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A new tumour marker tested in 98 patients with bladder carcinoma

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Summary

A new, indirect marker of human tumour has been tested in 98 patients with urothelial bladder carcinoma. The marker is detected by B5, a monoclonal antibody which agglutinates erythrocytes from tumour-bearing patients. Patients admitted for cystoscopy for diagnosis of bladder tumour, or for follow-up of known disease, were chosen to enable comparison between (a) B5 results and (b) visual assessment of tumour growth. Ninety per cent of those with new tumour (20) and, overall, 80% of patients with tumour (74), were B5 positive. These results were independent of tumour size and include very small recurrences, implying that B5 is a sensitive marker of tumour presence. The background incidence of B5 positive individuals is 18% in controls; a similar incidence occurs in patients who have been tumour-free for 9 months or more. Patients who had no visible tumour in this study, but who had tumour within 9 months, were often B5 positive (6/11). This may be due to the lifespan of erythrocytes causing a delay in change from B5 positive to B5 negative in those patients who will remain disease-free.

Introduction

A new indirect marker of malignancy which is present on human erythrocytes has recently been described *(1)*. This marker, the B5 antigen, is detected by a monoclonal antibody which is known to agglutinate whole red cells from tumour patients; normal individuals are rarely positive for B5 agglutination *(1)*. The high incidence of B5-positivity occured in a wide range of different tumour types. Here we have made a detailed study of a single tumour type, namely bladder tumours in patients at time of cystoscopy. This patient group was selected to enable us (a) to assay for B5 prior to treatment and (b) to compare the findings with a direct visual assessment of the tumour. We report the B5 results from 98 patients and compare these to other markers of urothelial tumour.

Patients and methods

PATIENTS

Ninety-eight consecutive patients admitted for cystoscopy were included in the study. Of these patients 20 had new bladder tumours and 78 were routine admissions for check cystoscopy. In the latter group 60 had been treated by transurethral resection alone, 7 had also been treated with intravesical chemotherapy and 11 had undergone radiotherapy. The patient's age, blood group and any relevant past medical history were noted. At operation the presence or absence of active tumour was recorded and appropriate treatment with cystodiathermy or transurethral resection undertaken. Control specimens were obtained from 249 healthy blood donors and hospital inpatients with non-malignant conditions.

METHODS

Heparinised blood samples were collected from patients on admission. These samples were stored at 4 °C until assayed. Repeat assays of any single whole blood sample over a 7-day storage period gave consistent haemagglutination results.

For assay, 1 ml of whole blood was washed three times in 20 ml of phosphate-buffered saline (PBS pH 7.4) at room temperature. A small volume of washed, packed erythrocytes was then diluted to 1% in PBS containing 4% foetal calf serum (FCS) and 25 microlitres of this suspension was added to 25 microlitres of B5 antibody in a 'U' well haemagglutination plate. In controls 1% erythrocytes were added to PBS containing 4% FCS or to culture supernatant containing an irrelevant antibody. The plates were covered with film and left for at least 2 hours at room temperature before being read. Haemagglutination was scored positive or negative using an inverted microscope to view the pellets directly under low power. Each sample was also scored by direct examination of cells gently resuspended and transferred onto a glass slide. In the latter method clusters of less than ten cells were regarded as negative and the rest scored positive.

Results

The cystoscopy findings and the results of the B5 haemagglutination test are summarised in Tables I and II. We found a correlation of 80% between tumour presence and B5 positivity. This was in marked contrast to the 18% incidence of B5 positivity seen in the control group. In those tumour patients where no recurrent tumour was seen, some 46% (11/24) were B5 positive, which is significantly higher than the control group.

Discussion

Reliable markers of human tumours have obvious value in clinical practice, and here we compare those in current use with our B5 results. A completely specific marker for malignancy, if one exists, has yet to be described, but there are changes in the tumour cell surface which are useful both in histological screening and in typing of tumours. For

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TABLE I *B5 haemagglutination*

	B5 haemagglutination	
	+ve	−ve
A. Controls	46	203
B. Bladder tumour patients		
1. Visible tumour		
(a) New tumour	18	2
(b) Previous tumour: resection	34	7
(c) Previous tumour: radiotherapy	4	4
(d) Previous tumour: chemotherapy	3	2
Totals	59	15
2. No tumour seen (follow-up patients)		
(a) Previous tumour: resection	8	11
(b) Previous tumour: radiotherapy	2	1
(c) Previous tumour: chemotherapy	1	1
Totals	11	13

B5 haemagglutination in controls, and in patients with new bladder tumours or under follow-up for previous known bladder tumours. The results are presented according to the presence or absence of tumour at cystoscopy and are further subdivided according to previous treatment. Positive agglutination with B5 was not related to any of the common blood group antigens. In the assay controls, none of the erythrocytes tested against irrelevant antibody or the PBS diluent were agglutinated.

