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Prediction of Superficial Bladder Cancer by Nuclear Image Analysis

P.K. Lipponen, M.J. Eskelinen, K. Jauhiainen, E. Harju, R. Terho and H. Haapasalo

A cohort of 270 superficial transitional cell bladder tumours (Ta-T1) was followed-up for over 8 years. WHO grade, papillary status and six nuclear factors were related to progression, recurrence-free survival (RFS) and bladder cancer-related survival (BS) during the follow-up period. Mean nuclear area (NA), standard deviation of nuclear area (SDNA), nuclear perimetry (PE), standard deviation of nuclear perimetry (SDPE), shortest nuclear axis (D_{min}) and longest nuclear axis (D_{max}) were significantly related to WHO grade and papillary status ($P < 0.0001$). All the nuclear factors were related significantly to progression in univariate analysis ($P < 0.01$) whereas in a multivariate analysis WHO grade ($P < 0.0001$) and papillary status ($P = 0.048$) included independent prognostic information. RFS was related to PE ($P = 0.009$), SDPE ($P = 0.013$), D_{min} ($P = 0.021$), D_{max} ($P = 0.028$) and SDNA ($P = 0.029$). In papillary tumours SDPE ($P = 0.007$) and D_{min} ($P = 0.024$) predicted RFS. BS was related to WHO grade, papillary status, NA, SDNA, PE, D_{max}, D_{min} (all $P < 0.0001$) and to SDPE ($P = 0.003$). In papillary tumours PE ($P < 0.0001$), D_{max} ($P = 0.0022$), D_{min} ($P = 0.0027$), WHO grade ($P = 0.0036$), NA ($P = 0.0005$), SDNA ($P = 0.0355$) and SDPE ($P = 0.0718$) predicted BS. In multivariate analysis SDPE ($P = 0.029$) predicted RFS and survival was related to WHO grade ($P < 0.001$) and PE ($P = 0.014$) independently. In papillary tumours only D_{max} ($P = 0.001$) predicted survival independently. The results show that superficial papillary transitional cell bladder tumours can be efficiently categorised into prognostic groups by nuclear image analysis and the results provide a new classification system for superficial papillary bladder tumours. Tumours with high nuclear factor values should be considered for radical primary therapy and adjuvant therapy after transurethral resections.
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INTRODUCTION

A LOT OF WORK has been done to develop a reliable and reproducible grading system for transitional cell bladder cancer [1–5]. The importance of relative grading is emphasised in superficial bladder tumours since at present the majority of newly detected bladder tumours belong to Ta-T1 categories. However, close to 20% of superficial tumours become invasive and disseminate [6]. The reliable and accurate identification of this subgroup of bladder tumours would be of great help to the urologist in making decisions on early radical surgical therapy and adjuvant therapy. At present the decisions on treatment of bladder cancer [7] are based on histological grade [8] and clinical stage [9]. However, both of these methods are variable. Recently introduced histoquantitative methods have led to a number of promising results [1–5] hopefully leading towards a quantitative grading system for transitional cell bladder tumours. Of the histoquantitative methods both morphometry [1, 2, 4] and flow cytometry [1, 2, 5, 10] have been tested but the materials have usually included a limited number of patients with short follow-up. Thus the aim of this study is to establish the potential of nuclear image analysis as a prognostic method in superficial transitional cell bladder cancer in a cohort of 27 patients. The potential of image analysis is compared to established predictors in a multivariate analysis and a proposal is made on the use of nuclear image analysis in grading of superficial bladder cancer.

