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Cytological and DNA Characteristics of Hyperplasia/Inflammation and Cancer of the Prostate

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Abstract—*A study has been made of the correlation between objectively measured nuclear DNA content (by image analysis cytometry) and visual pathologic/cytologic diagnoses in hyperplasia/inflammation and well, moderately and poorly differentiated carcinomas of the prostate. The DNA measurement data were described by four statistical description methods—namely mean deviation from 2C, percentage > 4.25 C, DNA index and entropy. In* 36% (5/14) *of well differentiated and* 13% (1/8) *of moderately differentiated cancer it was not possible by objective measurement methods to demonstrate any difference between hyperplasia/inflammation and cancer. On the other hand one or more of the statistical descriptors was significantly different from hyperplasia/inflammation in* **all** *of those cancers visually diagnosed as poorly differentiated. The results underline the need for improvement of diagnostic criteria of prostate cancer—in particular well differentiated—in order to correctly understand the epidemiology (true incidence), survival and effects of treatment on the disease.*

INTRODUCTION

A LARGE NUMBER of presentations of DNA characteristics of human malignancies have now accumulated indicating a large variability of DNA patterns in human cancers, confirming results of earlier reports [1–3]. The earlier studies were done microspectrophotometrically [4] but the ease with which measurements can be done with flow cytometry (FCM) has greatly increased the number of publications in the field [5]. More recently, image analysis cytometry (IAC) on fine-needle biopsies has gained increasing popularity for DNA measurements since it was first described [6].

Malignancy grading of prostate cancer on fine-needle biopsies has shown large differences in prognosis between cancers of high and low degree of differentiation so that highly differentiated prostatic carcinomas seem to have approximately the same survival curves as the normal population [7]. DNA studies on prostate cancer [8] have confirmed the results of the above-mentioned studies on tumours in general. Attempts to grade malignancy based on different DNA histogram patterns have also been made. These studies have shown that irregular DNA patterns are correlated with higher malignancy grade [9–11] although it is still not clear how this knowledge can be applied in the individual case at the time of diagnosis.

The reproducibility of visual prostate cancer grading has been reported to be between 71 and 90% (intraobserver reproducibility) and 38–86% (interobserver reproducibility) [12–15]. These results are analogous with what has been reported for breast cancer grading [16]. According to these observations it is obvious that it is difficult to apply visual diagnostic criteria with absolute reproducibility and significance. This raised the following question: How strong is the correlation between the visual diagnosis of cancer of various degrees of differentiation and densitometric (DNA) criteria of cancer? Are these expensive and technically difficult measurements of DNA content going to add significantly to our diagnostic acumen in the management of prostatic cancer and contribute to our knowledge of which patients with this disease require treatment?

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MATERIAL AND METHODS

Fine-needle aspiration (FNA) biopsy procedure

FNA biopsy according to the Franzén procedure was employed [17]. The syringe holder is designed to fit disposable syringes and to be operated by one hand. The curved needle guide has a ring-like holder at the distal end for insertion of the index finger.

The adjustable plate will allow a firm fixation of the guide in the palm during the aspiration procedure. The needle is a 22-gauge 20 cm long, flexible, nondisposable needle. Gloves and finger cots are required as well. Local anaesthesia or bowel preparation was not used.

A careful digital examination of the prostate identified locations for biopsy. The needle guide was then mounted on the freshly gloved left index finger (for right-handed persons). The finger cot was used to cover the distal part of the guide. This prevented faecal material from entering the needle when introduced into the rectum. In addition the cot helps to keep the guide in position. The target was located with the index finger. The needle, attached to the syringe, was then inserted into the guide and the needle tip was pushed into the target. Full suction was applied and the needle was quickly moved back and forth several times with a rotating motion. The negative pressure was released with the needle tip still in the target. After withdrawal from the prostate, the needle was detached from the syringe and the aspirate was pressed out on slides. Smears were then prepared and air-dried or fixed in ethanol. One of the slides was fixed in 4% phosphate-buffered formaldehyde for at least 30 min. This slide was stained according to the Feulgen procedure.

