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Acknowledgements—We thank Ms Angela Palumbo for help in preparation of the manuscript.

This study was supported by Public Health Service Grants from the National Cancer Institute, National Institute of Health, the Department of Health and Human Services and by a grant from the T.J. Martell Foundation.

The following CALGB members participated in the study: Mark Green, University of California at San Diego, San Diego (CA-11789); Michael Perry, University of Missouri, Columbia (CA-12046); Bruce Peterson, University of Minnesota, Minneapolis (CA-16450); Robert Cooper, Bowman-Gray School of Medicine, Winston-Salem (CA-03927); Joseph Aisner, University of Maryland Cancer Center, Baltimore (CA-31983); Raymond Weiss, Walter Reed Army Medical Center, Washington D.C. (CA-26806); Rose Ruth Ellison, Columbia University, New York (CA-12011); Richard T. Silver, New York Hospital–Cornell Medical Center, New York (CA-07968); Sameer Rafla, Maimonides Hospital, New York (CA-25119); James F. Holland, Mount Sinai School of Medicine, New York (CA-04457); Arlan Gottlieb, Upstate Medical Center of Syracuse, Syracuse (CA-21060); Mary Costanza, Central Massachusetts Oncology Group, Worchester (CA-37135); James R. Anderson, Harvard School of Public Health, Boston (CA-33601); Robert Carey, Massachusetts General Hospital, Boston (CA-12449); Louis Leone, Rhode Island Hospital, Providence (CA-08025); Gibbons Cornwell, Dartmouth-Hitchcock Medical Center, Hanover (CA-04326); J.L. Hutchinson, McGill Cancer Center, Montreal (CA-31809); and Harvey Golomb, University of Chicago, Chicago (CA-41287).

Eur J Cancer, Vol. 26, No. 10, pp. 1060–1063, 1990.
Printed in Great Britain

0277–5379/90 $3.00 + 0.00
Pergamon Press plc

Prediction of Superficial Bladder Cancer by Histoquantitative Methods

Pertti K. Lipponen, Matti J. Eskelinen and Martti Sotarauta

A retrospective clinicopathological study was done of 136 T1 bladder cancer patients, mean follow-up 10 years. With interactive morphometry, mean nuclear area, mean standard deviation of nuclear area (SDNA) and the mean area of the 10 largest nuclei (NA10) were measured in biopsy specimens from primary tumours. Volume corrected mitotic index (M/V index) was estimated in the same sections. Histological grading was done according to WHO and clinical staging according to UICC. Progress in bladder cancer was observed in 26 cases. Progressing tumours had significantly higher M/V values ($P = 0.0038$) than tumours without progression. By χ^2 statistics NA10 ($P = 0.08$) and M/V index ($P = 0.0024$) were related to invasive potential. Tumours with high NA10 values ($P = 0.0065$) and high M/V index values ($P = 0.0104$) eventually metastasised. Nuclear area ($P = 0.0025$), NA10 ($P = 0.0053$), histological grade ($P = 0.0071$), NA ($P = 0.0563$) and M/V index ($P = 0.0979$) predicted bladder cancer-related survival, in that order. The recurrence rate or recurrence-free period were not related to histological indices. The results suggest the use of these morphometric features instead of histological grading in the prediction of T1 bladder tumours.
Eur J Cancer Vol. 26, No. 10, pp. 1060–1063, 1990.

INTRODUCTION

MOST DIAGNOSED bladder cancers are superficial and overall prognosis is good. However, bladder cancer has a high potential for recurrence and about one-sixth of initially superficial tumours develop into invasive recurrences [1]. The decision on treatment of superficial tumours is generally based on clinical stage [2] and histological grade [3, 4]. Subjective grading, however, is not reproducible [5, 6] and the prediction of recurrence and invasion in T1 bladder tumours is difficult and unsatisfactory with subjective grading [7]. Flow cytometry [8–12], mitotic activity [13], nuclear morphometry [8, 9, 14, 15], semiquantitatively assessed blood group antigens [12] and Lewis a antigen-related CA 50 [16] have been used to grade bladder cancer with promising results. Most of the studies with quantitative techniques have included all stage and grade categories and sub-

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Table 1. Morphometric variables in 136 T1 bladder cancer patients

	Range	Mean (SD)
NA (μm^2)	34.4–214.6	72.3 (27.5)
SDNA (μm^2)	7.3–99.0	21.7 (13.8)
NA10 (μm^2)	54.0–434.0	111.7 (48.4)
M/V index (/mm^2)	0–42	7.9 (9.8)

NA = nuclear area, SDNA = standard deviation of NA and NA10 = NA of 10 largest nuclei.

grouping has not generally been done [9, 11, 13, 14, 16]. So we studied the value of morphometric methods in prediction of T1 bladder cancer. In this subgroup a reliable prediction of invasion would be most important.

