Evaluation of NMP22 in bladder cancer patients sensitive to environmental toxins

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Abstract

Background. Bladder cancer (BC) is recognized as environmentally related. The interaction of environmental exposure to chemicals and genetic susceptibility seem to play important roles in BC development. In order to improve diagnosis and the recognition of BC risk, a group of markers which combine genetic susceptibility with detoxification and nuclear matrix protein (NMP22) is proposed.

Objectives. The aim of the study was to examine the utility of nuclear matrix protein (NMP22) as a diagnostic marker in BC in genetic susceptibility (NAT2 slow acetylators) combined with detoxification abilities (glutathione S-transferase GST and isoenzyme GST-π).

Material and methods. The NMP22 level in urine, N-acetyltransferase 2 (NAT2) genotype and GST activity in hemolysate blood, as well as isoenzyme GST-π level, were determined in the urine and serum of 43 patients with BC and from 25 non-cancer controls. NMP22 and isoenzyme GST-π levels were measured by ELISA. The NAT2 genotype was examined in DNA isolated from whole blood using the PCR (Polymerase Chain Reaction) technique, while the activity of GST was determined with the spectrophotometric method.

Results. In the BC group, NMP22 (p = 0.005) concentration, GST-π (p = 0.003) in urine and GST (p = 0.009) activity in blood were statistically significantly higher than in the healthy controls. The majority of BC patients were slow acetylators (NAT2 genotype). A correlation between the level of nuclear matrix protein NMP22 and GST was found in all BC group (p = 0.007) and also slow acetylators (p = 0.0147).

Conclusions. The results support the utility of a marker combination, which covers the genetic susceptibility to chemicals with the level of detoxification and nuclear matrix protein in BC patients. A relationship between NMP22 level in urine, GST level in blood and NAT2 genotype was observed. Also the isoenzyme GST-π in urine seems useful as a marker of BC.

Key words: bladder cancer, GST, NMP22, NAT2, toxins

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Bladder cancer (BC) is the ninth most common malignancy in the world, the sixth in the USA and the fifth in Europe. According to the Polish National Cancer Registry, it is the fourth cancer among men and the thirteenth among women. There is abundant evidence for the connection between exposure to chemicals and the development of bladder cancer. Since Ludwik Rehn made the groundbreaking observation of increased BC incidence among aniline dye factory workers, many other causative agents have also been identified.

Bladder cancer is responsible for the highest cost of therapy among all the malignancies and, therefore, there is an urgent need for improving its early diagnosis and monitoring. The current diagnostic methods are insufficient, invasive and expensive (cystoscopy, biopsy, urography, computed tomography). Among many proposed potential BC markers, such as bladder tumor antigen (BTA), bladder cancer specific nuclear matrix protein (BLCA), Lewis X antigen, Aurora A kinase, carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), ImmunoCyt test, UroVysion, etc., only a few tests have been approved for the routine diagnosis. The Food and Drug Administration (FDA) accepted BTA stat, BTA TRAK, UroVysion and NMP22BladderChek.

Our interest has been focused on nuclear matrix proteins as BC in respect of their engagement in chemically induced carcinogenesis. Nuclear Matrix Protein 22 (NMP22) is a non-histone chromatic protein that belongs to the bladder cancer-specific nuclear mitotic proteins. NMP22 is responsible for the proper position of chromatin during mitosis and final separation of daughter cells. It has an important role in ribonucleic acid (RNA) synthesis, deoxyribonucleic acid (DNA) transcription and replication, and in morphological changes of nuclear structure; therefore, it can also have a huge influence on the genes that are crucial in carcinogenesis. The sensitivity of NMP22 as a BC marker is high (the urinary level of NMP22 in patients with BC is up to 25-fold greater than in healthy ones), but the specificity remains insufficient, because false-positive results can be obtained due to urinary infections, urolithiasis and other bladder disorders.

Human N-acetyltransferase 2 (NAT2) is a phase 2 drug-metabolizing enzyme that plays an important role in the detoxification of many carcinogens present in the environment.

