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Prostatic carcinoma cell DNA content measured by flow cytometry and its relation to clinical outcome

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DNA content of prostate tumour cells has been measured by flow cytometry of cell suspensions prepared from fixed tissue by an enzyme disaggregation technique. Two classes of tumours have been identified: diploid tumours, with a DNA content similar to benign cells and aneuploid tumours with grossly abnormal DNA values. The prognosis for the aneuploid tumours was significantly worse than diploid tumours ($P < 0.001$). When ploidy is combined with histological grading, here using the Gleason numerical system, it is possible to predict which patients, whatever their age at diagnosis, will die from their tumour and which patients will probably die before their tumour kills them. With these facts it is possible to select patients for active or expectant treatment.

Keywords: Prostate cancer, DNA ploidy, flow cytometry

Although prostatic carcinoma is the commonest urological malignancy with 8000 new cases reported in the United Kingdom each year, its natural history is still poorly understood[1-4]. It is known that some tumours will run a long and uneventful course, the patient dying with, rather than from, his carcinoma. In some cases the tumour is an incidental finding at post mortem[1,5], whereas other tumours are highly aggressive, running a rapidly fatal course. Any treatment selected for a patient must take into account both the likely outcome of the untreated tumour and the patient's life expectancy. The disease is commonest in the elderly male population with an average age at diagnosis of 70 years, and this group is prone to death from a multiplicity of other causes. A decision to treat a particular patient must realistically be aimed at increasing his life expectancy without substantially reducing his quality of life.

Markers such as acid phosphatase and hormone receptors have been used to predict prognosis and response to treatment with only limited success[5,19]. Histological grading gives some indication of the patient's prognosis for a particular tumour and the Gleason numerical grading system has been devised to make this more specific[6]. Similarly, grading tumour cell nuclear patterns has also been shown to correlate with outcome[7]. Prostatic carcinoma cell DNA content has been studied using microdensitometry by which individual tumour cells are identified and DNA content measured in Feulgen stained sections. Those patients whose cancers had high DNA values tended to have a worse prognosis[8,9]. Chromosome studies on malignant cells arrested in metaphase have shown abnormal numbers of chromosomes present, confirming the findings of microdensitometry[10]. Both these methods are slow, however, and only small numbers of cells can be studied.

Using flow cytometry the DNA content of large numbers of cells from preserved tumour tissue has been measured[14] and for some tumour types this has been found to correlate with prognosis[11-13]. In this study we have investigated prostatic carcinoma by similar methods and related the result to clinical outcome.

Patients and methods

Patients

Seventy-two patients with newly diagnosed carcinoma of the prostate who underwent prostatectomy for outflow obstruction during the decade 1973 to 1983 were studied. Their disease was staged at the time of diagnosis by bimanual pelvic examination under anaesthetic and by bone scintigraphy; in some cases lymphangiography was performed.

Following the primary treatment, patients were followed up at regular intervals until death. In addition, to act as controls, cases of benign prostatic hyperplasia were selected from each year of the decade studied.

Tumours and histological grading

Tissue taken at the time of prostatectomy was fixed in buffered 10 per cent neutral formalin solution and, following dehydration, embedded in paraffin wax. Sections were cut and stained and graded histologically according to the Gleason classification[6]. Two numbers between 1 and 5 are ascribed to each tumour, one number of the main histological pattern and one for the main subsidiary pattern, compared to a standard. The sum of these two numbers is called the Gleason score, and allows for the variation in differentiation throughout a particular tumour.

Preparation of cell suspensions from fixed tissue

From each tumour block a 40 μm and a 6μm section were cut on a microtome. The 6 μm section was stained with haemotoxylin and eosin and areas of tumour identified morphologically, mapped and then dissected out from the thick section. This tissue was dewaxed in xylol and rehydrated through absolute, 95 per cent, 70 per cent and 40 per cent alcohol and finally in distilled water. Three millilitres of 0·5 per cent pepsin solution (Sigma P7012) at acid pH 1·5 were added and the mixture incubated in a waterbath at 37 C for 30 min with intermittent vortex mixing. The resulting cell suspension was centrifuged at 1500 r.p.m. for 10 min and the pepsin supernatant removed. The cells were washed and filtered through 38 μm pore nylon mesh to remove large solid matter, leaving a suspension containing 1 5 million cells.

