Prognosis of Papillary Thyroid Cancer

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PROGNOSIS OF PAPILLARY THYROID CANCER

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TABLE OF CONTENTS

I.
Abbreviations

AA	arachidonic acid
AGES	Age Gender Extent
AMES	Age Metastasis Extent Size
Bcl-2	B cell lymphoma gene-2
COX	cyclooxygenase
DAMES	DNAploidy Age Metastasis Extent Size
EIA	Enzyme Immuno Assay
HUCH	Helsinki University Central Hospital
LNM	Lymph Node Metastases
MACIS	Metastasis Age Completens of resection Invasion Size
MMP	Matrix metalloproteinase
MRND	Modified Radical Neck Dissection
NCCN	National Comprehensive Cancer Network
NPA	Human papillary thyroid cancer cell line
NS-398	N-(2-Cyclohexyloxy-4-nitrophenyl)-Methanesulfonamide
PBS	Phosphate-buffered saline
PGE_2	Prostaglandin E2
PMA	phorbol 12-myristate 13-acetate
PTC	Papillary Thyroid Cancer
PTEN	**P**hosphatase and **ten**sin homolog on chromosome 10
RAI	Radioactive iodine
RET	REaranged during Transfection
RT-PCR	Real-time quantitative Polymerase Chain Reaction
SAG	Sex Age Grade
TBP	TATA-binding protein
TNM	Tumour Node Metastasis
TSH	Thyroid Stimulating Hormone
UICC	Union Internationale Contre le Cancer (International Union Against Cancer)
VEGF	Vascular Endothelial Growth Factor

2. List of Original Publications

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I Voutilainen PE, Siironen P, Franssila KO, Sivula A, Haapiainen RK, Haglund CH. AMES, MACIS and TNM prognostic classifications in papillary thyroid carcinoma. Anticancer Research 2003;23(5b):4283-8.

II Siironen P, Ristimäki A, Nordling S, Louhimo J, Haapiainen R, Haglund C. Expression of COX-2 is increased with age in papillary thyroid cancer. Histopathology 2004;44(5):490-7.

III Siironen P, Nordling S, Louhimo J, Haapiainen R, Haglund C. Immunohistochemical expression of bcl-2, Ki-67, and p21 in papillary thyroid cancer; Ki-67 expression is increased in older patients. Tumor Biology 2005;26:50-56.

IV Siironen P, Louhimo J, Nordling S, Ristimäki A, Mäenpää H, Haapiainen R, Haglund C. Prognostic factors in papillary thyroid cancer; An evaluation of 601 consecutive patients. Tumor Biology 2005;26:57-64.

V Siironen P, Ristimäki A, Narko K, Nordling S, Louhimo J, Andersson S, Haapiainen R, Haglund C. VEGF-C and COX-2 expression in papillary thyroid cancer. In manuscript.

3. Abstract

As age is the most important predictor of survival in papillary thyroid cancer (PTC), our interest was to compare young and older patients and to discover any possible association between increasing age and higher incidence of poor prognostic factors. Our hypothesis was that expression of selected tumour markers between young and older patients differs and that the same marker could identify the high-risk patients also among young patients. Our aim was to find a marker or set of markers to predict individual patient outcome better than do existing staging methods.

We analyzed the medical records of consecutive patients who underwent surgery for papillary thyroid cancer at Helsinki University Central Hospital (HUCH) between 1957 and 1996 (n= 682). We compared AMES (Age-Metastasis-Extent-Size), MACIS (Metastasis-Age-Completens of resection-Invasion-Size) and TNM (Tumour-Node-Metastasis) staging systems in predicting carcinoma-specific mortality in 495 papillary thyroid carcinoma patients. For immunohistochemical studies, we retrospectively selected two patient groups. One included 108 patients selected by age (under 35 and over 55 years) to compare young and older PTC patients, and the other 36 matched pairs selected by tumour behaviour (aggressive versus completely recovered) to compare differently behaving tumours. We studied expression of the tissue markers COX-2, MMP-2, bcl-2, Ki-67, p21, and VEGF-C. Furthermore, we investigated by Real-time quantitative Polymerase Chain Reaction (RT-PCR) and Enzyme Immuno Assay (EIA) methods the relation of VEGF-C and of COX-2 expression in human papillary thyroid cancer cells (NPA).

In predicting prognosis, TNM classification was more reliable than AMES or MACIS.

Age over 45 years, tumour size > 4 cm, extrathyroidal extension of tumour, nodal metastases, distant metastases, and stage IV disease significantly correlated with survival.
Expression of COX-2, Ki-67, and VEGF-C was higher in older patients, and expression of these markers correlated with stage, but not with other clinical parameters or aggressive disease. Among the patients with positive lymph nodes, most tumours of older patients expressed VEGF-C, but most of those younger patients did not. Immunohistochemically, expression of COX-2 and VEGF-C correlated strongly with each other. In cell culture studies, both VEGF-C and COX-2 were induced by phorbol 12-myristate 13-acetate (PMA), but the selective COX-2 inhibitor N-(2-Cyclohexyloxy-4-nitrophenyl)-Methanesulfonamide (NS-398) did not reduce VEGF-C expression. Thus, correlation of COX-2 and VEGF-C was not so clear.
Stromal expression of MMP-2 correlated with the extent of the primary tumour and with advanced stage, but not with aggressive disease. Expression of p21 was higher in tumours extending beyond the thyroid capsule and in larger tumours, but expression did not correlate with aggressive disease. Expression of Bcl-2 correlated neither with clinical parameters nor aggressive disease.

Most patients with papillary thyroid cancer can be cured if treated according to best clinical practice. In older patients, PTC is a more aggressive disease. Lymph node metastases (LNM) seemed to be predictive for distant metastases and death in older patients, but not at all for those younger. Unexpectedly, however, some young low-risk patients do suffer from aggressive disease. How to recognize these young patients is the problem. Although we found elevated expression of COX-2, VEGF-C, and Ki-67 in those older, these three markers did not predict patient outcome. Better understanding of differences in tumour biology in young and older patients may help us to select those young patients who display the tumour behaviour of older patients.
Initial treatment and follow-up could be individualized according to age. Older lymph node-positive PTC patients may benefit from more radical lymph node dissection. These patients may also benefit from COX-2 selective inhibitors.

4. Introduction

Despite the generally indolent behaviour of papillary thyroid cancer, we need to identify patients who experience an unfavourable outcome. Currently, several risk-classification methods have been established to predict the prognosis of individual patient at the time of diagnosis, TNM classification being the most widely used prognostic scoring system for thyroid cancer (Hay et al. 1987, Cady and Rossi 1988, Pasieka et al. 1992, Akslen 1993, Hay et al. 1993, Noguchi et al. 1994, Sobin and Wittekind 1997). However, a small proportion of PTC patients and even those classified as low-risk can develop an aggressive disease, and consequently none of these classifications can be considered completely reliable.

To reduce the number of thyroid cancer recurrences, our aim was to find a marker or a set of markers to predict the outcome of an individual patient better than does TNM classification alone. Thus far, thyroglobulin is the only tumour marker being routinely used in PTC. The main use of thyroglobulin is to determine the effectiveness of thyroid cancer treatment and to monitor for recurrence, not to predict prognosis. With an ideal tumour marker, we could select high-risk patients for more aggressive therapy. These patients could probably benefit from more radical lymph node dissection and should also be under more intensive follow-up.

Our hypothesis was that expression of selected tumour markers between young and older PTC patients differs. PTC is an atypical cancer in two ways; first, its aggressiveness increases significantly in older patients, and second, lymph node metastasis does not indicate poor survival, especially in young patients. It has been supposed that PTC is a biologically different disease depending on age (Cady

1998). In young patients, PTC does not behave like normal cancers: Survival is excellent, and the cancer does not metastasize further than to the regional lymph nodes. In older patients, however, PTC behaves like other cancers: it metastasizes and may cause death. One present problem is that even though most young patients have a good prognosis, some of these low-risk patients suffer from early recurrence and even die of cancer. We assumed that if expression of a marker is altered in older patients, this marker could be helpful in selecting high-risk patients from the young patient group for more intensive therapy and follow-up.

In older patients, it has been suggested that tumours grow faster (Tubiana et al. 1985), distant metastases occur more frequently (Bacourt et al. 1986, Samaan et al. 1992), and extracapsular extension of the primary tumour is more common (Samaan et al. 1992, Coburn and Wanebo 1995, Kurozumi et al. 1998). Changes in the cell-cycle and apoptosis, or in the signalling pathways controlling them, allow cancer cells to escape the normal control of cell proliferation and cell death (Evan and Vousden 2001). Changes in cell proliferation and death (bcl-2, Ki-67, p21) could possibly explain the different behaviour of PTC between young and older patients. Moreover, cancer cells` ability to invade and form fatal metastases (COX-2, MMP-2, VEGF-C) is the reason for cancer deaths seen in older patients. Thus, our interest was to study the expression of COX-2, MMP-2, VEGF-C, bcl-2, Ki-67, and p21 in PTC.

5. Review of the Literature

Thyroid cancer is the most common endocrine cancer and accounts for 1% of cancer cases. Patients with thyroid cancer usually present with a solitary thyroid nodule. Of all thyroid nodules, only 5% are malignant. Of these, 80 to 95% are differentiated thyroid carcinomas (papillary and follicular), 5 to 10% medullary carcinomas, and the remaining 1 to 2% undifferentiated (anaplastic) thyroid carcinomas (Table 1). Medullary thyroid carcinoma arises from C cells, all other types from follicular cells (DeLellis et al. 2004). Among differentiated thyroid carcinomas, lymph node metastases are uncommon in follicular carcinoma (10%), in contrast to papillary carcinoma, in which lymph node metastases are a common finding (30-40%) (Emerick et al. 1993, Gilliland et al. 1997). Moreover, distant metastases are more common in patients with follicular carcinoma (30%), and the tumours are larger in size (Emerick et al. 1993). Medullary thyroid carcinoma is hereditary in about 25% of cases and spreads to the lymph nodes earlier than do differentiated thyroid carcinomas. In contrast to papillary, follicular, and medullary thyroid carcinomas, anaplastic thyroid carcinoma is one of the most aggressive neoplasms in humans (Ain 1998). Prognosis is very poor and survival is measured in months, the median survival from diagnosis ranging from 3 to 7 months (McIver et al. 2001, Sugitani et al. 2001). A small proportion of patients enjoy long-term survival (Ain 1998, Nilsson et al. 1998).

Table 1. Thyroid cancer types, occurrence, peak onset ages, and 10-year survival rates (Gilliland et al. 1997, Hundahl et al. 1998, McIver et al. 2001). Sporadic medullary carcinoma occurs in older patients (40 to 60 years) in contrast to hereditary medullary carcinoma.

Thyroid cancer type	Occurrence (%)	Peak onset age (years)	10-year survival (%)
Papillary	70-80	30-50	93-98
Follicular	10-15	40-60	85-93
Medullary	5-10	40-60/ 30-50	75-80
Anaplastic	1-2	60-80	3-14

5.1 Epidemiology

Papillary thyroid carcinoma is the most common malignant tumour of the thyroid gland, accounting for 70 to 80% of all thyroid cancers (Gilliland et al. 1997, Hundahl et al. 1998). It is two to four times as frequent in women as in men (DeLellis et al. 2004). These tumours may arise at any age, but are most common between 30 and 50 years, with mean age at diagnosis being 45 to 50. In Finland, the incidence of papillary thyroid cancer has increased. According to the Finnish Cancer Registry (www.cancer.fi), the number of new thyroid cancer cases in 2003 was 374 (98 male, 276 female), which accounts for 1% of all cancers. In Finland, thyroid cancer is the 14th most common cancer in females.

5.2 Aetiology

Oncogenes

The role of oncogenes in thyroid cancer is a subject of intense investigation; several genes have been implicated in the molecular carcinogenesis of PTC.

The most commonly described genetic changes in PTC are the rearrangements of the RET (REarranged during Transfection) proto-oncogene (RET/PTC) (Santoro et al. 1992, Sugg et al. 1998) which is located in chromosome 10q11.2 (Myers et al. 1995). RET/PTC rearrangements are found in 20 to 30% of sporadic adult papillary carcinomas (Santoro et al. 1992, Bongarzone et al. 1998), and in 45 to 60% of tumours from children and young adults (Bongarzone et al. 1996, Fenton et al. 2000). The RET/PTC rearrangements have been associated with

young age and previous exposure to ionizing radiation (Kazakov et al. 1992). The reported frequency of RET-positive PTC cases in Chernobyl is 51 to 76%, which is higher than in most series of spontaneous thyroid cancers (Fugazzola et al. 1995, Klugbauer et al. 1995, Thomas et al. 1999). The correlation between RET/PTC rearrangements and the clinical outcome is obscure. It has been suggested that RET/PTC may be associated with greater likehood of metastatic spread and worse prognosis (Jhiang and Mazzaferri 1994, Sugg et al. 1996).
The other known genetic changes occurring in papillary carcinoma are TRK rearrangements, RAS mutations, BRAF mutations, and p53 mutations. TRK gene chromosomal rearrangements are found in 10% of papillary carcinomas (Bongarzone et al. 1998, Musholt et al. 2000), RAS mutations in less than 10% (Namba et al. 1990, Hara et al. 1994, Ezzat et al. 1996), and BRAF mutations in up to 70% (Kimura et al. 2003, Nikiforova et al. 2003, Soares et al. 2003). In a study of Nikiforova et al. (2003), BRAF-positive patients were older and had a higher incidence of extrathyroidal extension. P53 gene mutations occur in many carcinomas (Hollstein et al. 1991). In thyroid carcinoma, p53 mutations are frequently found in anaplastic carcinoma, but rarely in papillary thyroid carcinoma (Ito et al. 1992, Donghi et al. 1993, Ho et al. 1996, Farid 2001). This suggests that the p53 mutations play an important role in progression from differentiated thyroid carcinomas to anaplastic carcinoma.

Thyroid irradiation
Radiation exposure leads to increased risk for thyroid cancer (Kazakov et al. 1992), but it does not affect the prognosis or the aggressiveness of the tumour. Irradiation during childhood has been associated with the greatest risk for acquiring papillary thyroid cancer. After the Chernobyl disaster, childhood thyroid carcinoma showed a great increase (up to 100-fold) in Belarus and Ukraine (Kazakov et al. 1992).

Familial
The frequency of familial nonmedullary thyroid cancer has been shown to vary between 2.5 and 6.3% (Loh 1997). About 6% of PTC show a familial component (Lupoli et al. 1999). One gene has been identified, a phosphatase and tensin homolog on chromosome 10 (PTEN); germ line mutations have occurred in 80% of patients with Cowden syndrome (multiple hamartoma syndrome) (Liaw et al. 1997, Marsh et al. 1998). Cowden´s syndrome is, however, associated more often with follicular thyroid cancer, and rarely with papillary thyroid cancer (DeLellis et al. 2004). Patients with familial adenomatous polyposis appear to have an increased incidence of papillary thyroid cancer (Cetta et al. 2000).

5.3 Histology

Usual type. The typical papillary carcinoma is referred to as the usual type. The diagnosis of PTC is based on nuclear features which should be present in a significant proportion of the neoplasm (Vickery et al. 1985). Papillary architecture is not always present. Nuclei appear "optically clear" or resemble the eyes of the comic-strip character "Little Orphan Annie". Grooves may also be evident in the nuclei, which result from folds in the nuclear membrane. The papillae of the usual PTC have fibrovascular cores covered by cells with nuclei that overlap and appear to be "piled" one on the other.
Most papillary carcinomas are of this usual type and about 25% belongs to histological variants (Carcangiu et al. 1985, Akslen and LiVolsi 2000). The most important variants are microcarcinoma, the follicular variant, the diffuse sclerosing variant, oncocytic variant (Hürthle cell PTC), the tall cell and the columnar cell variants.

Variants of papillary carcinomas

Microcarcinoma. Microcarcinoma is a papillary carcinoma 1 cm or less in diameter. These lesions are of very low malignancy, and distant metastases are exceptionally rare. The indolent behaviour of these lesions is shown by their frequency as incidental findings in autopsy studies, being 6 to 7% in USA, Portugal, and Sweden (Sampson et al. 1974, Sobrinho-Simoes et al. 1979, Bondeson and Ljungberg 1984), 24% in Japan (Fukunaga and Yatani 1975), and 35% in a study from Finland (Harach et al. 1985).
Follicular variant. The follicular variant of PTC is the most common subtype of PTC after the usual type. It is composed of follicles and has characteristic papillary type nuclei (Chem and Rosai 1977). Thus, diagnosis of PTC is made when papillae are absent. These lesions behave biologically similarly to the usual type. In addition to common follicular variant, two other types of follicular variant have been described. The macrofollicular variant (Albores-Saavedra et al. 1991) is less aggressive and the diffuse follicular variant more aggressive than common follicular variant (Ivanova et al. 2002). The diffuse follicular variant occurs mainly in young females. In series of eight patients, all developed metastases in lungs, bones or both, two of them died of PTC (Sobrinho-Simoes et al. 1990).
Diffuse sclerosing variant. This variant was initially described by Crile and Fisher in 1953. It occurs predominantly in young individuals and is characterized by diffuse involvement of one or both lobes, dense sclerosis, abundant psammoma bodies, typical papillary carcinoma elements, foci of squamous metaplastic change, and a patchy lymphocytic infiltrate (Vickery et al. 1985). Metastases both to cervical nodes and lungs are more frequent than in the usual type (Carcangiu and Bianchi 1989).

Oncocytic variant (Hürthle cell PTC). Oncocytic variant is characterized by the presence of oncocytes (also called oxyphilic cells), which are large polygonal cells with hyperchromatic nuclei and an eosinophilic granular cytoplasm (DeLellis et al. 2004). This oncocytic variant may have a papillary or follicular architecture. Diagnosis is based on the classical nuclear features of papillary thyroid carcinoma. It seems to behave in a fashion analogous to the usual type of papillary carcinoma (Berho and Suster 1997, Cheung et al. 2000, Ludvikova et al. 2001).

Tall cell variant. Tall cell variant is defined as PTC in which a minimum of 30% of the cells have a height at least twice their width, indicating a more aggressive growth pattern (Johnson et al. 1988). The tall cell variant is associated with a higher incidence of recurrence and mortality (Prendiville et al. 2000). It occurs more often in older patients (Ruter et al. 1997).

Columnar cell variant. Thyroid tumours with columnar features were first described by Evans in 1986. They are rare, aggressive and have high mortality (Sobrinho-Simoes et al. 1988, Gaertner et al. 1995). The papillae are lined by tall columnar cells showing pseudostratification. The nuclei are elongated or oval, and are rich in chromatin, unlike the usual type. Columnar cell variant is an aggressive tumour associated with a fatal outcome.

5.4 Staging

Many staging systems have been developed to predict the prognosis of the PTC patient. These systems quantify various characteristics of the tumour and the patient. The essence of all staging methods is that age is a major factor that determines prognosis. Young patients rarely die of their disease.
The most commonly used staging method is International Union Against Cancer (UICC) TNM classification (Sobin and Wittekind 2002). Other common staging methods are AGES, MACIS, AMES, DAMES and SAG. The Mayo clinic described a prognostic scoring system based on patient age, tumour grade, extent, and size (AGES) (Hay et al. 1987) and a model including five variables abbreviated by metastasis, age, completeness of resection, invasion, and size (MACIS) (Hay et al. 1993). Cady et al. described a completely clinical classification based on age of patient, presence of distant metastases, extent and size of the tumour (AMES) (Cady and Rossi 1988). Pasieka et al. modified AMES risk-group classification to include DNA ploidy with AMES (DAMES) (Pasieka et al. 1992). Akslen et al. demonstrated that histologic grade is a strong and independent prognostic factor and introduced SAG score (sex, age, and grade) (Akslen 1993). These staging methods are described below.

TNM. TNM classifies all patients under 45 years in a low-risk category regardless of whether lymph nodes are involved, some of the tumour is not removed, or even regardless of distant metastases. In our study, we used TNM staging 1997 (Sobin and Wittekind 1997).
A new TNM classification of malignant tumours has been published (Sobin and Wittekind 2002). Compared to the 1997 5th edition some changes have been made in thyroid carcinoma classification. Major changes are that T1 includes tumours sized 2 cm or less and that T3 includes tumours with minimal extrathyroid extension.

Table 2 UICC age-related TNM staging (1997) for papillary and follicular thyroid carcinoma.

Stage	Age <45 year	Age >/=45 year
I	T1-4N0,1M0	T1N0M0
II	T1-4N0,1M1	T2-3N0M0
III		T4N0M0 or T1-4N1M0
IV		T1-4N0,1M1

T1 ≤ 1cm
T2 > 1cm, ≤ 4cm
T3 > 4cm
T4 Extrathyroidal
N1 Positive neck lymph node
M1 Positive distant metastasis

AGES (Hay et al. 1987, based on 860 patients). The prognostic score (PS) was generated by regression analysis. PS = 0.05 x age in years (if age 40 or more) or 0 (if less than 40), +1 (if grade 2) or +3 (if grade 3 or 4), +1 (if extrathyroid) or +3 (if distant spread), +0.2 x tumour size (maximun diameter in centimetres). The 860 patients were divided into four risk groups according to the score. Group 1 (86%) had scores 0 to 3.99, and had 25-year mortality from cancer of 2%. Group 2 (7%) had scores 4 to 4.99 and 25-year mortality of 24%. Groups 3 and 4, with scores 5 to 5.99 and 6+, had 25-year mortality rates of 49% and 93% respectively. Patients in Group 1 were considered to be at "minimal" risk of cancer mortality.

AMES (Cady and Rossi 1988, based on 821 patients). The risk-group definition is completely clinical. It can be used at the operating table to select conservative surgical procedures for low-risk patients. Cady and Rossi offered a multifactorial system for the identification of low-risk patients who made up 89.4% of all patients seen between 1961 and 1980 and who had a death rate of only 1.8%. The high-risk group constitutes 11% of cases but carries a 46% mortality rate. Low-risk group: A. All younger patients without distant metastases (men <41 years; women <51 years). B. All patients with: 1. intrathyroidal papillary cancer, *and* 2. primary cancers <5 cm in diameter, *and* 3. no distant metastases. High-risk group: A. All patients with distant metastases. B. All older patients with: 1. extrathyroidal papillary cancer, *and* 2. primary cancers 5 cm in diameter or larger regardless of extent of disease.

DAMES (Pasieka et al. 1992, based on 73 patients). Patients with euploid tumours that were AMES low risk were considered to be DAMES low risk; patients with euploid tumours that were AMES high risk became intermediate risk, and patients with aneuploid tumours that were AMES high risk became DAMES high risk. Three patients were in the DAMES high-risk group. Distant metastases developed in all three, who died from thyroid cancer within 24 months. In the low-risk group (n=48), distant metastases developed in four patients, no death occurred from cancer. In the intermediate group (n= 22), 12 had residual or recurrent disease, or distant metastases, with one death from cancer.