Correspondence to P. Lipponen.
P. Lipponen is at the Department of Pathology, M. J. Eskelinen is at the Department of Surgery, University of Kuopio, 70211-Kuopio, Finland; K. Jauhiainen is at the Department of Surgery, St Michels Central Hospital; E. Harju is at the Department of Surgery, Jyväskylä Central Hospital; R. Terho is at the Department of Surgery, Savonlinna Central Hospital; and H. Haapasalo is at the Department of Pathology, University of Tampere, Finland.
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PATIENTS AND METHODS

Patients

Altogether 537 patients with a newly diagnosed transitional cell bladder cancer were included in a multicentre study at Kuopio University Hospital, Jyväskylä Central Hospital, Savonlinna Central Hospital and Mikkeli Central Hospital in Eastern Finland. Of these patients 270 presented with a superficial tumour [Ta ($n = 23$); T1 ($n = 247$)] and they were included in the present analysis. The patients were diagnosed, treated and followed-up during 1965–1991. The treatment and follow-up investigations were done according to standard practice [7] which was, however, tailored individually. The initial staging of tumours was based on results of excretory pyelography, transurethral biopsy, cytological examination of voided urine and bimanual palpation under anaesthesia. In many of the invasive muscle tumours during the most recent years a computerised tomography (CT) or ultrasound examination was done. Screening for metastasis included chest radiography, laboratory tests, abdominal ultrasound, and when appropriate, bone scintigraphy and lymphography. Tumour, nodes and metastasis classification was done according to UICC 1978 [9] and was based on the above-mentioned examinations added with the pathologists reports. The follow-up investigations were done at 3-month intervals during the first 2 years and thereafter at 6-month intervals. If a recurrent growth was observed the follow-

Table 1. The pertinent clinical data of patients

Number of patients	270
Mean (S.E.) age at diagnosis, years	66.3 (0.7)
Sex (females/males)	60/210
Mean (S.E.) follow-up, years	8.7 (0.3)
Papillary status papillary/nodular	240/30
WHO grade 1/2/3	156/87/27
Primary therapy	
No treatment	3
Transurethral resection	267
Intravesical chemotherapy	61
Other therapy during follow-up*	
Partial cystectomy	16
Cystectomy	11
Cystectomy and radiotherapy	8
Radiotherapy	8

*Note that the same patient may have been subject to several modes of therapy and most of the recurrent tumours were treated by transurethral resections which are not included in the table.

up programme was recommenced. The treatment of recurrent tumours was based on the same principle as the treatment of primary tumours. The causes of death were verified from patient files, autopsy reports and death certificates. The pertinent clinical data of patients is summarised in Table 1. Progression was defined as an increase in T-, N- or M-categories as defined in patient files.

Histological methods

The histological samples were preoperative biopsy specimens. The samples were fixed in buffered formalin (pH 7.0), embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin or Van Gieson stains for grading. The samples were graded histologically according to WHO [8] by one observer. The distribution of cases into WHO grades and T-categories is shown in Table 1. The papillary status of tumours was recorded and the tumours were divided into papillary and nodular types (Table 1).

Nuclear image analysis

In nuclear image analysis the IBAS 1&2 analyser system was used. The images of most atypical well-preserved microscopic fields were selected subjectively and focused on a video screen through a video camera attached to the microscope (magnification 40×). A mean of 60 nuclei were traced using a digitiser tablet and a mouse connected to the computer. The computer automatically calculated mean nuclear area (NA), standard deviation of nuclear area (SDNA), nuclear perimetry (PE), standard deviation of nuclear perimetry (SDPE), largest nuclear diameter (D_{max}) and shortest nuclear diameter (D_{min}), all of which were used in the further analysis.

Statistical analysis

In basic statistical calculations the SPSS/PC+ programme package was used in a Toshiba T3200 computer and the statistical tests used are indicated in connection with the results when appropriate. The univariate survival analysis was based on life-table method with the statistics by Lee and Desu [11]. Survival analysis included only deaths due to bladder cancer. Recurrence-free survival (RFS) was defined as the time elapsed between primary therapy and first verified recurrent growth in the bladder. In univariate analysis of continuous variables several group limits were tested and the limits showing the best discriminating potential were selected. Multivariate survival analysis [12] was done with the BMDP(2L) programme. Nuclear factors were used as continuous variables in multivariate analysis. The analysis was conducted in two phases. The first analysis included all cases and in the second analysis only papillary tumours were studied.