FNA biopsy specimens were obtained from 11 well-established European and American centres in order to avoid bias of methodology; four slides per centre were analysed. These slides represented hyperplasia and/or inflammation and cancer of well, moderate and poor degree of differentiation according to cytopathological criteria.

Measuring instrumentation and procedure

The cells were measured through a 537 nm interference filter and the image was digitized with a CCD camera (Panasonic CCV, Model V 56/6) after shading correction. The measurement was done so that the cells selected by the segmentation procedure could be approved by the observer. The microscopic field of vision could be seen on a TV monitor and a decision to digitize the image could be made by the observer, who then could—on another monitor—observe the segmentation result and select or de-select objects of interest. This was programmed so that selection/de-selection of objects could be done by pointing at them with an electronic arrow on the display or at their respective position on a histogram over the objects density ('DNA') at the side of the display. The design considerations of this research system have been given elsewhere [18]. The system has been developed as part of a research project and is not commercially available.

Recorded features

In cases of cancer or non-cancerous diseases at least 20 control cells (granulocytes and lymphocytes) were measured. These cells were used in order to define the position of 2 C on the actual slide.

The second procedure consisted in the measurement of at least 200 cancer cells or other cells (on the slides from benign diseases) from each fine-needle biopsy as defined by an experienced cytopathologist.

The third procedure consisted in the production of statistics over the cancerous (or non-cancerous) cell population. The details of these statistical procedures are given in the Appendix of this paper.

RESULTS

Figure 1 is an illustration of the way the measurement data are presented to the observer on the display monitor. Figures 2–5 illustrate the relationship between the diagnosis and the statistical descriptors of the distribution of the measurement data of the tumour cell population as illustrated by the histograms in Fig. 1.

Mean deviation from 2 *C* (Fig. 2) in well differentiated cancer was in the same range as hyperplasias in 6/14 patients (29%). In moderately differentiated cancers the corresponding number was 1/8 (13%). In poorly differentiated cancers it was 0/13 (0%).

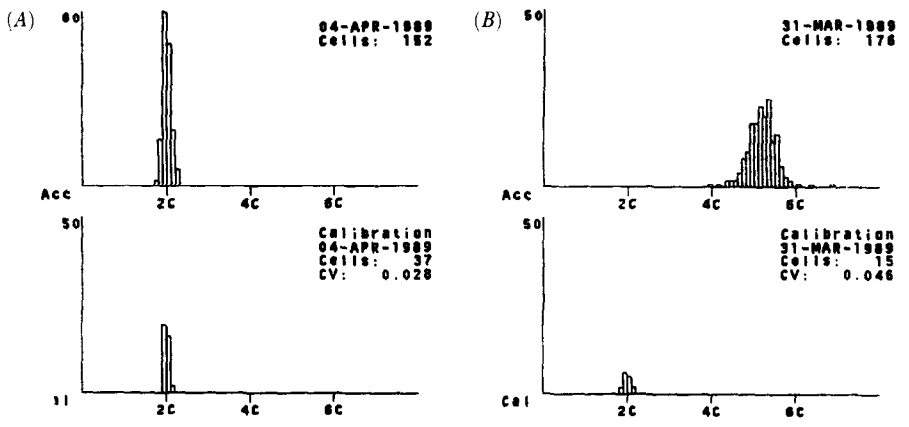
Per cent > 4.25 *C* (Fig. 3) in well differentiated cancer was in the same range as hyperplasias (0–2%) in 6/14 patients and in 2/8 moderately differentiated cancers. In poorly differentiated cancers the corresponding figure was 1/13.

DNA index (Fig. 4) in well differentiated cancer was in the same range as hyperplasias (1.0–1.2) in 9/14 patients and in 6/8 moderately differentiated cancers. In poorly differentiated prostate cancer it was in the same range as hyperplasias in 6/13 patients.

Entropy (Fig. 5) in well differentiated prostate cancer was in the same range as hyperplasias (204–306) in 7/14 patients and in 1/8 moderately and in none of the poorly differentiated prostate cancers.

In five of the well differentiated and one of the moderately differentiated prostate cancers it was not possible to demonstrate any deviation from the non-cancerous (hyperplasia/inflammation) range with respect to *all four* of the above-mentioned statistical variables, which describe the distribution of objective measurement data obtained by image analysis cytometry (IAC).