The NAT2 gene, located on chromosome 8p22, is autosomal dominant. This enzyme exhibits genetic polymorphisms. Different acetylation phenotypes within the population are the result of mutations in the NAT2 gene. Polymorphisms of NAT2 confer slow, intermediate, or fast acetylator phenotypes. The alleles NAT2*5, NAT*6 and NAT*7 are typical for slow acetylators. There are consistent reports on the connection of the NAT2 slow acetylator polymorphisms with higher BC risk, both independently and in association with smoking or occupational exposures, especially to arylamin. There are no reports on the specificity of NMP22 in the slow acetylators group.

It seems interesting to explain whether the specificity of the NMP22 marker increases in the BC group of genetically susceptible to environmental toxins slow acetylators with the N-acetyltransferase 2 (NAT2) genotype. The purpose of the study was to select the NAT2 genotype group from BC patients and to examine the NMP22/NAT2 correlation. Many environmental toxins, especially aromatic hydrocarbons, are removed from the body after splitting with glutathione by glutathione S-transferase (GST), so looking for a relationship of BC with environmental exposure, the NMP22/GST correlations were also examined as well as the utility of GST isoenzyme (GST-π) as a marker in BC. These enzymes play an important role in the detoxification of environmental chemicals capable of causing BC. GST-π excretion in urine is an important marker of the distal part of renal tubules and loop of Henle damage.

The aim of the study was to examine the utility of the nuclear matrix protein (NMP22) as a diagnostic marker of BC in genetic susceptibility (NAT2 slow acetylators) combined with detoxification abilities (GST and isoenzyme GST-π). Because bladder cancer is described as an environmentally related cancer, it seems necessary to include markers connected with genetic susceptibility and detoxification.

**Material and methods**

**Patients and clinical samples**

After their informed consent had been given, first morning urine and blood samples were collected from people who suffered from BC hospitalized in the Department and Clinic of Urology and Urological Oncology of the University Hospital in Wroclaw. The BC group consisted of 43 patients (35 men – M and 8 women – W) aged 36–87 years (mean age 69) with histopathologically proved bladder cancer. The BC group was divided into 4 subgroups based on growth – T (stage): Ta (n = 21), T1 (n = 13), T2 (n = 6) and TIS (n = 3). The majority of patients were smokers (67%). About 70.6% among them were urban dwellers and 38.2% reported having had contact with pesticides. Exposure related to the occupation or the place of residence in the industrial area was declared by 41.2% (date from questionnaire). The control group included 25 healthy volunteers (18 men and 7 women) aged 54–81 years (mean age 65) without any urinary tract diseases. The project received the permission of the Bioethics Committee of Wroclaw Medical University (no. KB-292/2013).

Mid-stream morning urine samples were collected in polystyrene containers (Aptaca, Italy) and a urine screening analysis was performed using the dipstick test (Combur 10 Test M, Roche Diagnostics GmbH, Mannheim). Next, the urine samples were centrifuged...
for 10 min (1438 × g at 4°C). The obtained supernatant was removed to Eppendorf tubes and stored at −80°C for further investigation.

Blood samples were collected from patients and controls into 2 plastic tubes (BD Vacutainer, USA), one with sodium citrate as an anticoagulant and one without (serum). The tubes were centrifuged at 1438 × g for at least 10 min at 4°C. The supernatant (plasma and serum) was frozen at −80°C until analyzed. The erythrocytes obtained after plasma isolation were hemolyzed in cold phosphate buffered saline and centrifuged at 30065 × g for 15 min at 4°C. The obtained lysate was used for GST measurement.

**Immunoenzymatic quantitative analysis (ELISA)**

**NMP22 concentration in urine**

NMP22 concentration was assessed by an enzyme-linked immunosorbent assay (ELISA Kit, Shanghai Sunred Biological Technology Co; CRL; sensitivity 0.25 ng/mL), using NMP22 specific antibodies labeled with biotin, and combined with streptavidin-horseradish peroxidase conjugate to form an immune complex. The absorbance of the samples was then measured with a STAT FAX 2100 spectrophotometer at λ = 450 nm.\(^5\) The obtained NMP22 level was calculated in relation to the urine creatinine level that had been previously estimated by Jaffe’s routine method. Under alkaline conditions, creatinine reacts directly with picric ions to form a reddish complex, the absorbance of which can be measured at \(\lambda = 520\) nm.