TABLE II

	B5 + ve
1. New tumours	90% (18/20)
2. Recurrent tumours	76% (41/54)
(a) No previous radiotherapy or chemotherapy	83% (34/41)
(b) Previous radiotherapy or chemotherapy	54% (7/13)
Total	80% (59/74)

Incidence, expressed as a percentage, of B5 positivity in those patients found to have urothelial tumours. The background rate of B5 positively in the general population (18%) should be noted.

example, bladder tumour cells may express carcinoembryonic antigen (CEA), or lose their normal expression of common blood group antigens A, B and H *(2–7)*. Other tumour markers are indirect and may occur in blood or urine of patients with bladder cancer. For example, CEA may be shed from the tumour and has been extensively investigated. The serum levels of CEA have proved useful in the hands of some workers in following response to treatment or assessing prognosis *(8,9)* but have been found to be of little value by others *(10, 11)*. Urinary CEA levels appear more reliable and have been successfully related to the prediction of both patient survival time and recurrence-free interval in those with invasive tumours treated with radiotherapy *(8, 12–14)*. A major disadvantage of this technique is the presence of misleading elevated CEA levels with infected urine (a common occurrence in such patients) or in urinary diversions. Other indirect markers of bladder cancer include alpha fetoprotein, foetal haemoglobin, tissue polypeptide antigen, urinary cancer related glycoprotein (EDC1) and urinary immunoglobulins *(15–18)* but these are of dubious value.

No single marker has yet been reported with the necessary sensitivity and specificity to be used alone as an indicator of the presence of active tumour and the need for multiple assays of a combination of markers has been emphasised *(3)*. Here we have found a good correlation between active tumour and the expression of the new tumour marker, B5 antigen. We have been able to predict the presence of active tumour at cystoscopy in 80% of our patients; moreover, of 20 patients with new bladder tumours, 90% were B5 positive. There is good evidence from studies in other patients with malignancy that successful treatment is followed by a switch from being B5 positive to B5 negative *(19)*. Furthermore, one of the patients in this study was tested on two occasions with an interval of 3 months. On the first occasion, when B5 haemagglutination was strongly positive, a tumour was seen at cystoscopy and destroyed with diathermy: on the second occasion there was no tumour visible in the bladder and B5 haemagglutination had markedly decreased to a weak positive. We anticipate a lag period in the clearance of B5 positive erythrocytes following successful tumour resection due to the 120-day lifespan of circulating red cells. Since such a lag might contribute to the high incidence (45%) of patients who were B5 positive while apparently free of tumour recurrence, we checked the date when the tumour had been last seen. Of the 'false-positive' patients 6 out of 11 had been known to have tumour within 9 months of testing, whereas of the correct negative patients 10 out of 13 patients had been disease-free for over 9 months. Those patients persistently B5 positive, but apparently tumour-free, may be naturally B5 positive (as in 18% of controls). Alternatively the presence of an unstable urothelium or occult tumour elsewhere may be the cause.

Our results show that B5 is particularly valuable in the prediction of the presence of new tumour or in monitoring patients who have undergone previous surgical resection only. Previous chemotherapy or radiotherapy apparently decreases the reliability of the investigation. The conventional pattern of follow-up entails regular cystoscopy to detect recurrent tumour in the urinary bladder and repeated intravenous urograms to inspect the upper tracts. The interval between examinations is extended as the tumour-free interval becomes longer. In patients becoming B5 negative after tumour resection, regular B5 assays, if they become positive again, may predict the presence of recurrent tumour and hence indicate the need for early cystoscopy. In the case of a negative cystoscopy with a positive B5 examination there would be an indication for further examination of the upper tracts. In those centres where patients who have been disease-free for 5–10 years are followed using urinary cytology, B5 would provide a useful adjunct in management.

The assay for B5 is very simple and requires a small blood sample only. Furthermore, our results with B5 in bladder tumour patients applied irrespective of the size of tumour seen, including very small recurrences. This indicates a degree of assay sensitivity which is clearly desirable. Since the B5 marker is independent of tumour type, similar sensitivity is likely to apply to other tumours. Thus we conclude that B5 might provide a reliable and sensitive indicator for follow-up of tumour patients in general, and also to monitor those individuals known to be at risk of developing malignancy.

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Notes on books

The Treatment and Research in Burns by Yang Zhi-Jun, Sheng Zhi-Yong and Shi Ji-Xiang. 541 pages, illustrated. Science Press/Wiley Medical, Chichester. £47.50.

This is the proceedings of the International Burn seminar held in Shanghai in June 1981. The first section is devoted to papers on experiences in the management of major burns. In the second section the results of research into burns are presented and the third section is devoted to the plastic and reconstructive surgery for the results of burns.

Photographic Atlas of the Human Body by Branislav Vidic and F R Suarez. 464 pages, illustrated. C V Mosby/Blackwell Scientific, Oxford. £36.00.

This atlas is built up from photographs of carefully produced dissections sometimes clarified by full scale line drawings with labelling. It proceeds systematically through the body showing various regions. The quality of the colour makes it extremely valuable as a reference manual for anatomists and surgeons.

Vascular Surgical Techniques edited by R M Greenhalgh. 321 pages, illustrated. Butterworths, London. £39.50.

This book displays the techniques used in vascular surgery and illustrates them excellently with simple line drawings. The first section is devoted to basic principles followed by a systematic review of vascular surgery in different sites. Each chapter written by an acknowledged expert is followed by a list reference for further reading.

Ophthalmic Surgery edited by T A Rice, R G Michels and W J Stark. Rob and Smith's Operative Surgery. 445 pages, illustrated. 4th edition. Butterworths, London. £65.00.

Although opthalmic surgery is changing rapidly the authors have given a review of present day methods in this specialist branch of surgery dealing systematically with various diseases and with various techniques. As in previous editions this is excellently produced and superbly illustrated.

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