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### The angiogenic factor, vascular endothelial growth factor, is detectable in urine and is overexpressed in the urine of patients with bladder cancer

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**Introduction:** Vascular endothelial growth factor (VEGF) may be a prime regulator of tumour angiogenesis. VEGF mRNA is overexpressed in bladder tumours, and overexpression correlates with recurrence of pT1 tumours. The urine of patients with bladder cancer is angiogenic but it is currently not known if VEGF is present in urine. Our hypothesis is that VEGF may be detectable in urine, and may prove to be a clinically useful tumour marker in a number of tumour types.

**Materials and methods:** Freshly voided urine (5 mL) was obtained from 51 subjects; 27 had bladder cancer, whereas 15 had a history of bladder cancer but had no evidence of active disease in the bladder. Nine subjects were controls who had no urological disease. A Quantikine™ ELISA was used to determine the concentration of urinary VEGF. Values were normalized for creatinine content and expressed as pg/g.

**Results:** Of 27 tumours, 23 were superficial (eight pTa and 15 pT1), whilst four were invasive. The median VEGF concentration was 4-fold higher in the urine of patients with tumours compared to those with no evidence of active disease, at 325 pg/g creatinine (range 27–4425) versus 89 pg/g creatinine (range 21–1500;  $P < 0.01$ ; Mann-Whitney U-test). There was no statistically significant difference in median urinary VEGF concentration between patients with a clear cystoscopy and the control patients (89 pg/g creatinine, range 21–1500, versus 85 pg/g creatinine, range 73–144;  $P < 1.0$ ).

**Conclusion:** This pilot study has shown for the first time that the angiogenic factor VEGF is detectable in urine and is found in excess in the urine of patients with bladder cancer. The significance and clinical usefulness of urinary VEGF measurements deserve further evaluation in patients with bladder cancer and in patients with other tumours (e.g. > 90% of renal cell carcinomas express VEGF).

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### Metabolism of bladder cancer

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**Introduction:** It has been suggested that the ability to metabolize the drug debrisoquine correlates with a propensity to develop TCC and furthermore, with the grade of the disease. The enzyme involved is a cytochrome P-450, gene locus 2D6. This gene is polymorphic and inherited in Mendelian fashion. Possession of a normal allele renders the individual a fast metaboliser. We present the results of a study in which the genetic metabolic status was determined by a blood-based genetic assay.

**Patients and methods:** A total of 126 patients with TCC and 132 controls participated in the study. Genetic analysis was carried out on DNA isolated from the leucocytes using PCR and gel electrophoresis. Direct reading of the banding pattern on the gel enabled genotypic classification.

**Results:** The percentage of homozygotes, heterozygotes and recessive genetic constitutions at the CYP2D6 locus in the normal group was 66.7, 27.3 and 6.0, respectively. Similar percentages were obtained for the bladder cancer group as a whole. There was an increase in the proportion of heterozygotes with progression from normal to G1, G2 and G3 (poorly differentiated) tumours. There was a significant difference between the normal group and those with G3 tumours ( $X^2 = 7.65$ ,  $P = 0.03$ ).

**Conclusion:** The heterozygous state of the CYP2D6 genotype is significantly associated with aggressive disease. It may be that the CYP2D6 is in linkage disequilibrium with a gene(s) that controls tumour differentiation.

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### Acetylation phenotype and the risk of bladder cancer

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**Introduction:** Bladder carcinogens such as beta-naphthylamine are metabolised via the N-acetyltransferase enzyme system. The activity of this enzyme may be an important factor in determining the risk of developing TCC of the bladder. Enzyme activity is determined by a genetic polymorphism such that fast acetylators (FA) are thought to be at lower risk than slow acetylators (SA). In this study, a simple blood test was used to categorize (phenotype) individuals according to their acetylation status and to relate this to the risk of bladder cancer occurrence. We also aimed to determine the interaction between metabolic phenotype and variables such as occupational exposure, smoking and alcohol consumption. Such a multifactorial study has not been reported to date.

