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The Changing Importance of Prognostic Factors in Bladder Cancer During a Long-term Follow-up

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A cohort of 505 patients with a transitional cell bladder cancer were followed up for over 9 years and clinical, histological and morphometric factors were related to survival. Several survival analyses were done by moving the start of the follow-up so that the first analysis started at the time of primary therapy and the last one after 9 years follow-up. T-category, WHO grade, papillary status and the density of tumour infiltrating lymphocytes had independent short-term prognostic value whereas mitotic index and standard deviation (S.D.) of nuclear area were independent long-term predictors up to 7 years after diagnosis. In papillary tumours S.D. of nuclear area and mitotic index were independent long-term predictors in contrast to T-category and WHO grade which were both short-term prognostic factors. In superficial tumours only mitotic index had independent long-term prognostic value. The results show that the prognostic information from the primary tumour biopsy specimen has long-term prognostic significance in transitional cell bladder cancer. The results particularly emphasize the importance of factors related to cancer cell proliferation as long-term predictors.
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INTRODUCTION

PREVIOUS ANALYSES of prognostic factors have shown that the clinical stage of transitional cell bladder cancer (TCC) is the main determinant of survival [1, 2]. However, there are significant differences in survival among stage categories which indicates that also the biological characteristics of bladder tumours affect prognosis, particularly in superficial tumours [3]. These biological characteristics include cancer cell proliferation [2, 4], various nuclear features [2, 3], histological differentiation [4], growth architecture [2] and the expression of certain antigens on cell surfaces [5, 6]. All the analyses presented so far have started the follow-up after diagnosis or after treatment. In other human tumours the prognostic significance of various features related to the primary tumour influence on prognosis during a long-term period after treatment [7] whereas some indicators of malignancy lose their prognostic value within a few years of follow-up. In breast cancer, factors related to histological differentiation, e.g. tubule formation and components of intraductal growth, and certain nuclear features have long-term prognostic value whereas proliferation indices seem to have only short-term prognostic value [8]. As far as the author is aware results of corresponding analyses have not been previously reported in transitional cell bladder tumours. In this analysis the potential of clinical, histological and morphometric factors was tested to predict survival during different time periods after primary therapy in a cohort of 505 patients with a transitional cell bladder cancer followed up for a mean of over 9 years.

PATIENTS AND METHODS

Patients

This study included 505 patients with a newly diagnosed TCC at four centres in eastern Finland. The patients were diagnosed, treated and followed up during 1965–1991. The female to male ratio was 109/396. The treatment and follow-up investigations were performed according to standard practice [9] which was, however, tailored individually. Most superficial Ta-T1 tumours were primarily treated by transurethral resections and in 61 cases adjuvant intravesical chemotherapy [10] was used. The primary therapy and the therapy during the follow-up in various clinical stage groups is summarised in Table 1. The 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 muscle-invasive tumours during the later years computed tomography or ultrasonography was performed. Screening for metastasis included chest radiography, laboratory tests, abdominal ultrasonography, and when appropriate, bone scintigraphy and lymphography. Tumour, nodes and metastasis classification was done according to UICC 1978 [11] and was based on the above-mentioned examinations and the pathologists reports (Table 2). As a rule, 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-up programe was restarted. 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, death certificates and from the files of the Finnish Cancer Registry.

Table 1. Treatment of patients during the follow-up

Type of therapy	Number of treatments Ta-T1	T2	T3-T4
No treatment	3	0	9
Transurethral resection	298	122	48
Partial cystectomy	14	19	15
Cystectomy	7	34	20
Cystectomy and radiation therapy	7	27	37
Radiation therapy	6	9	29
Intravesical chemotherapy	61	39	8

Note that the same patient may have been treated by several different methods during the follow-up.