DNA measurement by flow cytometry

Nuclear RNA was removed by incubating the cell suspension in 1 ml 0·05 per cent RNase solution (Sigma P4875). Following this the suspension was stained with 1 ml 0·05 per cent solution of the fluorochrome propidium iodide (Sigma P5264). This binds specifically to the DNA and in direct proportion to the amount of DNA present[19]. The suspension was then passed through a flow cytometer (FACS II Becton Dickinson Cell Sorter) with laser light set at excitation wavelength 480 nm and emission wavelength 560–630 nm and approximately 30 000 cells counted. The individual cell's fluorescence is measured by photomultipliers, processed electronically and plotted on a histogram on a 256 channel recorder. The data was stored on floppy disks on a Tektronix 4662 computer and a hard copy made with a Tektronix 4052 interactive digital plotter.

Results of tumour patterns were classified by comparison with their benign controls. The position of a peak relative to the channel numbers was proportional to the DNA content of those cells.

Statistical analysis

Outcome for different groups of patients were compared by life tables and Log rank analysis.

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Results

Patients

The average age at diagnosis was 69·1 ± 7·7 years (range 54–91 years). Forty-three patients (59·7 per cent) had advanced disease at diagnosis, either with evidence of metastases or T_3 or T_4 primary tumours. The majority of the patients (72·6 per cent) had some form of hormone manipulation as primary treatment and 21·9 per cent had radiotherapy with 6 patients having a combination of both therapies. Nine patients (12·3 per cent) had no further treatment after their prostatectomy.

Thirty-six patients (50 per cent) died from their carcinoma, the length of survival ranging from 6 months to 12 years. Of the remaining patients, 15 died from unrelated causes and 21 are alive 2–12 years after diagnosis.

Flow cytometry

Benign standards. Benign prostatic tissue produced a consistent pattern whatever year the tissue was taken from. A major narrow peak containing approximately 93 per cent of the sample counted and a second smaller peak, twice the channel number of the first peak, contained 5 per cent of the cells (*Figure 1*). This second peak represents small clumps of cells comprising more than two cells or several small fragments that have been incompletely disaggregated[16].