MACIS (Hay et al. 1993, based on 1779 patients). The prognostic score in the MACIS model = 3.1 (if aged or = 40 years), + 0.3 x tumour size (in centimetres), +1 (if incompletely resected), +1 (if locally invasive), +3 (if distant metastases present). Twenty-year cause-specific survival rates for patients with MACIS less than 6, 6 to 6.99, 7 to 7.99, and 8+ were 99%, 89%, 56%, and 24%, respectively.

SAG (Akslen et al. 1993, based on 263 patients). The SAG score represents the sum of the following features: sex (female, 0; male 1), age (younger than 70 years, 0; 70 and older, 1), grade (histologic grade 1, 0; histologic grade 2, 1). Histologic grade, based on absence or presence of the three microscopic key features: *v*ascular invasion, marked nuclear *a*typia, and tumour *n*ecrosis (VAN features). Grade 1 (low grade) was recorded when none of the VAN features were present, Grade 2 (high grade), was recorded when any of the VAN features were present. Three patient groups at different risk for cancer death emerged: SAG I-III (SAG I: score 0; SAG II: score 1; SAG III: score 2-3). Estimated risk for thyroid cancer death in 15 years in three groups were: SAG I (n= 97) 1.7%, SAG II (n= 55) 12.0%, and SAG III (n= 21) 61.0%.

5.5 Treatment

Patients with thyroid cancer should be managed by regionally designed multidisciplinary teams comprising a surgeon, endocrinologist, and oncologist with the support of a pathologist, medical physicist, biochemist, radiologist, and specialist nurse (Kendall-Taylor 2003).

Thyroidectomy. The balance between complications and extent of surgery has been an issue in whether thyroidectomy or a more conservative unilateral approach should be recommended for low-risk papillary cancers. The most common significant complications of thyroid surgery are permanent recurrent laryngeal nerve palsy and permanent hypoparathyroidism. The disadvantage of remaining thyroid tissue after surgery is an argument against conservative treatment, because remnant thyroid tissue makes follow-up difficult. In addition, performing lobectomy alone results in a 5 to 10% recurrence rate in the contralateral thyroid lobe, higher tumour recurrence, and higher incidence of distant metastases (Massin et al. 1984, Samaan et al. 1985, Hay et al. 1987). Complication rates have improved in the hands of experienced surgeons, and the main argument against extensive surgery seems less important. A study of 5860 patients in the USA found that surgeons who performed more than 100 thyroidectomies annually had the lowest overall complication rates (4.3%) (Sosa et al. 1998). In that study, those who performed less than 10 cases annually had 4-fold higher complication rates. The rates of permanent hoarseness and hypoparathyroidism are much lower than immediately after surgery. A work by Pattou et al. (1998) found hypocalcemia in 5.4% of patients immediately after surgery, but a year later in only 0.5%. A study of seven surgical series found that after total thyroidectomy the average rates of permanent laryngeal nerve injury and hypoparathyroidism were 3% and 2.6% (Udelsman et al. 1996).

If the diagnosis of cancer is clear at the time of surgery, most clinicians in the USA and Europe agree that total or near-total thyroidectomy is the treatment of choice (DeGroot et al. 1994, Solomon et al. 1996, Sherman et al. 1998, Mazzaferri 1999, Cailleux et al. 2000, Hundahl et al. 2000). Lobectomy alone has shown to be adequate for papillary microcarcinoma (< 1cm) discovered after surgery for benign disease (Mazzaferri and Jhiang 1994, Baudin et al. 1998). Total thyroidectomy is, however, the optimal treatment for microcarcinoma patients with multiple foci (Baudin et al. 1998). Based on these recommendations, at Helsinki University Central Hospital all preoperatively diagnosed PTC patients undergo total thyroidectomy. If occult PTC (one focus) is diagnosed after goiter surgery, lobectomy is believed to be sufficient.

Lymph node dissection. Another issue is the extent of lymph node dissection. As the impact of lymph node metastasis is in dispute, so is also the extent of lymph

node dissection.

Most surgeons agree that prophylactic neck dissection is not routinely necessary, as postoperative radioactive iodine (RAI) ablation destroys remnant thyroid tissue and possible micrometastasis. Another arguments against lymph node dissection, is that lymph node metastases do not affect survival (McConahey et al. 1986, Shah et al. 1992). If the patient has palpable nodes, some authors recommend selective resection for involved nodes only (Mazzaferri and Young 1981, Hay et al. 1998), and others recommend modified radical neck dissection (MRND) (McGregor et al. 1985, McHenry et al. 1991, Simon et al. 1996, Mann and Buhr 1998, Sivanandan and Soo 2001). During recent years, however, consensus is emerging that compartment-oriented neck dissection minimizes neck recurrences and is superior to a node picking procedure (in which only the grossly abnormal lymph nodes are excised) (Sherman et al. 2005). The National Comprehensive Cancer Network (NCCN) recommends central neck dissection (level VI) and lateral neck dissection (levels II-V) for lymph node-positive PTC (Sherman et al. 2005).

In Japan, the standard practice for papillary thyroid carcinoma is subtotal thyroidectomy with modified radical neck dissection of the affected side (Yamashita et al. 1998, Uchino et al. 2004). Japanese surgeons recommend prophylactic systemic cervical node dissection, as they have found that this improves prognosis (Noguchi et al. 1998). The presence of gross nodal metastases has also been shown to be an important risk factor for survival (Yamashita et al. 1998). Japanese surgeons do not, however, use RAI ablation routinely because of rigid regulations for the use of radioisotopes in Japan (Yamashita et al. 1998).

Some centres, including our own, at present continue to do node picking procedure. At our department, if suspicion or evidence of lymph node involvement (biopsy, ultrasound, or at operation) exists, pathological lymph nodes are removed. This may change in future as more information becomes available.

Modified radical neck dissection. MRND is defined as removing all of the lymphatic tissue at levels I-V and preserving the sternocleidomastoid muscle, internal jugular vein, and spinal accessory nerve (Khatri and Loree 2002).

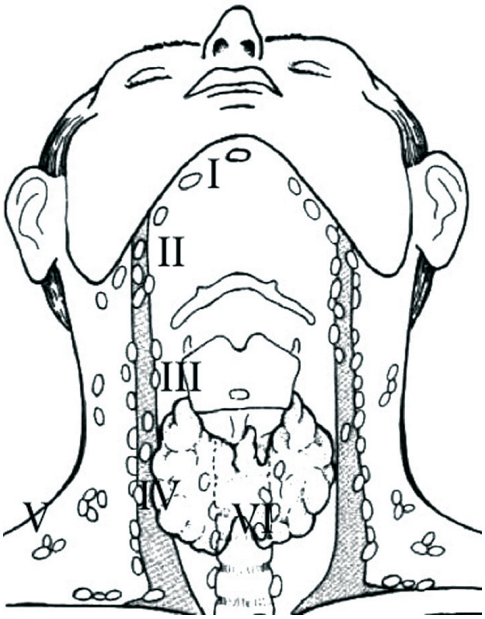


Figure 1 Cervical regional lymph nodes (level I-VI).

RAI ablation. Total thyroidectomy is followed by radioactive iodine ablation. Radioiodine therapy reduces local recurrence and improves survival (Mazzaferri and Kloos 2001). Thyroid cells are unique among all cells of the human body as they have the ability to absorb iodine. Iodine is a key component of the hormone thyroxin. Thyroid cells combine iodine and the amino acid tyrosine to make thyroxin. Every cell in the body depends upon thyroid hormones for regulation of its metabolism. Thyroid cells will absorb and concentrate also radioactive iodine, and the radioactivity destroys the cell. Because papillary and follicular thyroid cancer cells retain this ability to absorb iodine, radioiodine thus destroys any remaining normal thyroid tissue and occult microscopic carcinoma. This means that even metastatic disease in most cases will be treatable.

Treatment of local recurrences and distant metastases. Surgery is the first choice for neck recurrences, and regional recurrences are removed surgically if possible. After surgery, or if surgery is not suitable, local and regional recurrences detected by ^{131}I whole-body scans are treated with ^{131}I ablation with good results. External radiotherapy is recommend for those non-operable recurrences that do not concentrate ^{131}I, and recommended when surgery is done repeatedly (Schlumberger 1998, Sherman et al. 2005).

For metastatic disease, surgery, ^{131}I ablation, and external radiotherapy are recommended depending on the site of metastases and radioiodine uptake (Niederle et al. 1986, Schlumberger et al. 1986, Sherman et al. 2005). Surgery is recommended for all solitary metastases. Radioiodine is recommended for disseminated tumours that concentrate ^{131}I and external radiotherapy if the tumour does not concentrate ^{131}I. For skeletal metastases, external radiotherapy is the first choice.

Thyroxine. PTC responds to thyroid stimulating hormone (TSH) secreted by the pituitary. Thus a high dose of exogenous thyroid hormone, thyroxine, postoperatively results in decreased TSH levels and a lower impulse for any remaining cancer cells to grow.

Follow-up. Follow-up is based on measuring serum thyroglobulin levels. Thyroglobulin is a glycoprotein produced by normal or neoplastic follicular cells. It should not be detectable in the circulation, if the patient has undergone total thyroid ablation. Measurable thyroglobulin in the absence of antibodies indicate the presence of persistent or recurrent disease.

5.6 Prognosis

The vast majority of patients can expect to be cured if treated according to best practice. In series of over 15 000 patients, 10-year survival was 98% (Gilliland et al. 1997), and in a series of 53 000 patients, 93% (Hundahl et al. 1998). Despite the good prognosis, local or regional tumour recurrences occur in 5 to 20% of patients with PTC, depending on initial treatment and other prognostic variables. At the time of diagnosis, distant metastases are very uncommon, but during follow up 6 to 11% of PTC patients develop distant metastases (Table 11). Although patients may live for long time with distant metastases, this does significantly worsen prognosis. About one-third of patents with distant metastases survive for 10 years (Hoie et al. 1988, Ruegemer et al. 1988, Schlumberger et al. 1996). Age is important for both predicting development of distant metastases and for influencing long-term survival.

Age

Age is the most important prognostic variable. Unlike other types of cancer, the prognosis of papillary thyroid cancer depends largely on the age. Young people rarely die of their disease regardless of lymph node metastases or even distant metastases. For older patients, however, prognostic factors play a significant role in long-term disease-free survival. Ten-year survival for patients younger than 20 has been 99%, compared to 86% for patients 70 years and older (Gilliland et al.

1997). One study showed a clear cut age-related decrement in survival: 10-year survival was 92% in patients aged 21 to 50, 77% in those 51 to 70, and 48% in those over 71 (Coburn and Wanebo 1995).
The age at which a patient´s prognosis becomes poor has been a matter of debate. The cut-off age for poor prognosis has shown to be between 40 and 70 in prognostic staging classifications (AGES 40; AMES 41 in men, 51 in women; MACIS 40; SAG 70; TNM 45) (Hay et al. 1987, Cady and Rossi 1988, Akslen 1993, Hay et al. 1993, Sobin and Wittekind 1997). The UICC TNM system classifies all patients under 45 in the low-risk category independent of tumour size, extent of tumour, lymph node metastases, and distant metastases (Sobin and Wittekind 1997). All these cut-off ages are estimated. There seems to be "a grey area" between 40 and 70; patients over 70 have a poorer prognosis, but these grey area patients perhaps not.
Older PTC patients may represent a biologically distinct group. Relation of old age to other poor variables is uncertain. Coburn and Wanebo (n=382) showed that survival decreases and recurrence rates increase with age (Coburn and Wanebo 1995). They demonstrated that old patients show more high-risk factors such as extracapsular extension and vascular invasion. The interval between primary treatment and relapse was shorter in older patients, and authors suggest that tumours grow faster in patients over 45 (Tubiana et al. 1985). Bacourt et al. (1986) showed that large tumours, distant metastases and poorly differentiated forms occur mainly after 40. Samaan et al. (1992) found that the elderly more often have soft tissue involvement and bone metastases. Kurozumi et al. (1998) reported age to be related to extrathyroidal invasion. Thus it seems that older PTC patients have more pathological risk-factors, showing their tumours to be more aggressive.

Tumour size
Increasing tumour size is an accepted factor in poor prognosis (DeGroot et al. 1990, Akslen 1993, Hay et al. 1993). Survival is shorter, if the tumour is over 4 cm in diameter (Shaha et al. 1994). Furthermore, lymph node metastases are more frequent in patients with larger tumours (Gimm et al. 1998).

Multifocality
PTC has been shown to be multicentric in 20 to 80% of patients (Schlumberger 1998), the high variation probably reflecting the thoroughness of the pathologist. A few authors demonstrate multifocality as a strong factor for poor prognosis (Carcangiu et al. 1985, Mazzaferri and Jhiang 1994). In cases of microcarcinoma, the number of histologic foci has been shown to influence recurrence (Baudin et al. 1998).

Extent of tumour
Extrathyroid extension of the tumour has adverse effect on prognosis (Carcangiu et al. 1985, McConahey et al. 1986). Approximately one-third of patients who die

of PTC die of local disease (McConahey et al. 1986). Locally invasive papillary tumours are associated with risk for development of postoperative metastasis in cervical lymph nodes, risk for local recurrence, risk for development of postoperative distant metastases, and mortality (McConahey et al. 1986). Control of locally invasive tumour is therefore important.
The most important factor from the surgical standpoint is extrathyroidal extension of the tumour. Radical resection of aerodigestive structures is not believed to be necessary, because conservative surgery for invasive PTC has produced similar survival rates (McCaffrey et al. 1994, Czaja and McCaffrey 1997). In cases of gross intraluminal extension, life-threatening complications should be avoided by sacrificing aerodigestive tract structures (McCaffrey et al. 1994). In cases of minimal invasion of the tracheal cartilage, shaving tumour off the laryngotracheal structures is recommended. The goal of treatment is complete gross tumour removal with maximal preservation of function (Patel and Shaha 2005).
As for the role of age in the outcome after incomplete resection, the findings are contradictory. Older patients have a higher risk of death if the cancer is not grossly completely resected (Rossi et al. 1988). Other authors have, however, shown that among patients over 45, incomplete excision of tumour did not affect survival, as it did in younger patients (Andersen et al. 1995). They speculated that this could be due to the more aggressive course of the disease in these older patients.

Male gender
The influence of male gender for prognosis is a matter of controversy. Some studies have demonstrated an independent influence of gender on probability of relapse and survival (Byar et al. 1979, Tubiana et al. 1985, Akslen 1993). The majority of studies reveal, however, that gender is not an important prognostic factor (Hay et al. 1987, Simpson et al. 1987, Hay et al. 1993).

Histology
The diffuse sclerosing variant, tall cell variant, and columnar cell variant are more aggressive than the usual type. The diffuse sclerosing variant exhibits a greater incidence of lymph node metastases and lung metastases (Carcangiu and Bianchi 1989, Soares et al. 1989). The tall cell variant can invade blood vessels and metastasize via the blood stream as opposed to the usual variety of papillary carcinoma which spreads via the lymphatics rather than hematogenously (Prendiville et al. 2000). The columnar cell variant of thyroid papillary carcinoma is an aggressive tumour associated with widespread dissemination and a fatal outcome (Sobrinho-Simoes et al. 1988, Gaertner et al. 1995).
The prognostic value of histologic grade (nuclear atypia, tumour necrosis, and vascular invasion) has been superior when compared to histologic subclassification (Akslen and LiVolsi 2000). In one series of 128 PTC patients, each histologic subtype appeared in only a few cases, making firm conclusions difficult (Akslen and LiVolsi 2000). In one series of 401 patients, patients with extrathyroidal

vascular invasion had a higher incidence of distant metastases at diagnosis (40% vs. 4%), and patients with intrathyroidal vascular invasion were more likely to develop distant recurrence (20% vs. 3%) (Gardner et al. 2000).

PTC can transform to anaplastic thyroid carcinoma (Harada et al. 1977). Anaplastic carcinoma is rare, representing 1 to 2% of thyroid neoplasms, but it accounts for nearly half of all cases of mortality associated with thyroid cancer (Giuffrida and Gharib 2000). Approximately half of patients with anaplastic thyroid carcinoma have a history of nodular goiter or differentiated thyroid carcinoma (McIver et al. 2001). Poorly differentiated thyroid carcinoma occupies both morphologically and behaviourally an intermediate position between differentiated and anaplastic thyroid carcinomas (Sakamoto et al. 1983, Papotti et al. 1993), and some of these tumours appear to arise also from pre-existing PTC (DeLellis et al. 2004). The characteristic histology includes the presence of a solid pattern or a trabecular or scirrhous pattern or both (Sakamoto et al. 1983). The mean 5-year survival is about 50% (Sakamoto et al. 1983, DeLellis et al. 2004).

Lymph node metastases

The prognostic significance of lymph node metastases is in dispute. Primary lymph node metastases are present in approximately 30 to 40% of adult patients, and microscopic involvement of lymph nodes in as many as 80 to 90% of the cases (Gilliland et al. 1997, Noguchi et al. 1998). The lymph node metastases are more common among young patients whose prognosis is excellent. Many authors have reported that lymph node metastases are associated with an increased rate of locoregional recurrence of disease, but not with survival (Simpson et al. 1987, DeGroot et al. 1990, McHenry et al. 1991, Lin et al. 1999, Beasley et al. 2002). Some authors have, however, shown that in older patients, LNM have an adverse effect on survival (Harwood et al. 1978, Tubiana et al. 1985, Mazzaferri and Jhiang 1994, Passler et al. 2004). Cady et al. (1998) suggest that in young PTC patients, metastatic cells in regional lymph nodes do not have the capacity to grow in other organs. This hypothesis could explain the differing influence of lymph node metastases in older and young PTC patients.

Distant metastases

At presentation, distant metastases are very uncommon (Mazzaferri et al. 1977), but during follow up 6 to 11% of PTC patients develop them (Table 11). Distant metastases can have a serious impact on survival. Age at diagnosis of distant metastases is the most important factor affecting mortality (Ruegemer et al. 1988). The overall 10-year survival rate of patients with distant metastasis is 25 to 35% (Hoie et al. 1988, Ruegemer et al. 1988, Schlumberger et al. 1996). In PTC, it is, however, possible to cure distant metastases as, ^{131}I can destroy metastases, which take up iodine.

The lungs are the most common site of distant metastases (70-80%), and pulmonary involvement is usually micronodular (Samaan et al. 1985,

Schlumberger et al. 1986, Dinneen et al. 1995). Second in order are bone metastases seen in 0.7 to 2% of PTC patients (Hoie et al. 1988, Marcocci et al. 1989, Dinneen et al. 1995, Zettinig et al. 2002). The site of bone metastases is important for prognosis; metastasis to long bones has a worse prognosis than metastasis to the skull (Schlumberger et al. 1986). Mediastinium, brain, adrenal, skin, or liver are extremely rare sites for metastases.

Numerous studies have reported that patients with ^{131}I-accumulating metastases have far better prognosis than patients lacking such uptake (Samaan et al. 1985, Schlumberger et al. 1986, Hoie et al. 1988). Young patients with lung metastases that take up radioiodine have the most favourable prognosis (Dinneen et al. 1995, Schlumberger et al. 1996). In work by Pacini et al. (1994), micronodular diffuse lung metastases revealed by whole body scan without radiographic changes had the greatest chance of favourable response to radioactive iodine with complete response in most cases. Death from distant metastases is higher if lung metastases are macronodular and detectable by chest radiographs, if bone metastases are multiple, and if metastases to both lung and bone are present (Pacini et al. 1994). The occurrence of bone metastases significantly worsens survival, as bone metastases have a lower capacity to absorb radioiodine.

Recurrences

Local or regional tumour recurrences occur in 5 to 20% of patients with PTC (Schlumberger 1998), with a tendency to occur early. In work by Grant et al. (1988), risk was greatest within the first 5 years after the initial operation, and in work by Coburn et al. (1994), approximately 50% of the recurrences occurred less than 2 years after initial treatment. However, recurrence of disease may emerge many years after primary diagnosis, even as late as 41 years after the initial treatment (Schlumberger et al. 1986). Patients whose metastases appear within 5 years of treatment and those who have multiple sites of metastasis have a poorer prognosis (Wood et al. 1989). Moreover, younger age at primary operation or reoperation has been associated with a better prognosis (Voutilainen et al. 2001, Uruno et al. 2004). Voutilainen et al. (2001) showed that the prognosis for patients under 45 after recurrence was almost parallel to that of the normal reference population.

Some of the metastases are related to incomplete initial treatment, and others indicate an aggressive primary tumour (Schlumberger 1998). Treatment may be as important as tumour biology in predicting tumour recurrence. In series of 72 patients, reoperation was judged to have been preventable in 41 (57%) due to inadequate preoperative imaging, incomplete initial surgery, or absence of TSH suppression or due to all of these factors (Kouvaraki et al. 2004).

Cause of death

As age increases, risk of death from PTC also increases. In patients under 45, death from PTC is very uncommon. The cause of death in PTC is usually distant metastases, often lung metastases causing respiratory insufficiency or

compression of the great vessels. Local disease may cause massive haemorrhage from the major vessels of the neck or direct compression or invasion of the trachea (Smith et al. 1988, Kitamura et al. 1999). Patients who die of papillary thyroid cancer are usually over 45 at diagnosis, and have lymph node metastases, extrathyroid extension of tumour, or distant metastases (Ruegemer et al. 1988, Beasley et al. 2001).

Treatment

Adequate surgical treatment and radioactive iodine therapy for papillary thyroid cancer have improved long-term outcome, both in terms of recurrence and cancer death (Mazzaferri and Jhiang 1994). Time of primary operation is also important. Patients who die of PTC have had a longer delay of treatment than those who survived (18 months vs. 4 months) (Mazzaferri and Jhiang 1994).

5.7 Tumour markers in PTC

Tumour markers, usually proteins, can be detected in a solid tumour, in circulating tumour cells in peripheral blood, in lymph nodes, in bone marrow, or in other body fluids (ascites, urine, or stool). They are produced by the body in response to cancer growth or by the cancer tissue itself. Tumour markers can be used to screen a healthy population or a high-risk population for the presence of cancer, to make a diagnosis of cancer, to determine the prognosis of the patient, or to monitor the course in a patient during follow-up. The ideal tumour marker is specific for the cancer for which it is testing, is not presented in any other conditions, and the concentration changes with the amount of malignant tissue present.
Several studies have investigated tumour markers in papillary thyroid cancer, and some studies have reported them as promising for predicting prognosis, but no guaranteed markers for PTC have been established. At present, in PTC, thyroglobulin is the only tumour marker being routinely used. It is not useful as a diagnostic or prognostic marker. The main use of thyroglobulin is to determine the effectiveness of thyroid cancer treatment and to screen for recurrence.