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Table 2. The clinical characteristics of patients

Number of patients	505
Age at diagnosis (years)	
Mean	67.5
S.E.	0.5
Follow-up time (years)	
Mean	9.1
S.E.	0.2
Ta/T1	71/183
T2	144
T3	66
T4	41
Papillary/nodular	407/98
WHO grade 1/2/3	208/204/93
TIL grade 1/2/3	332/101/72
Recurrence	254
Died of bladder cancer/other	166/136

Histological methods

The histological samples were perioperative biopsy specimens from the primary tumours. 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 [12] by one observer in a blinded manner. The distribution of cases into WHO grades is shown in Table 2. The papillary status of tumours was recorded and the tumours were divided into papillary and nodular types (Table 2).

The density of tumour infiltrating lymphocytes (TIL) was graded into three categories: absent and weak, moderate, and dense [13]. Only TIL were scored; it was attempted to exclude polymorphonuclear leukocytes and plasma cells in the scoring process. TIL around blood vessels, in the centre and periphery of tumours and around invasive carcinoma cells were included in the scoring process and the general density of TIL was important. TIL were dense when the tumour margins and stroma contained a dense lymphocyte infiltrate [13]. TIL were weak when occasional lymphocytes were encountered in the stroma and the inflammatory cell reaction around the tumour consisted also of mainly occasional cells or regionally of a moderate cell reaction [13].

Morphometry

In nuclear image analysis the IBAS 1&2 analyser system was used in a blinded manner by one investigator as detailed previously [2]. The mean nuclear area (NA), standard deviation of nuclear area (SDNA), nuclear perimetry (PE) and standard deviation of nuclear perimetry (SDPE) were used in the further analysis. The mitotic figures were counted using an objective magnification of × 40 (field diameter 490 μm) from the most cellular areas of the tumour samples in a blinded manner. The mitotic activity was measured using the volume corrected mitotic index (M/V index) method which was originally described by Haapasalo and Collan [14].

Statistical methods

The determination of deaths due to bladder cancer was made by a critical review of the autopsy reports, death certificates and clinical data. Survival was calculated by counting as bladder cancer deaths only those patients who died with known metastases and censoring for non-cancer deaths. In basic statistics the SPSS/PC+ V 3.1 program package was used in a Toshiba T3200 computer. Survival analysis was done by the BMDP [2] (UCLA). The analyses were done at 1–3 year intervals and the follow-up in the first analysis started at the time of the primary therapy. The M/V index and nuclear morphometric variables were used as continuous variables in the analysis. The values in Tables 3–5 indicate the probability of no trend (P value) between prognostic variables and corrected survival in univariate analysis (step 0 in a stepwise multivariate analysis of the BMDP). The values in Table 6 indicate the probability of no independent prognostic value for the individual prognostic variables in Cox's multivariate analysis [15] starting at different timepoints during the follow-up. The multivariate analysis was done by adding 1–3 years to the year of treatment in each step and accordingly only the patients surviving at that time point were included in the multivariate model. The patients who had died before the new startpoint of the follow-up were automatically dropped out from the analysis (negative survival time is not acceptable in BMDP [2]. P values greater than 0.2 are indicated by the abbreviation ns.

RESULTS

All the variables included were significantly related to survival at the time of diagnosis (Table 3). The variables retained their

Table 3. The probability of no trend between the prognostic variables and survival in the entire cohort during the follow-up starting at different time points after the primary therapy

	Years after therapy							
Variable	0* (n=505)	1 (n=430)	2 (n=369)	3 (n=325)	4 (n=281)	5 (n=232)	8 (n=139)	10 (n=70)
T-category	<0.0001	<0.0001	<0.0001	0.0001	0.0039	0.0134	0.0400	ns
WHO grade	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	0.1505	ns
Papillary status	<0.0001	<0.0001	<0.0001	0.0052	0.0007	0.0095	ns	ns
TIL	0.0019	0.0013	0.0002	0.0019	0.0058	0.0191	0.1608	ns
NA	<0.0001	0.0002	0.0036	0.0048	0.0014	0.0015	0.0177	0.0950
SDNA	<0.0001	<0.0001	0.0001	0.0002	<0.0001	<0.0001	0.0142	0.0811
PE	<0.0001	0.0004	0.0032	0.0107	0.0031	0.0044	0.0328	0.1521
SDPE	0.0075	0.0338	0.0449	0.1432	0.1414	0.1895	ns	ns
M/V index	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0136	ns

*Time of treatment. ns, Not significant.

