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CELLULAR DNA PATTERN, S-PHASE FREQUENCY AND SURVIVAL IN PAPILLARY THYROID CANCER

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Abstract

A series of 150 patients with papillary thyroid cancer diagnosed in Iceland during the 30-year period from 1955 through 1984 was retrospectively analyzed. Flow cytometric analysis of archival paraffin-embedded material was used to study the prognostic significance of cellular DNA content and s-phase frequency. DNA-aneuploidy was found in 12% of the tumors. It was significantly more common in the elderly, in moderately and poorly differentiated carcinomas, in males and in tumors with a high proportion of s-phase cells. Multivariate analysis using stepwise Cox's model showed aneuploidy, age at diagnosis, lymph node metastasis and tumor extension beyond the thyroid capsule as independent prognostic factors. The frequency of cells in s-phase was generally low (mean 2.7%). Patients with high s-phase frequency (>2.5%) had less favorable prognosis than patients with low values (≤2.5%).

Key words: Thyroid, neoplasms; papillary carcinoma, prognostic factors, DNA pattern, s-phase frequency.

Compared to most other malignant neoplasms, papillary thyroid cancer has a relatively favorable prognosis with 10-year survival rates varying in different series from 60 to 95% (4). A number of factors have been suggested to be of prognostic importance, including age, gross extent of disease, sex and histologic grade (2, 11, 14, 18). In the individual patient, however, the prognosis of papillary thyroid cancer is difficult to predict from clinical and microscopic analysis alone and additional prognostic indicators are needed. Aneuploidy is a well recognized feature of human tumors and may be correlated to the biologic behavior of a variety of malignancies. Recent studies of thyroid cancer (4, 10) have indicated that DNA aneuploidy is inversely correlated to the prognosis. In some other types of cancer, including breast cancer, the proliferative index (proportion of s-phase cells) has been shown to be associated with the prognosis as well (15).

Material and Methods

From 1955 to 1984 about 300 cases of papillary thyroid cancer were diagnosed in Iceland. Among these 150 tumors with adequate material for microscopic analysis were selected for the present investigation. Four cases were excluded from the statistical analysis for technical reasons. The selection was not strictly random but there is no special reason to believe that biases were introduced by omitting approximately half of the material.

The patients had been treated in different hospitals in Iceland during this period. The follow-up period ended in December 1985. Forty-two patients were male and 104 were female. Mean age at diagnosis was 51 years (range 15–88 years). The follow-up ranged from one to 30 years (mean 9.0 years). Forty-three patients (29%) died during the observation period. Twenty-six patients died of thyroid cancer and 17 from other causes (7 with and 10 without signs of recurrence) and were withdrawn from the survival analysis at the date of death. These data were based on clinical and autopsy evidence.

All cases were reclassified by the same pathologist (SE) according to the WHO criteria (6); 100 were considered to be well differentiated, 45 moderately and 1 poorly differentiated.

According to the UICC (1978) postsurgical classification (8), 2 cases were classified as pT0, 23 as pT1, 55 as pT2, 36 as pT3 and 30 as pT4. In 9 cases diagnosed by surgical biopsy, the local extent was estimated from clinical findings. Forty-two patients had regional lymph node metastases and 6 patients had distant metastasis at the time of diagnosis.

Of the 146 patients analyzed, 137 underwent operation: 39 had total thyroidectomy, 40 subtotal thyroidectomy, 46

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hemithyroidectomy and 12 tumor extirpation only. Thirty-three of these patients received postoperative radioiodine for ablation of the thyroid remnant and 13 patients received external radiotherapy postoperatively. Nine patients were inoperable and in these patients only biopsy was performed. Some of these patients were treated with radioiodine and/or external irradiation. Continuous thyroxine treatment was given to most patients.