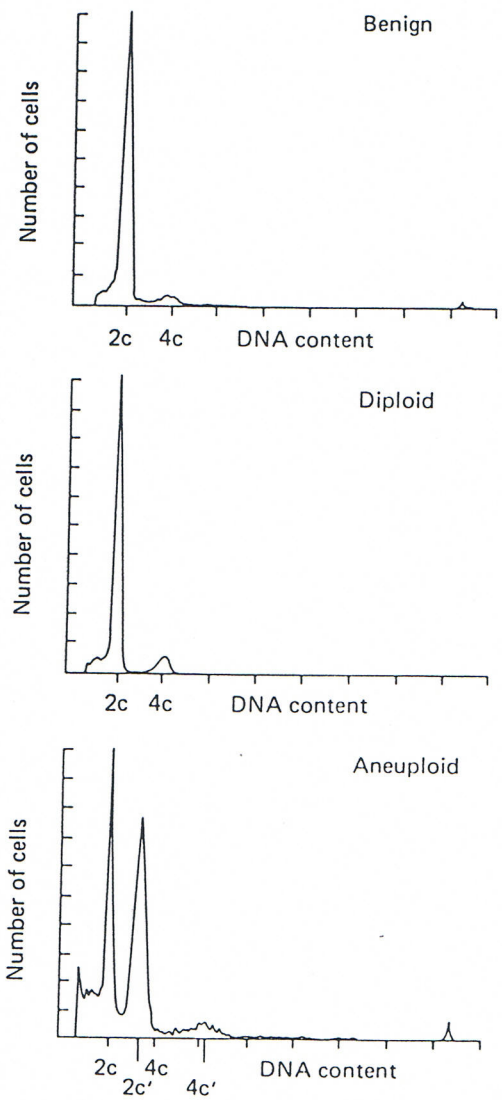

Figure 1 *Patterns of DNA content of prostatic cells measured by flow cytometry*

Malignant prostatic tumours. Two basic patterns were obtained from the cell suspension prepared from the carcinomas on the flow cytometer (*Figure 1*). The first type was very similar to the benign tissue patterns. These diploid tumours have a tumour cell DNA content in the same range as benign cells which contain a normal diploid chromosome complement. The second pattern was distinguished by the presence of additional peaks. These are produced by tumour cells with high values of DNA and are called aneuploid tumours (*Figure 1*). Those tumours with a second peak that was potentially ambiguous were classified as aneuploid if the second peak represented greater than 15 per cent of the cells counted or was shifted from the benign tetraploid value (4 C) by greater than 0·25 C. Of the aneuploid tumours, the position of the aneuploid peak (the DNA ploidy index) varied from 3·2 C to 4·0 C where the diploid value is given as 2 C. Of the 72 tumours studied 35 were classed as diploid and 37 aneuploid.

Correlation between stage and clinical outcome

Forty-three patients (60 per cent) had advanced disease at the time of diagnosis and of these 26 (60·4 per cent) died from their carcinoma 6 months to 12 years after diagnosis. Five patients died from unrelated causes and 12 patients are still alive 2 to 12 years post-diagnosis.

Twenty-nine patients had local disease (T_1M_0 or T_2M_0) at presentation of whom 10 died from their carcinoma (34·5 per cent), 7 from unrelated causes, and 12 are still alive 2 to 12 years later.

Correlation between Gleason score, ploidy and outcome

Thirty-three patients (46 per cent) had a Gleason score of 7 or greater with an average for all patients of 6·3 ± 1·9 (*Figure 2*). Aneuploid tumours had a higher Gleason score with a mean of 7·1 ± 1·7 compared to diploid tumours mean 5·8 ± 1·9, but this difference did not reach statistical significance (*Figure 3*). The tumours were therefore divided into 2 groups: a poorly differentiated group with Gleason score 7 or greater and a moderately differentiated group with Gleason score 6 and less.

From the life tables plotted it can be seen that the poorly differentiated tumours had a significantly worse prognosis compared to the moderately differentiated tumours ($\chi^2 = 16{\cdot}59$, $P < 0{\cdot}001$) (*Figure 4*). Similarly, the outcome for aneuploid tumours was significantly worse than for diploid tumours ($\chi^2 = 12{\cdot}98$, $P < 0{\cdot}001$) (*Figure 5*).

Combining these two variables, the Gleason score and the tumour ploidy gives four groups (*Table 1*). The average time to death in months from time of diagnosis is shown. Patients with poorly differentiated aneuploid tumours had an average of 22·4 months, much worse than patients with moderately