Table 2. The range and mean (SD) values of nuclear variables in WHO grades

Variable	Range	Grade 1 (n = 156)	Grade 2 (n = 87)	Grade 3 (n = 27)	P*
NA (μm²)	20–209	66 (16)	87 (29)	103 (43)	<0.0001
SDNA (μm²)	6–100	17 (6)	26 (14)	41 (25)	<0.0001
PE (μm)	23–57	32 (4)	36 (6)	41 (7)	<0.0001
SDPE (μm)	2–35	4 (2)	5 (2)	7 (3)	<0.0001
D_{max} (μm)	8–21	11 (1)	13 (2)	14 (3)	<0.0001
D_{min} (μm)	4–13	7 (1)	8 (1)	9 (1)	<0.0001

*Analysis of variance.

RESULTS

The range and mean values of nuclear factors in WHO grades is shown in Table 2. In nodular tumours nuclear factors were significantly higher than in papillary ones ($P < 0.0001$). Progression of bladder tumours was significantly related to several histological and nuclear features ($P = 0.023$ –<0.0001), whereas in a logistic multivariate regression analysis WHO grade predicted independently progression in T- ($P < 0.001$) and N-categories ($P < 0.001$). Progression in M-category was related independently to WHO grade ($P < 0.001$) and papillary status ($P = 0.048$). In the entire cohort RFS was significantly related to nuclear perimetry (Fig. 1) followed by other nuclear factors (Table 3). WHO grade or papillary status were not significantly related to RFS. In univariate survival analysis all the variables predicted survival significantly (Table 3). The most important predictors of survival were WHO grade (Fig. 2) and papillary status and of the nuclear factors PE (Fig. 3) was the best predictor of survival (Table 3). In papillary tumours only PE was related significantly to RFS (Table 4). In papillary tumours

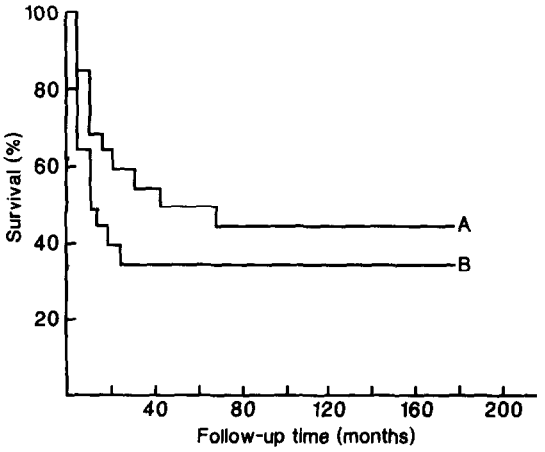

Fig. 1. Recurrence-free survival subdivided according to nuclear perimetry ($P = 0.0091$). Curve A: PE ≤ 40 μm, $n = 227$; curve B: PE > 40 μm, $n = 43$.

Table 3. The recurrence-free survival (RFS) and survival (BS) of patients in the entire cohort subdivided according to histological and morphometric variables. The number of patients in each category and the percentage (%) of patients free of recurrence and percentage of patients surviving at 5 and 10 years are shown

Variable	n	RSF (%) 5 years	RSF (%) 10 years	χ^2 P	BS (%) 5 years	BS (%) 10 years	χ^2 P
WHO grade							
Grade 1	156	(50)	(50)		(95)	(90)	
Grade 2	87	(40)	(40)	3.8	(90)	(75)	46.0
Grade 3	27	(40)	(40)	0.1488	(65)	(35)	<0.0001
Papillary status							
Papillary	240	(50)	(45)	2.3	(95)	(90)	31.1
Nodular	30	(35)	(35)	0.1295	(70)	(40)	<0.0001
NA (μm^2)							
≤90	205	(50)	(45)	5.1	(95)	(90)	26.2
>90	65	(40)	(40)	0.0232	(70)	(50)	<0.0001
SDNA (μm^2)							
≤30	225	(50)	(45)	4.7	(95)	(90)	22.1
>30	45	(35)	(35)	0.0291	(75)	(50)	<0.0001
PE (μm)							
≤40	227	(50)	(45)	6.8	(95)	(90)	29.0
>40	43	(35)	(35)	0.0091	(75)	(45)	<0.0001
PESD (μm^2)							
≤6	215	(50)	(45)	6.1	(95)	(90)	13.0
>6	55	(35)	(35)	0.0129	(80)	(60)	0.0003
D_{max} (μm)							
≤13.4	200	(50)	(45)	4.8	(95)	(90)	22.1
>13.4	70	(40)	(40)	0.0283	(75)	(55)	<0.0001