PATIENTS AND METHODS

Our retrospective study was based on clinical follow-up of 136 consecutive patients with T1 bladder cancer. The patients were treated and followed up during 1965–1990, a mean (S.D.) follow-up period of 10.1 (4.2) years (range 5–25). The mean age of the 27 females at the time of diagnosis was 68.4 (9.5) and of the 109 males, 62.8 (15.5). Treatment and follow-up were mainly done by two urologists [17]. Follow-up was every 3 months during the first 2 years and thereafter every 6 months. If recurrence occurred the follow-up programme was started again. Recurrences were treated as for primary tumours [17]. Clinical staging of tumours was done according to UICC criteria [2] by palpation, cystoscopy, ultrasound, computerised tomography, lymphography, routine laboratory tests and X-ray. The recurrence rate was calculated as (the number of recurrences divided by months of follow-up) times 100. The recurrence-free period was the time from treatment to the first observed recurrence in the bladder. Progression was defined as an increasing T, N or M category during follow-up. Most patients who died underwent necropsy to ascertain the extent and metastasis of tumours.

The histological samples were obtained by preoperative biopsy before any treatment was given. The samples were fixed in buffered formalin (pH 7), embedded in paraffin, cut into 5 μm sections and stained with haematoxylin-eosin or Van Gieson-iron-haematoxylin stains. The samples were graded histologically by a board-certified pathologist according to WHO recommendations [3].

For morphometric measurements we used the "IBAS 1&2" image analyser. The most atypical fields were selected subjectively. One investigator measured the nuclei (mean 74, range 55–85) with a magnetic digitiser plate. Various morphometric features were automatically calculated by the computer. For further analysis the mean (S.D.) nuclear area (NA), mean S.D. of NA (SDNA) and the mean area of the 10 largest nuclei (NA10) in each section were selected.

Mitoses were counted with an objective magnification of × 40 (field diameter 490 μm). The mitotic figures were identified using the same criteria as described in earlier contributions (13, 18). The volume corrected mitotic index (M/V index) was estimated [18].

*Table 2. Relation between morphometric variables and histological grade**

Grade	NA (μm^2)	SDNA (μm^2)	NA10 (μm^2)	M/V index (/mm^2)
I (n = 72)	60.6 (14.9)	16.5 (5.0)	94.2 (22.9)	4.3 (6.9)
II (n = 52)	80.9 (27.6)	23.8 (13.7)	122.7 (54.5)	10.2 (10.4)
III (n = 12)	105.1 (44.0)	43.4 (24.4)	170.0 (72.7)	19.3 (10.8)

*Significant difference between grades for each variable: $P < 0.0001$.

Table 3. M/V index and NA10 in progressing tumours and in tumours showing metastasis during mean follow-up of 10 years

Type of progress	M/V index (/mm^2)	P	NA10 (μm^2)	P
T category				
No progress (n = 110)	6.7 (9.1)		110.3 (49.7)	
Progress (n = 26)	12.8 (10.9)	0.0038	117.8 (42.5)	0.4793
M category				
No progress (n = 117)	6.9 (9.1)		107.9 (47.9)	
Progress (n = 19)	13.7 (11.8)	0.0047	135.5 (45.6)	0.0206

RESULTS

72 patients were grade I (GI), 52 were GII and 12 were GIII. Morphometric variables are shown in Table 1. Morphometric features were significantly related to histological grading: high grade tumours had larger mean values and wider variation (Table 2).

Progressing (in T category) tumours (n = 26) had on average higher NA, NA10 and SDNA values and significantly higher M/V indices (Table 3). Tumours eventually developing lymph-node involvement (P = 0.0103) or metastatic disease had significantly higher M/V indices as well (Table 3). Also, the NA10 value predicted lymph-node involvement (P = 0.0372) and metastasis (Table 3).

The results related to progression with χ^2-statistics are shown in Tables 4 and 5. In linear multiple regression analysis, the M/V index was the only independent factor predicting progression in T category (P = 0.0038), lymph-node involvement (P = 0.0103) and metastasis (P = 0.0047). The recurrence rate (mean 3.0 [4.4]) was higher in tumours with high NA, NA10, SDNA and M/V index values but the differences were not statistically significant.

