According to the data obtained (Table 2) there was stated that none of the indices studied has sufficient sensitivity and specificity to determine the number of tumour foci in patients with nonmuscle invasive BC (sensitivity <75%, specificity <65%).

There was developed a statistical model in the form of regression equations to enhance the efficiency of the prognosis of the presence or absence of multiple tumour foci. To develop the model there were studied the correlation relations between the analyzed indices according to Spearman. The correlation coefficient being $R<0.5$, the correlation was considered to be close that required the exclusion of one index of the pair from the analysis. Close correlation was found in the pairs: UBC–CRP; UBC–FGF; p185–p55; TPS–TPA, sICAM–TNF-α, while TPA, CRP, p55, FGF, TNF-α were excluded from the analysis. The indices minimally correlating among themselves were included in regression equations: UBC–p185 ($R=0.25$; $p=0.028$); TPS–sICAM ($R=0.25$; $p=0.026$), and on their base there were set up two regression equations, according to which there

Table 2

The characteristics of ROC-curves of the studied indices

Index	Sensitivity, %	Specificity, %	Cutoff point in measurement units	AUC	p
UBC, mcg/ml	73.8	59.0	19.3	0.68±0.02	<0.0001
TPS, IU/ml	71.0	49.6	97.1	0.64±0.02	<0.0001
p185, ng/ml	68.8	52.9	4.6	0.64±0.02	<0.0001
TPA, IU/ml	68.2	49.6	130.9	0.62±0.02	<0.0001
sICAM, ng/ml	68.1	51.9	123.9	0.61±0.02	<0.0001
СРБ, mg/dL	65.3	53.2	0.6	0.61±0.02	<0.0001
p55, ng/ml	64.0	54.4	3.4	0.62±0.02	<0.0001
FGF, pg/ml	63.9	51.5	132.0	0.60±0.02	<0.0001
TNF-α, pg/ml	62.3	60.8	10.7	0.62±0.02	<0.0001

Table 3

Prognosis of the presence of multiple tumour foci in patients with BC using logistic regression equations

Index	Regression equation	Statistical significance of model p
UBC, mcg, p185, ng/ml	Z_1= exp [–2.1779+(0.215678)×p185+(0.046401)×UBC]/(1+exp [–2.1779+(0.215678)× p185+ (0.046401)×UBC])	<0.00001
sICAM, ng/ml, TPS, IU/ml	Z_2= exp [–4.3158+(0.004084)×sICAM +(0.039214)×TPS]/(1+exp [–4.3158+(0.004084)× sICAM+(0.039214)×TPS])	<0.00001

Notes: Z — probability of determination of multiple tumour foci (expressed in significant figures from 0 to 1); exp — constant value = 2.71.

were performed the prognosis of the presence of multiple tumour foci in patients with nonmuscle invasive BC (Table 3).

Using regression equation Z_1 one should determine the concentration of tumour marker UBC in urine and p185 — in blood serum of patients with BC, and enter the values in Z_1 equation. And using regression equation Z_2 one should determine the concentration of TPS and sICAM in blood serum of patients with BC, and solve Z_2 equation.

The comparison of the results obtained when using the developed equations with histological findings was drawn in reclassification of learning sample (552 patients with bladder cancer). ROC-analysis was performed to obtain Z_1 and Z_2 values.

Using regression equation Z_1 240 patients from 286 (84%) were correctly referred to the group of patients with multiple tumour foci, and 165 patients from 266 (62%) were correctly referred to the group of patients with a single tumour focus. Thus, the sensitivity of the technique was 84%, specificity — 62%. AUC for Z_1 was 0.72±0.02, $p<0.0001$; cutoff point — 0.42.

Using regression equation Z_2 232 patients from 286 (81%) were correctly referred to the group of patients with multiple tumour foci, and 146 patients from 266 (55%) were correctly referred to the group of patients with a single tumour focus. Thus, the sensitivity of the method was 81%, specificity — 55%. AUC for Z_2 was 0.69±0.02, $p<0.0001$; cutoff point — 0.47.

Therefore, in $Z_1>0.42$ or $Z_2>0.47$ we may say about the presence of multiple tumour foci in patients with BC. In $Z_1<0.42$ or $Z_2<0.47$ one may state that BC patient has a single tumour focus.

Conclusion. Statistical analysis of the results of the study carried out using modern investigation techniques of a wide range of laboratory parameters in biological fluids of patients with nonmuscle invasive bladder cancer for the purpose of estimation of their significance to determine the number of tumour foci revealed four indices maximally correlating with the number of tumour foci, and minimally — among themselves, and on their bases enabled to develop two regression equations. The equations permit to make prognoses of the presence of multiple tumour foci in patients with nonmuscle invasive bladder cancer preoperatively, the sensitivity of the method being 81–84%. It enables clinicians in due time to carry out a thorough follow-up examination (photodynamic diagnosis) in order to reveal multifocal tumour growth that, finally, makes it possible to increase the efficiency of transurethral resections of nonmuscle invasive bladder cancer, avoid reoperations, and reduce the patients' length of hospital stay.

References

1. Rolevich A.I., Sukonko O.G., Krasnyy S.A. et al. *Onkourologiya — Oncourology* 2010; 1: 59–64.

2. Hall M.C., Chang S.S., Dalbagni G. et al. Guideline for the management of nonmuscle invasive bladder cancer (stages T_a, T_1 and T_{is}): 2007 update. *J Urol* 2007; 178: 2314–2330.

3. Yagudaev D.M., Bulgakova N.N., Sorokatyy A.E. et al. *Urologiya — Urology* 2007; 2: 53–58.

4. Dindyal S., Nitkunan T., Bunce C. The economic benefit of photodynamic diagnosis in non-muscle invasive bladder cancer. *Photodiagnosis Photodin Ther* 2008; 5(2): 153–158.

5. Prokhorova V.I., Derzhavets L.A., Lappo S.V. et al. Endogennye faktory riska raka mochevogo puzyrya. V kn.: *Materialy II kongressa Rossiyskogo obshchestva onkourologov*. 4–5 oktyabrya 2007 [Endogenic risk factors of urinary bladder cancer. In: The reports of the II Congress of Russian Oncourologists Society. October, 4-5, 2007]. Moscow; 2007.

6. Kushlinskiy N.E., Gershteyn E.S., Lyubimova N.V. *Vestnik Moskovskogo onkologicheskogo obshchestva — Herald of Moscow oncological society* 2007; 1: 5–7.

7. Glybochko P.V., Shakhpazyan N.K., Ponukalin A.N. et al. *Klinicheskaya laboratornaya diagnostika — Clinical Laboratory Diagnostics* 2011; 5: 16–20.

8. Spirina L.V., Kondakova I.V., Usynin E.A. et al. *Sibirskiy onkologicheskiy zhurnal — Siberian Journal of Oncology* 2008; 4(28): 65–70.

9. Szarvas T., Jeger T., Droste F. et al. Serum levels of angiogenic factors and their prognostic relevance in bladder cancer. *Pathol Oncol Res* 2008; 9: 228–232.