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DNA cytophotometry in malignant thyroid tumors – use of different evaluation schemes for prognostic statements

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Summary. Quantitative evaluation of nuclear DNA has been found to provide information on diagnosis and prognosis in a number of malignant tumours and borderline lesions. Using 53 carcinomas of the thyroid with varying differentiation we examined the prognostic information obtained by DNA cytophotometry with respect to clinical outcome, applying three different evaluation schemes. DNA cytophotometry allowed the discrimination of euploid carcinomas with good prognosis from aneuploid tumours with bad prognosis and a generally shortened life span. However, the encapsulated variants of follicular and papillary thyroid carcinomas, with their generally excellent prognosis, exhibited DNA histograms similar to those of their widely invasive counterparts. Thus, the favourable prognosis of these minimal invasive subtypes may primarily be related to the tumours' encapsulation and cannot be ascribed to a particular DNA content. We conclude that DNA cytophotometry can provide additional prognostic information for the individual patient suffering from thyroid carcinoma and may probably lead to an individualization of the therapeutic strategies.

Key words: Thyroid carcinoma – DNA-cytophotometry – Prognosis

Introduction

The prognostic significance of DNA measurements has been demonstrated in many malignant tumour entities, including carcinomas of the breast (Auer et al. 1980, 1984; Mellin et al. 1985; Mikuz 1985; Feichter et al. 1985; Erhardt and Auer 1986a, b), ovary (Friedlander et al. 1983, 1984; Erhardt et al. 1984), colon (Forsslund et al. 1984), endometrium (Moberger et al. 1984), and others (Zetterberg and Auer 1984; Ljungberg et al. 1986; Matsuura et al. 1986; Franzén et al. 1986). The correlations found with different schemes of classification have demonstrated a progressive deterioration of prognosis for tumours with increasing percentages of aneuploid cells, while carcinomas with an euploid DNA distribution generally follow a benign course. Thyroid tissue under various pathological conditions has also been investigated according for its DNA-content and several authors have stated the value of quantitative DNA determinations in providing additional information on the prognosis of thyroid lesions (Cohn et al. 1984; Auer et al. 1985; Bengtsson et al. 1984; Greenbaum et al. 1985; Bondeson et al. 1986). As in malignant tumours of different origin, euploidy indicates a favourable prognosis while aneuploid DNA patterns coincide with relatively short survival. The diagnostic value of DNA cytophotometry in discriminating benign from malignant thyroid lesions, however, has been interpreted in a number of ways: while some authors deny the diagnostic significance of DNA measurements in thyroid lesions (Haemmerli 1970; Auer et al. 1985; Bondeson et al. 1986) others found them helpful in demonstrating malignancy (Bengtsson et al. 1984; Greenbaum et al. 1985). The latter has been demonstrated for ovarian carcinomas of questionable behaviour, the so called borderline tumours of the ovary (Sachs et al. 1974; Bodecker et al. 1984; Dietel et al. 1986a). These can be subdivided on the basis of cytophotometric data into clearly malignant tumours with aneuploid DNA distribution patterns and correspondingly bad prognosis, and in euploid lesions with favourable outcome (Dietel et al. 1986b). We therefore investigated the DNA content of various histological

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types of thyroid carcinomas and correlated the results to the clinical outcome of disease, in order to evaluate the diagnostic and prognostic relevance of this technique. Special attention was paid to the subgroup of encapsulated thyroid carcinomas, which generally follow a benign course of disease (Schröder et al. 1984a, b). Using this material we examined the applicability of several interpretation schemes for DNA cytophotometry; typing of the histogram according to Auer (Auer et al. 1980), determination of the P90 value (Forsslund et al. 1984), and calculating the MG-index according to Böcking (Böcking et al. 1984).

Material and methods

Fifty-three thyroid carcinomas of different types were taken from the surgery pathology files of the Institute of Pathology, University of Hamburg, for determination of DNA-content (Table 1). The histology was reevaluated prior to cytophotometrical analysis.

Follow-up information was available for all patients for up to 17 years (median 4.4 years).

For cytophotometric determination of DNA content 4 µm sections of routinely paraffin embedded tissues were used. After dewaxing, RNA was destroyed by hydrolysis in 1N hydrochloric acid at 60° C for 15 min. Afterwards, Schiff's reagent was applied for 1 h and the sections were subsequently treated with 1% sodium metabisulphite. Coverslips were mounted with Eukitt (refraction index 1.494). Single cell DNA-measurement was performed in the scanning mode on a LEITZ-MPV-cytophotometer based on a LEITZ-ORTHOPLAN microscope. The measuring spot was 2.54 µm², the steps of the scanning process were 0.5 µm wide. Absorption of the probes was determined at a wave length of 560.0±9.5 nm. The number of cells examined was 50 to 100 for each case. Data were processed on-line by a EUROCOS-computer using commercial software (Leitz, Hamburg), specially adopted by one of us (H.A.). Determination of diploid DNA values was performed using fibrocytes of adjacent non-tumourous tissues; the mean ±2 standard deviations of their cellular DNA content was defined as 2c region.