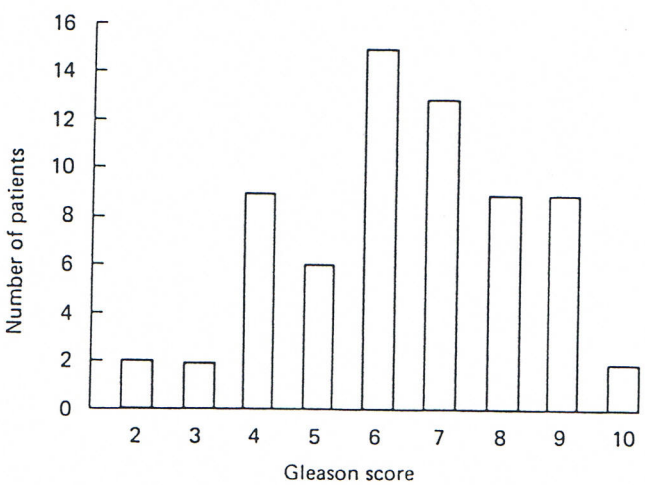

Figure 2 *Frequencies of Gleason scores for all tumours studied*

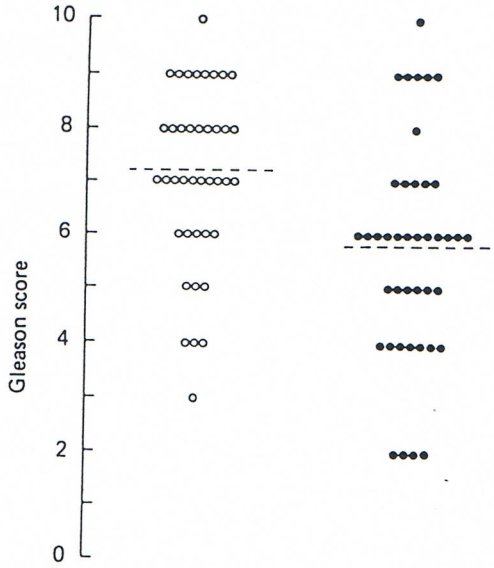

Figure 3 *Gleason scores for (○) aneuploid and (●) diploid tumours. Mean ± s.e.m.: aneuploid, 7·1 ± 1·7; diploid, 5·8 ± 1·9*

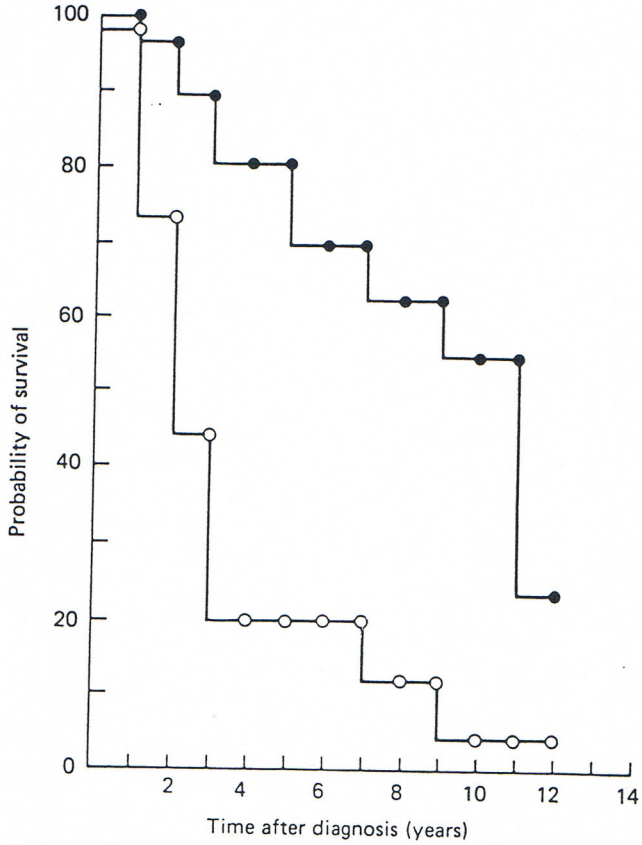

Figure 4 *Probability of survival for patients with (●) well differentiated (Gleason 2–6; n = 35) and (○) poorly differentiated (Gleason 7–10; n = 37), tumours. $\chi^2 = 16{\cdot}59$, P < 0·001*

differentiated diploid tumours, with an average of 104·5 months to death ($\chi^2 = 25{\cdot}54$, $P = 0{\cdot}000012$). Similarly, the 3 year survival figures for these groups (*Table 2*) show all the patients in the poor prognosis group had died from their cancers at 3 years compared to only 3 out of 17 patients in the best prognosis group.

Discussion

The clinical outcome of our patients is typical of carcinoma of the prostate in general. Patients who died from the disease did so after a time ranging from a few months to over a decade following their diagnosis irrespective of tumour grade or tumour stage. In patients whose tumours had no extracapsular spread, 35 per cent died from their disease 1 to 12 years after diagnosis. Those patients with extracapsular tumour spread had a similar range of years survival, some dying from cancer and others of unrelated cancers many years later. It is because of this problem that some reliable tumour marker is needed to predict which tumours are likely to result in the patient's rapid death and which are likely to run a relatively benign course.