**GST-π activity in urine and serum**

The concentration of GST-π in urine (ELISA kit, EKF Diagnostics; Ireland; sensitivity 2.0 ng/mL) and GST-π in serum (ELISA kit, Immunodiagnostic AG; Germany; sensitivity 10.7 ng/mL) was assessed by an enzyme-linked immunosorbent assay. This analysis was conducted according to the manufacturer’s instructions. The calibration curves were prepared using a purified standard for each protein assessed. Curve fitting was accomplished following the manufacturer’s instructions (range 0–40 ng/mL). The absorbance was measured spectrophotometrically using the STAT FAX 2100 spectrophotometer at \(\lambda = 450\) nm.\(^12,13\) The obtained level of GST-π in urine was then calculated in relation to the urine creatinine level measured previously by Jaffe’s routine method.

**Polymerase chain reaction (PCR), electrophoresis and acetylation genotype evaluation**

The NAT2 polymorphisms and allelic status were determined by the polymerase chain reaction/restriction fragment/length polymorphism.

In order to determine the type of acetylation, the NAT2 genotype was examined in DNA isolated from whole blood using a Blood Mini kit (A&A Biotechnology, Poland). DNA was isolated according to the manufacturer’s protocol supplied with the kit reagents. The polymorphic nucleotides at position C481T, A803G, G857A and G590A of the NAT2 coding region were analyzed by digesting the PCR products with the endonucleases KpnI, Dde, BamHI, TaqI, respectively.

The obtained DNA was dissolved in 200 µL of 10 mM Tris-HCl buffer (pH = 8.5) and stored in the refrigerator until analysis. Amplification of the NAT2 gene fragment was carried out using a MixPlus 2xPCR kit (A&A Biotechnology, Poland). Primers used in the reaction had the sequence: NAT2 F 5’GCT AGC GGG GGA TCC TCT TC 3’ and NAT2 R 5’TGT GAT GGT TAC ACA ACA AGG G 3’. The amplification primers were used at a concentration of 10 µM each, and the previously obtained DNA was used in an amount of 1 µL. Reactions were carried out according to the program: 94°C – 4 min, 34 cycles: (94°C – 30 s, 59°C – 30 s, 72°C – 45 s), 72°C – 5 min, 8°C – 10 s. The correctness of amplification for NAT2 gene fragments was checked on 2% agarose gel. The restriction digestion of obtained NAT2 gene fragments was carried out in 0.2 mL tubes according to the following scheme: 0.2 µL of enzyme, 0.8 µL of Fast Digest buffer, 2 µL of PCR product and 5 µL of H2O (Milli-Q system). The reaction was performed for 30 min at 37°C or 60°C for the KpnI, Ddel, BamHI and TaqI enzymes. The wild-type allele (NAT2*4) was identified after complete digestion by KpnI, TaqI, BamHI and Ddel. Genetic variation in the NAT2 gene changed the recognition sites for KpnI, TaqI, BamHI and Ddel restriction enzymes. NAT2*5, NAT2*6, and NAT2*7 alleles were identified by the presence of C481T, G590A, G857A and A803G genetic variations, respectively.

According to the obtained results, the BC patients were divided into three categories: I – with slow acetylation genotype SA (*5/*5,*5/*6,*5/*7,*6/*6,*6/*7); II – intermediate acetylation genotype IA (*4/*5,*4/*6); III – fast acetylation genotype FA (*4/*4).