Table 1 is a correlation matrix that illustrates the intercorrelation between the variables used to objectively characterize the various cancers. As can be seen they are all intercorrelated and also



Mean deviation from 2C:	0,10	3,21
Percentage > 4.25 C:	0,0	98,9
DNA-Index:	1,00	2,63
Entropy:	2,04	3,73

Fig. 1. Illustration of how measurement results are presented after IAC (image analysis cytometry). A is from a patient with hyperplasia/inflammation. The lower histogram shows the distribution of control cells (granulocytes), which determine the position of 2 C (normal chromatin content). The upper histogram shows the distribution of the prostate cells in the specimen. The numbers at the bottom are the statistical variables extracted, which describe the distribution characteristics in mathematical terms. B illustrates the distribution of prostate cells in a patient with a cytopathological diagnosis of poorly differentiated prostate cancer.

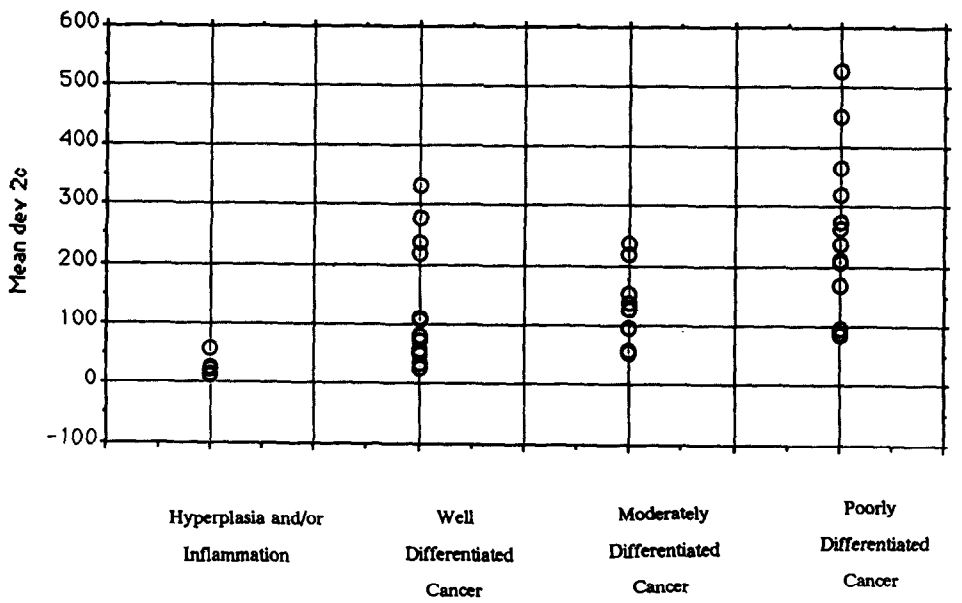


Fig. 2. Illustration of the distribution of 'mean deviation from 2 C' among patients with non-cancerous disease and patients with prostate cancer of various degrees of differentiation according to cytopathological criteria. In 6/14 patients with well and 1/8 with moderately and 0/13 with poorly differentiated cancer of the prostate the values were within the same range as non-cancerous disease.