TUMOURIGENESIS

Tumourigenesis is a multistep process leading to progressive transformation of normal cells into cancer cells. During this process, cells lose their normal ability to sense and repair DNA damage and to regulate cell cycle progression and apoptosis. They acquire abnormal patterns of growth- signalling, abnormal angiogenesis, and abnormal invasive growth.

Ki-67: proliferation

The Ki-67 antibody was named after its site of production in Kiel, Germany, and because the clone producing the antibody was grown in the 67th well of a 96-well microtitre plate (Gerdes et al. 1983). It recognizes an antigen which is associated with the cell nucleus and is expressed in all phases of the cell cycle except G0, with expression associated with tumour grade and number of mitoses (Gerdes et al. 1984). The proportion of proliferating cells is important for predicting the growth potential and aggressiveness of the tumour. The proliferation marker Ki-67 is a prognostic marker in various cancers, with aggressive tumours displaying high expression (Brown and Gatter 1990, Endl and Gerdes 2000).

Ki-67 and thyroid. Most authors have demonstrated low Ki-67 expression in differentiated thyroid carcinomas, but higher expression in those undifferentiated (Wallin et al. 1992, Katoh et al. 1995, Basolo et al. 1997, Okayasu et al. 1998,

Tallini et al. 1999, Yoshida et al. 1999, Saiz et al. 2002). In differentiated thyroid cancers, the percentage of positive cells has varied between 0.2 and 3 (Wallin et al. 1992, Basolo et al. 1997) and in the undifferentiated between 33 and 52 (Katoh et al. 1995, Basolo et al. 1997). Sugitani et al. (1998) found that in a small group of PTC microcarcinoma patients, Ki-67 expression was higher in those with lymph node metastases and with noncapsulated tumours, and moreover, patients who died of cancer showed the highest Ki-67 expression. Others have found no correlation with clinical parameters (Katoh et al. 1995, Tallini et al. 1999).

p21: growth inhibition

Cyclin-dependent kinases (CDKs) make up a large family of proteins that function in a variety of key regulatory pathways including control over the cell cycle. Uncontrolled CDK activity is often the cause of human cancer. p21 (also known as Cip1, WAF1, SD11) is a cyclin-dependent kinase inhibitor transcriptionally activated by p53 in response to DNA damage. It is believed that wild-type (non-mutant) p53 activates the production of p21. Wild-type p53 is not detectable, as it is degraded rapidly; however, after mutation, the half-life of p53 is prolonged, making immunohistochemical detection possible. The most common genetic change in human cancers is p53 mutation (Hollstein et al. 1991), and also p53-independent pathways have been proposed (Parker et al. 1995). The first CDKI to be isolated was p21 (Xiong et al. 1992, el-Deiry et al. 1993, Harper et al. 1993). It is a proliferation inhibitor and plays an important role in preventing tumour development. In addition, p21 also acts as an inhibitor of apoptosis, counteracting its tumour-suppressive functions. Its expression is commonly altered in human tumours (Thor et al. 1984, Viola et al. 1985, Viola et al. 1986, Gomyo et al. 1997, Lu et al. 1998, Natsugoe et al. 1999, Shoji et al. 2002), and it has been associated with good prognosis in oesophageal, uterine, gastric, and pulmonary cancer (Gomyo et al. 1997, Lu et al. 1998, Natsugoe et al. 1999, Shoji et al. 2002) and with poor prognosis in breast cancer (Barbareschi et al. 1996).

p21 and the thyroid. Many researchers have demonstrated that p21 plays no significant role in regulating the progression of thyroid carcinoma (Johnson et al. 1987, Mizukami et al. 1995, Ito et al. 1996). Some authors have, however, shown that p21 associates with clinical parameters in PTC (Basolo et al. 1994, Akslen and Varhaug 1995, Okayasu et al. 1998). In a study by Akslen and Varhaug (1995), expression of p21 protein was increased in PTC patients with lymph node metastases, but no significant association appeared between p21 protein immunostaining and patient survival. Other researchers showed that p21 correlates with tumour aggressiveness in PTC, because its expression was significantly higher in fatal cases (Basolo et al. 1994). Anaplastic thyroid carcinomas and poorly differentiated papillary carcinomas show higher immunostaining than do well-differentiated PTCs (Okayasu et al. 1998). Thus, thyroid and breast cancer have revealed an association between over-expression of

p21 and bad outcome (Basolo et al. 1994, Akslen and Varhaug 1995, Barbareschi et al. 1996, Okayasu et al. 1998), in contrast to most other human tumours, in which over-expression of p21 is associated with good outcome (Gomyo et al. 1997, Lu et al. 1998, Natsugoe et al. 1999, Shoji et al. 2002). Differing patterns of p21 expression in various cancers might be explained by the differing mechanisms regulating cell proliferation and apoptosis in differing epithelial systems (el-Deiry et al. 1995). Expression of p21 is regulated by p53, but acts also via p53-independent pathways (Michieli et al. 1994). In PTC, p53 mutation is rare, as it is in breast cancer (Steele et al. 1998).

Bcl-2: apoptosis

Bcl-2 (B cell lymphoma gene-2) (Tsujimoto et al. 1984) oncoprotein, localized in the mitochondria, prolongs cell survival by inhibiting apoptosis (Hockenbery et al. 1990). Its over-expression is classically associated with malignant lymphomas and poor prognosis (Yunis et al. 1989), but its expression is also associated with good prognosis; thus, the precise mechanism of bcl-2 remains unknown. In addition to lymphoma, over-expression associates with poor prognosis in prostate and uterine cancer (Saegusa et al. 1995, Bubendorf et al. 1996), and in contrast, with good prognosis in ovarian, breast, oesophageal, and lung cancer, and in melanoma (Pezzella et al. 1993, Joensuu et al. 1994, Ofner et al. 1995, Tron et al. 1995, Herod et al. 1996, Ohbu et al. 1997, Martin et al. 2003). Differing roles of bcl-2 in various neoplasia could be explained by tissue-specific expression of bcl-2-binding proteins which determine the functional activity of bcl-2 (Boise et al. 1993). Moreover, it has been speculated that bcl-2 may retard the rate of proliferation in solid tumours, resulting in slow-growing tumours with a favourable outcome.

Bcl-2 and thyroid. Cytoplasmic bcl-2 staining is seen in normal thyroid epithelium. In thyroid carcinomas, bcl-2 expression correlates with degree of cell differentiation (Pilotti et al. 1994, Pollina et al. 1996, Moore et al. 1998, Puglisi et al. 2000). Bcl-2 appears to be associated with less aggressive behaviour as well differentiated, and poorly differentiated thyroid tumours express bcl-2; in cases of undifferentiated carcinomas, however, its expression is low. No correlation between bcl-2 and prognostic factors has appeared in papillary thyroid cancer, but in medullary thyroid carcinoma, lack of bcl-2 expression is an independent predictor of worse prognosis (Viale et al. 1995).

VEGF-C: angiogenesis

Vascular endothelial growth factor-C (VEGF-C) is a member of the VEGF family of polypeptide growth factors which play key roles in angiogenesis and lymphangiogenesis. VEGF-C, found in 1996 in human prostate cell lines (Joukov et al. 1996), binds vascular endothelial growth factor receptor –3

(VEGFR-3), which is specifically expressed on lymphatic vessels and stimulates lymphangiogenesis (Alitalo and Carmeliet 2002, Stacker et al. 2002). VEGF-C shows high expression in cancers metastasizing to the lymph nodes (Jeltsch et al. 1997, Mandriota et al. 2001) including breast, lung, colorectal, pancreatic, prostate, oesophageal, and head and neck cancers (Kurebayashi et al. 1999, Tsurusaki et al. 1999, Niki et al. 2000, George et al. 2001, Kitadai et al. 2001, P et al. 2001, Skobe et al. 2001, Tang et al. 2001, Duff et al. 2003, Hanrahan et al. 2003, Homer et al. 2003, Onogawa et al. 2004).

VEGF-C and the thyroid. Papillary thyroid cancer expresses VEGF-C (Bunone et al. 1999, Fellmer et al. 1999, Shushanov et al. 2000, Tanaka et al. 2002, Tanaka et al. 2002a, Hung et al. 2003). Some of these studies have demonstrated a correlation between lymph node metastasis and high VEGF-C (Bunone et al. 1999, Tanaka et al. 2002a, Tanaka et al. 2002b), but no correlation exists with other clinical parameters. Tanaka et al. (2002b) studied expression of VEGF-C immunohistochemically in 25 patients with recurrent PTC with distant metastases and in 50 with nonrecurrent PTC. VEGF-C correlated with lymph node involvement, but not with other clinical factors. Bunone et al. (1999) had a smaller number of PTC patients (68 thyroid tumours including 26 PTCs) and showed that of 26 PTC patients, 24 had lymph node metastases, and of those, 21 expressed VEGF-C, as analyzed by RT-PCR and immunohistochemistry. Another RT-PCR analysis showed similar results: 48 of 50 PTCs expressed VEGF-C, and patients with nodal involvement showed higher expression of VEGF-C mRNA (Tanaka et al. 2002a).

MMP-2: invasion and metastasis

Matrix metalloproteinases (MMPs) form a continuously growing family of zinc-dependent endopeptidases that degrade extracellular matrix components and facilitate invasion (Egeblad and Werb 2002). MMPs are classified into subfamilies based on their substrate preferences. Until now, 21 members of the MMP gene family have been discovered (Vihinen and Kähäri 2002). MMP-2 (Gelatinase A, 72 kDa type IV collagenase) is a metalloproteinase that specifically cleaves type IV collagen, the major structural component of basement membrane (Liotta et al. 1979). It is therefore closely associated with the malignant behaviour of tumour cells. MMP-2 expression is linked to invasiveness in several human neoplasms including breast, colon, ovarian, lung, prostate, kidney, bladder, hepatocellular carcinomas and melanoma (Sier et al. 1996, Young et al. 1996, Talvensaari-Mattila et al. 1998, Kitamura et al. 1999, Ogata et al. 1999, Hofmann et al. 2000, Chan et al. 2001, Ross et al. 2003, Vasala et al. 2003). The MMP-2 has been localized immunohistochemically to tumour cells and to stromal cells (Grigioni et al. 1994).

MMP-2 and thyroid. In 1992, Campo et al. demonstrated that malignant thyroid

tumours including PTC express MMP-2, and in PTC staining was diffuse and intracytoplasmic. More aggressive variants like the tall cell variant of PTC and invasive follicular carcinoma show stronger expression than do low-grade tumours (Campo et al. 1992). In 1996, results of Zedenius et al. supported these findings, however, the number of papillary carcinomas was low. They demonstrated that four of five PTC showed a strong hybridization signal and in all cases the signal for MMP-2 was seen in the stroma surrounding invading tumour cells. Later, Nakamura et al. (1999) demonstrated that among seven different MMPs, only MMP-2 production was higher than normal in PTC tissue. MMP-2 was immunolocalized in carcinoma cells and stromal fibroblasts. Maeta et al. (2001) showed in a large number of cases (n=86 PTC) that expression of MMP-2 was associated with large tumour size, high stage, high intrathyroidal invasion, capsular invasion, high vascular invasion, and lymph node metastasis. Immunolocalization of MMP-2 was mainly to tumour cells and less frequently to stromal mesenchymal cells.

COX-2: invasion and metastasis

Cyclooxygenase-2 (COX-2) is the key enzyme in the conversion of arachidonic acid (AA) into prostanoids. Two COX isoforms have been cloned. COX-1 is produced constitutively, while COX-2 is induced rapidly in response to growth factors, hormones, cytokines, and tumour promoters. These inflammatory mediators are thought to play a critical role in the initiation and maintenance of cancer cell survival and growth. COX-2 is up-regulated in several malignant epithelial neoplasms and it plays an important role in carcinogenesis. Expression of COX-2 correlates with poor prognosis in colorectal (Sheehan et al. 1999), breast (Ristimäki et al. 2002), oesophageal (Buskens et al. 2002), cervical (Gaffney et al. 2001), and ovarian cancer (Denkert et al. 2002, Erkinheimo et al. 2004). In colon carcinogenesis, the COX-2 enzyme is the rate-limiting step. Selective COX-2 inhibitors have been shown to prevent intestinal tumour formation and inhibit colon carcinoma growth in animal models (Oshima et al. 1996, Sheng et al. 1997). COX-2 supports tumour growth by inhibiting apoptosis (Tsujii and DuBois 1995), stimulating angiogenesis (Tsujii et al. 1998, Masferrer et al. 2000), and increasing tumour invasion and metastatic potential (Tsujii et al. 1997, Kakiuchi et al. 2002, Niki et al. 2002).

COX-2 and the thyroid. Recent results indicate that COX-2 expression is upregulated also in human thyroid carcinoma (Cornetta et al. 2002, Nose et al. 2002, Specht et al. 2002). Association of COX-2 and thyroid disease was first reported by Di Paola et al. in 1997. They demonstrated that indometacin, a cyclooxygenase inhibitor, inhibits thyroid cell proliferation in patients with Graves` disease. Based on their findings, they suggested that thyroid growth might depend on activation of the cyclooxygenase pathway. In 1999, Smith et al. examined COX-2 expression in thyroid cancer and found that normal,

autoimmune and neoplastic human thyroid epithelium express COX-2. In addition, they demonstrated COX-2 expression in the human thyrocyte cell line KAT-50. In contrast, Berg et al. (2000) reported that COX-2 gene expression is inducible only upon stimulation with cytokines, and the normal thyroid does not express COX-2. The pro-inflammatory cytokines, interleukin-1β (IL-1β) and Tumour Necrosis Factor-α (TNF-α), raised COX-2 expression in a human thyroid cell line. Cornetta et al. (2002) supported these results, showing that COX-2 is expressed in papillary and follicular thyroid tumours and in Hashimoto`s thyroiditis, but not in normal thyroid or in goiter. Interestingly, they also showed that anaplastic thyroid carcinomas do not express COX-2. They suggested that COX-2 may be involved in the progression of normal thyroid to differentiated carcinoma, whereas poorly differentiated tumours do not require COX-2 for growth. Recently, it was shown that follicular adenomas, papillary and follicular carcinomas and chronic lymphocytic thyroiditis over-express COX-2 (Nose et al. 2002). Normal thyroid epithelium showed weak expression of COX-2. Specht et al. (2002) also demonstrated that COX-2 is up-regulated in human papillary thyroid cancer, but not in benign thyroid nodules; COX-2 mRNA was detected in fine needle aspiration, and they suggested that COX-2 could serve as a marker of cancer.

COX-2 inhibitors and cancer. Although COX-2 inhibitors were designed to relieve pain, several reports suggest that they may offer some protection against cancer, especially colon cancer. The first two trials were promising. After 6 months, the patients receiving 400 mg of celecoxib twice a day had a 28% reduction in the mean number of colorectal polyps as compared with a reduction of 4.5% in the placebo group (Steinbach et al. 2000). In another study, at 9 months, the polyp number in the rofecoxib group decreased by 6.8% from the baseline values, whereas that in the placebo group increased by 3.1%; polyp size was also reduced in the rofecoxib group as compared with the placebo group (Higuchi et al. 2003). These two studies reported no harmful side effects. Several clinical trials are now underway to determine whether COX-2 inhibitors have chemopreventive effects. Just recently, serious cardiovascular side effects were reported in two large trials (Bresalier et al. 2005, Solomon et al. 2005). Due to this, both rofecoxib and valdecoxib were removed from the market. It is not yet known whether these side effects seen with rofecoxib and valdecoxib are a general effect of COX-2 inhibitors or a specific effect of these two drugs.

Other markers

A number of other markers have been studied to confirm diagnosis and to predict prognosis of papillary thyroid carcinoma. Galectin-3 and mesothelioma antibody (HBME-1) have proven to be sensitive diagnostic markers for PTC (Weber et al. 2004, Cvejic et al. 2005, de Matos et al. 2005). Promising prognostic tumour markers include cyclin D1, p27, retinoblastoma gene product (pRb), and E-cadherin. Overexpression of cyclin D1 and underexpression of p27 have been shown to be predictors of lymph node metastases in PTC (Khoo et al. 2002, Lantsov et al. 2005). Ito et al. (2005) demonstrated that papillary microcarcinoma cases of clinically apparent metastases showed increased cyclin D1, pRb and Ki-67, together with decreased p27. Lack of E-cadherin expression was demonstrated to be an adverse prognostic factor (von Wasielewski et al. 1997). Moreover, loss of E-cadherin immunostaining has been correlated with development of distant metastases and lymph node metastases (Scheumman et al. 1995, Naito et al. 2001).

6.
Aims of the Study

The purpose of this study was to find clinical or biological markers to predict the outcome of the individual PTC patient.

The specific aims of the present study were:

- To analyze all patients who underwent surgery for PTC at HUCH between 1957 and 1996

- To compare TNM, AMES, and MACIS staging systems in predicting the prognosis of PTC

- To find any possible association between increasing age and higher incidence of poor prognostic factors by comparing expression of COX-2, MMP-2, bcl-2, Ki-67, p21, and VEGF-C in young and older PTC patients

- To find markers to predict tumour behaviour by comparing the expression of COX-2, MMP-2, bcl-2, Ki-67, p21, and VEGF-C in non-aggressive and aggressive tumours

7.
Patients and Methods

7.1 Patients

Study I
This series comprised 495 patients undergoing surgery for PTC at Helsinki University Central Hospital between 1967 and 1994. Medical records were studied retrospectively. All patients were followed up until the end of November 1999. Any death certificates were available from the Population Registry of Finland.

Studies II, III, and V
To compare young and older patients, we analyzed 259 consecutive PTC-surgery patients at HUCH during a 10-year period (1987-1996). The cut-off age varies among scoring systems, but all cut-off ages are estimates. We selected all patients under age 35 for a young patient group and all patients over 55 for an older group (cut-off age of TNM classification +/- 10 years). The eventual total chosen with adequate clinical data was 108, and the tumour specimens of these patients were studied immunohistochemically.

Study IV
This series comprised 601 consecutive patients who underwent surgery for PTC at HUCH between 1973 and 1996. Clinical data were collected retrospectively. Clinical outcome was checked at the end of December 2002, and survival data at the end of November 2003. Survival data and cause of death were obtained from the Population Registry of Finland. Mean follow-up was 14.5 years (range 5.0-29.3) for those last known to be alive, and 10.1 (range 0.1-28.0) for the deceased.

From this initial series we selected 36 matched pairs for immunohistochemical

study to compare non-aggressive and aggressive tumours. All tumours were of the usual type. Each individual pair had similar clinical characteristics at diagnosis, but during follow-up their disease behaved differently, one patient recovered completely after primary surgery, and the other suffered from aggressive disease (aggressive disease vs completely-recovered pair). These pairs were matched by age (within 10 years), gender, timing of primary operation (within 10 years), T stage, N stage, tumour size, and primary RAI ablation (yes/no). Disease was defined as aggressive if a need existed for more than three radioactive iodine ablations (with recurrences detected with scintigraphy), surgery for recurrent disease more than once within 6 months after the primary operation, development of distant metastases 6 months after the primary operation, primary distant metastases, or death from PTC.

Non-published data

In addition we analyzed clinical data of patients who underwent surgery for PTC at HUCH between 1957 and 1972. We excluded these patients from Studies II to V, because the method of fixation of samples changed in 1973, and samples from the previous period were unsuitable for immunohistochemistry. The other reason for exclusion was that only after 1973 the standard treatment for PTC at HUCH was total thyroidectomy.

7.2 Methods

Immunohistochemistry (Studies II-V)

All tissue specimens were obtained from PTC operations performed at HUCH from 1973 to 1996. Formalin-fixed, paraffin-embedded archival tissue blocks were freshly cut into 4-μm sections onto slides and dried for 12 to 24 hours at 37°C. Freshly cut sections were deparaffinized in xylene and rehydrated through graded alcohol and deionized water. Then sections were heated in a microwave oven in 0.3% citrate buffer (pH 6.0) for 4 x 5 min, and washed with phosphate-buffered saline (PBS) for 2 x 5 min. Endogenous peroxidase activity of the tissues was inactivated by 30 minutes incubation in methanol containing 1.6% hydrogen peroxide followed by PBS wash for 3 x 5 min. To block nonspecific binding sites, the sections were then treated with normal horse serum for 15 minutes. The antibodies for COX-2, MMP-2, bcl-2, Ki-67, p21, and VEGF-C were used for overnight incubation (Table 3). After overnight incubation with the primary antibody, the sections were first reacted for 30 min with biotinylated anti-mouse IgG diluted in PBS containing 1% normal horse serum or anti-rabbit IgG diluted in PBS containing 1% normal goat serum, and then for 30 min in avidin-biotinylated peroxidase complex (Vectastain ABC Kits, Vector Laboratories, Inc., Burlingame, CA, USA). Between each step in the staining procedure, sections were washed three times with PBS for 5 minutes. Staining was visualized with 3-amino-9-ethyl-carbazole (A5754; Sigma, St. Louis, MO, USA), 0.2 mg/ml in 0.5 M acetate buffer containing 0.03% hydrogen peroxide (pH 5.0) for 15 minutes. Finally, the sections were washed thoroughly in tap water, counterstained lightly with Meyer´s haematoxylin, washed, and mounted in aqueous mounting media (Aquamount; BDH, Poole, UK).

Formalin-fixed, paraffin-embedded specimens of colorectal cancer known to be positive for COX-2, MMP-2, Bcl-2, and p21 served as positive controls. Specimens of breast cancer served as positive controls for VEGF-C and Ki-67. As the negative control we used PBS instead of primary antibody.

Table 3 Antibodies, their dilutions and sources.

Antibody	Dilution	Manufacturer	Study
COX-2 Mouse anti-human monoclonal antibody	1:200	160112, Cayman Chemical, Ann Arbor, MI, USA	II, IV
MMP-2 mouse monoclonal antibody antibody	1:700	Neomarkers, Fremont, CA, USA	II, IV
Bcl-2 mouse anti-human monoclonal antibody	1:400	Dako, clone 124, Glostrup, Denmark	III, IV
Ki-67 rabbit polyclonal antibody	1:500	Dako	III, IV
P21 mouse monoclonal antibody	1:200	Novo Castra Laboratories, Newcastle-Upon-Tyne, UK	III, IV
VEGF-C rabbit polyclonal antibody	1:500	Zymed Laboratories, Inc., South San Francisco, CA, USA	IV, V

Scoring

The scoring of COX-2 was performed independently and in a blinded manner by two investigators (AR and PS), and when scores were discrepant, scoring was determined after discussion. Scoring of MMP-2, VEGF-C, Bcl-2, Ki-67, and p21 was performed by pathologist (SN) and by an investigator (PS) blinded to the clinical data.