**Spectrophotometric method of enzymatic activity evaluation**

The activity of GST was determined with the spectrophotometric method (GST Assay Kit, Sigma, USA) based on the conjugation of the thiol group of glutathione with 1-chloro-2,4-dinitrobenzene (CDNB). The absorbance of the colored product was measured with a STAT FAX 2100 spectrophotometer at \(\lambda = 340\) nm.\(^15\) The hemoglobin level in hemolysates was also evaluated using routine Drabkin’s method and then the GST level was calculated in relation to the amount of hemoglobin in the sample.
Statistical analysis

Statistical analysis was conducted with Statistica PL software (v. 10.0). The variability of distribution was checked with the Lilliefors and the Kolomogorov-Smirnov tests. For variables with normal and non-normal distribution, Student’s t-test and U Mann-Whitney test were used, respectively. Correlations were assessed by the Pearson/Spearman correlation analysis. A p-value of less than 0.05 was considered statistically significant.

Results

Nuclear matrix protein 22

There was a statistical difference, an almost 2 times higher level of NMP22, expressed as ng/mg creatinine, in patients with bladder cancer than in the control group (p = 0.005) (Fig. 1). The concentration of NMP22 was up to 20% higher in the group of smokers compared to non-smokers. Women had more than 1.5 times higher concentration of NMP22 compared to men, but this difference was not statistically significant (Fig. 1). No correlation between the level of NMP22, BMI, erythrocyturia and proteinuria was found. The analysis of the association between NMP22 concentration and cancer stage was not completed because of the small size of subgroups (e.g. 21 persons with Ta, 13 with T1, 6 with T2, 3 with TIS); however, the mean urinary level of NMP22 was the highest in T1 stage (14.34 ng/mg cr.) and TIS (14.82 ng/mg cr.) and it was almost 2 times lower in Ta (9.89 ng/mg cr.) (Fig. 1).

N-acetyltransferase 2 genotype (NAT2)

The analysis of the NAT2 genotype in BC patients revealed the presence of 4 mutations (Table 1).

1. C481T mutation revealed the presence of a wild type genotype (wt) in 1 patient, the genotype with 1 mutated allele (ht) in 33 patients, and 2 mutated alleles (mt) in 9 patients. The frequency of the mutant C481T allele of the patients was 97.7%
2. A803G mutation corresponding values were respectively wt – 15; ht – 21; mt – 7. The frequency of the mutant A803G allele of the patients was 65.1%
3. G857A mutation corresponding values were respectively wt – 34; ht – 9; mt – 0. The frequency of the mutant G857A allele of the patients was 20.9%
4. G590A mutation corresponding values were respectively wt – 16; ht – 24; mt – 3. The frequency of the mutant G590A allele G590A of the patients was 62.8%.

There were 8 different genotypes of NAT2 in the examined group of BC patients. Three people (6.9%) with NAT2 *4/*5, *5/*6, *5/*7, *6/*6 and *6/*7 as slow acetylators (SA). The obtained results indicated that the slow acetylation genotype dominated in the BC patient group (Table 2).

The correlation between NMP22 concentration and acetylation genotype was examined. No significant correlation was found, although the mean NMP22 urinary levels in each group – fast (FA), intermediate (IA) and slow (SA) acetylators – were different, the highest in slow acetylators (12.44 ng/mg cr.) and the lowest in fast acetylators (5.58 ng/mg cr.) (Table 3, Fig. 1).

No statistically significant correlation was found between the acetylation genotype and cancer stage. However, in the group of slow acetylators the predominant stages were Ta (42.9%) and T1 (36%).

Glutathione S-transferase and isoenzyme π

The analyses of glutathione S-transferase (GST) activity and its isoenzyme π in the blood of BC patients and the control group were shown (Table 1). There was a statistically significant increase of GST activity in the BC group compared with the control group (3.20 and 2.39 μmol/g Hb, respectively) (p = 0.009). The analyses of the correlation