Table 4. Progression of bladder cancer (T category)

	No.	No progress	Progress	χ^2	P
Grade					
I	72	60	12		
II	52	42	10	1.8	0.3968
III	12	8	4		
M/V index (/mm^2)					
≤ 9	96	84	12		
> 9	40	26	14	9.2	0.0024
NA10 (μm^2)					
≤ 117	87	74	13		
> 117	49	36	13	2.7	0.0990

Table 5. Metastatic spread of bladder cancer (M category)

	No.	No progress	Progress	χ^2	*P*
Grade					
I	72	66	6		
II	52	44	8	9.6	0.0080
III	12	7	5		
M/V index (/mm²)					
≤ 9	91	84	7		
> 9	45	33	12	9.0	0.0027
NA10 (μm²)					
≤ 117	87	81	6		
> 117	49	36	13	10.5	0.0015

Recurrence-free period (mean 51.8 [49.2] months) was not related significantly to these morphometric variables although a similar trend was found as with recurrence rate. Crude survival was not predicted with any of the variables included in this study. Bladder cancer related survival could be predicted significantly by NA (Fig. 1), NA10 (Fig. 2) and histological grade (Fig. 3) in that order. M/V index (Fig. 4) and SDNA (χ^2 = 3.6, P = 0.0563, group limit 18 μm²) were just above statistical significance.

DISCUSSION

Classical prognostic factors in superficial tumours are usually unsatisfactory, as highlighted by our results [7]. Notwithstanding, treatment is generally still based on stage and histological grade [17]. Our patients were initially treated with electrocoagulation or transurethral resection and there was no significant relation between primary treatment, prophylactic chemotherapy [19], development of invasive recurrences or survival. Histoquantitative techniques are superior to classical prognostic factors [8] although patient selection may have had a role. Our cases were consecutive in one hospital under the care of two urologists.

NA and NA10 were powerful predictors of survival, and M/V index significantly predicted progression. These quantitative variables were better prognostic factors than grading and the results were confirmed in a multivariate analysis. Blomjous *et al.* [8] found similar results, except for NA10 and M/V index which were not included in their analysis. Progression in bladder tumours was related more firmly to M/V index and NA10 than to NA or SDNA. The rate of growth, as expressed in high mitotic activity [13], DNA aneuploidy [8–12], large nuclei and wide variation in nuclear size [8, 9, 11, 14, 15], also applies in bladder cancer. We conclude that high mitotic activity indicates an increased risk of invasion in bladder cancer, as has been also demonstrated in other epithelial tumours [20–22]. More aggressive treatment and more intensive follow-up should be focused on patients whose bladder tumour has a high M/V index and NA10. The difference in the predictive value of NA and M/V index in survival and progression analysis was probably due to treatment and different numbers of progressing tumours (n = 26) and tumours causing a cancer death (n = 12). Most deaths in those elderly patients were due to cerebrovascular accidents or myocardial infarction.

In previous studies stereological estimates of mean nuclear volume and DNA aneuploidy were significantly correlated [11], and the relation between large mean NA and aneuploidy has also been shown with planar morphometry [8]. The relation

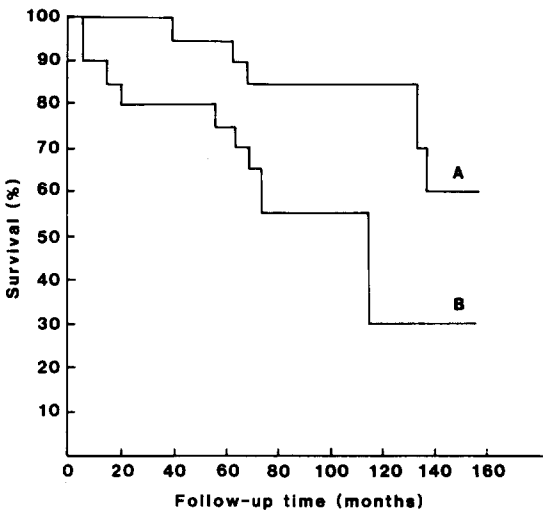

Fig. 2. Bladder cancer related survival by NA10. A = NA10 ≤ 117 μm² (n = 87), B = NA10 > 117 μm² (n = 49) (χ^2 = 7.7, P = 0.0053).