**Patients and methods:** Subjects were recruited from the haematuria clinic, those with benign causes of haematuria being used as controls. Epidemiological data were recorded in a standard questionnaire and patients received 100 mg of Dapsone. In an 8 h sample, the ratio of mono-acetyl dapsone/dapsone (MDR) was measured as a marker of acetylation phenotype. Patients with liver or renal disease were excluded from the study. There were 85 controls and 107 cases with a similar age and sex distribution in each group recruited over a 3-year period.

**Results:** In a univariate analysis no difference in MDR was found between cases and controls. However, after a subanalysis for grade (G1/2 vs G3) it was found that SA status correlated highly with the risk of low-grade tumour formation (relative risk ratio 3.66; CI 1.42–9.4). In a logistic regression analysis, patients who were both smokers and SA had a 5.3-fold increased risk of TCC over smokers (CI 1.3–22.2) who were FA. Likewise, subjects who had had significant occupational exposure and who were SA had a 4.6-fold increased risk of bladder cancer over other subjects with similar exposure but who were FA.

**Conclusion:** The results suggest a protective role for fast acetylation and this simple blood test may be used in counselling, to advise individuals in appropriate cases of the extreme risks attached to smoking or occupational exposure because of their phenotype.

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### Telomerase activity in transitional cell carcinoma; its implications for non-invasive detection of bladder cancer

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**Introduction:** The inability of the DNA replication machinery to replicate chromosome termini (telomeres) leads to progressive shortening of chromosomes upon continuous cell division, ultimately leading to loss of telomeric function and chromosome destabilization. A ribonucleoprotein called telomerase is necessary to repair telomeric losses. In adult humans, only germline cells show telomerase activity to ensure the transmission of full length chromosomes to the progeny. In contrast, the telomeres of somatic cells shorten until they reach senescence. Telomerase activity has been reported in a wide range of malignancies including those of the gastrointestinal tract, breast and lung.

**Patients and methods:** Telomerase activity was examined in 48 bladder carcinomas, 17 non-neoplastic bladders and two dysplastic lesions, using a PCR-based assay designated 'telomeric repeat amplification protocol'. Telomerase activity was also studied in lysates of exfoliated cells in urine samples from 30 patients with bladder carcinoma and 82 patients with no evidence of malignant disease.

**Results:** Telomerase activity was present in 41 (85%) of 48 bladder carcinomas; in the normal bladder tissues examined there was no telomerase activity. The dysplastic lesions showed weak telomerase activity. It was detected with various intensities in 20 of the 30 urine samples (67% sensitivity) from patients with bladder carcinoma whilst two of 82 non-malignant urine samples showed weak activity (97.6% specificity).

**Conclusion:** These results suggest that telomerase activity may be a useful diagnostic marker in the non-invasive detection of bladder cancer.

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### Random mucosal biopsies in superficial bladder carcinoma – better at first-check cystoscopy?

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**Introduction:** The role of random mucosal biopsies in the management of superficial bladder carcinoma remains unclear. Routine random biopsies in all patients at presentation would involve many biopsies.

**Patients and methods:** During a 3-year period, a total of 172 patients with TCC of the bladder underwent routine random mucosal biopsies (RMB) at presentation. Histological examination showed muscle invasion in 17 (16 patients with high grade, and one patient with moderate grade, none with well differentiated tumours). In the 155 patients with only superficial tumour (Ta-T1), 53 patients had well differentiated (G1) tumour, 57 showed moderate grade (G2) and the remaining 45 presented with high-grade (G3) carcinoma.

**Results:** RMBs were normal in 48 of the 53 patients in the G1 group; one patient showed evidence of dysplasia and four patients showed carcinoma *in situ* (CIS). In the G2 group, RMBs were normal in 40 and 17 patients showed abnormal mucosa (dysplasia 15, CIS 2), 19 patients with high-grade tumour (G3) showed evidence of CIS at presentation. There were three patients with recurrence(s) at first check cystoscopy and with abnormal mucosa on RMB in the G1 group (all with CIS), five in the G2 group (both patients with CIS, three with dysplasia) and 13 in the G3 group.