Cells from paraffin-embedded tissue from these patients were investigated with flow cytometry. To obtain an adequate number of cells for analysis, the size of the tumors had to be at least 3–4 mm in diameter. The paraffin blocks were prepared as described by Schutte et al. (16) and stained with 0.13 mg/ml propidium iodide according to Vindelöv et al. (20). Analyses were performed as described earlier (7) using a Leitz MPV flow (Leitz, Welzlar, FRG) and a Monroe OC8888 microcomputer.

The coefficient of variation of the stem-line peaks and the proportion of cells in s-phase were calculated as described by Stål et al. (17). No correction was made for baseline noise (nuclear fragments) as it was insignificant in most histograms.

An average of 22500 cells was measured (range 17000–59000). The mean coefficient of variation was 4.8 (range 2.6–9.8). In cases with stem-line peaks lying close to each other (DNA index 1.1), the coefficient of variation was not calculated (3 cases). Twenty patients had coefficients of variation >6.0 and <10. Two thirds of these cases were diploid. Histograms with more than 20% G2/M phase cells were considered to have an aneuploid DNA stem-line in the tetraploid region (one case). Estimation of the DNA index was calculated by using the peak of the lowest DNA value as an internal reference. The DNA-histograms had to have 2 peaks of G1 to be considered aneuploid. About 50% of the measurements were repeated to optimize the analysis.

Statistical methods. Survival rate, corrected for intercurrent death, was estimated by the actuarial method. Differences in survival rate between groups were tested by generalized Wilcoxon statistics. The χ^2 test was used with contingency tables. The relative importance of prognostic factors was analyzed using Cox's proportional hazard model. Bivariate correlation between covariates was evaluated by Pearson's correlation coefficient. A p-value less than 5% is referred to as significant.

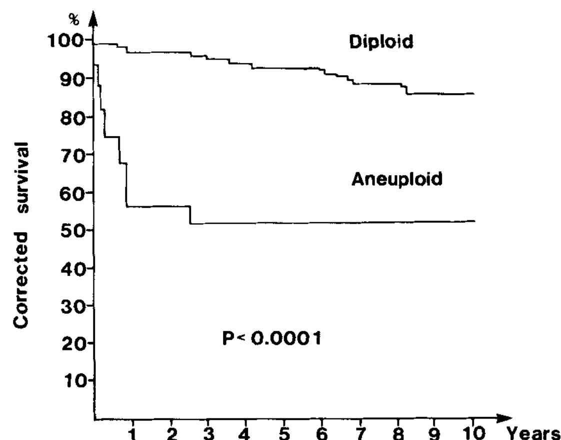

Fig. 1. Survival corrected for causes of death other than thyroid cancer in 129 diploid and 17 aneuploid papillary thyroid cancers.

Table 1

Occurrence of DNA aneuploidy according to age, nodal status, size of primary tumor, sex, s-phase frequency and histologic grade

Variables	Total	Aneuploid		p-value
	No.	No.	%	χ^2
Age				
≤50	75	3	4	
51–70	44	7	16	
>70	27	7	26	p<0.01
Nodal status				
pN0	104	9	9	
pN 1–3	42	8	19	NS
Primary tumor				
pT0–3	116	11	9	
pT4	30	6	20	NS
Sex				
Male	42	10	24	
Female	104	7	7	p<0.005
S-phase				
≤2.5%	87	2	2	
>2.5%	59	15	25	p<0.001
Histologic grade				
G1	100	5	5	
G2 and G3	46	12	26	p<0.001

Results

Of 146 tumors, 17 were aneuploid (12%). The mean DNA index for these tumors was 1.45 (range 1.1–3.0).

Table 1 shows the occurrence of DNA aneuploidy in relation to other possible prognostic parameters. Aneuploidy was more common in males (p<0.005) and in the elderly (p<0.01). It was more common in tumors with a growth beyond the thyroid capsule and in tumors with a lymph node metastasis but these latter differences were not significant. Also, aneuploidy was more common in tumors with a high s-phase frequency and a high histologic malignancy grade.