Evaluation of the data was done using three different schemes:

(I) Classifying the histograms according to Auer (Auer et al. 1980) in four different types. Type I with a single peak of diploid DNA values and type II with a diploid peak and/or a tetraploid peak are summarized as euploid DNA distributions. Type III with a sizable amount of cells with DNA values between 2c and 4c representing proliferating cells and type IV demonstrating a broad DNA distribution ranging from 2c to beyond 8c are classified as aneuploid.

(II) Determining the P90-values (Forsslund et al. 1984). This is the percentage of tumour cells with DNA values higher than an empirically determined amount not exceeded by 90% of the control cells.

(III) Calculating MG (Böcking et al. 1984); the MG determines the percentage of clearly hypertetraploid (in this case cells with DNA values greater than 5c) cells (5cER) and the variation of DNA-distribution within the tumour (2cDI). The product of these two parameters is normalized to a range of 0 to 3, this being comparable to the generally applied grading of malignancies.

Statistical evaluations were done using the Chi-square-test,

Table 1. Clinical data, histological diagnosis, and DNA-cytophotometric evaluation of 53 cases of thyroid carcinomas

Age/sex	Diagnosis[a]	Follow-up[b]	Type[c]	P90[c]	MG[c]
70/m	AC	0.1+	IV	100	1.14
70/m	MC	0.2+	III	71	0.00
74/w	AC	0.2+	IV	100	2.56
76/w	AC	0.2+	IV	83	1.34
55/w	AC	0.2+	IV	96	1.97
58/m	AC	0.2+	IV	100	2.53
58/m	WIPC	0.4+	III	57	0.00
67/w	AC	0.5+	IV	97	2.45
61/m	WIFC	0.8+	III	60	0.00
75/m	AC	0.8+	IV	94	1.99
85/m	AC	1.0+	IV	94	1.15
61/m	MC	2.0+	IV	57	1.52
55/w	WIPC	2.5+	IV	97	1.28
26/w	WIFC	3.0+	III	60	0.29
38/m	MC	3.4+	III	83	0.34
46/w	WIPC	3.5+	III	77	0.00
58/m	WIPC	4.2+	IV	100	1.76
42/w	MC	5.0+	III	66	0.64
56/m	MC	5.3+	III	94	0.52
54/m	MC	9.0+	IV	93	0.53
71/w	WIFC	12.4+	III	70	0.00
78/w	WIPC	2.4 R	III	27	0.00
72/w	MC	3.0 R	II	56	0.00
42/m	WIPC	3.2 R	II	31	0.00
80/w	WIPC	4.0 R	III	64	0.45
59/w	WIFC	5.0 R	I	61	0.00
55/m	WIPC	6.0 R	III	64	0.22
31/w	EFC	7.0 R	III	91	1.07
58/w	WIFC	7.0 R	III	67	0.00
67/w	WIFC	7.0 R	IV	100	0.98
60/w	WIFC	9.0 R	III	76	0.42
48/m	MC	10.0 R	IV	100	0.95
16/w	EPC	2.2	III	91	1.16
69/w	WIFC	4.0	I	57	0.00
31/m	EPC	4.0	II	24	0.00
63/w	OPC	4.0	III	60	0.00
22/w	OPC	4.0	III	40	0.00
57/m	MC	4.2	III	54	0.00
22/w	EFC	4.9	III	100	1.20
40/m	OPC	5.0	II	25	0.00
61/w	EFC	5.5	III	100	1.04
57/m	OPC	6.0	II	15	0.00
45/w	EFC	6.0	III	80	0.30
71/w	WIFC	6.5	II	36	0.44
28/w	OPC	7.0	II	20	0.00
51/w	EFC	7.0	III	99	1.24
36/w	MC	7.2	II	60	0.63
38/w	EFC	7.8	II	74	0.41
21/m	EPC	9.0	I	14	0.00
83/m	WIFC	9.7	III	76	0.00
52/w	MC	11.8	IV	84	1.45
66/w	EPC	13.0	III	40	0.00
44/w	WIPC	17.0	III	49	0.57

[a] AC = anaplastic carcinoma; MC = medullary carcinoma; WIFC = follicular carcinoma, widely invasive; WIPC = papillary carcinoma, widely invasive; EFC = follicular carcinoma, encapsulated; EPC = papillary carcinoma, encapsulated; OPC = papillary carconima, occult