Life table curves of tumours classified by the Gleason system show a clear difference between those with high grade poorly differentiated tumours that have a poor prognosis and the better prognosis, moderately differentiated tumours. Life table curves for the ploidy classification also show aneuploid tumours to have a significantly worse prognosis when compared to the diploid tumours. Although the aneuploid tumours tend to have a higher Gleason score compared to the diploid tumours it is possible to combine these largely independent markers to produce four groups from the original 72 patients. The poorly differentiated aneuploid group of patients were all dead from

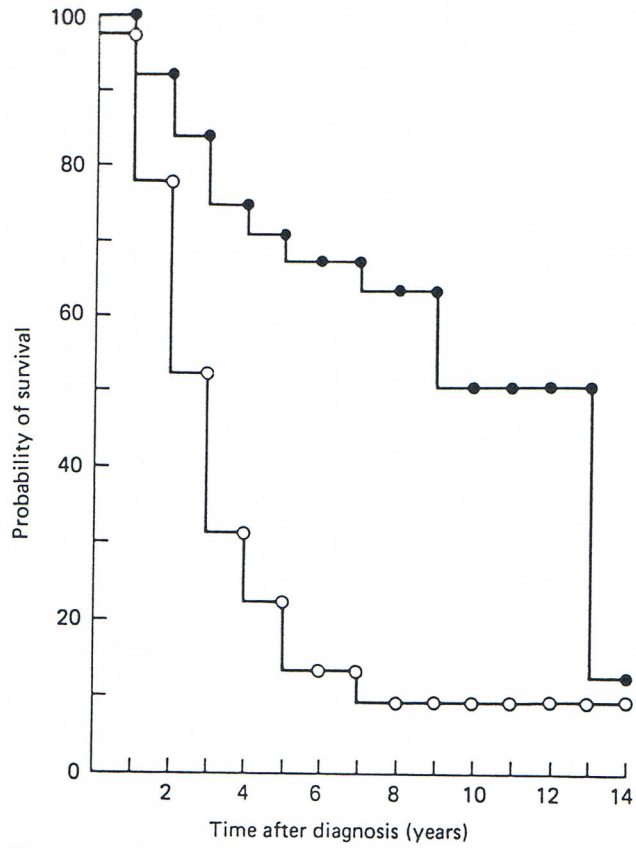

Figure 5 *Probability of survival for patients with (●) diploid (n = 35) and (○) aneuploid (n = 37) tumours. $\chi^2 = 12{\cdot}98$, P < 0·001*

Table 1 *Mean survival in months*

	Aneuploid	Diploid
Gleason 7–10	22·4 ± 10·1	55·2 ± 46·8
Gleason 2–6	68·0 ± 49·0	104·5 ± 46·8

Table 2 *Predicting deaths from cancer at 3 years (overall 60 per cent)*

	Aneuploid	Diploid
Gleason 7–10	100 per cent $n = 19$	60 per cent $n = 10$
Gleason 2–6	33 per cent $n = 6$	17 per cent $n = 18$

their cancers at 3 years with an average survival of 22·4 months, regardless of any treatment used, whereas in the moderately differentiated diploid group of patients only 3 patients had died from cancer by 3 years and the average life expectancy was over 8 years.

One of the problems in assessing how effective a treatment is for prostatic carcinoma is that the outcome of the untreated disease is unknown. Radical prostatectomy for local tumours that may have remained latent for a decade is an unnecessary treatment and assessing the success of such procedures is difficult, particularly in view of the known complications such as urinary incontinence and impotence[12]. Conversely, patients with an aggressive lesion with a high Gleason score and of the aneuploid type seem to die within a few years of diagnosis whatever the treatment. It may be that no effective treatment exists to combat this aggressive type of tumour. A policy of controlled clinical trials may be a more useful method of managing these tumours knowing their prognosis is so poor. Conversely, those tumours in the good prognosis group may best be treated by observation alone, reserving treatment for lesions when they become symptomatic.

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