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DNA Ploidy, S Phase Fraction and G_2 Fraction as Prognostic Determinants in Prostatic Adenocarcinoma

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Abstract. The results of DNA flow cytometry (FCM), histological features and clinical stage of prostatic adenocarcinoma were correlated to outcome in 91 patients during a mean follow-up period of 15.6 years. Aneuploidy was detected in 33 (36%) tumours, and 59 (64%) tumours were diploid. Eighteen (20%) tumours showed a tetraploid DNA index. The frequency of tetraploidy increased towards high-stage and high-grade tumours. Aneuploidy, high S phase fraction (SPF) and G_2 fraction were significantly related to clinical stage, histological grade and perineural infiltration. Progressing tumours (T category) had higher SPF values ($p = 0.0248$), and progression in N ($p = 0.0122$) and M categories ($p = 0.0021$) was related to high G_2 fraction as well. In T_{1-2} tumours, DNA ploidy ($p = 0.0280$) and SPF ($p = 0.0230$) predicted progression, whereas histological grade had no significant predictive value. The clinical stage (T) predicted crude survival ($p = 0.0005$). Results show that FCM gives prognostic information in prostatic adenocarcinoma beyond that of histological grading.

Introduction

Treatment schemes for prostatic adenocarcinoma are generally based on clinical stage and histological grade [1–3] of the tumour. Several studies have shown that these conventional methods [4] are unable to divide the patients accurately into various treatment groups. Sophisticated subjective grading methods [1, 5] have led to improved accuracy in grading of human prostatic adenocarcinoma. However, many of the grading systems [1] are complicated to use and, accordingly, they are not widely applied in clinical practice. Thus, there is an urgent need to establish additional histological and/or immunological characteristics which could predict tumour progression more accurately, intracapsular T_{1-2} tumours in particular.

Recently used quantitative measurements like nuclear morphometry [6–9], stereological calculations of the nuclear volume [10], as well as mitotic activity measurements [5, 9] have shown to be of some predictive value in prostatic adenocarcinoma. More lately, DNA flow cytometry (FCM) [11–20] has been tested in grading of prostatic cancer, and the results seem promising. However, the experience of all these quantitative methods is still too limited to establish their value more generally.

In the present paper, FCM was applied to assess tumour progression and patient survival in a series of 91 patients with prostatic adenocarcinoma.

Materials and Methods

Patients and Follow-Up

The study comprises a total of 91 patients with prostatic adenocarcinoma, treated and followed up at our clinic from 1968 to 1989 for 15.7 ± 3.3 years (mean ± SD; range 5–24). The age of patients at diagnosis was 71.6 ± 7.5 years (mean ± SD; range 53–89). Clinical staging of the tumours was completed according to the UICC

[21]. Follow-up and treatment were done according to standard practice [22], mostly by 2 urologists. A total of 32 patients underwent orchiectomy, and 20 patients were treated with radical prostatectomy. Hormonal therapy was given in 47 patients (polyestradiol-phosphate in 45, ethinylestradiol in 2, LHRH analog in 1). Seven patients were treated with irradiation.

Tumour Grading

Histological biopsy specimens were fixed in buffered formalin (pH 7), embedded in paraffin, sectioned at 5 µm and stained with HE. Each tumour was graded histologically into one of the three malignancy grades: well, moderately or poorly differentiated [23]. Perineural infiltration (PNI) of the tumour was scored either 0 (absent) or 1 (present).

Flow Cytometry

FCM was done from paraffin blocks using 50-µm-thick sections [24]. The deparaffinised sections were treated with 10 µg/ml proteinase K (Sigma) for 30 min at room temperature. After filtration, nuclei were treated with 10 µg/ml RNase and stained with 25 µg/ml ethidium promide (Sigma) for at least 1 h. DNA was determined with a FACS Star (Becton Dickinson) instrument using an emission at 488 nm at 200 mW. Total emission above 560 nm was recorded. As staining intensity of fixed nuclei varied from one sample to another, no internal standard was included. The lowest peak was given a DNA index (DI) value 1 and DI of other peaks was calculated using this reference. Tumours with a DI of 1.00–1.49 were recorded as diploid, and tumours with a DI > 1.5 were considered aneuploid. Tumours with a DI between 1.75 and 2.00 were considered near tetraploid or tetraploid. S phase fraction (SPF) and G_2 fraction were calculated using the built-in programme of the FACS Star. SPF and G_2 fractions were available in 87 of the 91 cases.

Statistical Analysis

In statistical calculations, SPSS/PC+ program package was used in an Amstrad PC1640HD20 computer.

Results

Fifty-seven (63%) tumours were diploid and 34 (37%) aneuploid. A total of 19 (21%) tumours was tetraploid or near tetraploid and the remaining 15 (16%) were non-tetraploid aneuploid.