COX-2. COX-2 immunoreactivity was assessed by intensity of staining and percentage of positive area. Intensity of the staining was scored 0 to 3 (absent; mild; moderate; strong), and the area of positivity was estimated as percentage of the total area of the tumour (under 10%, between 10 and 49%, and over 50%). The final score was a combination of these two variables as follows. Score 0: negative staining or intensity 1 under 10%; score 1: intensity 1 between 10 and 100% or intensity 2 to 3 under 10%; score 2: intensity 2 over 10% or intensity 3 under 50%; score 3: intensity 3 area over 50%. Positive expression of COX-2 was defined as intensity 2 to 3 seen over 10% of the total area (scores 2-3).

MMP-2, bcl-2, VEGF-C. Cytoplasmic immunoreactivity for MMP-2, bcl-2, and VEGF-C was evaluated according to extent of staining. Positive expression was defined as more than 10% of the tumour area stained. Epithelial expression of

MMP-2 (cancer cell) and stromal staining were evaluated separately.
Ki-67, p21. Nuclear immunostaining for Ki-67 and p21 was estimated, and immunoreactivity was expressed as the percentage of positive tumour cell nuclei per tumour; p21 expression was graded as negative (≤ 5%) or positive (> 5% of tumour cells stained) and the cut-off value for Ki-67 expression was 1%. In the work of Räty et al. (2002), findings with a grid-counting and with simple estimation of percentage of stained cells (Ki-67) were similar. As expression of Ki-67 in differentiated thyroid tumours is low (Wallin et al. 1992, Katoh et al. 1995, Basolo et al. 1997, Okayasu et al. 1998, Tallini et al. 1999, Yoshida et al. 1999, Saiz et al. 2002), we defined the cut-off value for positivity as 1%.

Cell culture (Study V)

The cells were grown in RPMI reagent supplemented with 10% fetal calf serum (Promo Cell, Heidelberg, Germany), 2mM L-glutamine, and antibiotics (BioWhittager, Verviers, Belgium). Cells were incubated with phorbol 12-myristate 13-acetate (PMA) (10 ng/ml; Sigma), NS-389 (5-10 μM; Cayman), arachidonic acid (10 μM; Sigma) for selected time periods.

PGE_2 and VEGF-C EIA. Prostaglandin E2 (PGE_2) levels were measured from cell culture medium by Enzyme Immuno Assay (EIA) from Cayman Chemical Co and VEGF-C production by EIA from Zymed (San Francisco, CA, USA) according to manufacturer´s instructions.

RT-PCR. Total RNA from NPA cells was isolated by use of Trizol Reagents (Invitrogen, Carlsbad, CA, USA) and RNA (1 μg) was converted to cDNA with Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA), RNasin (Promega), 2'deoxynucleoside 5'triphosphates (Amersham Pharmacia Biotech Inc, Buckinghamshire, England), and random primers (Invitrogen). COX-2 and VEGF-C mRNA expression was quantified by using TaqMan real-time RT-PCR and Gene Amp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA). VEGF-C primers and TaqMan probe sequences were by Hung et al. (2003) and were purchased from Sigma Chemical Co. Human TATA-binding protein (TBP) served as an endogenous control to normalize the expression of VEGF-C and COX-2 mRNA levels. COX-2 and TBP Assays-on-Demand were purchased from Applied Biosystems. Each reaction was run in triplicate and relative quantitation was performed by the comparative CT method (Applied Biosystems User Bulletin #2).

7.3 Statistical analysis

Statistical analysis was performed with SPSS software (SPSS Inc., Chicago, IL, USA). A p-value less than 0.05 was regarded as statistically significant. The following test were used:

I Carcinoma-specific survival curves for each classification systems were calculated by the product-limit method, and log-rank test values served in comparisons. The proportion of variance in the outcome explained by each staging classification was calculated by consideration of each classification separately as a linearly increasing prognostic factor in the Cox proportional hazards regression model.

II, **III**, **V** The chi-square test (or Fisher´s exact test when applicable).

IV The chi-square test (or Fisher´s exact test when applicable), the Kaplan-Meier method, and the log-rank test.

8.
Results

The individual publications are referred to by their Roman numerals. For additional tables and figures, see each individual article.

8.1 Classification systems (Study I)

We compared AMES, MACIS, and TNM classifications to study which classification is the most reliable in predicting PTC patient prognosis.
According to these classification systems, the low-risk group included 85.8% of patients (n= 425) in the TNM classification, 89.7% (n= 444) in the AMES, and 89.9% (n= 445) in the MACIS system. According to the TNM system, there were three carcinoma deaths among low-risk Stage I patients. According to the MACIS system, six patients with scores up to 5.99 (low-risk) died of thyroid carcinoma. In the AMES system, as many as 12 fatal cancers occurred in the low-risk group.
The TNM classification showed the highest mortality ratio between low-risk and high-risk patients at 5, 10, and 15 years after diagnosis. It was superior to the other staging systems when compared by the chi-square value of the log rank test, both in four prognostic groups and in comparison of low-risk and high-risk patients. Cancer-specific survival seemed to be most reliably predicted by the TNM classification.
In this series of 495 PTC patients, the most significant predictors of survival were presence of distant metastases ($p < 0.0001$), of nodal metastases ($p = 0.004$), age ($p = 0.0087$), and diameter of the primary tumour ($p = 0.0189$) in patients over 45 years old. In the subgroup of patients under 45, neither distant metastases, nor nodal metastases nor diameter of primary tumour were significant predictors of survival.

8.2 Outcome of 601 patients (Study IV)

Clinical characteristics of these 601 patients are shown in Table 4. Mean age was 45 years, and female to male ratio 3.8. Of the 601 patients, 479 (80%) recovered completely after primary surgery and had no problems during follow-up. During follow-up, tumours recurred in a total of 91 (15%) patients; 33 patients (5%) experienced distant metastases; 23 patients (4%) died of PTC.

In univariate survival analysis, age over 45, tumour size > 4 cm, extrathyroidal extension of tumour, nodal metastases, distant metastases, and stage IV disease significantly correlated with survival. Gender was not a significant prognostic factor.

When comparing patients under and over 45 years, the latter had more extrathyroidal disease, distant metastases, and their tumours were more aggressive during follow-up because each patient (n = 23) who died of PTC was over 45 at diagnosis. Lymph node metastases seemed to be more frequent among the patients under 45.

Table 4 Clinical characteristics of 601 papillary thyroid cancer (PTC) patients, with Tumour-Node-Metastases (TNM) stage according to the International Union Against Cancer (UICC) TNM classification of 1997.

Patient Characteristics	All patients (n= 601)	<45 years (n= 313)	>45 years (n= 288)
Mean age (range)	45 (5-83)		
Women	477	251	226
Men	124	62	62
Female/male ratio	3.8:1	4:1	3.6:1
	n (%)	**n (%)**	**n (%)**
Tumour size (cm)			
≤1	170 (28)	92 (29)	78 (27)
1-4	360 (60)	189 (60)	171 (60)
> 4	68 (12)	31 (10)	37 (13)
Extent of tumour			
T1 (≤ 10 mm)	165 (27)	91 (29)	74 (26)
T2 (> 10 mm, ≤ 40 mm)	310 (52)	170 (54)	140 (49)
T3 (> 40 mm)	48 (8)	29 (9)	19 (7)
T4 (beyond the capsule)	75 (13)	22 (7)	53 (19)
Nodal metastases	118 (20)	70 (22)	47 (16)
Distant metastases	13 (2)	3 (1)	10 (3)
TNM Stage			
I	381 (63)	311 (99)	70 (24)
II	136 (23)	2 (1)	134 (47)
III	75 (12)		75 (26)
IV	9 (2)		9 (3)
Neck recurrence	89 (15)	50 (16)	39 (14)
Distant recurrence	20 (3)	1 (0.3)	19 (7)
Recurrence	91 (15)	50 (16)	41 (14)
Death from PTC	23 (4)	0	23 (8)

Fatal cases (Tables 5-6). Of 601 patients, 23 died of PTC (4%). All of them were over 45. Mean age at initial treatment was 67.3 years and mean survival time from initial treatment to death 79 months. Total thyroidectomy was performed for 20 patients (87%) and primary RAI ablation was given for 17 patients (74%). Macroscopically curative surgery was carried out for 15 patients (65%). Primary distant metastases were present in 8 (35%), 3 of whom developed more distant metastases at other sites, and 13 new patients developed distant metastases during follow-up (56%). Histologically, of the 23, 22 tumours were of the usual type, and one was tall cell variant. In addition, one patient was diagnosed with anaplastic carcinoma at the time of death. Lungs were the commonest site of distant metastases; at the time of death, 16 patients (70%) had metastases in the lungs. Respiratory problems were the most common cause of death: widely spread multiple pulmonary metastases were responsible for death in 11 patients and airway obstruction due to local disease in 3. Extensive bone metastases were responsible for death in 4 patients and one developed liver coma due to liver metastases. The last 4 were in poor general health as a consequence of multi-organ metastases and specific cause of death could not be determined.

Table 5 Details of 23 patients dying of papillary thyroid cancer.

Patient Characteristics	n (%)
n of death	23/ 601 (4%)
Mean age at presentation, yrs (range)	67.3 (49-81)
Female/male ratio	2.3:1
Mean survival time, months (range)	79 (3-218)
Extent of tumour	
T1 (≤ 10 mm)	1 (4)
T2 (> 10 mm, ≤ 40 mm)	6 (26)
T3 (> 40 mm)	4 (17)
T4 (beyond the capsule)	12 (52)
Mean size, cm (range)	4,3 (1-10)
Nodal metastases	12 (52)
Distant metastases	8 (35)
Secondary distant metastases	13 (57)
Neck recurrence	16 (70)

Table 6 Site of primary and secondary distant metastasis in papillary thyroid cancer patients with fatal disease (n=23).

Site	Primary (n= 8)	Secondary (n= 13)	Total n (%)
Lung	5	10	15 (65)
Bone	3	5	8 (35)
Mediastinum	1	1	2 (9)
Liver	2	1	3 (13)
Kidney	1	0	1 (4)

8.3 Non-published data

We analyzed clinical data from patients who underwent surgery at HUCH from 1957 to 1972. These patients were excluded from immunohistochemical studies because the histological samples of the archives had been fixed in non-buffered formalin and the specimens were not reliable for immunohistochemical detection of antigens. In addition, treatment for PTC has changed over the years; since 1973 total thyroidectomy has been the standard method. Death from PTC has declined (Table 7).

Table 7 Death from papillary thyroid cancer (PTC) and treatment strategies between 1957 and 1996 at Helsinki University Central Hospital.

Operations, years	Number of patients	Deaths from PTC (%)	Total thyroid-ectomies (%)	Radioactive iodine ablations (%)
1957-1969	50	16 (32)	7 (14)	41 (82)
1970-1979	167	12 (7)	123 (74)	59 (35)
1980-1989	227	6 (2.6)	205 (90)	143 (63)
1990-1996	238	5 (2.1)	217 (91)	206 (87)

8.4 Immunohistochemistry (Studies II-V)

Of our two patient groups in immunohistochemistry, one included 108 patients selected by age (under 35 and over 55 years), and the other 36 matched pairs selected by behaviour of tumour (aggressive vs. completely recovered). Clinical characteristics of these four groups are shown in Table 8.

Table 8 Clinical characteristics of papillary thyroid cancer patients in four immunohistochemical groups. Tumour-Node-Metastases (TNM) stage according to International Union Against Cancer (UICC) TNM classification of 1997.

Patient Characteristics at presentation	Under 35 n (%)	Over 55 n (%)	36 matched pairs n (%)	
			Aggressive disease	Completely recovered
Study	**II, III, V**		**IV**	
n patients	59	49	36	36
Mean age	27.8	64	47.7	46.2
Women	48	38	25	27
Men	11	11	11	9
Female/male ratio	4.3:1	3.5:1	2.3:1	3:1
Extent of tumour*				
T1 (≤ 10 mm)	11 (19)	14 (29)	4 (11)	4 (11)
T2 (> 10 mm, ≤ 40 mm)	37 (63)	24 (49)	18 (50)	18 (50)
T3 (> 40 mm)	6 (10)	1 (2)	7 (19.5)	7 (19.5)
T4 (beyond the capsule)	5 (8)	10 (20)	7 (19.5)	7 (19.5)
Nodal metastases	20 (34)	7 (14)	12 (33)	12 (33)
Distant metastases	0	0	6	0
TNM Stage				
I	59 (100)	14 (29)	17 (47)	18 (50)
II	0	22 (45)	8 (22)	9 (25)
III	0	13 (26)	7 (20)	9 (25)
IV	0	0	4 (11)	0

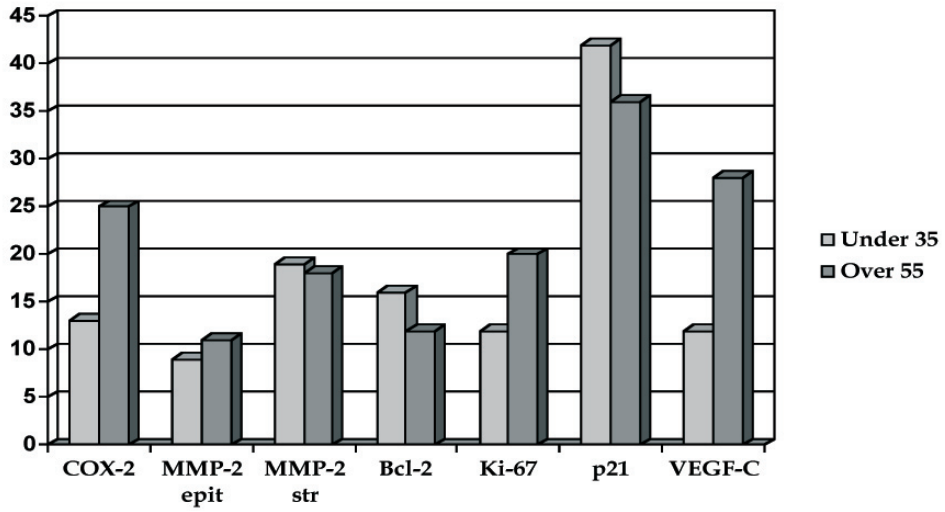
Studies II, III, V

Expression of tumour markers was studied in PTC in two age groups: under 35 and over 55 (Table 9 and Figure 2).

Table 9 Number of patients with positive expression of COX-2, MMP-2, bcl-2, ki-67, p21, and VEGF-C in papillary thyroid cancer in patients under 35 and over 55 at diagnosis.

	All patients n (%)	Under 35 n (%)	Over 55 n (%)	p-value young vs old
COX-2	38 (35)	13 (22)	25 (51)	0.002
Epithelial MMP-2	20 (19)	9 (15)	11 (22)	NS
Stromal MMP-2	37 (34)	19 (32)	18 (36)	NS
bcl-2	28 (26)	16 (27)	12 (24)	NS
Ki-67	32 (30)	12 (20)	20 (42)	0.017
p21	78 (72)	42 (71)	36 (74)	NS
VEGF-C	40 (38)	12 (21)	28 (57)	< 0.001

Figure 2. Number of patients with positive expression of COX-2, MMP-2, bcl-2, ki-67, p21, and VEGF-C in papillary thyroid cancer in patients under 35 and over 55 at diagnosis



COX-2. COX-2 expression was higher in older patients (p= 0.002) and correlated with high stage (p= 0.031), but not with other clinical parameters.
The staining pattern of COX-2 in tumour cells was cytoplasmic. Nonneoplastic thyroid epithelium adjacent to carcinoma was negative or only weakly positive. Most positive tumours were only moderately positive (33 of 38). In five patients, COX-2 expression was strong. All these patients had tumour sized 1 cm to 4 cm, and no lymph node or distant metastases. They were aged between 21 and 79, and had no recurrences during follow-up.

MMP-2. Stromal expression of MMP-2 correlated with the extent of primary tumour (p= 0.038), and in older patients with stage (p= 0.039).
Expression was higher in tumours sized 1 to 4cm and in extrathyroidal tumours and at stages II and III. This series included no stage IV tumours. Epithelial expression of MMP-2 did not correlate with clinical parameters.
MMP-2 was immunolocalized both to the cancer cells and stromal cells. Stromal staining was stronger: 37% of tumours expressed stromal MMP-2, but only 20% showed epithelial expression.

Bcl-2. Expression of Bcl-2 correlated neither with clinical parameters nor with recurrent disease.
The staining pattern was cytoplasmic. Of 28 positive cases, 19 were moderately positive and 9 strongly.

Ki-67. Ki-67 expression was higher in older patients (p=0.017) and correlated with stage (p=0.013), but not with other clinical parameters.
In the 32 Ki-67 -positive tumours, positive nuclear immunostaining was usually low (<10%), except in one tumour, which showed over 50% expression. This patient was a 30-year-old woman with a T2N0M0 stage tumour, and she had had no recurrences during follow-up.
Tumour-associated lymphocytes seemed to be positive for Ki-67. However, we did not collect data systematically from these observations.

p21. Expression of p21 was associated with large tumours (p= 0.010) and with tumours extending beyond the thyroid capsule (p= 0.007).
Positive nuclear immunostaining of p21 (>5%) occurred in as many as 78 of 108 (72%) tumours. The percentage of p21-positive cells ranged from 0% to 60%, with a median value of 14%.

VEGF-C. Expression of VEGF-C was higher in older patients (p < 0.001) and in older patients with lymph node metastases (p = 0.001).
Staining pattern was cytoplasmic. Lymph node metastases were present in 26 patients, with VEGF-C expression positive in 8. Among these lymph node-positive patients, VEGF-C immunoreactivity appeared in only 2 of 19 younger and in 6 of 7 older patients. Thus, most older node-positive patients were also VEGF-C-

positive, and conversely, most young patients with lymph node metastases did not express VEGF-C.
Of the 106, we classified 5 patients as at high-risk. These patients either died of cancer or developed distant metastases during follow-up. All these high-risk patients were older, and 4 of them expressed VEGF-C.

Associations between markers
COX-2 expression correlated strongly with VEGF-C expression ($p < 0.001$), and low expression of COX-2 correlated with low epithelial expression of MMP-2 ($p=0.047$). Stromal expression of MMP-2 did not correlate with expression of COX-2.
Expression of bcl-2 was associated with expression of p21 ($p=0.019$) with no strong correlation, as most tumours were bcl-2-negative (80 of 108, 74%) and p21-positive (78 of 108, 72%). Positive expression of Bcl-2 was associated with positive expression of p21 and conversely, negative expression of p21 correlated with negative expression of bcl-2.

Study IV

In Study IV, we had 36 matched pairs (aggressive disease versus completely recovered). No marker showed any significant difference between groups.
Of 72 tumours, 31% expressed COX-2 (aggressive 31%; completely recovered 28%), 4% epithelial MMP-2 (6%; 3%), and 21% expressed stromal MMP-2 (19%; 22%), 28% expressed VEGF-C (31%; 25%), 35% Bcl-2 (39%; 31%), 14% expressed Ki67 (11%; 17%), and 42% p21 (36%,;47%). Expression of VEGF-C and bcl-2 was slightly higher in the aggressive group. In contrast, Ki-67 and p21 showed slightly higher expression in the completely recovered group.
Expression of COX-2 and VEGF-C seemed to be higher in older patients, confirming the results of Studies II and V. COX-2 was expressed in 15 of 19 patients over 45, but in only 7 of 17 patients under 45. Numbers for VEGF-C were, correspondingly, 13 of 19 and 7 of 17.

Histology (Studies II-V)

In our series of 108, were three PTC variants, two diffuse sclerosing variants, and one oncocytic variant (Hürthle cell PTC). In our series of 72 patients, all tumours were of the usual type.
All three histologic variants were women under 35 and they were staged T2N0M0, T3N1M0, and T1N0M0. Two of them were cured by surgery, and one suffered from local recurrences (diffuse sclerosing variant T3N1M0). Expression of tumour markers in these patients is shown in Table 10.

Table 10 Expression of COX-2, MMP-2, Bcl-2, Ki-67, p21, and VEGF-C in two diffuse sclerosing variant and in one oncocytic variant. o = negative, + = positive expression.

Histologic variants	COX-2	MMP-2 epithelial	MMP-2 Stromal	Bcl-2	Ki-67	P21	VEGF-C
Diffuse sclerosing	+	o	+	o	+	+	o
Diffuse sclerosing	+	o	o	o	+	+	o
Oncocytic variant	o	+	+	o	+	+	o

Fatal cases (Studies II-V)

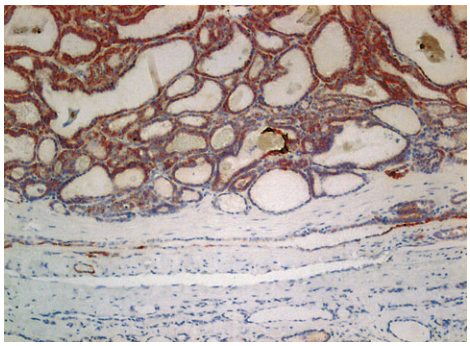
Combined data from the immunohistochemical series of 108 patients and of 72 patients included 13 fatal cases (Table 11). Half of the fatal cases expressed COX-2 and VEGF-C; expression of other markers was lower.

Table 11 Expression of COX-2, MMP-2, Bcl-2, Ki-67, p21, and VEGF-C in 13 fatal cases. o = negative, + = positive expression.

	COX-2	MMP-2 epithelial	MMP-2 stromal	Bcl-2	Ki-67	P21	VEGF-C
1	o	o	o	o	o	o	o
2	+	o	o	o	+	o	o
3	+	o	o	o	o	o	o
4	o	o	o	+	o	o	+
5	+	o	o	+	o	o	+
6	+	o	o	o	o	o	o
7	o	o	o	o	o	o	o
8	o	o	o	+	o	o	o
9	+	o	o	o	o	o	+
10	+	o	o	+	o	+	+
11	o	o	+	+	o	+	o
12	o	o	o	o	+	+	+
13	+	o	+	o	+	o	+
Total n (%)	7 (54)	o	2 (15)	5 (38)	3 (23)	3 (23)	6 (46)

8.5 Cell culture (Study V)

We showed that NPA cells expressed VEGF-C and COX-2 mRNA and that their expression was induced by PMA. At the protein level, after AA incubation, PMA induced PGE_2 production, and the COX-2 selective inhibitor NS-398 blocked both basal and PMA-induced PGE_2 synthesis. Moreover, PMA raised VEGF-C protein production after 24 h incubation by 2.7-fold, but NS-398 reduced neither basal nor PMA-induced VEGF-C expression.