Table 4. The probability of no trend between the prognostic variables and survival in papillary tumours during the follow-up starting at different timepoints after the primary therapy

Variable	Years after therapy 0* (n=405)	1 (n=366)	2 (n=316)	3 (n=284)	4 (n=245)	5 (n=203)	8 (n=98)	10 (n=62)
T-category	<0.0001	<0.0001	<0.0001	0.0084	ns	ns	ns	ns
WHO grade	<0.0001	<0.0001	<0.0001	0.0040	0.0479	0.0079	0.0736	0.1023
TIL	0.1112	0.1076	0.0616	ns	ns	ns	0.2000	ns
NA	0.0006	0.0024	0.0047	0.0152	0.0052	0.0004	0.0159	0.0082
SDNA	0.0001	0.0020	0.0025	0.0058	0.0023	0.0001	0.0102	0.0012
PE	0.0007	0.0042	0.0039	0.0368	0.0139	0.0015	0.0296	0.0235
SDPE	0.1798	ns	ns	ns	ns	ns	ns	ns
M/V index	<0.0001	<0.0001	0.0001	0.0005	0.0025	0.0029	0.1548	ns

*Time of treatment. ns, Not significant.

Table 5. The probability of no trend between the prognostic variables and survival in Ta-T1 tumours during the follow-up starting at different time points after the primary therapy

Variable	Years after therapy 0* (n=253)	1 (n=233)	2 (n=214)	3 (n=197)	4 (n=173)	5 (n=140)	7 (n=80)	10 (n=39)
WHO grade	<0.0001	0.0007	0.0045	0.0085	0.0164	0.0045	ns	ns
Papillary status	0.0001	0.1547	0.1939	0.1425	0.0975	0.1390	ns	ns
TIL	0.0073	0.0094	0.0165	0.0620	0.0613	0.0813	0.1063	ns
NA	<0.0001	0.0006	0.0017	0.0060	0.0071	0.0020	0.0116	ns
SDNA	0.0001	0.0010	0.0014	0.0016	0.0012	0.0001	0.0132	ns
PE	<0.0001	0.0005	0.0022	0.0104	0.0112	0.0048	0.0211	ns
SDPE	0.0153	0.0214	0.0124	0.0043	0.0037	0.0002	0.0334	ns
M/V index	<0.0001	<0.0001	0.0001	0.0005	0.0003	0.0031	ns	ns

*Time of treatment. ns, Not significant.

significant relation to survival after 5 years follow-up except SDPE which had prognostic value during 2 postoperative years (Table 3). At 5 years the M/V index and SDNA as determined in the primary tumour biopsy specimens were the most important predictors followed by WHO grade. After 8 years follow-up T-category, NA, SDNA, PE and M/V index were related significantly to survival whereas after 10 years follow-up only NA and SDNA were weakly related to survival. The independent predictors of survival in the multivariate analysis are shown in Table 6. The results show that T-category has independent prognostic value for up to 4 postoperative years. Papillary status and the density of lymphocyte infiltrates have prognostic value only immediately after primary therapy (Table 6) whereas the M/V index and SDNA have long-term prognostic value.

In papillary tumours T-category had prognostic value up to 3 years (Table 4) whereas WHO grade and M/V index predicted survival during 5 postoperative years (Table 4). Nuclear factors had prognostic value even after 10 years follow-up (Table 4). Also in papillary tumours M/V index and SDNA have independent long-term prognostic value in multivariate analysis (Table 4).