Stratification of the tumours according to DNA ploidy, clinical stage, histological grade and PNI is shown in table 1. Altogether, 65% of grade III tumours and 65% of T_{3-4} tumours were aneuploid, whereas 72% of T_{1-2} tumours were diploid (table 1). Similarly, 9 of 17 T_4 tumours were tetraploid or near tetraploid, whereas none of the T_1 tumours showed a tetraploid or near tetraploid DNA histogram. Respectively, 12 of 41 grade II–III tumours were tetraploid versus 5 of 41 grade I tumours. Lack of PNI was significantly associated with diploid tumours ($p < 0.0001$).

Table 1. DNA pattern as related to clinical stage, histological grade and perineural infiltration

Feature	Number	Diploid	Aneuploid	χ^2	p
Clinical stage					
T_1	10	8	2		
T_2	33	23	10	10.4	0.0348
T_3	28	19	9		
T_4	17	5	12		
T_x	3	2	1		
Total	91	57	34		
Histological grade					
I	41	32	9		
II	21	12	9	10.9	0.0043
III	20	7	13		
Total	82	51	31		
Perineural infiltration					
PNI–	46	38	8	18.6	<0.0001
PNI+	36	13	23		
Total	82	51	31		

Table 2. SPF and G_2 percentage related to clinical stages, histological grades and in PNI

Feature	Number	SPF (SD)	p[a]	G_2 (SD)	p[a]
Stage					
T_1	10	3.8 (3.4)		1.4 (0.7)	
T_2	32	6.8 (7.6)	0.0796	3.0 (3.4)	0.0781
T_3	25	5.4 (4.4)		3.8 (2.4)	
T_4	17	10.2 (7.8)		4.2 (2.9)	
T_x	3	4.0 (2.7)		1.4 (0.8)	
Total	87				
Grade					
I	41	5.0 (5.9)		2.4 (2.1)	
II	21	5.3 (3.1)	0.0004	3.2 (1.9)	0.0082
III	20	12.1 (9.2)		4.9 (4.4)	
Total	82				
PNI					
PNI–	46	4.2 (8.8)	0.0001	2.5 (2.1)	0.0094
PNI+	36	10.0 (8.8)		4.1 (3.5)	
Total	82				

[a] Analysis of variance; for PNI: Student's t test.

Table 2 depicts SPF and G_2 percentage related to stage, grade and PNI. Grade III tumours showed significantly higher SPF and G_2 values than grade I–II tumours. Similarly, T_4 tumours showed also higher SPF and G_2 values than the tumours confined within the

Table 3. SPF and G_2 in progressing and non-progressing tumours

Feature	Non-progressing tumours	Progressing tumours	p
T category	n = 57	n = 9	
SPF	5.8 (4.0)	10.7 (13.0)	0.0248
G_2	3.4 (3.3)	2.7 (1.7)	0.5550
N category	n = 49	n = 2	
SPF	6.1 (6.4)	4.3 (1.9)	0.6947
G_2	2.5 (2.0)	6.4 (2.9)	0.0122
M category	n = 48	n = 14	
SPF	6.7 (6.9)	5.8 (3.3)	0.653
G_2	2.6 (2.1)	4.8 (2.7)	0.0021

Progression in clinical stage could be precisely assessed in all cases. Cases with incomplete data were excluded from the analysis. Numbers in parentheses are standard deviations.

Table 4. Progression in clinical stage (T category) of T_{1-2} tumours subdivided according to DNA ploidy and SPF

Feature	Non-progressing tumours	Progressing tumours	p
DNA ploidy			
Diploid	22	3	0.0614
Aneuploid	6	4	
Total	28	7	
SPF			
SPF < 10%	24	4	0.0183
SPF > 10%	4	3	
Total	28	7	

Table 5. DI, SPF and G_2 in progressing and non-progressing T_{1-2} tumours

Feature	Non-progressing tumours	Progressing tumours	p
T category	n = 28	n = 7	
DI	1.1 (0.3)	1.5 (0.5)	0.0280
SPF	4.9 (3.4)	12.0 (14.6)	0.0230
G_2	2.7 (3.6)	2.7 (1.9)	0.9800

Numbers in parentheses are standard deviations.

prostatic capsule. SPF (mean ± SD) values were higher in aneuploid (9.8 ± 8.6%) than in diploid (4.6 ± 3.8%) tumours (p = 0.0001), but no significant difference in G_2 values was found between aneuploid and diploid tumours (3.1 ± 2.1 versus 3.3 ± 3.3%; p = 0.7118).