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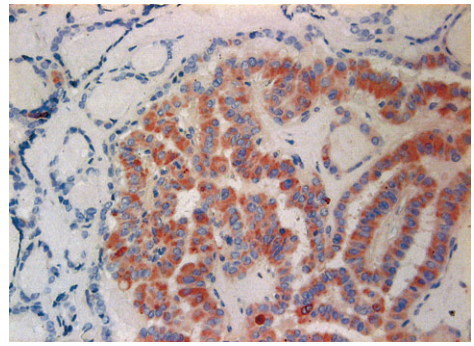

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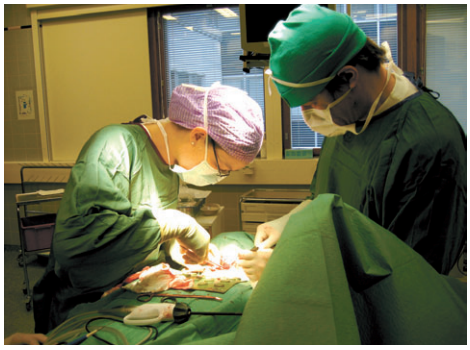

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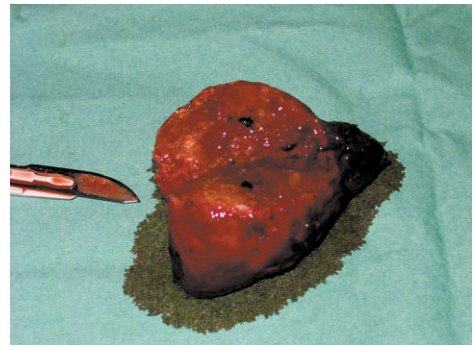

D.

Figure 3. A-B. Immuhohistochemical staining of COX-2 in papillary thyroid carcinoma. C. Senior and junior surgeon performing thyroidectomy for patient with papillary thyroid cancer. D. 3-cm tumour in the left thyroid lobe.

9. Discussion

9.1 Classification systems

In our series of 495 patients, the TNM classification was the most reliable for predicting outcome of individual patients. Thus, we agree with another study that showed no statistically significant superiority of any system over the TNM classification (Brierley et al. 1997). TNM classification is the only classification system that does not ignore the effect of lymph node metastases on survival, and we assume that perhaps this fact made it superior to others. In the subgroup of patients over 45 years old, the most significant predictors of survival were presence of distant metastases, nodal metastases, age, and diameter of the primary tumour, whereas in patients under 45, those factors were not significant predictors. Similarly, the TNM system classifies all patients under 45 into the low-risk category (Stage I-II), despite lymph node metastases or distant metastases. In patients over 45, however, the TNM classification takes lymph node metastases into account in predicting prognosis.

According to the MACIS system, six of our patients who died of thyroid carcinoma were in the low-risk group. Of these six, five were over 45 and five had cervical node metastases. Based on the AMES system, 12 fatal cancers occurred in the low-risk group, and also, the majority (83%) of these patients were older (over 45) and had nodal metastases (67%). Thus, old age together with lymph node metastases seems to have an adverse effect on survival, and the TNM classification focus on those points.

Improving knowledge as to the effect on prognosis of primary tumour size and local invasion has justified some changes in TNM classification. In the new TNM classification (Sobin and Wittekind 2002), tumours under 2 cm belong to T1; in contrast, in the earlier TNM classification (Sobin and Wittekind 1997), only tumours under 1 cm belonged to T1. The new classification also includes tumours with minimal invasion in T3, in contrast to the 1997 TNM classification, in which all tumours extending beyond the thyroid capsule belonged in the T4 group.

9.2 Outcome of 601 patients

We analyzed a large series of PTC patients. Although their overall prognosis is good, a small group of PTC patients suffer from numerous recurrences or distant metastasis; some even die. The most important factor that affects prognosis is old age. In our study, all (n=23) patients who died of cancer were over 45, showing that young age alone was enough to protect them.
Recurrent disease, however, occurs in young patients. Therefore, although PTC is among the most curable cancer types, it can be a distressing disease for those young patients suffering from frequent recurrences of lymph nodes. Patient's awareness of the remaining cancer and the constant anguish of yet another recurrence can be very depressing. Also, from the aesthetic point of view, scars in the neck region from repeated operations on lymph node metastases contribute to lack of confidence. In our study, 50 of the 89 (56%) patients who suffered from local recurrences were under 45.

Table 12 compares clinical characteristics of our series of 601 PTC patients to those of series in previous studies. In our series, the mean age 45 at diagnosis is quite similar to that reported by most others, but different from that of Mazzaferri et al. (1994) who reported a lower mean age. In that series, patients were under the care of the US Air Force and Ohio State University hospital; thus most of them were fit for work.
The predominance of PTC in females was higher in our series than in others. As males may represent a more aggressive course of disease, our lower rate of distant metastases compared to those of some others (Carcangiu et al. 1985, Samaan et al. 1992, Gilliland et al. 1997) may relate to our sex ratio of 3.8:1.
As for nodal metastases, present in 15 to 66% in other studies, the incidence was low: 20%. The incidence of lymph node involvement depends not only on extent of surgery but also on the diligence of the pathologist, as microscopic involvement has been shown to be as high as 80 to 90% (Noguchi et al. 1998). Gross nodal metastases are present in approximately 30 to 40% of patients (Gilliland et al. 1997, Noguchi et al. 1998).
Our death rate of 4% is the average figure, Mazzaferri et al. (1994) and Samaan et al. (1992) had higher death rates, 8% and 9%. Patients in those series were collected from 1950 to 1989, whereas other series included patients also from the 1990s, except Hay et al. (1945-1985). Death rates declined in the 1990s, probably as a consequence of increased use of total thyroidectomy and RAI ablation.
The incidence of tumour recurrence in our study was the lowest (15%). The recurrence rates in other series were between 20 and 33%. Sutton et al. (1988) suggested that differences in recurrence rates may relate to increased use of radionuclide neck scans and neck imaging with high-resolution ultrasonography. The differences in recurrence rate may also be due to differences in surgical strategy and postoperative treatment strategy.

Table 12 Clinical characteristics of our series of 601 PTC patients and other series (Carcangiu et al. 1985, Hay 1990, Samaan et al. 1992, Mazzaferri and Jhiang 1994, Simon et al. 1996, Gilliland et al. 1997, Tzavara et al. 1999).

Author	n	Mean age (years)	Women: men ratio	Nodal metas-tases (%)	Distant metas-tases (%)	Distant recur-rences (%)	Any recur-rence (%)	Death (%)
Carcangiu	241	41.3	2.6:1	54	6.5	11.5		5.8
Hay	1500	44	2:1	38	2	4	20	5.5
Mazzaferri	1077	35.7	2.2:1	46	2	5	21	8
Samaan	1289	40.6	2.3:1	38	5	6	23	9
Simon	176	43.6	2:1	66	3	7	33	3.9
Tzavara	677	44	3.2:1	15	2	6	21	2
Gilliland	11857	39	3.1:1	35	3.7			2
Range		35-44	2.1-3.1:1	15-66	2-6.5	4-11.5	20-33	2-9
Siironen	**601**	**45**	**3.8:1**	**20**	**2**	**3**	**15**	**4**

Based on our results for the outcome of 601 patients, we agree that age, extent of primary tumour, tumour size, and distant metastases are useful predictors of poor prognosis (Cady and Rossi 1988, Hay et al. 1993). Contradicting many others' findings, in our series, nodal metastases had an unfavourable effect on survival.

The prognostic significance of lymph node status is controversial. It is believed that papillary thyroid cancer is the only human cancer with no relationship between lymph node metastases and survival (Bacourt et al. 1986, DeGroot et al. 1990, Samaan et al. 1992). Consistent with our results, however, some authors have found that LNM has a significant adverse effect on survival (Harwood et al. 1978, Sellers et al. 1992, Scheumann et al. 1994). In our series, LNM incidence was almost the same in patients under and over 45 years, and lower than in most other series for younger patients. In our series, as the incidence of LMN was low for younger patients, the strong positive influence of young age had no favourable effect on survival in patients with nodal metastases. The other reason could be the differing follow-up times. Our mean follow-up was 14.5 years for those last known to be alive, and 10.1 years for those who had died. The mean follow-up time in those studies showing a prognostic significance for LNM is usually over 15 years (Harwood et al. 1978, Tubiana et al. 1985, Sellers et al. 1992, Shaha et al. 1994), whereas the average follow-up time in studies showing no influence is often under 10 to 15 years (Bacourt et al. 1986, DeGroot et al. 1990, Samaan et al. 1992, Akslen 1993, Hay et al. 1993). Relapses can occur as late as 30 years after primary treatment, and overall survival at 10 years therefore seems insufficient (Tubiana et al. 1985). Thus, the clinical significance of lymph

node metastases in PTC might not be obvious after short-term follow-up. It is also notable that in our series, only gross nodal metastases were noted. Since we do not routinely perform lymph node dissection but only remove pathological lymph nodes, small lymph node metastases may have gone unnoticed. Thus, our positive lymph nodes probably represent only the most aggressive metastases, those which may influence survival in contrast to micrometastases.

Our findings support the fact that older PTC patients represent a biologically distinct group (Cady 1998). Many authors have reported that older PTC patients have more pathological risk factors (Tubiana et al. 1985, Bacourt et al. 1986, Samaan et al. 1992, Andersen et al. 1995, Coburn and Wanebo 1995, Kurozumi et al. 1998). In our series of 601 patients, extrathyroidal invasion of tumour, distant metastases, and aggressive disease were more common among those over 45. Moreover, all patients who died of cancer were over 45. Similarly, others have shown that extrathyroidal invasion is related to older age (Samaan et al. 1992, Coburn and Wanebo 1995, Kurozumi et al. 1998). Also, authors have reported that distant metastases occur more frequently in those older (Bacourt et al. 1986, Samaan et al. 1992). Andersen et al. (1995) found that among the patients over 45 incomplete resection did not significantly affect survival, and suggested that it was due to the more aggressive course of disease in older patients.

In our study, male gender did not affect survival, but was associated with aggressive disease. Many studies agree that male gender has no influence on survival (Hay et al. 1987, Simpson et al. 1987, Hay et al. 1993). Others have, however, demonstrated an independent influence of gender on probability of relapse and survival (Byar et al. 1979, Tubiana et al. 1985, Akslen 1993). We believe that PTC in males is a more aggressive disease, at least their tumours seem to recur more easily than those of women.

9.3 Tumour markers (immunohistochemistry and cell culture)

Our study included two patient series for immunohistochemistry: 108 patients aged under 35 and over 55, and 36 matched pairs. In matched-pair patients, the overall expression of all markers seemed lower. This difference may be attributable to the differing age and TNM stage distribution in these two groups.

COX-2, Ki-67 and VEGF-C

Higher expression in older patients. COX-2, Ki-67, and VEGF-C expression was higher in older PTC patients. Others have demonstrated a similar tendency for Ki-67 (Yoshida et al. 1999), but not for COX-2 or VEGF-C. In contrast to our results, COX-2 expression has been higher in younger patients in the study of Ito et al. (2003), which divided patients into those under and over 54 years old. Contradictory results may depend on the different cut-off limits for age and the fact that our study comprised a larger number of PTC specimens. In addition, we found that among lymph node-positive patients, VEGF-C expression was positive in most older patients, but only a few young ones expressed VEGF-C. Moreover, more than half the older lymph node-positive patients either died of cancer or developed distant metastases. To our knowledge, only two works have shown VEGF-C expression in thyroid tumours to be higher in patients with nodal involvement (Bunone et al. 1999, Tanaka et al. 2002a). In other studies, correlations of LNM with VEGF-C have appeared (Shushanov et al. 2000, Hung et al. 2003). To the best of our knowledge, relations between old age, LNM and VEGF-C expression have not been studied.

Increased expression of COX-2 in our study may reflect the more aggressive behaviour of the tumours of older PTC patients, because COX-2 inhibits apoptosis (Tsujii and DuBois 1995), stimulates angiogenesis (Tsujii et al. 1998, Masferrer et al. 2000), and increases tumour invasion and metastatic potential (Tsujii et al. 1997, Kakiuchi et al. 2002, Niki et al. 2002). Moreover, increased expression of Ki-67 means increased proliferative activity and thus faster-growing, aggressive tumours. However, Ki-67 recognizes proliferating cells and tells whether a cell is in the cycle, but it provides no information as to the length of the cell cycle. Thus, we cannot draw very strong conclusions about tumour behaviour based only on Ki-67 expression status (Brown and Gatter 1990). Increased VEGF-C expression has been shown to promote distant metastasis in breast cancer (Skobe et al. 2001). We think that up-regulation of VEGF-C in older lymph node-positive patients can lead to increased distant dissemination of tumour cells. Possibly due to VEGF-C, most older lymph node-positive patients either developed distant metastases or died of cancer. This supports other reports that nodal metastases indicate poor prognosis in older PTC patients (Harwood et al. 1978, Tubiana et al. 1985, Mazzaferri and Jhiang 1994, Passler et al. 2004). Our sample size was, however, small and inadequate for drawing final conclusions. We noticed a trend that VEGF-C could associate with distant dissemination of tumour cells in older patients.

Lymph node dissection for older patients? Based on our findings that lymph node metastases impacted survival, and that all patients who died of PTC were older, and that immunohistochemical expression of VEGF-C was higher in those older, we agree that older PTC patients may benefit from prophylactic lymph node dissection (Passler et al. 2004).

At present, in our department as in most other Nordic hospitals, only palpable lymph nodes or lymph nodes suspected to be metastatic at ultrasound are removed (Mazzaferri and Young 1981). This recommendation is due to an agreement that lymph node metastases have no influence on survival (McConahey et al. 1986, Shah et al. 1992) and thus the neck dissection may not be necessary. However, the literature is controversial regarding the impact of lymph node metastases. In the United States, the latest NCCN recommendation is to perform central and lateral neck dissection for PTC patients with lymph node metastases (Sherman et al. 2005). Our findings support that recommendation in cases of older lymph node-positive PTC patients.

COX-2 inhibitors for older patients? As we found that COX-2 expression is higher in older patients and older PTC patients have a worse prognosis, inhibiting COX-2 by selective COX-2 inhibitors may improve the prognosis in older PTC patients. In fact, promising findings in colon carcinogenesis have shown that COX-2 inhibitors may offer some protection against colon cancer (Steinbach et al. 2000, Higuchi et al. 2003). However, further studies were affected when life-threatening cardiovascular side effects were reported in two trials (Bresalier et al. 2005, Solomon et al. 2005). It is not known whether these side effects were dose dependent or related to rofecoxib and valdecoxib.

No correlation with patient outcome. In our series of matched pairs (aggressive versus completely recovered), COX-2, Ki-67, and VEGF-C expression did not correlate with aggressive disease. Among the 13 fatal cases from the two series, COX-2 expression was positive in 7, Ki-67 in 3, and VEGF-C in 6 tumours. Thus, our data do not confirm those of Sugitani et al. (1998) who found that in a small group of PTC microcarcinoma patients, Ki-67 expression is higher in those with lymph node metastases and with noncapsulated tumours, and moreover, patients who die of cancer show the highest Ki-67 expression. Expression of COX-2 and VEGF-C in our fatal cases was not higher than the expression of these markers in other older patients (Study II and V). One explanation for this could be that increased expression of these markers in older patients is only a reflection of a type of cancer biologically different from that in young patients. Cancer cells in older patients are more aggressive and invade distant organs and cause death. In young PTC patients, cancer remains in most cases in the neck region. In our series of 601 patients, no patients under 45 at initial diagnosis died of PTC.

Correlation of COX-2 and VEGF-C. In our immunohistochemical study, expression of COX-2 and VEGF-C correlated strongly. In cell culture studies, NPA cells expressed both COX-2 and VEGF-C, but COX-2 selective inhibitor NS-398 did not reduce VEGF-C expression, indicating that VEGF-C expression was not dependent on COX-2-derived prostanoids.
To the best of our knowledge, only three works in the literature have shown a correlation between COX-2 and VEGF-C (Byeon et al. 2004, Kyzas et al. 2004,

Su et al. 2004). In thyroid carcinoma, no correlation between COX-2 and VEGF-C has been reported.

A novel correlation between COX-2 and VEGF-C occurs in human lung adenocarcinoma (Su et al. 2004); in this work, they studied human lung adenocarcinoma cell lines and 59 patients who underwent surgery for lung adenocarcinoma. Immunohistochemical analysis of 59 lung adenocarcinoma specimens revealed that VEGF-C expression was significantly associated with the expression of COX-2 supporting our results in immunohistochemistry. They also showed that "high VEGF-C and COX-2 expression patients" were more likely to have lymph node metastasis and a shorter survival rate. Moreover, in contrast to our cell culture studies, Su et al. (2004) showed that in lung adenocarcinoma cell lines, COX-2 and its derived prostanoids are able to up-regulate the VEGF-C gene through the EP1 and HER-2/Neu-dependent pathway. A difference in tissue distribution of the prostanoid receptors may be one reason for these contradictory findings. However, we think that the same factors may control the expression of these two markers in PTC via a PGE_2-independent pathways, as both COX-2 and VEGF-C were induced by PMA.

In addition, a correlation between COX-2 and VEGF-C was shown in two immunohistochemical studies (Byeon et al. 2004, Kyzas et al. 2004). In a study of head and neck carcinoma (n= 70), increased expression of both markers also correlated with the presence of lymph node metastases and advanced clinical stage (Kyzas et al. 2004). In another work, as well, expression of VEGF-C in oesophageal squamous cell carcinoma was higher in lymph node-positive patients (Byeon et al. 2004). Of their 31 oesophageal squamous cell carcinoma patients, 21 had lymph node metastases, and all of them expressed VEGF-C (Byeon et al. 2004).

P21

Views differ regarding the importance of p21 in PTC. In our study of 108 patients, p21 expression was higher in large tumours and in tumours infiltrating through the thyroid capsule. Therefore, we hypothesized that p21 may be a sign of a more aggressive tumour. In our matched-pair group of 72 patients, expression did not, however, correlate with aggressive disease. In addition, of 13 fatal cases, only 3 expressed p21. These findings argue against Basolo et al. (1994) who showed that p21 correlates with tumour aggressiveness in PTC, because its expression was significantly higher in fatal cases. This inconsistency may be explained by too small patient series: In Basolo et al. (n=45) the number of fatal cases was only 14, in our series 13. In another study, the authors suggested that over-expression of p21 in thyroid tumours has a relationship to thyroid tumorigenesis, particularly in late events (Okayasu et al. 1998). In that study, anaplastic thyroid carcinomas and poorly differentiated papillary carcinomas showed higher immunostaining than did well-differentiated PTCs. As thyroid cancer is a slow-growing tumour, our finding of higher p21 in large tumours and in extrathyroidal tumours supports

the role of p21 in late events in thyroid tumours (Okayasu et al. 1998). In contrast to reports showing the importance of p21 in thyroid tumours (Basolo et al. 1994, Akslen and Varhaug 1995, Okayasu et al. 1998), many researchers agree that p21 expression has no clinical significance in thyroid cancer (Johnson et al. 1987, Mizukami et al. 1995, Ito et al. 1996). Based on our results, we could not suggest p21 as a prognostic marker in PTC, but it may take part in tumour progression.

Bcl-2

In thyroid cancer, Bcl-2 expression associates with less aggressive histology (Pilotti et al. 1994, Pollina et al. 1996, Basolo et al. 1997, Moore et al. 1998). High bcl-2 reflects a slow growth pattern, thus favouring a good prognosis. Lack of bcl-2 expression has been a sign of aggressive disease in medullary thyroid cancer, (Viale et al. 1995), but not in other thyroid cancer forms (Pollina et al. 1996). We agree, as in our studies expression correlated neither with clinical parameters nor with aggressive disease. Compared with works by others (Pilotti et al. 1994, Pollina et al. 1996, Basolo et al. 1997, Moore et al. 1998), the overall expression in our patient series was lower. This discrepancy may in part be explained by our use of different antibodies (Pilotti et al. 1994, Pollina et al. 1996, Basolo et al. 1997) or different cut-off values for positive expression (Pollina et al. 1996, Basolo et al. 1997, Moore et al. 1998). Our cut-off for positive expression was 10%, in contrast to others who had lower cut-offs and reported higher positivity for bcl-2 (Pollina et al. 1996, Basolo et al. 1997, Moore et al. 1998). Ito et al. (2003) had the same antibodies and same cut-off as we did and expression was lowest 50.8% of all (Pollina et al. 1996, Basolo et al. 1997, Moore et al. 1998). Expression in our studies were 26% (Study III) and 35% (Study IV).

MMP-2

In our series, increased stromal expression of MMP-2 correlated with advanced stage and with tumours extending beyond the thyroid capsule. Expression of MMP-2 in PTC has been shown to associate with large tumour size, high stage, high intrathyroidal invasion, capsular invasion, high vascular invasion, and lymph node metastasis (Maeta et al. 2001). Thus we confirmed some of these findings. Maeta et al. had 86 PTC cases in their immunohistochemical study, in which the final score was a combination of epithelial and stromal staining in tumour and in non-tumour tissue. Tumour cell expression was higher than in stromal cells.

In our study, we analyzed epithelial and stromal staining separately. Similar to findings of Campo et al. (1992) and Zedenius et al. (1996), stromal staining was stronger than epithelial staining in contrast to other study (Maeta et al. 2001). Higher expression of MMP-2 in advanced stage and in extrathyroidal disease may relate to the poor outcome in our series. In matched pair patients, however, MMP-2 expression did not correlate with aggressive disease. Moreover, among

13 fatal cases, only 2 showed positive stromal staining; none of the fatal cases showed positive epithelial staining for MMP-2. In our study, the overall number of positive cases was similar to that of Nakamura et al. (1999), but lower than in other studies (Campo et al. 1992, Zedenius et al. 1996, Maeta et al. 2001). We cannot adequately explain this discrepancy: It may be attributable to the fact that we analyzed epithelial and stromal staining separately. Based on our observations, we would not consider MMP-2 to be a prognostic marker for PTC, as expression was quite low, and statistical significances were poor.
Correlation between COX-2 and MMP-2 agrees with previous results (Tsujii et al. 1997, Callejas et al. 2001, Dohadwala et al. 2002, Miyata et al. 2003). Tsujii et al. (1997) showed that in human colon cancer cells, activation of MMP-2 is modulated by COX-2. Dohadwala et al. (2002) reported that overexpression of COX-2 increases MMP-2 expression and facilitates invasion of lung cancer cells. A relationship has also been found between COX-2 and MMP-2 expression in foetal hepatocytes (Callejas et al. 2001). That study suggests that COX-2 is a key component in the secretion of MMP-2. Miyata et al. (2003) demonstrated a correlation between COX-2 and MMP-2 in patients with renal cell carcinoma in which expression of COX-2 correlated with high T, N, and M stage, high tumour grade, and expression of MMP-2. Furthermore, COX-2 inhibitors reduce colon cancer metastasis and MMP-2 production in animal models (Nagatsuka et al. 2002, Yao et al. 2003).