Table 1. The analysis of four NAT2 SNP tested of BC patients

<table>
<thead>
<tr>
<th>NAT2 polymorphisms</th>
<th>C481T KpnI n (%)</th>
<th>A803G DdeI n (%)</th>
<th>G857A BamHI n (%)</th>
<th>G590A TaqI n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>1 (2.3)</td>
<td>15 (34.9)</td>
<td>34 (79.1)</td>
<td>16 (37.2)</td>
</tr>
<tr>
<td>ht</td>
<td>33 (76.7)</td>
<td>21 (48.3)</td>
<td>9 (2.9)</td>
<td>24 (55.8)</td>
</tr>
<tr>
<td>mt</td>
<td>9 (2.9)</td>
<td>7 (16.3)</td>
<td>0 (0)</td>
<td>3 (1.29)</td>
</tr>
</tbody>
</table>

wt – wild type; ht – heterozygotus mutant; mt – homozygotus mutant; SNP – single nucleotide polymorphisms; KpnI, DdeI, BamHI, TaqI – restriction enzymes.
between the urinary level of NMP22 and the blood level of GST in BC patients showed a statistically significant positive correlation \( r = 0.41 \) \( p = 0.007 \) (Fig. 2). Also, a significant difference in the isoenzyme GST-\( \pi \) activity, but only in urine, not in the serum, was noted for BC patients in comparison to the control group (C) \( p = 0.003 \). The mean level of GST-\( \pi \) in urine in the BC and C group was 28.98 and 4.6 ng/mg creatinine, respectively (Table 3). A positive NMP22/GST-\( \pi \) urine level correlation was noted only for T1 stage (13 cases), not for the whole BC group \( r = 0.818 \); \( p = 0.001 \).

The correlation between NAT2 genotype and the activity of GST and GST-\( \pi \) in BC patients was also assessed (Table 3). The level of GST was lower in the SA group (28 patients) than in IA (12 patients) and FA (3 patients) acetylators (2.98; 3.54 and 3.98 ng/g Hb respectively) (Table 3), but the differences were not statistically significant, including in relation to the activity of isoenzyme GST-\( \pi \) (Table 3).

With regard to NAT2 genotype, a NMP22/GST-\( \pi \) correlation was found only in the slow acetylator group \( r = 0.46 \); \( p = 0.0147 \). No significant NMP22/GST-\( \pi \) correlation in SA, IA or in FA acetylators was found.

### Table 2. The analysis of genotype and acetylation status in BC patients

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Acetylation status</th>
<th>Number: n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>*4/*4</td>
<td>fast</td>
<td>3 (6.9)</td>
</tr>
<tr>
<td>*4/*5</td>
<td>intermediate</td>
<td>4 (9.3)</td>
</tr>
<tr>
<td>*4/*6</td>
<td>intermediate</td>
<td>8 (18.6)</td>
</tr>
<tr>
<td>*5/*5</td>
<td>slow</td>
<td>6 (13.6)</td>
</tr>
<tr>
<td>*5/*6</td>
<td>slow</td>
<td>12 (27.9)</td>
</tr>
<tr>
<td>*5/*7</td>
<td>slow</td>
<td>3 (6.9)</td>
</tr>
<tr>
<td>*6/*6</td>
<td>slow</td>
<td>3 (6.9)</td>
</tr>
<tr>
<td>*6/*7</td>
<td>slow</td>
<td>4 (9.3)</td>
</tr>
</tbody>
</table>