The results with D_{min} were similar to those by D_{max}.

survival was predicted by PE (Table 4), D_{max} (Fig. 4) and NA (Table 4) in that order. In multivariate survival analysis SDPE was independently related to RFS whereas none of the factors predicted RFS significantly in papillary tumours (Table 5). Independent predictors of survival were WHO grade and SDPE whereas in papillary tumours D_{max} included all of the available prognostic information (Table 5).

DISCUSSION

Superficial transitional cell bladder tumours are a heterogeneous group of neoplasms with a distinctly different clinical behaviour [2, 4, 6]. Until now superficial tumours are categorised into prognostic groups by different subjective grading systems [3, 8] which all suffer from the lack of reproducibility. The overall agreement in grading systems between observers is usually between 60 and 70% [3] which in practice means that every third case is falsely classified. In nuclear morphometry of transitional cell bladder tumours the measurement variation is practically nil [14] and the overall grading efficiency in a two-grade system is about 90% [15], when nuclear area is used in grading which means that only every tenth case is falsely classified. Accordingly, the present analysis used a two-grade

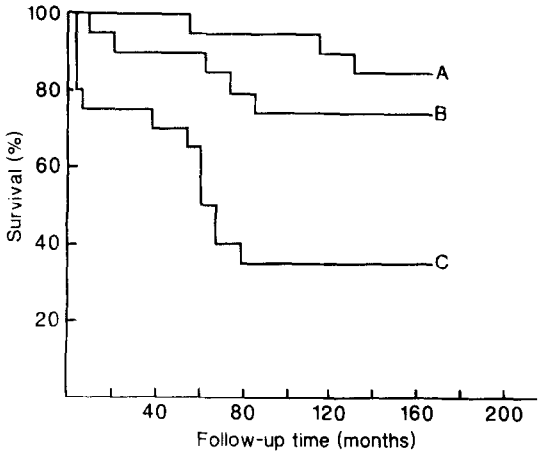

Fig. 2. Survival of patients categorised according to WHO grade ($P < 0.0001$). Curve A: Grade 1, $n = 156$; Curve b: Grade 2, $n = 87$; Curve C: Grade 3, $n = 27$.

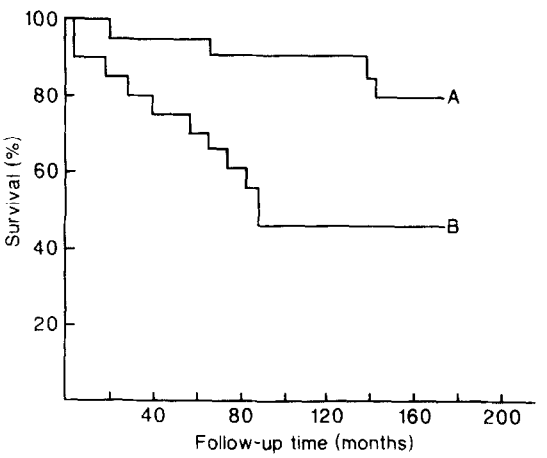

Fig. 3. Survival of patients subdivided according to nuclear perimetry ($P < 0.0001$). Curve A: PE ≤ 40 μm, $n = 227$; curve B: PE > 40 μm, $n = 43$.