9.4 Non-published data

Deaths from PTC have declined dramatically during the last few decades (Table 6). Evidence that survival is longer for those patients treated after 1970 than for those treated earlier, deserves comment. In our opinion, increased use of total thyroidectomy and postoperative RAI ablation for PTC patients from the 1970s on might be the reason for better survival. Treatment factors have been shown to be as important as tumour biology in predicting tumour recurrence (Kouvaraki et al. 2004). Performing only lobectomy results in recurrences in the remnant thyroid lobe, a higher tumour recurrence rate, and higher incidence of distant metastases (Massin et al. 1984, Hay et al. 1987, Mazzaferri and Kloos 2001). Radioiodine therapy reduces recurrences and thyroid cancer-related mortality (Mazzaferri and Kloos 2001).

10.
Summary and Conclusions

The major findings were:

- Age over 45, tumour size > 4 cm, extrathyroidal extension of tumour, nodal metastases, distant metastases, and stage IV disease correlated significantly with survival.

- TNM classification was more reliable than AMES or MACIS classifications in predicting prognosis of PTC. TNM is the only staging method that takes into account lymph node metastases.

- Expression of COX-2, Ki-67, and VEGF-C was higher in older PTC patients, which could explain the more aggressive behaviour of PTC in the older age group. Most older lymph node-positive patients expressed VEGF-C and did not manage well. In contrast, most young lymph node-positive patients did not express VEGF-C and managed well, supporting the idea that older patients have different disease.

- Expression of MMP-2, bcl-2, and p21 did not differ between young and older PTC patients. Stromal expression of MMP-2 correlated with extent of tumour and with advanced stage. In tumours extending beyond the thyroid capsule and in large tumours, p21 expression was higher. Bcl-2 expression did not correlate with clinical parameters.

- In a series of matched pair groups (aggressive versus completely recovered), immunohistochemical expression of COX-2, MMP-2, bcl-2, Ki-67, p21, and VEGF-C did not differ between groups, indicating that none of the markers could predict tumour behaviour.

- Immunohistochemically, expression of COX-2 and VEGF-C correlated strongly. In cell culture, this correlation was not so clear, because the COX-2 selective inhibitor NS-398 did not reduce VEGF-C expression. However, because both COX-2 and VEGF-C were induced by tumour promoter (PMA), the same factors may control them both.

A tendency for improved outcomes of PTC patients over the past few years is a consequence of the increasing use of total thyroidectomy and ^{131}I ablation, and most patients with papillary thyroid cancer can be cured if treated according to best clinical practice.

In the present series of 601 PTC patients, all patients who died of PTC were over 45. Young age thus protects against cancer death in PTC. In older patients, PTC takes a more malignant course. Lymph node metastases are predictive for distant metastases and death in older patients, but not in younger ones. In young patients, PTC does not behave like most other types of cancer, as it does not cause death.

Initial treatment and follow-up could possibly be individualized according to age. Older PTC patients, especially those with lymph node metastases, may benefit from more radical lymph node dissection and from more intensive follow-up. As COX-2 was elevated in older patients, there is a chance that these patients may also benefit from COX-2 selective inhibitors. However, serious cardiovascular side effects in clinical trials have reduced the excitement over using these drugs in chemoprevention. In PTC, COX-2 inhibitors could serve as adjuvant therapy, and in that setting, some side effects can be acceptable.

Unexpectedly, also some young low-risk patients may suffer from aggressive disease. The problem is how to recognize these young patients from the large pool of patients who recover completely after primary treatment. Although we found elevated expression of COX-2, VEGF-C, and Ki-67 in those older, these three markers did not predict outcome, and thus we cannot use these proteins as tumour markers for predicting prognosis. Higher COX-2, VEGF-C, and Ki-67 in those older may only reflect the fact that PTC here is biologically different. Better understanding of differences in tumour biology at all ages may help us to choose those young patients who represent the tumour behaviour of older patients.

To date, no ideal marker yet exists to predict the prognosis of the PTC patient. In future, identification of genetic variations that correlate with the poor prognosis may help us to focus on a subgroup with papillary thyroid cancer who will already benefit from more aggressive treatment at the onset of the disease.

II.
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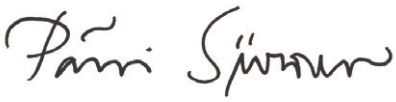

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12.
References

Ain KB. Anaplastic thyroid carcinoma: behavior, biology, and therapeutic approaches. Thyroid 1998;8(8):715-26.

Akslen LA. Prognostic importance of histologic grading in papillary thyroid carcinoma. Cancer 1993;72(9):2680-5.

Akslen LA and LiVolsi VA. Prognostic significance of histologic grading compared with subclassification of papillary thyroid carcinoma. Cancer 2000;88(8):1902-8.

Akslen LA and Varhaug JE. Oncoproteins and tumor progression in papillary thyroid carcinoma: presence of epidermal growth factor receptor, c-erbB-2 protein, estrogen receptor related protein, p21-ras protein, and proliferation indicators in relation to tumor recurrences and patient survival. Cancer 1995;76(9):1643-54.

Albores-Saavedra J, Gould E, Vardaman C and Vuitch F. The macrofollicular variant of papillary thyroid carcinoma: a study of 17 cases. Hum Pathol 1991;22(12):1195-205.

Alitalo K and Carmeliet P. Molecular mechanisms of lymphangiogenesis in health and disease. Cancer Cell 2002;1(3):219-27.

Andersen PE, Kinsella J, Loree TR, Shaha AR and Shah JP. Differentiated carcinoma of the thyroid with extrathyroidal extension. Am J Surg 1995;170(5):467-70.

Bacourt F, Asselain B, Savoie JC, D'Hubert E, Massin JP, Doucet G, Leger A and Garnier H. Multifactorial study of prognostic factors in differentiated thyroid carcinoma and a re-evaluation of the importance of age. Br J Surg 1986;73(4):274-7.

Barbareschi M, Caffo O, Doglioni C, Fina P, Marchetti A, Buttitta F, Leek R, Morelli L, Leonardi E, Bevilacqua G, et al. p21WAF1 immunohistochemical expression in breast carcinoma: correlations with clinicopathological data, oestrogen receptor status, MIB1 expression, p53 gene and protein alterations and relapse-free survival. Br J Cancer 1996;74(2):208-15.

Basolo F, Pinchera A, Fugazzola L, Fontanini G, Elisei R, Romei C and Pacini F. Expression of p21 ras protein as a prognostic factor in papillary thyroid cancer. Eur J Cancer 1994;30A(2):171-4.

Basolo F, Pollina L, Fontanini G, Fiore L, Pacini F and Baldanzi A. Apoptosis and proliferation in thyroid carcinoma: correlation with bcl-2 and p53 protein expression. Br J Cancer 1997;75(4):537-41.

Baudin E, Travagli JP, Ropers J, Mancusi F, Bruno-Bossio G, Caillou B, Cailleux AF, Lumbroso JD, Parmentier C and Schlumberger M. Microcarcinoma of the thyroid

gland: the Gustave-Roussy Institute experience. Cancer 1998;83(3):553-9.
Beasley NJ, Lee J, Eski S, Walfish P, Witterick I and Freeman JL. Impact of nodal metastases on prognosis in patients with well-differentiated thyroid cancer. Arch Otolaryngol Head Neck Surg 2002;128(7):825-8.
Beasley NJ, Walfish PG, Witterick I and Freeman JL. Cause of death in patients with well-differentiated thyroid carcinoma. Laryngoscope 2001;111(6):989-91.
Berg J, Stocher M, Bogner S, Wolfl S, Pichler R and Stekel H. Inducible cyclooxygenase-2 gene expression in the human thyroid epithelial cell line Nthy-ori3-1. Inflamm Res 2000;49(4):139-43.
Berho M and Suster S. The oncocytic variant of papillary carcinoma of the thyroid: a clinicopathologic study of 15 cases. Hum Pathol 1997;28(1):47-53.
Boise LH, Gonzalez-Garcia M, Postema CE, Ding L, Lindsten T, Turka LA, Mao X, Nunez G and Thompson CB. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993;74(4):597-608.
Bondeson L and Ljungberg O. Occult papillary thyroid carcinoma in the young and the aged. Cancer 1984;53(8):1790-2.
Bongarzone I, Fugazzola L, Vigneri P, Mariani L, Mondellini P, Pacini F, Basolo F, Pinchera A, Pilotti S and Pierotti MA. Age-related activation of the tyrosine kinase receptor protooncogenes RET and NTRK1 in papillary thyroid carcinoma. J Clin Endocrinol Metab 1996;81(5):2006-9.
Bongarzone I, Vigneri P, Mariani L, Collini P, Pilotti S and Pierotti MA. RET/NTRK1 rearrangements in thyroid gland tumors of the papillary carcinoma family: correlation with clinicopathological features. Clin Cancer Res 1998;4(1):223-8.
Bresalier RS, Sandler RS, Quan H, Bolognese JA, Oxenius B, Horgan K, Lines C, Riddell R, Morton D, Lanas A, et al. Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 2005;352(11):1092-102.
Brierley JD, Panzarella T, Tsang RW, Gospodarowicz MK and O'Sullivan B. A comparison of different staging systems predictability of patient outcome. Thyroid carcinoma as an example. Cancer 1997;79(12):2414-23.
Brown DC and Gatter KC. Monoclonal antibody Ki-67: its use in histopathology. Histopathology 1990;17(6):489-503.
Bubendorf L, Sauter G, Moch H, Jordan P, Blochlinger A, Gasser TC and Mihatsch MJ. Prognostic significance of Bcl-2 in clinically localized prostate cancer. Am J Pathol 1996;148(5):1557-65.
Bunone G, Vigneri P, Mariani L, Buto S, Collini P, Pilotti S, Pierotti MA and Bongarzone I. Expression of angiogenesis stimulators and inhibitors in human thyroid tumors and correlation with clinical pathological features. Am J Pathol 1999;155(6):1967-76.
Buskens CJ, Van Rees BP, Sivula A, Reitsma JB, Haglund C, Bosma PJ, Offerhaus GJ, Van Lanschot JJ and Ristimäki A. Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. Gastroenterology 2002;122(7):1800-7.
Byar DP, Green SB, Dor P, Williams ED, Colon J, van Gilse HA, Mayer M, Sylvester RJ and van Glabbeke M. A prognostic index for thyroid carcinoma. A study of the E.O.R.T.C. Thyroid Cancer Cooperative Group. Eur J Cancer 1979;15(8):1033-41.
Byeon JS, Jung HY, Lee YJ, Lee D, Lee GH, Myung SJ, Yang SK, Hong WS, Kim JH, Min YI, et al. Clinicopathological significance of vascular endothelial growth factor-C and cyclooxygenase-2 in esophageal squamous cell carcinoma. J Gastroenterol Hepatol 2004;19(6):648-54.

Cady B. Presidential address: beyond risk groups--a new look at differentiated thyroid cancer. Surgery 1998;124(6):947-57.
Cady B and Rossi R. An expanded view of risk-group definition in differentiated thyroid carcinoma. Surgery 1988;104(6):947-53.
Cailleux AF, Baudin E, Travagli JP, Ricard M and Schlumberger M. Is diagnostic iodine-131 scanning useful after total thyroid ablation for differentiated thyroid cancer? J Clin Endocrinol Metab 2000;85(1):175-8.
Callejas NA, Casado M, Diaz-Guerra MJ, Bosca L and Martin-Sanz P. Expression of cyclooxygenase-2 promotes the release of matrix metalloproteinase-2 and -9 in fetal rat hepatocytes. Hepatology 2001;33(4):860-7.
Campo E, Merino MJ, Liotta L, Neumann R and Stetler-Stevenson W. Distribution of the 72-kd type IV collagenase in nonneoplastic and neoplastic thyroid tissue. Hum Pathol 1992;23(12):1395-401.
Carcangiu ML and Bianchi S. Diffuse sclerosing variant of papillary thyroid carcinoma. Clinicopathologic study of 15 cases. Am J Surg Pathol 1989;13(12):1041-9.
Carcangiu ML, Zampi G, Pupi A, Castagnoli A and Rosai J. Papillary carcinoma of the thyroid. A clinicopathologic study of 241 cases treated at the University of Florence, Italy. Cancer 1985;55(4):805-28.
Cetta F, Montalto G, Gori M, Curia MC, Cama A and Olschwang S. Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. J Clin Endocrinol Metab 2000;85(1):286-92.
Chan CC, Menges M, Orzechowski HD, Orendain N, Pistorius G, Feifel G, Zeitz M and Stallmach A. Increased matrix metalloproteinase 2 concentration and transcript expression in advanced colorectal carcinomas. Int J Colorectal Dis 2001;16(3):133-40.
Chem KT and Rosai J. Follicular variant of thyroid papillary carcinoma: a clinicopathologic study of six cases. Am J Surg Pathol 1977;1(2):123-30.
Cheung CC, Ezzat S, Ramyar L, Freeman JL and Asa SL. Molecular basis off hurthle cell papillary thyroid carcinoma. J Clin Endocrinol Metab 2000;85(2):878-82.
Coburn M, Teates D and Wanebo HJ. Recurrent thyroid cancer. Role of surgery versus radioactive iodine (I131). Ann Surg 1994;219(6):587-93; discussion 593-5.
Coburn MC and Wanebo HJ. Age correlates with increased frequency of high risk factors in elderly patients with thyroid cancer. Am J Surg 1995;170(5):471-5.
Cornetta AJ, Russell JP, Cunnane M, Keane WM and Rothstein JL. Cyclooxygenase-2 expression in human thyroid carcinoma and Hashimoto's thyroiditis. Laryngoscope 2002;112(2):238-42.
Crile G, Jr. and Fisher ER. Simultaneous occurrence of thyroiditis and papillary carcinoma; report of two cases. Cancer 1953;6(1):57-62.
Cvejic D, Savin S, Petrovic I, Paunovic I, Tatic S, Krgovic K and Havelka M. Galectin-3 expression in papillary microcarcinoma of the thyroid. Histopathology 2005;47(2):209-14.
Czaja JM and McCaffrey TV. The surgical management of laryngotracheal invasion by well-differentiated papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg 1997;123(5):484-90.
de Matos PS, Ferreira AP, de Oliveira Facuri F, Assumpcao LV, Metze K and Ward LS. Usefulness of HBME-1, cytokeratin 19 and galectin-3 immunostaining in the diagnosis of thyroid malignancy. Histopathology 2005;47(4):391-401.
DeGroot LJ, Kaplan EL, McCormick M and Straus FH. Natural history, treatment, and

course of papillary thyroid carcinoma. J Clin Endocrinol Metab 1990;71(2):414-24.
DeGroot LJ, Kaplan EL, Straus FH and Shukla MS. Does the method of management of papillary thyroid carcinoma make a difference in outcome? World J Surg 1994;18(1):123-30.
DeLellis RA, Lloyd RV and Heitz PU (2004). World Health Organization Classification of Tumours. Tumours of Endocrine Organs. Lyon, IARCPress.
Denkert C, Kobel M, Pest S, Koch I, Berger S, Schwabe M, Siegert A, Reles A, Klosterhalfen B and Hauptmann S. Expression of cyclooxygenase 2 is an independent prognostic factor in human ovarian carcinoma. Am J Pathol 2002;160(3):893-903.
Di Paola R, Menzaghi C, De Filippis V, Corda D and Di Cerbo A. Cyclooxygenase-dependent thyroid cell proliferation induced by immunoglobulins from patients with Graves' disease. J Clin Endocrinol Metab 1997;82(2):670-3.
Dinneen SF, Valimaki MJ, Bergstralh EJ, Goellner JR, Gorman CA and Hay ID. Distant metastases in papillary thyroid carcinoma: 100 cases observed at one institution during 5 decades. J Clin Endocrinol Metab 1995;80(7):2041-5.
Dohadwala M, Batra RK, Luo J, Lin Y, Krysan K, Pold M, Sharma S and Dubinett SM. Autocrine/paracrine prostaglandin E2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. J Biol Chem 2002;277(52):50828-33.
Donghi R, Longoni A, Pilotti S, Michieli P, Della Porta G and Pierotti MA. Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. J Clin Invest 1993;91(4):1753-60.
Duff SE, Li C, Jeziorska M, Kumar S, Saunders MP, Sherlock D, O'Dwyer ST and Jayson GC. Vascular endothelial growth factors C and D and lymphangiogenesis in gastrointestinal tract malignancy. Br J Cancer 2003;89(3):426-30.
Egeblad M and Werb Z. New functions for the matrix metalloproteinases in cancer progression. Nat Rev Cancer 2002;2(3):161-74.
el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B. WAF1, a potential mediator of p53 tumor suppression. Cell 1993;75(4):817-25.
el-Deiry WS, Tokino T, Waldman T, Oliner JD, Velculescu VE, Burrell M, Hill DE, Healy E, Rees JL, Hamilton SR, et al. Topological control of p21WAF1/CIP1 expression in normal and neoplastic tissues. Cancer Res 1995;55(13):2910-9.
Emerick GT, Duh QY, Siperstein AE, Burrow GN and Clark OH. Diagnosis, treatment, and outcome of follicular thyroid carcinoma. Cancer 1993;72(11):3287-95.
Endl E and Gerdes J. The Ki-67 protein: fascinating forms and an unknown function. Exp Cell Res 2000;257(2):231-7.
Erkinheimo TL, Lassus H, Finne P, van Rees BP, Leminen A, Ylikorkala O, Haglund C, Butzow R and Ristimäki A. Elevated cyclooxygenase-2 expression is associated with altered expression of p53 and SMAD4, amplification of HER-2/neu, and poor outcome in serous ovarian carcinoma. Clin Cancer Res 2004;10(2):538-45.
Evan GI and Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001;411(6835):342-8.
Evans HL. Columnar-cell carcinoma of the thyroid. A report of two cases of an aggressive variant of thyroid carcinoma. Am J Clin Pathol 1986;85(1):77-80.
Ezzat S, Zheng L, Kolenda J, Safarian A, Freeman JL and Asa SL. Prevalence of activating ras mutations in morphologically characterized thyroid nodules. Thyroid 1996;6(5):409-16.

Farid NR. P53 mutations in thyroid carcinoma: tidings from an old foe. J Endocrinol Invest 2001;24(7):536-45.
Fellmer PT, Sato K, Tanaka R, Okamoto T, Kato Y, Kobayashi M, Shibuya M and Obara T. Vascular endothelial growth factor-C gene expression in papillary and follicular thyroid carcinomas. Surgery 1999;126(6):1056-61; discussion 1061-2.
Fenton CL, Lukes Y, Nicholson D, Dinauer CA, Francis GL and Tuttle RM. The ret/PTC mutations are common in sporadic papillary thyroid carcinoma of children and young adults. J Clin Endocrinol Metab 2000;85(3):1170-5.
Fugazzola L, Pilotti S, Pinchera A, Vorontsova TV, Mondellini P, Bongarzone I, Greco A, Astakhova L, Butti MG, Demidchik EP, et al. Oncogenic rearrangements of the RET proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. Cancer Res 1995;55(23):5617-20.
Fukunaga FH and Yatani R. Geographic pathology of occult thyroid carcinomas. Cancer 1975;36(3):1095-9.
Gaertner EM, Davidson M and Wenig BM. The columnar cell variant of thyroid papillary carcinoma. Case report and discussion of an unusually aggressive thyroid papillary carcinoma. Am J Surg Pathol 1995;19(8):940-7.
Gaffney DK, Holden J, Zempolich K, Murphy KJ, Dicker AP and Dodson M. Elevated COX-2 expression in cervical carcinoma: reduced cause-specific survival and pelvic control. Am J Clin Oncol 2001;24(5):443-6.
Gardner RE, Tuttle RM, Burman KD, Haddady S, Truman C, Sparling YH, Wartofsky L, Sessions RB and Ringel MD. Prognostic importance of vascular invasion in papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg 2000;126(3):309-12.
George ML, Tutton MG, Janssen F, Arnaout A, Abulafi AM, Eccles SA and Swift RI. VEGF-A, VEGF-C, and VEGF-D in colorectal cancer progression. Neoplasia 2001;3(5):420-7.
Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U and Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. J Immunol 1984;133(4):1710-5.
Gerdes J, Schwab U, Lemke H and Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31(1):13-20.
Gilliland FD, Hunt WC, Morris DM and Key CR. Prognostic factors for thyroid carcinoma. A population-based study of 15,698 cases from the Surveillance, Epidemiology and End Results (SEER) program 1973-1991. Cancer 1997;79(3):564-73.
Gimm O, Rath FW and Dralle H. Pattern of lymph node metastases in papillary thyroid carcinoma. Br J Surg 1998;85(2):252-4.
Giuffrida D and Gharib H. Anaplastic thyroid carcinoma: current diagnosis and treatment. Ann Oncol 2000;11(9):1083-9.
Gomyo Y, Ikeda M, Osaki M, Tatebe S, Tsujitani S, Ikeguchi M, Kaibara N and Ito H. Expression of p21 (waf1/cip1/sdi1), but not p53 protein, is a factor in the survival of patients with advanced gastric carcinoma. Cancer 1997;79(11):2067-72.
Grant CS, Hay ID, Gough IR, Bergstralh EJ, Goellner JR and McConahey WM. Local recurrence in papillary thyroid carcinoma: is extent of surgical resection important? Surgery 1988;104(6):954-62.
Grigioni WF, D'Errico A, Fiorentino M, Baccarini P, Onisto M, Caenazzo C, Stetler-Stevenson WG, Garbisa S and Mancini AM. Gelatinase A (MMP-2) and its mRNA detected in both neoplastic and stromal cells of tumors with different invasive

and metastatic properties. Diagn Mol Pathol 1994;3(3):163-9.
Hanrahan V, Currie MJ, Gunningham SP, Morrin HR, Scott PA, Robinson BA and Fox SB. The angiogenic switch for vascular endothelial growth factor (VEGF)-A, VEGF-B, VEGF-C, and VEGF-D in the adenoma-carcinoma sequence during colorectal cancer progression. J Pathol 2003;200(2):183-94.
Hara H, Fulton N, Yashiro T, Ito K, DeGroot LJ and Kaplan EL. N-ras mutation: an independent prognostic factor for aggressiveness of papillary thyroid carcinoma. Surgery 1994;116(6):1010-6.
Harach HR, Franssila KO and Wasenius VM. Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study. Cancer 1985;56(3):531-8.
Harada T, Ito K, Shimaoka K, Hosoda Y and Yakumaru K. Fatal thyroid carcinoma. Anaplastic transformation of adenocarcinoma. Cancer 1977;39(6):2588-96.
Harper JW, Adami GR, Wei N, Keyomarsi K and Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell 1993;75(4):805-16.
Harwood J, Clark OH and Dunphy JE. Significance of lymph node metastasis in differentiated thyroid cancer. Am J Surg 1978;136(1):107-12.
Hay ID. Papillary thyroid carcinoma. Endocrinol Metab Clin North Am 1990;19(3):545-76.
Hay ID, Bergstralh EJ, Goellner JR, Ebersold JR and Grant CS. Predicting outcome in papillary thyroid carcinoma: development of a reliable prognostic scoring system in a cohort of 1779 patients surgically treated at one institution during 1940 through 1989. Surgery 1993;114(6):1050-7.
Hay ID, Grant CS, Bergstralh EJ, Thompson GB, van Heerden JA and Goellner JR. Unilateral total lobectomy: is it sufficient surgical treatment for patients with AMES low-risk papillary thyroid carcinoma? Surgery 1998;124(6):958-64; discussion 964-6.
Hay ID, Grant CS, Taylor WF and McConahey WM. Ipsilateral lobectomy versus bilateral lobar resection in papillary thyroid carcinoma: a retrospective analysis of surgical outcome using a novel prognostic scoring system. Surgery 1987;102(6):1088-95.
Herod JJ, Eliopoulos AG, Warwick J, Niedobitek G, Young LS and Kerr DJ. The prognostic significance of Bcl-2 and p53 expression in ovarian carcinoma. Cancer Res 1996;56(9):2178-84.
Higuchi T, Iwama T, Yoshinaga K, Toyooka M, Taketo MM and Sugihara K. A randomized, double-blind, placebo-controlled trial of the effects of rofecoxib, a selective cyclooxygenase-2 inhibitor, on rectal polyps in familial adenomatous polyposis patients. Clin Cancer Res 2003;9(13):4756-60.
Ho YS, Tseng SC, Chin TY, Hsieh LL and Lin JD. p53 gene mutation in thyroid carcinoma. Cancer Lett 1996;103(1):57-63.
Hockenbery D, Nunez G, Milliman C, Schreiber RD and Korsmeyer SJ. Bcl-2 is an inner mitochondrial membrane protein that blocks programmed cell death. Nature 1990;348(6299):334-6.
Hofmann UB, Westphal JR, Zendman AJ, Becker JC, Ruiter DJ and van Muijen GN. Expression and activation of matrix metalloproteinase-2 (MMP-2) and its co-localization with membrane-type 1 matrix metalloproteinase (MT1-MMP) correlate with melanoma progression. J Pathol 2000;191(3):245-56.
Hoie J, Stenwig AE, Kullmann G and Lindegaard M. Distant metastases in papillary thyroid cancer. A review of 91 patients. Cancer 1988;61(1):1-6.