### Table 3. The values of NMP22, GST, GST-\( \pi \) in subgroups depending on the acetylation genotypes

<table>
<thead>
<tr>
<th>Acetylation genotype/ values in BC and C</th>
<th>NMP22 [ng/mg creat.] (urine)</th>
<th>GST [( \mu )mol/g Hb] (blood)</th>
<th>GST-( \pi ) [ng/mL] (serum)</th>
<th>GST-( \pi ) [ng/mg creat.] (urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>( \bar{x} ) 12.44</td>
<td>11.61</td>
<td>2.96</td>
<td>159.51</td>
</tr>
<tr>
<td>SD</td>
<td>11.61</td>
<td>1.09</td>
<td>216.82</td>
<td>29.72</td>
</tr>
<tr>
<td>IA</td>
<td>( \bar{x} ) 11.22</td>
<td>10.91</td>
<td>3.54</td>
<td>99.89</td>
</tr>
<tr>
<td>SD</td>
<td>10.91</td>
<td>1.53</td>
<td>214.43</td>
<td>35.13</td>
</tr>
<tr>
<td>FA</td>
<td>( \bar{x} ) 5.58</td>
<td>1.07</td>
<td>3.98</td>
<td>72.97</td>
</tr>
<tr>
<td>SD</td>
<td>1.07</td>
<td>2.45</td>
<td>205.30</td>
<td>32.36</td>
</tr>
<tr>
<td>BC</td>
<td>( \bar{x} ) 11.48*</td>
<td>10.95</td>
<td>3.20*</td>
<td>142.00</td>
</tr>
<tr>
<td>SD</td>
<td>10.95</td>
<td>1.32</td>
<td>225.37</td>
<td>35.17</td>
</tr>
<tr>
<td>C</td>
<td>( \bar{x} ) 5.15</td>
<td>2.03</td>
<td>2.39</td>
<td>68.07</td>
</tr>
<tr>
<td>SD</td>
<td>2.03</td>
<td>0.84</td>
<td>192.08</td>
<td>4.62</td>
</tr>
</tbody>
</table>

* statistically significant activity in comparison to control group; SA – slow acetylation; IA – intermediate acetylation; FA – fast acetylation in BC (bladder cancer patients group); C – control group.

### Discussion

Cystoscopy with the cytology of urine has been a standard procedure in the detection of bladder cancer. It is also used in the current surveillance protocol every 3 months for 1–3 years of therapy, every 6 months for the next 1–3 years, and then annually thereafter. This is costly and invasive to the patient. The detection of BC is still an important global medical problem. In the last years, several new urine markers have been developed for urothelial carcinoma detection. They should be non-invasive, have high sensitivity, high specificity and a positive predictive value to avoid unnecessary cystoscopies. NMP22 was considered as a promising marker, accepted by the FDA, but critical opinions are also published nowadays. The NMP22 test has been evaluated as a non-invasive (urine), qualitative (ELISA) and quantitative (Bladder Check) method; the latter in particular is accepted for disease monitoring. It was observed that a bladder cancer patient has a higher amount of NMP22 in urine. However, the specificity of the marker NMP22 was low, because this protein is released from dead urothelial cells and could give false positive results, e.g. in an inflammatory state. The review of bladder cancer markers led to the conclusion that there is no single one that could be recommended for reducing cystoscopy frequency. It seems that the best way to improve BC detection is to look for a combination of tumor markers. Bladder cancer is an environmentally related cancer. Many chemicals are able to induce BC. Tobacco smoking is a major cause of BC in humans. The interaction of environmen-
The relationship between environmental exposure and genetic susceptibility seems to be an important cause of BC. An increased risk of BC was observed for smokers with NAT2 slow acetylation genotype. The genetic polymorphisms of enzymes involved in detoxification (activation) or the detoxification of environmental toxins play a significant role in the individual susceptibility to BC, particularly in regards to smokers. There are some reports on the association of NAT2 slow acetylator genotype and the risk of BC in the exposure to aromatic amines, but there are also reports where the association was not found. The slow acetylator status was proved as a genetic risk factor for arylamine-induced bladder cancer for the European (Caucasian) population, but not for the Chinese population.

In our study a significantly higher (over twofold) urinary level of NMP22 was noted in patients with BC compared to the control group. The majority of patients (61%) were slow acetylators. Although we did not find a significant correlation between NMP22 level and NAT2 genotype, an upward trend was observed: from the lowest NMP22 concentration in fast acetylators to the highest NMP22 concentration in the urine of slow acetylators. The study was limited by a small numbers in the subgroups. It was also found that the frequency of NAT2 slow acetylator genotype was significantly higher in BC patients than in the normal group. Also, in our group of BC patients, the slow acetylator genotype was predominant.