In superficial tumours papillary status had prognostic value during 1 postoperative year (Table 5) whereas the density of tumour infiltrating lymphocytes, WHO grade and mitotic frequency had prognostic value 5 years after treatment (Table 5). All four nuclear factors had prognostic value during 7 postoperative years and only M/V index has significant long-term prognostic value (Table 6). The results in T1 tumours alone are similar to the results in Ta-T1 tumours (Table 6).

DISCUSSION

When the results of this analysis are evaluated, one should realise that the number of patients after 9 years follow-up is substantially smaller than in the beginning of the follow-up. Lower numbers of patients at the end of the follow-up may skew the prognostic estimates and accordingly some caution is needed when the results are interpreted after 7 years follow-up.

The results confirm previous findings in that the clinical stage at the time of diagnosis is the main determinant of survival followed by various cellular features related to tumour malignancy [1, 2, 5, 13]. The importance of the extent of the primary tumour as a long-term prognostic factor was established in TCC which is in line with previous reports in other neoplastic diseases [7, 8]. Thus it seems that the risk of metastatic behaviour is directly correlated to the extent of the primary tumour since the mechanisms of metastatic spread are also related to tumour size [16]. The long-term prognostic value of pelvic lymph node involvement was not separately assessed since it is not routinely staged histologically in TCC.

Nodular growth architecture refers to lowered survival probability and the potential of this feature seems to be prognostically relevant at least 5 years after diagnosis. Nodular growth pattern

Table 6. The independent prognostic variables in the entire cohort, in papillary (PAP), in Ta-T1 and T1 tumours during the follow-up starting at different time points after the primary therapy. The risk ratio (RR) related to prognostic factors at the time of diagnosis is also given

Variable	RR (95% CI)	Years after therapy 0*	1	2	3	5	7	10
All cases								
T-category	1.93 (1.63–2.27)	<0.001	<0.001	<0.001	0.015	0.085	ns	ns
WHO grade	1.54 (1.11–2.15)	<0.001	0.013	<0.001	ns	ns	ns	ns
M/V index	1.02 (1.00–1.03)	0.005	<0.001	ns	<0.001	0.006	0.014	ns
TIL	0.70 (0.56–0.88)	0.007	ns	ns	ns	ns	ns	ns
Papillary status	0.63 (0.41–0.95)	0.020	ns	ns	ns	ns	ns	ns
SDNA	1.01 (0.99–1.02)	0.093	ns	ns	0.011	<0.001	ns	0.081
PAP								
T-category	2.27 (1.81–2.85)	<0.001	<0.001	<0.001	0.092	ns	0.055	ns
M/V index	1.02 (1.01–1.03)	<0.001	<0.001	ns	<0.001	0.096	ns	ns
WHO grade	1.52 (1.04–2.24)	0.065	ns	0.002	ns	ns	ns	ns
TIL	0.78 (0.59–1.04)	0.079	ns	0.064	ns	ns	ns	ns
SDNA		ns	ns	ns	ns	<0.001	0.016	0.001
SDPE		ns	ns	ns	ns	ns	ns	ns
Ta-T1								
M/V index	1.06 (1.04–1.08)	<0.001	<0.001	<0.001	<0.001	ns	ns	ns
PESD	0.81 (0.66–0.99)	0.024	ns	ns	ns	ns	ns	ns
PE	1.13 (1.04–1.23)	0.039	ns	ns	ns	ns	ns	ns
SDNA		ns	ns	ns	ns	<0.001	ns	ns
NA		ns	ns	ns	ns	ns	0.012	ns
T1								
PE	1.08 (1.02–1.15)	<0.001	0.056	0.056	ns	ns	0.008	ns
M/V index	1.04 (0.75–1.43)	0.022	<0.001	<0.001	0.002	<0.001	ns	ns