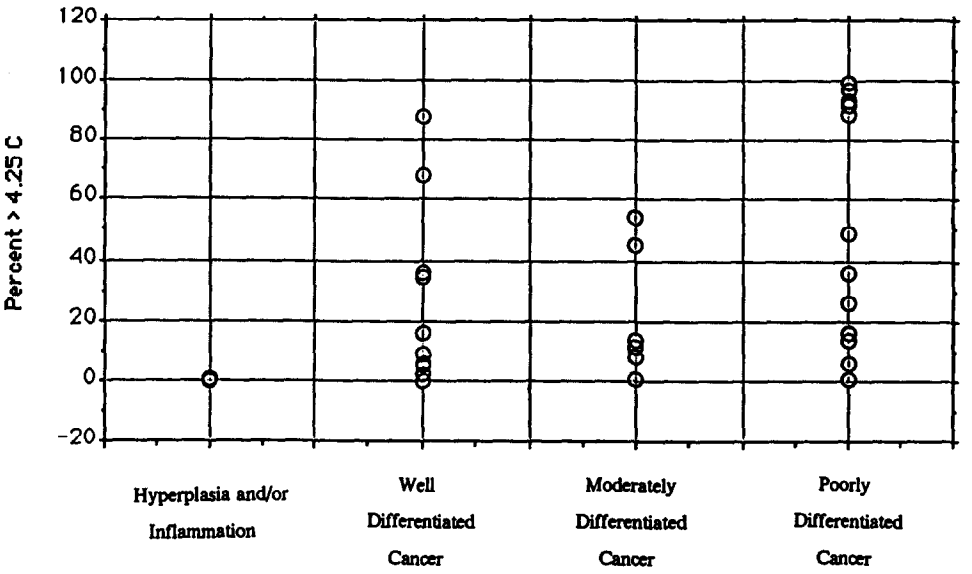


Fig. 3. Illustration of the distribution of 'percentage > 4.25 C'. In 6/14 patients with well, in 2/8 with moderately and 1/13 with poorly differentiated cancer the values were within the same range as in non-cancerous lesions.

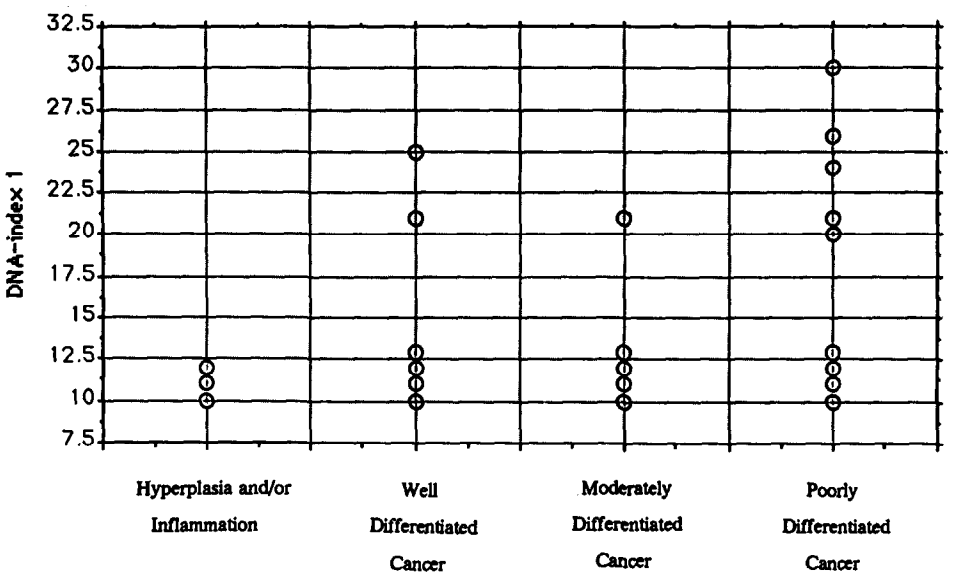


Fig. 4. Illustration of the distribution of 'DNA index' (× 10). This value—which was the least discriminatory of all descriptors—was within the same range as non-cancerous disease in 9/14 of well, 6/8 moderately and 6/13 poorly differentiated prostate cancers.

Table 1. Illustration of the intercorrelation between the analysed variables with inclusion of two 'dummies'—cytology record number and birth number. Also in this analysis entropy and diagnosis are most closely correlated with each other

	Birthnr	Cytnr	Mean de...	Percent ...	DNA-ind...	Entropy	Diagnosi...
Birthnr	1						
Cytnr	-.094	1					
Mean dev...	-.244	.202	1				
Percent >...	-.292	.281	.868	1			
DNA-inde...	-.185	.277	.849	.791	1		
Entropy	-.165	.157	.736	.634	.471	1	
Diagnosis...	-.089	-.069	.634	.509	.438	.671	1