Hollstein M, Sidransky D, Vogelstein B and Harris CC. p53 mutations in human cancers. Science 1991;253(5015):49-53.
Homer JJ, Prentice MG, Cawkwell L, Birchall M, Greenman J and Stafford ND. Angiogenesis and the expression of vascular endothelial growth factors A and C in squamous cell carcinoma of the piriform fossa. Arch Otolaryngol Head Neck Surg 2003;129(10):1110-4.
Hundahl SA, Cady B, Cunningham MP, Mazzaferri E, McKee RF, Rosai J, Shah JP, Fremgen AM, Stewart AK and Holzer S. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the United States during 1996. U.S. and German Thyroid Cancer Study Group. An American College of Surgeons Commission on Cancer patient care evaluation study. Cancer 2000;89(1):202-17.
Hundahl SA, Fleming ID, Fremgen AM and Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995 [see comments]. Cancer 1998;83(12):2638-48.
Hung CJ, Ginzinger DG, Zarnegar R, Kanauchi H, Wong MG, Kebebew E, Clark OH and Duh QY. Expression of vascular endothelial growth factor-C in benign and malignant thyroid tumors. J Clin Endocrinol Metab 2003;88(8):3694-9.
Ito T, Seyama T, Mizuno T, Tsuyama N, Hayashi T, Hayashi Y, Dohi K, Nakamura N and Akiyama M. Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res 1992;52(5):1369-71.
Ito Y, Kobayashi T, Takeda T, Komoike Y, Wakasugi E, Tamaki Y, Tsujimoto M, Matsuura N and Monden M. Expression of p21 (WAF1/CIP1) protein in clinical thyroid tissues. Br J Cancer 1996;74(8):1269-74.
Ito Y, Uruno T, Takamura Y, Miya A, Kobayashi K, Matsuzuka F, Kuma K and Miyauchi A. Papillary microcarcinomas of the thyroid with preoperatively detectable lymph node metastasis show significantly higher aggressive characteristics on immunohistochemical examination. Oncology 2005;68(2-3):87-96.
Ito Y, Yoshida H, Nakano K, Takamura Y, Miya A, Kobayashi K, Yokozawa T, Matsuzuka F, Matsuura N, Kakudo K, et al. Bag-1 expression in thyroid neoplasm: its correlation with Bcl-2 expression and carcinoma dedifferentiation. Anticancer Res 2003;23(1B):569-76.
Ito Y, Yoshida H, Nakano K, Takamura Y, Miya A, Kobayashi K, Yokozawa T, Matsuzuka F, Matsuura N, Kuma K, et al. Cyclooxygenase-2 expression in thyroid neoplasms. Histopathology 2003;42(5):492-7.
Ivanova R, Soares P, Castro P and Sobrinho-Simoes M. Diffuse (or multinodular) follicular variant of papillary thyroid carcinoma: a clinicopathologic and immunohistochemical analysis of ten cases of an aggressive form of differentiated thyroid carcinoma. Virchows Arch 2002;440(4):418-24.
Jeltsch M, Kaipainen A, Joukov V, Meng X, Lakso M, Rauvala H, Swartz M, Fukumura D, Jain RK and Alitalo K. Hyperplasia of lymphatic vessels in VEGF-C transgenic mice. Science 1997;276(5317):1423-5.
Jhiang SM and Mazzaferri EL. The ret/PTC oncogene in papillary thyroid carcinoma. J Lab Clin Med 1994;123(3):331-7.
Joensuu H, Pylkkänen L and Toikkanen S. Bcl-2 protein expression and long-term survival in breast cancer. Am J Pathol 1994;145(5):1191-8.
Johnson TL, Lloyd RV, Thompson NW, Beierwaltes WH and Sisson JC. Prognostic implications of the tall cell variant of papillary thyroid carcinoma. Am J Surg

Pathol 1988;12(1):22-7.
Johnson TL, Lloyd RV and Thor A. Expression of ras oncogene p21 antigen in normal and proliferative thyroid tissues. Am J Pathol 1987;127(1):60-5.
Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, Saksela O, Kalkkinen N and Alitalo K. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. Embo J 1996;15(7):1751.
Kakiuchi Y, Tsuji S, Tsujii M, Murata H, Kawai N, Yasumaru M, Kimura A, Komori M, Irie T, Miyoshi E, et al. Cyclooxygenase-2 activity altered the cell-surface carbohydrate antigens on colon cancer cells and enhanced liver metastasis. Cancer Res 2002;62(5):1567-72.
Katoh R, Bray CE, Suzuki K, Komiyama A, Hemmi A, Kawaoi A, Oyama T, Sugai T and Sasou S. Growth activity in hyperplastic and neoplastic human thyroid determined by an immunohistochemical staining procedure using monoclonal antibody MIB-1. Hum Pathol 1995;26(2):139-46.
Kazakov VS, Demidchik EP and Astakhova LN. Thyroid cancer after Chernobyl. Nature 1992;359(6390):21.
Kendall-Taylor P. Guidelines for the management of thyroid cancer. Clin Endocrinol (Oxf) 2003;58(4):400-2.
Khatri VP and Loree TR. A logical and stepwise operative approach to radical neck dissection. Arch Surg 2002;137(3):345-51.
Khoo ML, Beasley NJ, Ezzat S, Freeman JL and Asa SL. Overexpression of cyclin D1 and underexpression of p27 predict lymph node metastases in papillary thyroid carcinoma. J Clin Endocrinol Metab 2002;87(4):1814-8.
Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE and Fagin JA. High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res 2003;63(7):1454-7.
Kitadai Y, Amioka T, Haruma K, Tanaka S, Yoshihara M, Sumii K, Matsutani N, Yasui W and Chayama K. Clinicopathological significance of vascular endothelial growth factor (VEGF)-C in human esophageal squamous cell carcinomas. Int J Cancer 2001;93(5):662-6.
Kitamura H, Oosawa Y, Kawano N, Kameda Y, Hayashi H, Nakatani Y, Udaka N, Ito T and Miyazaki K. Basement membrane patterns, gelatinase A and tissue inhibitor of metalloproteinase-2 expressions, and stromal fibrosis during the development of peripheral lung adenocarcinoma. Hum Pathol 1999;30(3):331-8.
Kitamura Y, Shimizu K, Nagahama M, Sugino K, Ozaki O, Mimura T, Ito K and Tanaka S. Immediate causes of death in thyroid carcinoma: clinicopathological analysis of 161 fatal cases. J Clin Endocrinol Metab 1999;84(11):4043-9.
Klugbauer S, Lengfelder E, Demidchik EP and Rabes HM. High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. Oncogene 1995;11(12):2459-67.
Kouvaraki MA, Lee JE, Shapiro SE, Sherman SI and Evans DB. Preventable reoperations for persistent and recurrent papillary thyroid carcinoma. Surgery 2004;136(6):1183-91.
Kurebayashi J, Otsuki T, Kunisue H, Mikami Y, Tanaka K, Yamamoto S and Sonoo H. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. Jpn J Cancer Res 1999;90(9):977-81.
Kurozumi K, Nakao K, Nishida T, Nakahara M, Ogino N and Tsujimoto M. Significance

of biologic aggressiveness and proliferating activity in papillary thyroid carcinoma. World J Surg 1998;22(12):1237-42.
Kyzas PA, Stefanou D and Agnantis NJ. COX-2 expression correlates with VEGF-C and lymph node metastases in patients with head and neck squamous cell carcinoma. Mod Pathol 2004.
Lantsov D, Meirmanov S, Nakashima M, Kondo H, Saenko V, Naruke Y, Namba H, Ito M, Abrosimov A, Lushnikov E, et al. Cyclin D1 overexpression in thyroid papillary microcarcinoma: its association with tumour size and aberrant beta-catenin expression. Histopathology 2005;47(3):248-56.
Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, et al. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997;16(1):64-7.
Lin JD, Liou MJ, Chao TC, Weng HF and Ho YS. Prognostic variables of papillary and follicular thyroid carcinoma patients with lymph node metastases and without distant metastases. Endocr Relat Cancer 1999;6(1):109-15.
Liotta LA, Abe S, Robey PG and Martin GR. Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. Proc Natl Acad Sci U S A 1979;76(5):2268-72.
Loh KC. Familial nonmedullary thyroid carcinoma: a meta-review of case series. Thyroid 1997;7(1):107-13.
Lu X, Toki T, Konishi I, Nikaido T and Fujii S. Expression of p21WAF1/CIP1 in adenocarcinoma of the uterine cervix: a possible immunohistochemical marker of a favorable prognosis. Cancer 1998;82(12):2409-17.
Ludvikova M, Ryska A, Korabecna M, Rydlova M and Michal M. Oncocytic papillary carcinoma with lymphoid stroma (Warthin-like tumour) of the thyroid: a distinct entity with favourable prognosis. Histopathology 2001;39(1):17-24.
Lupoli G, Vitale G, Caraglia M, Fittipaldi MR, Abbruzzese A, Tagliaferri P and Bianco AR. Familial papillary thyroid microcarcinoma: a new clinical entity. Lancet 1999;353(9153):637-9.
Maeta H, Ohgi S and Terada T. Protein expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinase 1 and 2 in papillary thyroid carcinomas. Virchows Arch 2001;438(2):121-8.
Mandriota SJ, Jussila L, Jeltsch M, Compagni A, Baetens D, Prevo R, Banerji S, Huarte J, Montesano R, Jackson DG, et al. Vascular endothelial growth factor-C-mediated lymphangiogenesis promotes tumour metastasis. Embo J 2001;20(4):672-82.
Mann B and Buhr HJ. Lymph node dissection in patients with differentiated thyroid carcinoma--who benefits? Langenbecks Arch Surg 1998;383(5):355-8.
Marcocci C, Pacini F, Elisei R, Schipani E, Ceccarelli C, Miccoli P, Arganini M and Pinchera A. Clinical and biologic behavior of bone metastases from differentiated thyroid carcinoma. Surgery 1989;106(6):960-6.
Marsh DJ, Coulon V, Lunetta KL, Rocca-Serra P, Dahia PL, Zheng Z, Liaw D, Caron S, Duboue B, Lin AY, et al. Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. Hum Mol Genet 1998;7(3):507-15.
Martin B, Paesmans M, Berghmans T, Branle F, Ghisdal L, Mascaux C, Meert AP, Steels E, Vallot F, Verdebout JM, et al. Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer 2003;89(1):55-64.
Masferrer JL, Leahy KM, Koki AT, Zweifel BS, Settle SL, Woerner BM, Edwards DA,

Flickinger AG, Moore RJ and Seibert K. Antiangiogenic and antitumor activities of cyclooxygenase-2 inhibitors. Cancer Res 2000;60(5):1306-11.
Massin JP, Savoie JC, Garnier H, Guiraudon G, Leger FA and Bacourt F. Pulmonary metastases in differentiated thyroid carcinoma. Study of 58 cases with implications for the primary tumor treatment. Cancer 1984;53(4):982-92.
Mazzaferri EL. An overview of the management of papillary and follicular thyroid carcinoma. Thyroid 1999;9(5):421-7.
Mazzaferri EL and Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994;97(5):418-28.
Mazzaferri EL and Kloos RT. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab 2001;86(4):1447-63.
Mazzaferri EL and Young RL. Papillary thyroid carcinoma: a 10 year follow-up report of the impact of therapy in 576 patients. Am J Med 1981;70(3):511-8.
Mazzaferri EL, Young RL, Oertel JE, Kemmerer WT and Page CP. Papillary thyroid carcinoma: the impact of therapy in 576 patients. Medicine (Baltimore) 1977;56(3):171-96.
McCaffrey TV, Bergstralh EJ and Hay ID. Locally invasive papillary thyroid carcinoma: 1940-1990. Head Neck 1994;16(2):165-72.
McConahey WM, Hay ID, Woolner LB, van Heerden JA and Taylor WF. Papillary thyroid cancer treated at the Mayo Clinic, 1946 through 1970: initial manifestations, pathologic findings, therapy, and outcome. Mayo Clin Proc 1986;61(12):978-96.
McGregor GI, Luoma A and Jackson SM. Lymph node metastases from well-differentiated thyroid cancer. A clinical review. Am J Surg 1985;149(5):610-2.
McHenry CR, Rosen IB and Walfish PG. Prospective management of nodal metastases in differentiated thyroid cancer. Am J Surg 1991;162(4):353-6.
McIver B, Hay ID, Giuffrida DF, Dvorak CE, Grant CS, Thompson GB, van Heerden JA and Goellner JR. Anaplastic thyroid carcinoma: a 50-year experience at a single institution. Surgery 2001;130(6):1028-34.
Michieli P, Chedid M, Lin D, Pierce JH, Mercer WE and Givol D. Induction of WAF1/CIP1 by a p53-independent pathway. Cancer Res 1994;54(13):3391-5.
Miyata Y, Koga S, Kanda S, Nishikido M, Hayashi T and Kanetake H. Expression of cyclooxygenase-2 in renal cell carcinoma: correlation with tumor cell proliferation, apoptosis, angiogenesis, expression of matrix metalloproteinase-2, and survival. Clin Cancer Res 2003;9(5):1741-9.
Mizukami Y, Nonomura A, Michigishi T, Noguchi M, Nakamura S and Hashimoto T. Differential (Ha-, K- and N-) ras p21 expression in benign and malignant human thyroid tumors: an immunohistochemical study. Anticancer Res 1995;15(3):755-9.
Moore D, Ohene-Fianko D, Garcia B and Chakrabarti S. Apoptosis in thyroid neoplasms: relationship with p53 and bcl-2 expression. Histopathology 1998;32(1):35-42.
Musholt TJ, Musholt PB, Khaladj N, Schulz D, Scheumann GF and Klempnauer J. Prognostic significance of RET and NTRK1 rearrangements in sporadic papillary thyroid carcinoma. Surgery 2000;128(6):984-93.
Myers SM, Eng C, Ponder BA and Mulligan LM. Characterization of RET proto-oncogene 3' splicing variants and polyadenylation sites: a novel C-terminus for RET. Oncogene 1995;11(10):2039-45.
Nagatsuka I, Yamada N, Shimizu S, Ohira M, Nishino H, Seki S and Hirakawa K. Inhibitory effect of a selective cyclooxygenase-2 inhibitor on liver metastasis of

colon cancer. Int J Cancer 2002;100(5):515-9.
Naito A, Iwase H, Kuzushima T, Nakamura T and Kobayashi S. Clinical significance of E-cadherin expression in thyroid neoplasms. J Surg Oncol 2001;76(3):176-80.
Nakamura H, Ueno H, Yamashita K, Shimada T, Yamamoto E, Noguchi M, Fujimoto N, Sato H, Seiki M and Okada Y. Enhanced production and activation of progelatinase A mediated by membrane-type 1 matrix metalloproteinase in human papillary thyroid carcinomas. Cancer Res 1999;59(2):467-73.
Namba H, Rubin SA and Fagin JA. Point mutations of ras oncogenes are an early event in thyroid tumorigenesis. Mol Endocrinol 1990;4(10):1474-9.
Natsugoe S, Nakashima S, Matsumoto M, Xiangming C, Okumura H, Kijima F, Ishigami S, Takebayashi Y, Baba M, Takao S, et al. Expression of p21WAF1/Cip1 in the p53-dependent pathway is related to prognosis in patients with advanced esophageal carcinoma. Clin Cancer Res 1999;5(9):2445-9.
Niederle B, Roka R, Schemper M, Fritsch A, Weissel M and Ramach W. Surgical treatment of distant metastases in differentiated thyroid cancer: indication and results. Surgery 1986;100(6):1088-97.
Niki T, Iba S, Tokunou M, Yamada T, Matsuno Y and Hirohashi S. Expression of vascular endothelial growth factors A, B, C, and D and their relationships to lymph node status in lung adenocarcinoma. Clin Cancer Res 2000;6(6):2431-9.
Niki T, Kohno T, Iba S, Moriya Y, Takahashi Y, Saito M, Maeshima A, Yamada T, Matsuno Y, Fukayama M, et al. Frequent co-localization of Cox-2 and laminin-5 gamma2 chain at the invasive front of early-stage lung adenocarcinomas. Am J Pathol 2002;160(3):1129-41.
Nikiforova MN, Kimura ET, Gandhi M, Biddinger PW, Knauf JA, Basolo F, Zhu Z, Giannini R, Salvatore G, Fusco A, et al. BRAF mutations in thyroid tumors are restricted to papillary carcinomas and anaplastic or poorly differentiated carcinomas arising from papillary carcinomas. J Clin Endocrinol Metab 2003;88(11):5399-404.
Nilsson O, Lindeberg J, Zedenius J, Ekman E, Tennvall J, Blomgren H, Grimelius L, Lundell G and Wallin G. Anaplastic giant cell carcinoma of the thyroid gland: treatment and survival over a 25-year period. World J Surg 1998;22(7):725-30.
Noguchi S, Murakami N and Kawamoto H. Classification of papillary cancer of the thyroid based on prognosis. World J Surg 1994;18(4):552-7.
Noguchi S, Murakami N, Yamashita H, Toda M and Kawamoto H. Papillary thyroid carcinoma: modified radical neck dissection improves prognosis. Arch Surg 1998;133(3):276-80.
Nose F, Ichikawa T, Fujiwara M and Okayasu I. Up-regulation of cyclooxygenase-2 expression in lymphocytic thyroiditis and thyroid tumors: significant correlation with inducible nitric oxide synthase. Am J Clin Pathol 2002;117(4):546-51.
Ofner D, Riehemann K, Maier H, Riedmann B, Nehoda H, Totsch M, Bocker W, Jasani B and Schmid KW. Immunohistochemically detectable bcl-2 expression in colorectal carcinoma: correlation with tumour stage and patient survival. Br J Cancer 1995;72(4):981-5.
Ogata R, Torimura T, Kin M, Ueno T, Tateishi Y, Kuromatsu R, Shimauchi Y, Sakamoto M, Tamaki S, Sata M, et al. Increased expression of membrane type 1 matrix metalloproteinase and matrix metalloproteinase-2 with tumor dedifferentiation in hepatocellular carcinomas. Hum Pathol 1999;30(4):443-50.
Ohbu M, Saegusa M, Kobayashi N, Tsukamoto H, Mieno H, Kakita A and Okayasu I. Expression of bcl-2 protein in esophageal squamous cell carcinomas and its association with lymph node metastasis. Cancer 1997;79(7):1287-93.