Table 4. The recurrence-free survival (RFS) and survival (BS) of patients with papillary tumours subdivided according to histological and morphometric variables. The number of patients in each category and the percentage (%) of patients free of recurrence and percentage of patients surviving at 5 and 10 years are shown

Variable	N	RFS (%) 5 years	RFS (%) 10 years	χ^2 P	BS (%) 5 years	BS (%) 10 years	χ^2 P
WHO grade							
Grade 1	155	(55)	(45)		(95)	(95)	
Grade 2	77	(45)	(45)	2.3	(90)	(75)	11.3
Grade 3	8	(55)	(40)	0.3053	(85)	(65)	0.0036
NA (μm^2)							
≤90	193	(50)	(45)	3.3	(95)	(90)	12.2
>90	47	(45)	(45)	0.0676	(80)	(65)	0.0005
SDNA (μm^2)							
≤30	213	(50)	(45)	2.1	(95)	(90)	4.4
>30	27	(40)	(40)	0.1410	(80)	(70)	0.0355
PE (μm)							
≤40	212	(50)	(45)	7.2	(95)	(90)	17.2
>40	28	(40)	(35)	0.0072	(75)	(60)	<0.0001
PESD (μm^2)							
≤6	203	(50)	(45)	3.1	(95)	(90)	3.2
>6	37	(40)	(35)	0.0803	(90)	(80)	0.0718
D_{max} (μm)							
≤13.4	188	(50)	(45)	3.1	(95)	(90)	9.4
>13.4	52	(45)	(45)	0.0775	(85)	(40)	0.0022

The results with D_{min} were similar to those by D_{max}.

system in interpreting the results of nuclear image analysis. Other previously characterised prognostic factors in superficial tumours are the tumour size, multiplicity and the presence of concomitant urothelial lesions [6]. These latter variables were not available in this retrospective analysis for all cases and thus they were not included. This cohort of patients was gathered during a long time period in rural area in Eastern Finland and the fraction of superficial tumours in the original cohort was lower than in corresponding cohorts diagnosed recently.

The nuclear factors and subjective grading are significantly interrelated which has been observed in previous morphometric analyses of bladder tumours as well [4]. Accordingly papillary status and nuclear factors were also interrelated, most of the nodular lesions represent grade 3 histological atypia [8].

The assessment of progression in T-category in a retrospective cohort may be problematic. Although, WHO grade, papillary status and nuclear factors predicted progression significantly, which has been confirmed by other groups as well [2, 16]. The progression in N- and M-categories was accurately predicted by nuclear factors as well as by conventional histological methods. However, in a separate analysis of papillary tumours alone the prognostic power of quantitative methods was significantly lower than in the entire cohort. This is due to the intimate relationship between nodular growth pattern and large nuclear factor values [4]. Nodular tumours owe a high intrinsic malignancy related to rapid proliferation [4, 17] and other as yet unknown characteristics which may act as confusing factors in this morphometric analysis. Also in this analysis nodular tumours could not be

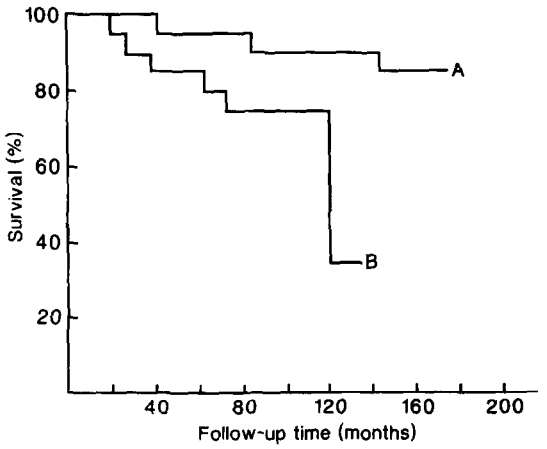

Fig. 4. Survival of papillary tumours subdivided according to longest nuclear axis (P = 0.0022). Curve A: D_{max} ≤ 13.4 μm, n = 188; curve B: D_{max} > 13.4 μm, n = 52.