*Time of treatment. The risk ratio and its 95% confidence interval (CI) is calculated by using the coefficients and their standard errors (multivariate regression model). The RR corresponds to the time of treatment.

is associated with several malignant cellular features in TCC including the lack of blood group antigens at cell surfaces [17] and rapid cellular proliferation [18] which all refer to increased metastatic potential. It is highly probable that the nodular bladder tumours comprise a separate subgroup with respect to their basic malignant features and growth regulation. Hypothetically, the relationship between papillary and nodular bladder tumours is analogous to the relationship between sex steroid receptor-positive and -negative breast tumours. Sex steroid receptor-negative breast tumours are rapidly proliferating [19] and they exhibit several other malignant cellular features [19] like nodular bladder tumours. However, biochemical differences in growth regulation have not been described as yet between nodular and papillary TCC. The frequent expression of oncogenes and their protein products in high grade tumours already support the presence of such biochemical differences in growth regulation [20] and improved methodologies may reveal the underlying basic differences between papillary and non-papillary tumours.

The prognostic importance of TIL has been established previously in TCC [13 ,21], as well as in other human tumours [22]. The prognostic importance of TIL is intimately related to proliferation rate in TCC since in papillary tumours alone, which are slowly proliferating [18], TIL had no significant long-term prognostic value. In contrast, the prognostic potential of TIL is highest in nodular tumours and in muscle-invasive tumours which are usually rapidly proliferating. A similar association between TIL, proliferation rate and prognosis has been previously described at least in melanomas [22] and in breast cancer [23]. The results clearly show that the prognosis of malignant subsets of bladder tumours is efficiently modulated by the host defence mechanisms [24] and the stimulation of the host defence mechanisms has been already used successfully in the treatment of TCC [25]. The effect of the immunostimulators is particularly efficient in the treatment of superficial tumours [25].

In superficial tumours the malignant potential of bladder tumours is the main determinant of the risk of invasion, since the multivariate analysis showed no independent prognostic value for TIL. The histological analysis showed that superficial tumours in general are rarely surrounded by dense TIL which suggests that immune defence mechanisms are not activated. The effect of immunostimulators [25] in the treatment of superficial TCC is probably based on the activation of the immune system which concurs with the findings from experimental studies [26]. It was unexpected that the effect of TIL was present only immediately after primary therapy which may be related to the treatment and to alternations in host immune response as a function of time.

The prognostic significance of WHO grade, nuclear factors and M/V index can be evaluated together, since grading combines information from nuclear structure and mitotic frequency [12]. The potential of these factors to predict survival has already been established in several univariate and multivariate analyses [1–3, 13, 18]. This analysis showed that the potential of SDNA lasted up to 10 years after therapy and similar results have been previously reported in breast cancer [7]. The mitotic index and WHO grade were highly significant prognostic factors up to 8 years postoperatively, whereas in breast cancer the prognostic value of these features lasts only 3 years postoperatively [8]. The real significance of these factors in relation to each other is

difficult to establish, however, since they all are highly significantly interrelated in TCC. The results of multivariate analysis are also dependent on the frequency distribution of these factors in relation to each other and accordingly one of these three features was usually selected among the independent predictors. However, the prognostic potential of SDNA was present after 10 years in papillary tumours which suggests that variation in nuclear shape and size reflects intrinsic malignant potential of bladder epithelium as observed also in other neoplastic cells [27].

What do the results mean in practice? The recurrence of bladder tumours is common and the factors having long-term independent prognostic value reflect the potential of bladder tumours to have invasive recurrences since most of the bladder tumours are superficial at diagnosis. Accordingly, the same factors also reflect metastatic potential, which was clearly demonstrated in this analysis. Accordingly, a rapidly proliferating tumour at the onset is a continuous metastatic threat even after a long-term follow-up. One should also realise that the impact of rapidly proliferating tumours on prognosis may be mediated through the presence of malignant concomitant lesions in the surrounding bladder epithelium [28].

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