[b] + = died from thyroid carcinoma, R = recurrent disease

[c] details see Material and methods

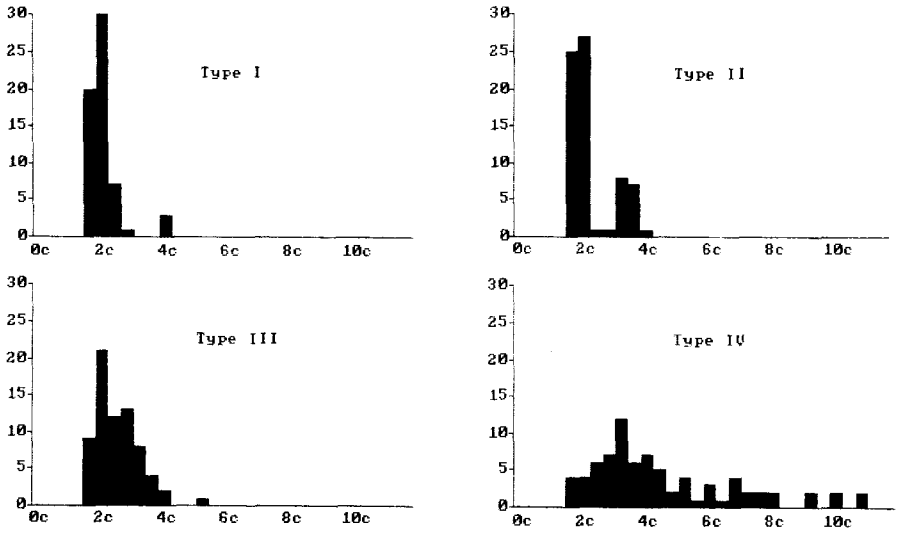

Fig. 1. Representative histograms of thyroid carcinomas demonstrating the four different DNA distribution patterns according to Auer

survival curves were calculated using the life-table method of Cuttler and Ederer (1958).

Results

Clinical outcome and results of the DNA-measurements are summarized in Table 1. Examples of resulting histograms are given in Fig. 1.

77% of the thyroid carcinomas exhibited aneuploid DNA distribution patterns according to the typing of histograms. 21 (51%) of these have died from the tumour within 12.4 years (median 1.0), additional 8 (20%) suffer from recurrent disease. Only 12 patients with aneuploid carcinomas are alive and free of disease; among these are 6 patients with encapsulated carcinomas. None of the 12 malignant thyroid tumours with euploid DNA-distribution resulted in fatal outcome; 75% of these patients are free of disease for at least 4 years.

Encapsulated follicular (EFC) and papillary (EPC) carcinomas, despite their excellent prognosis when compared with their widely invasive counterparts (WIFC, WIPC), did not show euploid DNA values to a greater extend than their widely invasive counterparts. The respective numbers of euploid/aneuploid cases are: WIFC 2/6, EFC 1/5; WIPC 1/8, EPC 2/2.

Statistical evaluation with exclusion of the encapsulated carcinomas revealed a significantly impaired prognosis ($p \ll 0.01$) of euploid thyroid carcinomas as compared to aneuploid tumours. Both other evaluation schemes resulted in a comparably evident discrimination of tumours with good prognosis from tumours with fatal outcome (Table 2). The respective cut-off levels for worsening of prognosis were determined as 70% (P90) and 1.0 (MG). Each of the evaluation schemes discriminated well between a group of patients with good prognosis vs. another group with relatively bad prognosis. The respective graphs are given in Fig. 2.

Table 2. Statistical analysis of three different evaluation schemes of quantitative DNA determination

Evaluation			Chi-Square-Test
Type	I/II	vs. III/IV	$p \leq 0.0005$
P90	<70	vs. ≥ 70	$p \leq 0.005$
Böcking-Index	< 1.00	vs. ≥ 1.00	$p \leq 0.0005$

Discussion

Quantitative cytophotometric determination of nuclear DNA has been recommended for determination of cell behaviour from cytological smears of a number of organs (Böcking et al. 1984). Furthermore, in cases of questionable behaviour as determined by histology alone, the so called borderline cases, DNA cytophotometry can provide substantial diagnostic information and thus help to plan therapy (Sachs et al. 1974; Erhardt et al. 1985; Dietel et al. 1986b). The use of quantification of DNA for diagnostic decisions in cases of thyroid tumours is less certain. The data presented in this study demonstrate that quantitative values of nuclear DNA in thyroid malignancies of different histological types cover a wide range from euploidy to aneuploidy. Since discrimination between an euploid benign and an euploid malignant lesion is impossible, additional diagnostic information is only gained in cases where the presence of aneup-