The examination of NAT2 mutation deserves special attention, because of its role in detoxification by N-acetyltransferase activation, especially through the NAT2 regulated pathway. It is also known that slow acetylators are more sensitive to aromatic amines and other agents present in the environment. Examination of NAT2 mutation deserves special attention, because of its role in detoxification by N-acetyltransferase activation, especially through the NAT2 regulated pathway. It is known that the NAT2 slow genotype is characterized by the decreased acetylation of aromatic amines, which increases the risk of bladder cancer, especially in heavy smokers.

The aim of our study was to examine whether the combination of markers which join the genetic susceptibility to chemicals with the level of detoxification and NMP22 is useful in BC diagnosis and whether the specificity of NMP22 is high enough in BC patients with the slow acetylation genotype NAT2 and could be helpful in early diagnosis of BC. To our knowledge no one has examined the specificity of NMP22 in regards to the group with a genetic susceptibility to environmental toxins. It seemed interesting to assess the relation between genetic susceptibility and the activity of enzymes which play an important role in detoxification (GST, GST-π) and urinary NMP22 quantity. There was a preliminary study evaluating the utility of NMP22 for prophylactic screening of early recurrence of disease for patients with genetic susceptibility. There is some evidence of the greater importance of NMP22 estimation in risk groups, e.g. smokers and the elderly. Investigations of Cui et al. indicate that the NAT2 slow acetylator genotype can decrease the detoxification of tobacco mutagens, such as arylamines, and be synergistic with other metabolizing enzymes. The authors suggest that individual genetic variations affect the metabolic activity of enzymes involved in arylamine detoxification could modify the risk of bladder cancer. Also, the role of nicotine-induced cell proliferation is reported.

Glutathione S-transferase is involved in the biotransformation of many substances qualified as risk factors for BC, specifically aromatic and halogenated hydrocarbons. GST catalyzes the conjugation of electrophilic toxins with glutathione. Some studies have reported that the GSTT1 null genotype was associated with an increased risk for BC. Thus, changes in the expression and activity of GST influence the individual resistance or susceptibility to xenobiotic-induced damage. According to Saygili et al., the stimulation of GST expression in people with BC is a response to DNA damage caused by various environmental factors. The results of our study showed significantly higher total GST activity in the BC group in comparison to healthy volunteers. Although no statistically significant differences were found for the isoenzyme, GST-π in serum and a significant difference in the GST-π level in urine suggest that isoenzyme π is also involved in the examined processes. The investigations have revealed increased detoxification among BC patients and suggest that GST could be an additional environmental related marker in BC. The statistically positive correlation between NMP22 and GST points to a relationship between the intensity of detoxification and nuclear matrix protein level. The examination of NMP22/GST correlation in the 3 acetylator groups SA, IA and FA gave a statistically significant result only in the SA group (r = 0.4, p = 0.015). This suggests higher specificity of the marker NMP22 for slow acetylators, i.e. BC people susceptible to environmental toxins. This research should be continued. Simic et al. indicate that the upregulation of various GST classes, especially π and θ isoforms, might have important consequences for transitional cell carcinoma (TCC) of bladder growth by providing a reduced cellular environment and the inhibition of apoptotic pathways (by inhibition of JNK kinase). Moreover, the occurrence of low GST-π type of conjugation can be considered as a risk factor of TCC. For people with lowered GST-π activity, exposure to arsenic and PAH is more dangerous than for others. The role of hereditary polymorphisms of NAT and GST genes involved in the etiology of neoplasm of the urinary tract is still controversial. Some authors state that both NAT and GST polymorphisms are
responsible for the different ability to metabolize carcinogens, especially those in tobacco smoke. The correlation between total GST activity in serum and NMP22 concentration in urine suggests that these parameters may be useful for assessing the exposure to carcinogens among BC patients susceptible to environmental toxins and probably for evaluating the predisposition to BC development. Thus, further research including a larger number of people should be carried out.

Further prospective research is needed to confirm these relationships and to prove the usefulness of these parameters in the prognosis of BC development and/or evaluation of BC morbidity, which is crucial in the aspect of race NAT2 polymorphism and the susceptibility to BC.

References