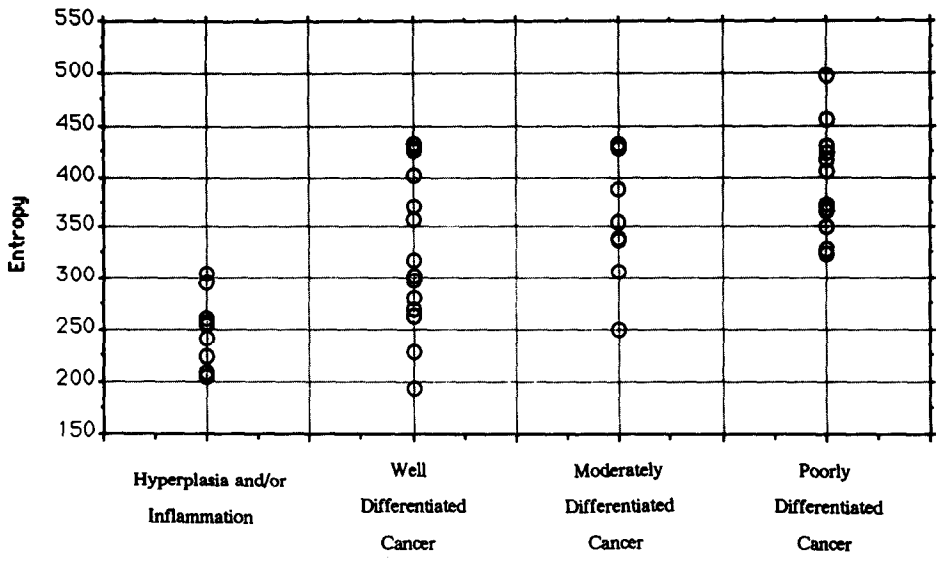


Fig. 5. Illustration of 'entropy' which was within the same range as non-cancerous lesions in 7/14 patients in well, 1/8 in moderately and 0/13 in poorly differentiated prostate cancers.

correlated with diagnosis—in particular entropy and diagnosis are correlated with each other. Birth number and cytology record number were used as 'dummies' in order to set the level of the statistical 'background noise'.

Figure 6 illustrates via an orthogonal plot after factor analysis (which we also have used previously in a study on breast cancer [16]) that entropy and diagnosis are closely correlated with each other.

DISCUSSION

Within its small volume, the prostate gland harbours cancer more frequently than any organ elsewhere in the human body. In addition it is also a common site of hyperplasia and has an unusually high incidence of inflammatory lesions. The prostate gland is probably one of the most frequently diseased internal organs. A high proportion of prostatic lesions are clinically silent—a clinical experience which is true not least for well differentiated prostatic carcinoma.

Well differentiated prostatic carcinoma is part of a vast reservoir of unsuspected disease which is revealed first at autopsy. This reservoir which begins to accumulate from age 50, consists not only of carcinomas but also foci of chronic prostatitis, hyperplasia nodules and a grey zone of so-called premalignant changes.

The present study was aimed at shedding some light on the grey zone from hyperplasia to well differentiated carcinoma. It is known that—although large efforts have been done overcome it—the diagnostic criteria of the pathologic/cytologic diagnosis of prostate cancer are extremely difficult