The mean s-phase frequency was 2.7% (min 0.6, max 9.5, SD 1.6). The extreme value of 9.5% was found in a patient with an aneuploid tumor, histologic grade 3 who died of tumor one month after diagnosis. The patients were divided into 2 groups for statistical analysis: s-phase frequence 2.5% or less (n=87) and more than 2.5% (n=59).

Pair-wise correlation was calculated between all the possible negative prognostic factors (Table 2). Several

Table 2

Pair-wise correlation between prognostic factors of papillary thyroid carcinoma

	Age	S-phase (>2.5%)	DNA (aneupl.)	pT4	Grades 2 & 3	Male sex	Lymph node met.
Age	–						
S-phase (>2.5%)	0.27	–					
DNA (aneupl.)	0.27	0.39	–				
pT4	0.51	0.13	0.13	–			
Grades 2 & 3	0.31	0.15	0.29	0.18	–		
Male sex	0.18	0.27	0.24	−0.05	−0.10	–	
Lymph node met.	0.27	0.28	0.15	−0.24	−0.04	0.27	–

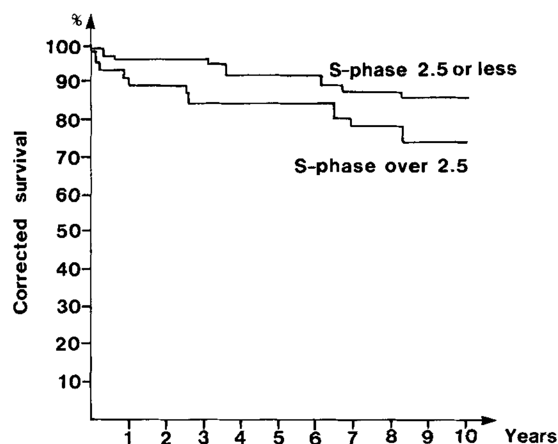

Fig. 2. Survival corrected for causes of death other than thyroid cancer in 87 patients with s-phase frequency of 2.5% or less and 59 patients with s-phase frequency over 2.5%.

high correlations were found, such as between age and tumor invasion beyond the thyroid capsule and between aneuploidy and s-phase frequency as mentioned above.

The crude survival rate at 5 years was 83% and at 10 years 71%. Survival curves, corrected for intercurrent death for diploid and aneuploid tumors and high and low s-phase frequency tumors, are shown in Figs 1 and 2 respectively. DNA aneuploidy was strongly associated with a shorter survival.

The variables tested in Cox's proportional hazard model are shown in Table 3 (age at diagnosis, lymph node status, primary tumor extent (pT), ploidy, s-phase, sex and histologic grade). The number of patients dead of thyroid cancer and the number of patients censored (alive or dead from intercurrent disease) are shown.

In order to study the relative importance and independency of DNA aneuploidy and s-phase frequency as prognostic factors these variables were compared to the others listed in Table 3 using the Cox's proportional hazard model. The results are shown in Table 4. The DNA content was independently associated with survival but the s-phase frequency was not. Age was the strongest independent prognostic factor, followed by lymph node status, aneuploidy and tumor extension beyond the thyroid capsule. Sex and histologic grade were significant prognostic factors only when considered alone.

Discussion

In a recent paper (4) it was suggested that the DNA pattern might predict with some accuracy which patients will succumb to their papillary thyroid cancer.

In our series, 18% of the patients died of thyroid cancer. In 12% of the cases an aneuploid DNA stem-line was found and 47% of these died of thyroid cancer during the observation period. During the same period 17% of the patients with diploid tumors died of tumor. Joensuu et al. (10) have recently reported aneuploidy in 24% of 82 papillary thyroid cancers and they also found higher survival rate in diploid tumors. In another report (12) only 5% of the papillary cancers were aneuploid. Based on these results it seems obvious that the prognostic information obtained by the DNA pattern is only relative.