**Conclusion:** Routine RMBs in superficial bladder carcinoma at presentation are unhelpful in the initial management. The initial grade of the tumour is a strong predictor of the presence of CIS in the normal mucosa. It is proposed that RMBs should be carried out at the time of the first check cystoscopy in patients with recurrent tumour only, especially with well to moderately differentiated tumours.

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### Carcinogenesis with nitrosamines and nitrites in paraplegics

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**Introduction:** Paraplegics have a 20-fold increased incidence of bladder cancer, and nitrosamines have been aetiologically implicated. This study assessed nitrite and nitrosamine excretion in paraplegics.

**Patients and methods:** Paraplegics, with a mean duration of injury of 17 years, were assessed in two groups of 12 each and compared to an age-matched control group of 12 volunteers. All patients followed a standardized dietary regimen. Group 1 (mean age 42.7 years) was made up of patients voiding spontaneously. Patients in Group 2 (mean age 41.5 years) had long-term indwelling catheters. 24-hour urinary nitrosamine excretion (ppb) urinary nitrite (ppm) and concurrent infections were assessed. Urinary pH, 24 h urinary nitrosamine excretion, 24 h urinary nitrite excretion and infections are presented as Tables showing the effect of catheter drainage (Table 1) and urinary infections (Table 2).

#### Results:

Table 1. Effect of catheter drainage

	Range control (mean)	Range catheter-free (mean)	Range catheters (mean)
Urinary pH	5.2–8.6 (6.2)	5.1–9.2 (6.5)	5.8–7.8 (6.5)
Nitrosamine – 24 h	125–1470 (481)	310–17720 (1385.5)	471–7142 (2364)
Nitrites – 24 h	0.03–204.2 (18)	0.94–714 (114.6)	0.89–929 (141.9)
Bacteriuria	none	3 pure, 5 mixed	5 pure, 6 mixed

Table 2. Effect of urinary infections

	-ve cultures (mean)	pure cultures (mean)	mixed cultures (mean)
24 h Nitrosamine	125–3710 (802)	310–17720 (4507)	47.5–7142 (5403)
24 h Nitrites	0.03–175 (12.8)	1.2–316.2 (77)	0.94–969.5 (229.9)

**Conclusion:** Nitrosamine excretion in normal controls and paraplegics is variable. The presence of an indwelling catheter and bacteriuria have an influence on nitrite and nitrosamine excretion and this correlates with tumour formation.

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### Epirubicin resistant bladder cancer and p53 oncogene status

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**Introduction:** We have previously reported an ablative effect of epirubicin in 48% of patients with TAG1 superficial bladder tumours. Wild-type p53 oncogene has been associated with growth regulation, DNA repair and drug-induced cell death (apoptosis). Wild-type p53 expression has usually been associated with high-grade and invasive bladder tumours. p53 mutation has been implicated in determining tolerance of cancer cells to drug-induced DNA damage and cell death. The aim of the study was to determine whether the mutation of p53 correlated with drug resistance and failure to undergo apoptosis.

**Materials and methods:** Tumour specimens were obtained from nine patients with recurrent superficial bladder cancer who had previously been treated with epirubicin. Epirubicin-sensitive and resistant bladder cancer cell lines were studied in parallel. All specimens were snap-frozen in liquid nitrogen and stored at –80°C. Both normal and chemoresistant bladder specimens were analysed using PCR and exon-specific single-strand conformation polymorphism (SSCP) analysis. p53 exons 2–10 were amplified using standard PCR conditions. Samples were analysed for DNA frame-shift mutation using PAGE.

**Results:** Eight of nine epirubicin-resistant bladder tumours showed p53 mutation.

**Conclusion:** The substantial increase in detection of p53 mutation in epirubicin-resistant bladder cancer relative to cancers before treatment is consistent with a possible role for wild-type p53 in conferring sensitivity to the drug epirubicin.