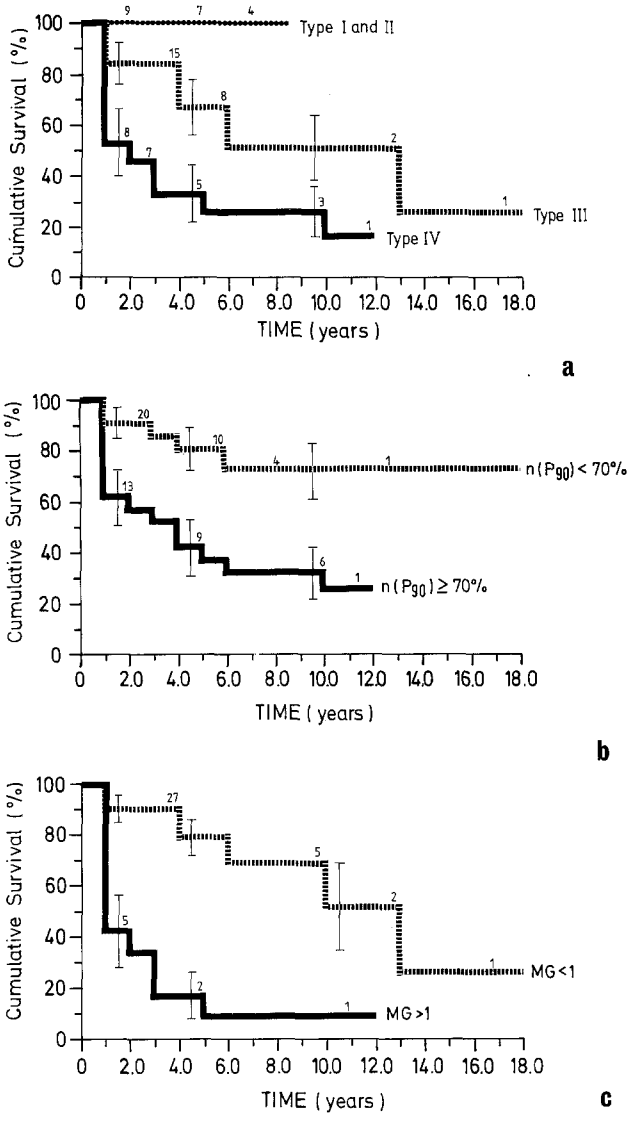

Fig. 2. Survival curves according to Cuttler and Ederer in relation to different evaluation schemes, demonstrating a discrimination in survival probability between euploid and aneuploid carcinoma types: (**a**) classification according to Auer, (**b**) determining P90 values, (**c**) calculating MG

loid cells allows the definitive diagnosis of malignancy.

However, prognosis has been demonstrated to be related to the ploidy of a given carcinoma in various organs (Auer et al. 1980; Caspersson et al. 1983; Zetterberg and Auer 1984; Ljungberg et al. 1986) including the thyroid (Cohn et al. 1984; Greenbaum et al. 1985). The results of the present study underline that careful analysis of nuclear DNA distribution can provide additional information concerning prognosis of the disease, since a strict correlation exist between degree of aneuploidy and risk of fatal outcome. The three different evaluation schemes used provided equivalent information with respect to the clinical course. Although the typing of histograms according to Auer is performed with a certain subjectivity in the evaluation, this method seems to be adequate for application to clinical routine. Determination of the P90 value or MG are somewhat more elaborate and can be used when mathematically exact values are necessary. The MG, however, revealed a certain limitation, since it is necessary to determine the percentage of cells exceeding 5c DNA values (5cER). Some highly malignant tumours with unfavorable outcome exhibited an aneuploid DNA distribution but were lacking cells which DNA exceeded 5c (type III histograms according to Auer). Hence, a MG of 0.00 was calculated, erroneously indicating a favourable prognosis. Since the MG is also used for diagnostic purposes these cases would have been classified as benign or suspect from their 2cDI (Böcking et al. 1984), but not as malignant.

A remarkable result of the quantitative DNA determination was the identity of the DNA distribution in encapsulated and widely invasive carcinomas of follicular and papillary type. The group of encapsulated carcinomas exhibited the same proportion of cases with highly aneuploid DNA distribution patterns as the widely invasive types. The extremely good prognosis of the encapsulated subtypes (Schröder et al. 1984a, b) seems to be dependent primarily on encapsulation and is obviously not a consequence of a less aggressive potential of the individual malignant cell. Consequently, DNA cytophotometry does not add further information concerning prognosis when encapsulation has been demonstrated histologically.

Our results confirm the validity of DNA determination in widely invasive thyroid carcinomas for an estimation of their prognosis. Discrimination between benign and malignant thyroid lesions is not possible on the basis of DNA quantification alone. The excellent prognosis of malignant thyroid tumours with type I and II histograms, however, should lead to conservative surgical therapy. As already suggested by Cohn et al. (1984a, b), preoperative determination of the degree of aneuploidy, performable using cytological smears from fine needle aspirates or punch biopsies, could possibly be used to restrict surgery to a hemithyroidectomy in cases with good prognosis.

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