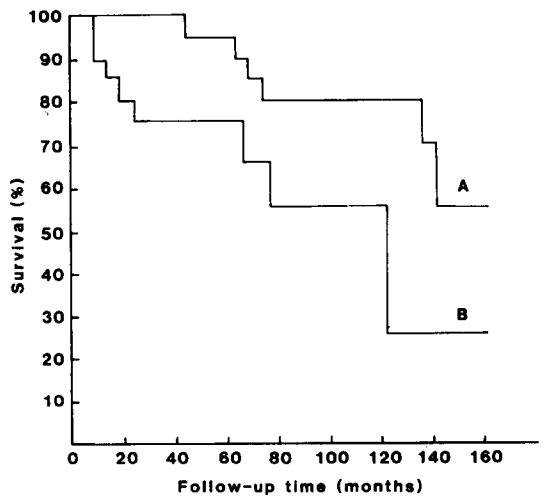

Fig. 1. Bladder cancer related survival by NA. A = NA ≤ 80 μm² (n = 91), B = NA > 80 μm² (n = 45) (χ^2 = 9.1, P = 0.0025).

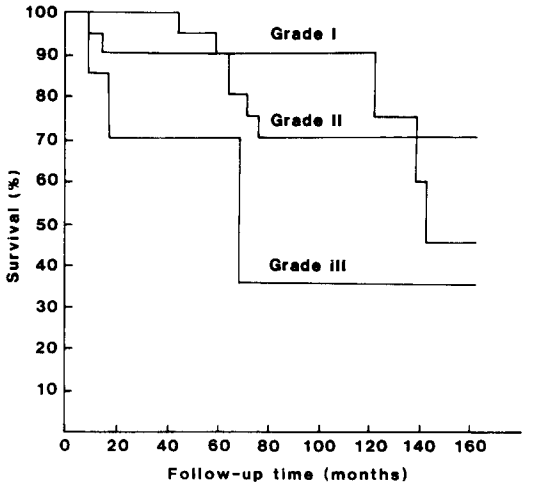

Fig. 3. Bladder cancer related survival by histological grades I (n = 72), II (n = 52) and III (n = 12) (χ^2 = 9.1, P = 0.0071).

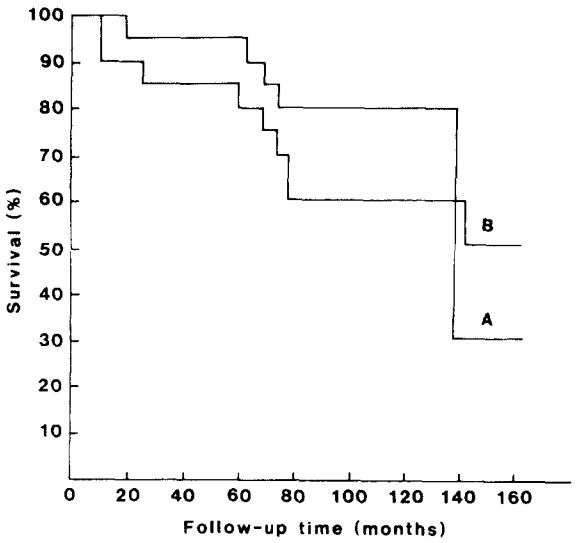

Fig. 4. Bladder cancer related survival of patients by M/V index. A = M/V index ≤ 9/mm² (n = 91), B = M/V index > 9/mm² (n = 45) (χ^2 = 2.7, P = 0.0979).

between NA10, progression and survival probably relies at least in part on quantitative DNA abnormalities since DNA aneuploidy has been related to increased invasive potential [8]. Stereologically estimated high nuclear volumes have also been related to increased risk of invasive recurrences [15].

Morphometric measurements are highly reproducible [5, 20, 23, 24]. The grading efficiency [25] in a two-grade system is about 90% and in a three-grade system, about 80% [23]. The superiority of morphometric grading is reflected in many clinically valuable results in grading other epithelial tumours [20–22]. However, these results were based on retrospective materials. Prospective results are not available yet, including in bladder cancer. Generally, individualised treatment based on clinical stage and histological grade instead of morphometric grading must have had an effect on bladder cancer behaviour. So, a prospective follow-up study should be done to evaluate the value of morphometric grading in a clinical context.

Compared with flow cytometry [8–10, 27, 28], morphometry does not need expensive instruments or specially trained operators. The estimation of M/V index in a routinely processed paraffin section and nuclear morphometry measurements takes 5-10 min. The identification of mitotic figures may sometimes be difficult [13, 20–22, 29], but grading variation due to the measurement itself is practically nil [24].

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