Some authors, using single cell cytophotometry have reported a clear difference in DNA-histograms between benign and malignant thyroid lesions and suggested that this could have a diagnostic value (1, 5). We have not been able to confirm these result (7). It seems obvious from our experience, that the diagnostic value of the DNA pattern in papillary thyroid cancer is limited, as 88% of the papillary cancers had diploid DNA content indistinguishable from normal thyroid cells. Similar observations have been reported by other authors (9, 13). DNA determination in thyroid cancer provides valuable prognostic, but little diagnostic, information.

The proportion of cells in the proliferative phase (s-phase) was generally low in our series and much lower than observed in many other tumor types. This has been reported earlier by Johannessen et al. (12) and they have even noted a lower proliferative fraction in some papillary carcinomas than in normal thyroid tissue, a finding that we confirmed. The low s-phase frequency explains why most papillary thyroid carcinomas possess such a low degree of clinical malignancy and growth rate. In an earlier report from our laboratory, DNA stem-line ploidy and

Table 3

Variables analyzed in Cox's step-wise proportional hazard model

	Total	No. censored		No. dead	p-value (Wilcoxon-test)
		Living	Intercurrent death		
Ag					
	75	72	1	2	
	44	25	5	14	<0.0001
	27	6	11	10	
us					
	104	84	12	8	
pN 1–3	42	19	5	18	<0.0001
DNA					
Diploid	129	98	13	18	
Aneuploid	17	5	4	8	<0.0001
Primary tumor					
pT0–3	116	94	8	14	
pT4	30	9	9	12	<0.0001
Sex					
Female	104	84	7	13	
Male	42	19	10	13	<0.006
S-phase					
≤2.5%	87	68	7	12	
>2.5%	59	35	10	14	<0.07
Histologic grade					
G1	100	80	7	13	
G2 & 3	46	23	10	13	<0.01

s-phase frequency were analyzed with 3 different methods, using fresh tissue as a standard (7). This showed a good correlation between different methods of ploidy determination. The s-phase frequency was generally low but the agreement between estimates on fresh and paraffin-embedded material was poor. Probably, current methods of estimations of low s-phase frequencies in archival material are not sufficiently reliable.

A few prognostic factors in papillary thyroid cancer have been established as independently associated with survival including age at diagnosis and invasion beyond thyroid capsule (4, 9, 18, 19). Joensuu et al. (10) found DNA ploidy associated with survival when considered alone, but not in Cox's stepwise analysis. In our series, DNA ploidy was independently associated with survival and thus had a strong prognostic significance. This difference may be explained by our greater number of patients and longer follow-up.

Not surprisingly, the extent of tumor as characterized by pTNM classification correlates to prognosis. Tumor invasion beyond the thyroid capsule has proved to be a significant unfavorable prognostic factor in almost all multivariate studies. Nodal involvement was inversely related to survival in our series as in some previously reported studies (14, 19). However, in several other series no such correlation could be found (3, 10, 18).

Our findings are similar to those reported by Joensuu et al. (10). They, however, found a proportion of aneuploid tumors twice as large as we did. They also found aneuploid tumors more often in females than in males, while in our series aneuploidy was more common in males, especially in older patients. The present study has confirmed the prognostic significance of the DNA pattern. Before the prognostic value of s-phase determination can be firmly established, further studies will need to be undertaken.

Table 4

Factors associated with survival in patients with papillary thyroid cancer (Cox's step-wise proportional hazard model)

Variables	Improvement		Coeff./SE	p-value
	χ^2	p-value		
Age at diagnosis	44.4	<0.0001	4.1	<0.0001
Nodal status (N0 vs. N1–3)	16.1	<0.001	3.8	<0.0001
DNA ploidy	6.2	<0.013	2.7	<0.004
Primary tumor (pT0–3 vs. pT4)	6.0	<0.014	2.8	<0.003

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