Okayasu I, Osakabe T, Onozawa M, Mikami T and Fujiwara M. p53 and p21(WAF1) expression in lymphocytic thyroiditis and thyroid tumors. Clin Immunol Immunopathol 1998;88(2):183-91.
Onogawa S, Kitadai Y, Tanaka S, Kuwai T, Kimura S and Chayama K. Expression of VEGF-C and VEGF-D at the invasive edge correlates with lymph node metastasis and prognosis of patients with colorectal carcinoma. Cancer Sci 2004;95(1):32-9.
Oshima M, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF and Taketo MM. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 1996;87(5):803-9.
P Oc, Rhys-Evans P and Eccles SA. Expression of vascular endothelial growth factor family members in head and neck squamous cell carcinoma correlates with lymph node metastasis. Cancer 2001;92(3):556-68.
Pacini F, Cetani F, Miccoli P, Mancusi F, Ceccarelli C, Lippi F, Martino E and Pinchera A. Outcome of 309 patients with metastatic differentiated thyroid carcinoma treated with radioiodine. World J Surg 1994;18(4):600-4.
Papotti M, Botto Micca F, Favero A, Palestini N and Bussolati G. Poorly differentiated thyroid carcinomas with primordial cell component. A group of aggressive lesions sharing insular, trabecular, and solid patterns. Am J Surg Pathol 1993;17(3):291-301.
Parker SB, Eichele G, Zhang P, Rawls A, Sands AT, Bradley A, Olson EN, Harper JW and Elledge SJ. p53-independent expression of p21Cip1 in muscle and other terminally differentiating cells. Science 1995;267(5200):1024-7.
Pasieka JL, Zedenius J, Auer G, Grimelius L, Hoog A, Lundell G, Wallin G and Backdahl M. Addition of nuclear DNA content to the AMES risk-group classification for papillary thyroid cancer. Surgery 1992;112(6):1154-9.
Passler C, Scheuba C, Prager G, Kaczirek K, Kaserer K, Zettinig G and Niederle B. Prognostic factors of papillary and follicular thyroid cancer: differences in an iodine-replete endemic goiter region. Endocr Relat Cancer 2004;11(1):131-9.
Patel KN and Shaha AR. Locally advanced thyroid cancer. Curr Opin Otolaryngol Head Neck Surg 2005;13(2):112-6.
Pattou F, Combemale F, Fabre S, Carnaille B, Decoulx M, Wemeau JL, Racadot A and Proye C. Hypocalcemia following thyroid surgery: incidence and prediction of outcome. World J Surg 1998;22(7):718-24.
Pezzella F, Turley H, Kuzu I, Tungekar MF, Dunnill MS, Pierce CB, Harris A, Gatter KC and Mason DY. bcl-2 protein in non-small-cell lung carcinoma. N Engl J Med 1993;329(10):690-4.
Pilotti S, Collini P, Rilke F, Cattoretti G, Del Bo R and Pierotti MA. Bcl-2 protein expression in carcinomas originating from the follicular epithelium of the thyroid gland. J Pathol 1994;172(4):337-42.
Pollina L, Pacini F, Fontanini G, Vignati S, Bevilacqua G and Basolo F. bcl-2, p53 and proliferating cell nuclear antigen expression is related to the degree of differentiation in thyroid carcinomas. Br J Cancer 1996;73(2):139-43.
Prendiville S, Burman KD, Ringel MD, Shmookler BM, Deeb ZE, Wolfe K, Azumi N, Wartofsky L and Sessions RB. Tall cell variant: an aggressive form of papillary thyroid carcinoma. Otolaryngol Head Neck Surg 2000;122(3):352-7.
Puglisi F, Cesselli D, Damante G, Pellizzari L, Beltrami CA and Di Loreto C. Expression of Pax-8, p53 and bcl-2 in human benign and malignant thyroid diseases. Anticancer

Res 2000;20(1A):311-6.
Ristimäki A, Sivula A, Lundin J, Lundin M, Salminen T, Haglund C, Joensuu H and Isola J. Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res 2002;62(3):632-5.
Ross JS, Kaur P, Sheehan CE, Fisher HA, Kaufman RA, Jr. and Kallakury BV. Prognostic significance of matrix metalloproteinase 2 and tissue inhibitor of metalloproteinase 2 expression in prostate cancer. Mod Pathol 2003;16(3):198-205.
Rossi RL, Cady B, Silverman ML, Wool MS, ReMine SG, Hodge MB and Salzman FA. Surgically incurable well-differentiated thyroid carcinoma. Prognostic factors and results of therapy. Arch Surg 1988;123(5):569-74.
Ruegemer JJ, Hay ID, Bergstralh EJ, Ryan JJ, Offord KP and Gorman CA. Distant metastases in differentiated thyroid carcinoma: a multivariate analysis of prognostic variables. J Clin Endocrinol Metab 1988;67(3):501-8.
Ruter A, Nishiyama R and Lennquist S. Tall-cell variant of papillary thyroid cancer: disregarded entity? World J Surg 1997;21(1):15-20; discussion 20-1.
Räty R, Franssila K, Joensuu H, Teerenhovi L and Elonen E. Ki-67 expression level, histological subtype, and the International Prognostic Index as outcome predictors in mantle cell lymphoma. Eur J Haematol 2002;69(1):11-20.
Saegusa M, Takano Y, Hashimura M, Shoji Y and Okayasu I. The possible role of bcl-2 expression in the progression of tumors of the uterine cervix. Cancer 1995;76(11):2297-303.
Saiz AD, Olvera M, Rezk S, Florentine BA, McCourty A and Brynes RK. Immunohistochemical expression of cyclin D1, E2F-1, and Ki-67 in benign and malignant thyroid lesions. J Pathol 2002;198(2):157-62.
Sakamoto A, Kasai N and Sugano H. Poorly differentiated carcinoma of the thyroid. A clinicopathologic entity for a high-risk group of papillary and follicular carcinomas. Cancer 1983;52(10):1849-55.
Samaan NA, Schultz PN, Haynie TP and Ordonez NG. Pulmonary metastasis of differentiated thyroid carcinoma: treatment results in 101 patients. J Clin Endocrinol Metab 1985;60(2):376-80.
Samaan NA, Schultz PN, Hickey RC, Goepfert H, Haynie TP, Johnston DA and Ordonez NG. The results of various modalities of treatment of well differentiated thyroid carcinomas: a retrospective review of 1599 patients. J Clin Endocrinol Metab 1992;75(3):714-20.
Sampson RJ, Woolner LB, Bahn RC and Kurland LT. Occult thyroid carcinoma in Olmsted County, Minnesota: prevalence at autopsy compared with that in Hiroshima and Nagasaki, Japan. Cancer 1974;34(6):2072-6.
Santoro M, Carlomagno F, Hay ID, Herrmann MA, Grieco M, Melillo R, Pierotti MA, Bongarzone I, Della Porta G, Berger N, et al. Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. J Clin Invest 1992;89(5):1517-22.
Scheumann GF, Gimm O, Wegener G, Hundeshagen H and Dralle H. Prognostic significance and surgical management of locoregional lymph node metastases in papillary thyroid cancer. World J Surg 1994;18(4):559-67; discussion 567-8.
Scheumman GF, Hoang-Vu C, Cetin Y, Gimm O, Behrends J, von Wasielewski R, Georgii A, Birchmeier W, von Zur Muhlen A, Dralle H, et al. Clinical significance of E-cadherin as a prognostic marker in thyroid carcinomas. J Clin Endocrinol Metab 1995;80(7):2168-72.
Schlumberger M, Challeton C, De Vathaire F, Travagli JP, Gardet P, Lumbroso JD,

Francese C, Fontaine F, Ricard M and Parmentier C. Radioactive iodine treatment and external radiotherapy for lung and bone metastases from thyroid carcinoma. J Nucl Med 1996;37(4):598-605.
Schlumberger M, Tubiana M, De Vathaire F, Hill C, Gardet P, Travagli JP, Fragu P, Lumbroso J, Caillou B and Parmentier C. Long-term results of treatment of 283 patients with lung and bone metastases from differentiated thyroid carcinoma. J Clin Endocrinol Metab 1986;63(4):960-7.
Schlumberger MJ. Papillary and follicular thyroid carcinoma. N Engl J Med 1998;338(5):297-306.
Sellers M, Beenken S, Blankenship A, Soong SJ, Turbat-Herrera E, Urist M and Maddox W. Prognostic significance of cervical lymph node metastases in differentiated thyroid cancer. Am J Surg 1992;164(6):578-81.
Shah JP, Loree TR, Dharker D, Strong EW, Begg C and Vlamis V. Prognostic factors in differentiated carcinoma of the thyroid gland. Am J Surg 1992;164(6):658-61.
Shaha AR, Loree TR and Shah JP. Intermediate-risk group for differentiated carcinoma of thyroid. Surgery 1994;116(6):1036-40; discussion 1040-1.
Sheehan KM, Sheahan K, O'Donoghue DP, MacSweeney F, Conroy RM, Fitzgerald DJ and Murray FE. The relationship between cyclooxygenase-2 expression and colorectal cancer. Jama 1999;282(13):1254-7.
Sheng H, Shao J, Kirkland SC, Isakson P, Coffey RJ, Morrow J, Beauchamp RD and DuBois RN. Inhibition of human colon cancer cell growth by selective inhibition of cyclooxygenase-2. J Clin Invest 1997;99(9):2254-9.
Sherman SI, Ball DW, Beenken SW, Byrd D, Clark OH, Daniels GH, Dilawari RA, Ehya H, Farrar WB, Gagel RF, et al. NCCN Oncology Practice Guidelines v.1.2005. National Comprehensive Cancer Network. Available at: http://www.nccn.org/physician_gls/f_guidelines.html.
Sherman SI, Brierley JD, Sperling M, Ain KB, Bigos ST, Cooper DS, Haugen BR, Ho M, Klein I, Ladenson PW, et al. Prospective multicenter study of thyrois]carcinoma treatment: initial analysis of staging and outcome. National Thyroid Cancer Treatment Cooperative Study Registry Group. Cancer 1998;83(5):1012-21.
Shoji T, Tanaka F, Takata T, Yanagihara K, Otake Y, Hanaoka N, Miyahara R, Nakagawa T, Kawano Y, Ishikawa S, et al. Clinical significance of p21 expression in non-small-cell lung cancer. J Clin Oncol 2002;20(18):3865-71.
Shushanov S, Bronstein M, Adelaide J, Jussila L, Tchipysheva T, Jacquemier J, Stavrovskaya A, Birnbaum D and Karamysheva A. VEGFc and VEGFR3 expression in human thyroid pathologies. Int J Cancer 2000;86(1):47-52.
Sier CF, Kubben FJ, Ganesh S, Heerding MM, Griffioen G, Hanemaaijer R, van Krieken JH, Lamers CB and Verspaget HW. Tissue levels of matrix metalloproteinases MMP-2 and MMP-9 are related to the overall survival of patients with gastric carcinoma. Br J Cancer 1996;74(3):413-7.
Simon D, Goretzki PE, Witte J and Roher HD. Incidence of regional recurrence guiding radicality in differentiated thyroid carcinoma. World J Surg 1996;20(7):860-6; discussion 866.
Simpson WJ, McKinney SE, Carruthers JS, Gospodarowicz MK, Sutcliffe SB and Panzarella T. Papillary and follicular thyroid cancer. Prognostic factors in 1,578 patients. Am J Med 1987;83(3):479-88.
Sivanandan R and Soo KC. Pattern of cervical lymph node metastases from papillary carcinoma of the thyroid. Br J Surg 2001;88(9):1241-4.
Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, Velasco P, Riccardi L, Alitalo

K, Claffey K and Detmar M. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 2001;7(2):192-8.
Smith SA, Hay ID, Goellner JR, Ryan JJ and McConahey WM. Mortality from papillary thyroid carcinoma. A case-control study of 56 lethal cases. Cancer 1988;62(7):1381-8.
Smith TJ, Jennings TA, Sciaky D and Cao HJ. Prostaglandin-endoperoxide H synthase-2 expression in human thyroid epithelium. Evidence for constitutive expression in vivo and in cultured KAT-50 cells. J Biol Chem 1999;274(22):15622-32.
Soares J, Limbert E and Sobrinho-Simoes M. Diffuse sclerosing variant of papillary thyroid carcinoma. A clinicopathologic study of 10 cases. Pathol Res Pract 1989;185(2):200-6.
Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, Maximo V, Botelho T, Seruca R and Sobrinho-Simoes M. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene 2003;22(29):4578-80.
Sobin L and Wittekind C (1997). TNM Classification of Malignant Tumours. New York, Wiley-Liss, Inc.
Sobin LH and Wittekind C (2002). TNM Classification of Malignant Tumours. New York, Wiley-Liss.
Sobrinho-Simoes M, Nesland JM and Johannessen JV. Columnar-cell carcinoma. Another variant of poorly differentiated carcinoma of the thyroid. Am J Clin Pathol 1988;89(2):264-7.
Sobrinho-Simoes M, Soares J, Carneiro F and Limbert E. Diffuse follicular variant of papillary carcinoma of the thyroid: report of eight cases of a distinct aggressive type of thyroid tumor. Surg Pathol 1990;3:189-203.
Sobrinho-Simoes MA, Sambade MC and Goncalves V. Latent thyroid carcinoma at autopsy: a study from Oporto, Portugal. Cancer 1979;43(5):1702-6.
Solomon BL, Wartofsky L and Burman KD. Current trends in the management of well differentiated papillary thyroid carcinoma. J Clin Endocrinol Metab 1996;81(1):333-9.
Solomon SD, McMurray JJ, Pfeffer MA, Wittes J, Fowler R, Finn P, Anderson WF, Zauber A, Hawk E and Bertagnolli M. Cardiovascular risk associated with celecoxib in a clinical trial for colorectal adenoma prevention. N Engl J Med 2005;352(11):1071-80.
Sosa JA, Bowman HM, Tielsch JM, Powe NR, Gordon TA and Udelsman R. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. Ann Surg 1998;228(3):320-30.
Specht MC, Tucker ON, Hocever M, Gonzalez D, Teng L and Fahey TJ, 3rd. Cyclooxygenase-2 expression in thyroid nodules. J Clin Endocrinol Metab 2002;87(1):358-63.
Stacker SA, Achen MG, Jussila L, Baldwin ME and Alitalo K. Lymphangiogenesis and cancer metastasis. Nat Rev Cancer 2002;2(8):573-83.
Steele RJ, Thompson AM, Hall PA and Lane DP. The p53 tumour suppressor gene. Br J Surg 1998;85(11):1460-7.
Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, et al. The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med 2000;342(26):1946-52.
Su JL, Shih JY, Yen ML, Jeng YM, Chang CC, Hsieh CY, Wei LH, Yang PC and Kuo ML. Cyclooxygenase-2 induces EP1- and HER-2/Neu-dependent vascular endothelial

growth factor-C up-regulation: a novel mechanism of lymphangiogenesis in lung adenocarcinoma. Cancer Res 2004;64(2):554-64.
Sugg SL, Ezzat S, Rosen IB, Freeman JL and Asa SL. Distinct multiple RET/PTC gene rearrangements in multifocal papillary thyroid neoplasia. J Clin Endocrinol Metab 1998;83(11):4116-22.
Sugg SL, Zheng L, Rosen IB, Freeman JL, Ezzat S and Asa SL. ret/PTC-1, -2, and -3 oncogene rearrangements in human thyroid carcinomas: implications for metastatic potential? J Clin Endocrinol Metab 1996;81(9):3360-5.
Sugitani I, Kasai N, Fujimoto Y and Yanagisawa A. Prognostic factors and therapeutic strategy for anaplastic carcinoma of the thyroid. World J Surg 2001;25(5):617-22.
Sugitani I, Yanagisawa A, Shimizu A, Kato M and Fujimoto Y. Clinicopathologic and immunohistochemical studies of papillary thyroid microcarcinoma presenting with cervical lymphadenopathy. World J Surg 1998;22(7):731-7.
Sutton RT, Reading CC, Charboneau JW, James EM, Grant CS and Hay ID. US-guided biopsy of neck masses in postoperative management of patients with thyroid cancer. Radiology 1988;168(3):769-72.
Tallini G, Garcia-Rostan G, Herrero A, Zelterman D, Viale G, Bosari S and Carcangiu ML. Downregulation of p27KIP1 and Ki67/Mib1 labeling index support the classification of thyroid carcinoma into prognostically relevant categories. Am J Surg Pathol 1999;23(6):678-85.
Talvensaari-Mattila A, Paakko P, Höyhtyä M, Blanco-Sequeiros G and Turpeenniemi-Hujanen T. Matrix metalloproteinase-2 immunoreactive protein: a marker of aggressiveness in breast carcinoma. Cancer 1998;83(6):1153-62.
Tanaka K, Kurebayashi J, Sonoo H, Otsuki T, Yamamoto Y, Ohkubo S, Yamamoto S and Shimozuma K. Expression of vascular endothelial growth factor family messenger RNA in diseased thyroid tissues. Surg Today 2002a;32(9):761-8.
Tanaka K, Sonoo H, Kurebayashi J, Nomura T, Ohkubo S, Yamamoto Y and Yamamoto S. Inhibition of infiltration and angiogenesis by thrombospondin-1 in papillary thyroid carcinoma. Clin Cancer Res 2002b;8(5):1125-31.
Tang RF, Itakura J, Aikawa T, Matsuda K, Fujii H, Korc M and Matsumoto Y. Overexpression of lymphangiogenic growth factor VEGF-C in human pancreatic cancer. Pancreas 2001;22(3):285-92.
Thomas GA, Bunnell H, Cook HA, Williams ED, Nerovnya A, Cherstvoy ED, Tronko ND, Bogdanova TI, Chiappetta G, Viglietto G, et al. High prevalence of RET/PTC rearrangements in Ukrainian and Belarussian post-Chernobyl thyroid papillary carcinomas: a strong correlation between RET/PTC3 and the solid-follicular variant. J Clin Endocrinol Metab 1999;84(11):4232-8.
Thor A, Horan Hand P, Wunderlich D, Caruso A, Muraro R and Schlom J. Monoclonal antibodies define differential ras gene expression in malignant and benign colonic diseases. Nature 1984;311(5986):562-5.
Tron VA, Krajewski S, Klein-Parker H, Li G, Ho VC and Reed JC. Immunohistochemical analysis of Bcl-2 protein regulation in cutaneous melanoma. Am J Pathol 1995;146(3):643-50.
Tsujii M and DuBois RN. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase 2. Cell 1995;83(3):493-501.
Tsujii M, Kawano S and DuBois RN. Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A 1997;94(7):3336-40.
Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M and DuBois RN. Cyclooxygenase

regulates angiogenesis induced by colon cancer cells. Cell 1998;93(5):705-16.
Tsujimoto Y, Finger LR, Yunis J, Nowell PC and Croce CM. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. Science 1984;226(4678):1097-9.
Tsurusaki T, Kanda S, Sakai H, Kanetake H, Saito Y, Alitalo K and Koji T. Vascular endothelial growth factor-C expression in human prostatic carcinoma and its relationship to lymph node metastasis. Br J Cancer 1999;80(1-2):309-13.
Tubiana M, Schlumberger M, Rougier P, Laplanche A, Benhamou E, Gardet P, Caillou B, Travagli JP and Parmentier C. Long-term results and prognostic factors in patients with differentiated thyroid carcinoma. Cancer 1985;55(4):794-804.
Tzavara I, Vlassopoulou B, Alevizaki C, Koukoulis G, Tzanela M, Koumoussi P, Sotsiou F and Thalassinos N. Differentiated thyroid cancer: a retrospective analysis of 832 cases from Greece. Clin Endocrinol (Oxf) 1999;50(5):643-54.
Uchino S, Noguchi S, Yamashita H and Watanabe S. Modified radical neck dissection for differentiated thyroid cancer: operative technique. World J Surg 2004:28(12):1199-203.
Udelsman R, Lakatos E and Ladenson P. Optimal surgery for papillary thyroid carcinoma. World J Surg 1996;20(1):88-93.
Uruno T, Miyauchi A, Shimizu K, Nakano K, Takamura Y, Ito Y, Miya A, Kobayashi K, Yokozawa T, Matsuzuka F, et al. Prognosis after reoperation for local recurrence of papillary thyroid carcinoma. Surg Today 2004;34(11):891-5.
Vasala K, Paakko P and Turpeenniemi-Hujanen T. Matrix metalloproteinase-2 immunoreactive protein as a prognostic marker in bladder cancer. Urology 2003;62(5):952-7.
Viale G, Roncalli M, Grimelius L, Graziani D, Wilander E, Johansson H, Bergholm U and Coggi G. Prognostic value of bcl-2 immunoreactivity in medullary thyroid carcinoma. Hum Pathol 1995;26(9):945-50.
Vickery AL, Jr., Carcangiu ML, Johannessen JV and Sobrinho-Simoes M. Papillary carcinoma. Semin Diagn Pathol 1985;2(2):90-100.
Vihinen P and Kähäri VM. Matrix metalloproteinases in cancer: prognostic markers and therapeutic targets. Int J Cancer 2002;99(2):157-66.
Viola MV, Fromowitz F, Oravez S, Deb S, Finkel G, Lundy J, Hand P, Thor A and Schlom J. Expression of ras oncogene p21 in prostate cancer. N Engl J Med 1986;314(3):133-7.
Viola MV, Fromowitz F, Oravez S, Deb S and Schlom J. ras Oncogene p21 expression is increased in premalignant lesions and high grade bladder carcinoma. J Exp Med 1985;161(5):1213-8.
von Wasielewski R, Rhein A, Werner M, Scheumann GF, Dralle H, Potter E, Brabant G and Georgii A. Immunohistochemical detection of E-cadherin in differentiated thyroid carcinomas correlates with clinical outcome. Cancer Res 1997;57(12):2501-7.
Voutilainen PE, Multanen MM, Leppäniemi AK, Haglund CH, Haapiainen RK and Franssila KO. Prognosis after lymph node recurrence in papillary thyroid carcinoma depends on age. Thyroid 2001;11(10):953-7.
Wallin G, Backdahl M, Christensson B, Grimelius L and Auer G. Nuclear protein content and Ki-67 immunoreactivity in nonneoplastic and neoplastic thyroid cells. Anal Quant Cytol Histol 1992;14(4):296-303.
Weber KB, Shroyer KR, Heinz DE, Nawaz S, Said MS and Haugen BR. The use of a combination of galectin-3 and thyroid peroxidase for the diagnosis and prognosis

of thyroid cancer. Am J Clin Pathol 2004;122(4):524-31.
Wood WJ, Jr., Singletary SE and Hickey RC. Current results of treatment for distant metastatic well-differentiated thyroid carcinoma. Arch Surg 1989;124(12):1374-7.
Xiong Y, Zhang H and Beach D. D type cyclins associate with multiple protein kinases and the DNA replication and repair factor PCNA. Cell 1992;71(3):505-14.
Yamashita H, Noguchi S, Murakami M, Watanabe S, Uchino S, Kawamoto H, Toda M and Nakayama I. Changing trends and prognoses for patients with papillary thyroid cancer. Arch Surg 1998:133(10):1058-65.
Yao M, Kargman S, Lam EC, Kelly CR, Zheng Y, Luk P, Kwong E, Evans JF and Wolfe MM. Inhibition of cyclooxygenase-2 by rofecoxib attenuates the growth and metastatic potential of colorectal carcinoma in mice. Cancer Res 2003;63(3):586-92.
Yoshida A, Nakamura Y, Imada T, Asaga T, Shimizu A and Harada M. Apoptosis and proliferative activity in thyroid tumors. Surg Today 1999;29(3):204-8.
Young TN, Rodriguez GC, Rinehart AR, Bast RC, Jr., Pizzo SV and Stack MS. Characterization of gelatinases linked to extracellular matrix invasion in ovarian adenocarcinoma: purification of matrix metalloproteinase 2. Gynecol Oncol 1996;62(1):89-99.
Yunis JJ, Mayer MG, Arnesen MA, Aeppli DP, Oken MM and Frizzera G. bcl-2 and other genomic alterations in the prognosis of large-cell lymphoma. N Engl J Med 1989;320(16):1047-54.
Zedenius J, Stahle-Backdahl M, Enberg U, Grimelius L, Larsson C, Wallin G and Backdahl M. Stromal fibroblasts adjacent to invasive thyroid tumors: expression of gelatinase A but not stromelysin 3 mRNA. World J Surg 1996;20(1):101-6.
Zettinig G, Fueger BJ, Passler C, Kaserer K, Pirich C, Dudczak R and Niederle B. Long-term follow-up of patients with bone metastases from differentiated thyroid carcinoma -- surgery or conventional therapy? Clin Endocrinol (Oxf) 2002;56(3):377-82.

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Original Publications