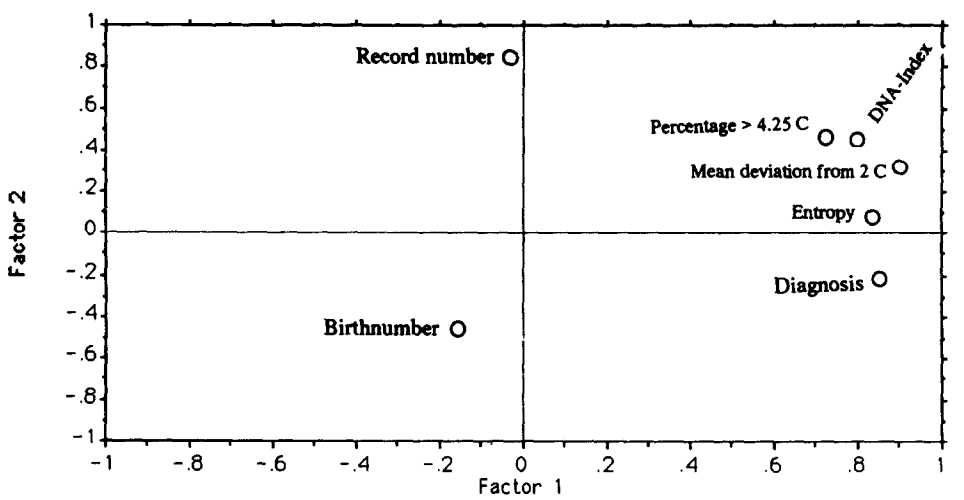


Fig. 6. Orthogonal plot after factor analysis. Entropy is situated closest to diagnosis of all the analysed variables. The 'dummy' variables—cytology record number and birth number—have a completely different strength and direction when compared with the others (eigenvalues > 1).

to apply reproducibly in the daily clinical routine. Over- or underdiagnosis of the disease will influence the incidence figures. Treatment results or decisions not to treat (in patients with well differentiated carcinoma) will be strongly influenced by the correctness of the diagnosis. Even incidence figures as high as 50% or more in elderly men have been reported based on histopathologic criteria only.

It would be a step in the right direction to find objective criteria, which could be used as supportive of the diagnosis in the grey zone from hyperplasia to well differentiated cancer. In the study presented here one can see that in six of the patients (five with well differentiated and one with moderately differentiated prostate cancer according to pathologic/cytologic diagnostic criteria) it was impossible to find any demonstrable difference with respect to statistics over variables describing densitometric features of the cell population (DNA distribution) after measurement of the cells with image analysis cytometry. Follow-up of the patients presented in this study with addition of a larger number of patients will reveal if there is a true survival difference between 'cancer' patients with normal DNA distributions when they are compared with patients not being normal when objective measurement methods are added to the conventional diagnostic procedure. We are now conducting such a study and hopefully this study will give some information about the need to support conventional pathology with image analysis cytometry to create better definitions of the disease subgroups. As an example—if there is a correlation between the objective criteria reported here and the clinical behaviour, 17% (6/35) of prostate cancer diagnoses today are due to overdiagnosis of the disease. This underlines the need for clarification.

Other measurable variables such as examination of nuclear protein content/nuclear size and/or presence of specific proliferation markers and oncogenes could possibly have a stronger correlation with the cytopathological diagnosis—a possibility, which we are going to explore further in the above-mentioned on-going study since it is possible that the DNA measurement procedure as it has been described in the present report, although the method distinguishes differences of 0.8–1.0 pg of DNA between cell nuclei, is too inexact to correctly identify the subtle properties of cells in the transition zone between normal and 'early' malignancy.

The present study has not indicated that these objective parameters are any better than routine histological/cytological examination in defining patients with prostate cancer who have a poor prognosis. The search must continue, however, for better objective indices in order that those patients whose cancers do need treating may be picked out at an early stage, i.e. when they have a small volume of disease, and clinicians have a better chance of curing them.

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APPENDIX, STATISTICAL PROCEDURES

Mean deviation from 2 C was calculated as previously described [9] and according to the formula:

$$SD = \sqrt{\frac{1}{N}\sum_{i-1}^{N}(c_i - 2c)^2}$$

where N = number of cells in the selected interval
c_i = DNA value for the ith cell
$2c$ = 2 C value.

Percentage of cells exceeding 4.25 *C*: Self explanatory.

The DNA index was calculated according to the formula:

$$DI = \frac{\frac{1}{N}\sum_{i=1}^{N} c_i}{2c}$$

where N = number of cells in the selected interval
c_i = DNA value for the ith cell
$2c$ = 2 C value.

Depending on if there were one or two discernible peaks in the histogram, one or two DNA indexes were calculated according to the above formula.

Entropy [18], which is a concept from mathematical information theory, was calculated according to the formula:

$$\text{Entropy} = -\sum_{i=1}^{N} pi \times \log 2(pi).$$

We have previously used this mathematical method to describe irregularities of chromatin distribution [6] in individual cell nuclei. The formula simply describes the informational content in the histogram so that it attains the value 0 if all measurement values of density (DNA in the present situation) become located in exactly the same channel or log 2 (80) = 6.33 if the measurement values were evenly distributed over all 80 (n = 80) channels, which is the number in the situation presented here.