Table 5. The independent predictors of recurrence-free survival (RFS) and survival (BS) in the entire cohort and in papillary tumours

RFS	β (S.E.)	β/S.E.	P	Hazard rate
Entire cohort				
PESD	0.059 (0.023)	2.579	0.029	1.06 (1.01–1.11)
BS				
WHO grade	0.992 (0.242)	4.099	<0.001	2.69 (1.66–4.37)
PE	0.062 (0.024)	2.520	0.014	1.06 (1.01–1.11)
Papillary tumours				
BS				
D_{max}	0.317 (0.085)	3.701	0.001	1.37 (1.16–1.63)

β = coefficient of the regression model. S.E. = standard error. β/S.E. describes the significance of β (z-value). Hazard rate with 95% confidence interval is given for individual factors.

categorised prognostically by means of grading or image analysis. However, in a multivariate analysis WHO grade was the most important independent predictor of progression which is in accordance with previous results [16]. Preliminary results, however, suggest that proliferation indices are potential predictors of progression and survival in superficial tumours [2, 4, 17]. Thus, the definite importance of subjective grading remains to be studied in relation to cell proliferation as well.

The recurrence of superficial transitional cell bladder tumours is frequent [6] as shown also by the results of this analysis. Previously analysed cohorts have shown the relationship between grading [18], mitotic frequency [19] and recurrence, and the present analysis established a significant relationship between several nuclear factors and RFS. Of the nuclear factors PE was the most important predictor of RFS which suggests a somewhat as yet unknown relationship between PE and malignancy. The PE has been a significant predictor also in other human tumours studied by image analysis [20]. Nuclear shape is under a genetic regulation [21] which suggest a relationship between nuclear shape and genetic alternation related to malignancy. However, none of these factors probably has clinical use in predicting recurrence since the curves were not widely separated. Again, in papillary tumours the prognostic potential of nuclear factors was lower than in the entire cohort due to the same reasons as outlined previously.

The results of survival analyses presented previously are in full agreement with the results of this analysis [1–5]. Usually NA and SDNA have been included in the survival analysis [1, 2] and also this analysis established their significant role in predicting survival. However, the present results stress the importance of shape factors like PE, SDPE and D_{max} which with high probability is related to cellular mechanisms related to intrinsic malignancy [21]. On the contrary the NA and SDNA may vary significantly being in any relevant relationship to malignancy. The amount of active DNA (euchromatin) and the amount of RNA contribute to nuclear size in addition to cell cycle kinetics. It is well known that many non-malignant cells have varying nuclear sizes depending on their functional status, compare, for example, a lymphocyte and a histiocyte.

Also in multivariate analysis shape factors included independent prognostic information. In papillary tumours only D_{max} had independent prognostic value which validates the previously presented suggestion on the relationship between nuclear shape and malignancy [21]. In the entire cohort the WHO grade was the most important predictor which is due to intimate relationship between nodular growth pattern and grade 3 histology. It is well known [4] and also supported by the results from this analysis that nodular growth pattern *per se* is a highly unfavourable prognostic sign.

The prognostic potential of different nuclear factors is probably quite similar albeit different factors predicted survival and recurrence-free survival in multivariate analysis. The multivariate methods are dependent on the particular sample analysed and the distribution of variables within that sample. Accordingly, other materials should be tested with these same methods to validate the present results and preferably in a prospectively followed-up cohort of patients. Therefore some caution is warranted in predicting the results by nuclear variables in comparison to WHO grading.

In conclusion the following statements seem justified. Nodular tumours have an unfavourable prognosis and accordingly should be treated as radically as possible in the primary phase. Papillary bladder tumours can be categorised into prognostic groups by nuclear image analysis more efficiently than by conventional histological grading. The results provide a morphometric classification system for papillary superficial transitional cell bladder tumours. Papillary tumours with large nuclear factor values should be considered as candidates for radical primary therapy and adjuvant intravesical therapy after transurethral resections. Moreover these patients should be monitored closely after primary therapy.

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