Correlation of urine cytology with ABO(H) antigenicity in transitional cell carcinoma of the bladder

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SUMMARY Cell surface ABO(H) antigenicity of superficial bladder tumours was assessed by the indirect immunoperoxidase test in 49 patients. Good correlation was obtained between surface antigenicity of tumours and the results of urine cytology. Malignant cells were detected cytologically in 22(56%) of cases with ABO(H) antigen negative tumours which are known to behave more aggressively than ABO(H) antigen positive ones. In contrast, malignant cells were found in the urine cytology of only one (10%) of patients with ABO(H) antigen positive tumours.

ABO(H) blood group antigen expression of urothelial transitional cell cancer has been extensively studied and its prognostic importance established.¹⁻³ Some workers have claimed that loss of cell surface blood group antigens indicates future invasive potential in superficial bladder cancer.⁴ Others have shown an increased incidence of recurrent disease in patients whose primary tumours were antigen negative.⁵ Most workers agree that loss of surface antigens represents increased biological aggressiveness of transitional cell tumours with a consequently less favourable clinical course. This study was conducted to determine if this heterogeneity of superficial bladder cancers was reflected in the findings of urine cytological analysis.

Patients and methods

Forty nine patients with superficial bladder cancer (pTa or pT1) were studied (41 were male and eight female, their mean age was 65.4 years, range: 50 to 85). All had undergone urine cytological analysis as part of their routine investigation. ABO(H) antigenicity of the resected primary tumours was performed by the indirect immunoperoxidase test using blood group specific monoclonal antibodies.²

Early morning specimens of urine were obtained. The cells were stained by the standard Papanicolaou technique and cytology was reported as normal or showing atypical or malignant cells.

Monoclonal antibodies anti-A, anti-B, and anti-H were used as primary antibodies. Normal rabbit serum in a dilution of 1/10 in Tris-buffered saline was used to block non-specific antigen sites. Peroxidase conjugated antimouse rabbit immunoglobulin G diluted 1/50 in Tris-buffered saline was used as the second antibody layer. Freshly prepared diaminobenzidine was added to visualise the antigen-antibody complex as a brown stain. To perform the test, 4 µm tissue sections were mounted on glass slides, dewaxed, and incubated with the primary blood group specific monoclonal antibody, followed by incubation with peroxidase conjugated antimouse rabbit IgG. The final reaction was produced by adding freshly prepared diaminobenzidine, counterstained with Mayer's haemalum, blued in Scott's tap water substitute, dehydrated, cleared and mounted with dibutylphthalate xylene.

The specificity of the monoclonal antibodies was confirmed by testing against isologous and non-isologous red cells. To exclude false positive results, a control slide was used with each test slide (the control slide was incubated with an inappropriate primary antibody while the rest of the procedure remained unchanged). To rule out false negative results, the built-in internal controls of the test slide were studied, which consisted of erythrocytes, vascular endothelium, and normal bladder epithelium (when included in the section). All these possess the appropriate blood group isoantigen on their respective surfaces and always stain positive if the immunoperoxidase test is correctly performed. If the internal

Table Correlation of ABH antigenicity of tumours with urine cytology

Tumours	Urine cytology			Total
	Normal	Atypical	Malignant	
Antigen positive	6(60%)	3(30%)	1(10%)	10
Antigen negative	5(13%)	12(31%)	22(56%)	39
Total	11	15	23	49

controls in the test slide were negative or the control slide showed positive staining, that particular test was considered to be invalid.

Results

The table shows that 60% of patients with antigen positive tumours had normal cytology with only 10% ($n = 1$) in whom frankly malignant cells were detected. In contrast, 56% of patients with antigen negative tumours had malignant cells in urine, with only 13% being normal ($\chi^2 = 11.55$, $df = 2$, $p = 0.003$).

Of the five patients with antigen negative tumours and normal urine cytology, two had well differentiated (G1) tumours, two had moderately differentiated (G2) tumours, and only one had a poorly differentiated (G3) neoplasm. The only patient with an antigen positive tumour and malignant urine cytology, surprisingly had a well differentiated (G1) tumour. Only a few malignant cells were noted in the urine cytology.

Discussion

These results show a clear correlation between ABO(H) antigenicity of transitional cell tumours and urine cytology findings. Patients with blood group antigen negative tumours were more likely to have detectable malignant cells in their urine compared with those with tumours that retained surface antigens. Similar results have been reported from other centres.⁶

Superficial bladder carcinomas are not homogeneous neoplasms. Their heterogeneity is evident in their differing clinical courses and varying response to treatment modalities such as transurethral resection, intravesical chemotherapy, and BCG immunotherapy. The concept that tumour characterisation is complete once grade and stage have been specified is no longer acceptable to modern urologists.⁷ Although

stage and grade remain the most commonly determined variables of tumour characteristics in clinical urology, increasing numbers of centres are now performing more sophisticated tests such as ABO(H) antigenicity and detection of abnormal chromosomes to obtain a more comprehensive understanding of tumour cell biology.

ABO(H) antigenicity has been shown to be related to tumour behaviour in superficial bladder cancer. Evidence has accumulated from previous work that patients with ABO(H) antigen negative superficial transitional cell tumours have an adverse clinical course both in terms of progression to invasive disease and increased risk of superficial recurrences.^{4,5} These reports have suggested that patients with ABO(H) antigen negative superficial tumours should undergo closer surveillance and early aggressive treatment. This short report reinforces the argument that ABO(H) antigenicity provides an important insight into the cell biology of transitional cell tumours.

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