Values of SPF and G_2 in progressing and non-progressing tumours are summarized in tables 3–5. Tumours presenting with pelvic lymph node metastasis (n = 3; 10.3 ± 4.7%) at diagnosis had significantly higher SPF values than tumours confined within the prostatic capsule (n = 64; 6.1 ± 5.9%; p = 0.0601). Progression in clinical stage (T) was related to SPF (table 4) in that progressing tumours had significantly higher SPF values (p = 0.0248). Moreover, progression in N (p = 0.0122) and M categories (p = 0.0021) was related to G_2 fraction (table 3). In T_{1-2} tumours (n = 35), progression was significantly related to DI (p = 0.0280) and SPF (p = 0.0230), too (table 5).

Results of survival analysis including all deaths are summarised in table 6. Figures show that only the extent of tumour (i.e., clinical stage) at diagnosis has significantly prognostic value, whereas histological and FCM parameters had no significant prognostic value.

Discussion

Tumour staging and grading are the standard methods in assessing the malignant potential of prostatic adenocarcinoma [1–3]. Disadvantages of these methods include that fact that many of them suffer from some subjectivity, and some are quite complicated. One of the most extensive and carefully conducted studies on the malignancy grading of prostatic cancer was presented by Gleason et al. [1]. It must be concluded, however, that

Table 6. Results of univariate survival analysis

Feature	p
Clinical stage (T)	0.0005
Histological grade	0.1603
G_2 fraction (group limit 5%)	0.2020
SPF (group limit 10%)	0.2070
DNA ploidy (diploid/aneuploid)	0.4110

For analysis, continuous variables were divided into two groups of approximately the same size.

their system is more complicated to use than morphometry [25] or FCM [26] in assessing the malignant potential of these tumours. Moreover, further subdivision of the tumours included in the various grades, particularly the large group of moderately differentiated carcinomas, has been achieved by means of FCM [27].

The present analysis showed a significant relation between aneuploidy, high SPF, high G_2, clinical stage and histological grade. The majority of T_4 as well as grade III tumours were aneuploid, in contrast to grade I tumours which were usually diploid. This is in full agreement with the previous results [20], including the recent findings by Al-Abadi et al. [28] who used single-cell cytophotometry in assessing the DNA content and cell phase of cancer cells. Distribution of DNA indexes was similar to that reported by Haugen et al. [29] who also found a significant proportion of tumours to have a nearly tetraploid or tetraploid DNA index. Also, in the present series, the frequency of tetraploidy increased with tumour stage and grade, in that some 50% of T_4 tumours were tetraploid or near tetraploid.

The reliability of PNI as a parameter of malignancy has been questioned by the detection of perineural glands in normal and hyperplastic prostates [30]. On the basis of the present findings, however, it seems justified to consider PNI as a sign of malignancy, because of its significant association with aneuploid tumours: PNI was found in 64% of aneuploid tumours and only 17% of diploid tumours.

Most studies published so far have focused on analysis of the relation between DNA ploidy and survival [11–20]. In the present series, tumour progression was analysed as well. Our analysis showed a significant relation between DNA ploidy, SPF and progression, particularly in T_{1-2} tumours. In similar analyses, the subjective histological grading had no significant predictive value. The results of FCM were similar to those obtained by using the volume-corrected mitotic index (M/V) [9]. In fact, the volume-corrected mitotic index and SPF are significantly correlated (correlation coefficient: $R = 0.415$, $p < 0.001$) in prostatic adenocarcinoma as well as in transitional cell bladder tumours [31], which clearly indicates that SPF can be semiquantitatively assessed by using conventional light microscopy.

Survival analysis of the present series showed that the extent of tumour (i.e., tumour stage) at diagnosis eventually determines disease outcome (table 6). This is consistent with previous studies [32]. Although patients with extracapsular tumour spread have usually had a shorter survival expectancy, the results of previous survival analyses have been variable [32, 33]. It is known that some tumours will run a long and uneventful course, the patient dying with, rather than of his carcinoma. This was the case in the present series as well, where only 9 patients died of their prostatic carcinoma. This precludes the survival predictions by DNA ploidy, SPF or G_2. Even in that group, results showed, however, a weak trend suggesting that patients with aneuploid tumours or tumours with SPF $> 10\%$ had a shorter expectancy of survival.

On the basis of the present results, it seems justified to conclude that FCM offers significant prognostic information in early stage prostatic adenocarcinoma. Results also indicate that FCM is an objective and relevant method of grading prostatic adenocarcinoma. However, in advanced cancers, the benefits of this technique are more limited as also demonstrated in other epithelial tumours [32]. The contribution of DNA ploidy and SPF to survival estimation can be conclusively assessed by using a multi-parameter analysis [5, 33] in which all prognostic factors known at present are included.

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