Clinical studies on treatment and follow-up in differentiated thyroid carcinoma

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Clinical studies on treatment and follow-up in differentiated thyroid carcinoma

Klinische studies naar de behandeling en de nazorg van patiënten met een gedifferentieerd schildkliercarcinoom
(met een samenvatting in het Nederlands)

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"Blijf mens, en denk niet dat je alles weet"

Oma Corrie Bouwmeester (29 november 1919 - 9 januari 1999), november 1998
Oom Jan Dekker (27 augustus 1925 - 24 december 1999), november 1999

Contents

Chapter One

Introduction and objectives

Introduction

Without radioiodine therapy, nuclear medicine might never have existed. One of the most spectacular contributions to the inception of the specialty was made in 1942 by Dr. Samuel M. Seidlin who, when faced with a patient who suffered from extensive functioning metastases of differentiated thyroid cancer 20 years after thyroidectomy, decided to try the newly available radioactive iodine in an attempt to ameliorate the patient's symptoms.[1] In those days metastasized cancer was usually fatal within a short time. For this patient, however, radioactive iodine therapy caused remission of the symptoms, regression of the metastatic masses, and improvement of the quality of life. The report (notably in the Wall Street Journal) of a potential cure for terminally ill patients fuelled the public imagination to a degree that it hit the political agenda. Effective on August 1, 1946, the Atomic Energy Act (AEA) made radioisotopes available for medical use in the United States of America. This date marks the beginning of 'atomic medicine', which was later named nuclear medicine.

During the treatment Dr. Seidlin introduced another method typical of modern nuclear medicine: he made a study of the radiopharmaceutical distribution. After administration of ^{131}I he used a Geiger counter to prove radionuclide uptake in the known metastases. Guided by the pain symptoms, he also scanned other locations, identifying two more previously unknown metastases in the process. Thus the concept of *in vivo* radionuclide scanning was introduced before the invention of the Anger-type gamma camera or even rectilinear scanners.

Dr. Seidlin did not abandon ^{131}I therapy for thyroid cancer after treating his initial patient. Until his death in 1955 he published several articles on the subject, dealing with radiation doses in blood as a result of ^{131}I therapy,[2] and with leukemia as a consequence of high cumulative activities of ^{131}I.[3,4] Like any medical intervention, radioiodine treatment had to reckon with potential side effects.

After more than 60 years radioiodine therapy is still a cornerstone of modern nuclear medicine. Notwithstanding this long history, many questions about the treatment, the follow-up, and the prognosis of patients with differentiated thyroid carcinoma (DTC) have remained unresolved. The advent of ^{131}I therapy is one of the factors that have improved the prognosis for those patients who cannot be cured by surgery alone. Ever since Dr. Seidlin's first observation, proper double-blind, randomized trials with cancer-related death as an endpoint became impossible to execute without ^{131}I co-treatment. Trials with very large numbers of patients are not unusual when it comes to ubiquitous diseases such as cardiovascular disease or diabetes mellitus. However, the

relatively low incidence of DTC in practice precludes the execution of such research. Most research in DTC is therefore performed either *in vitro* or in the form of population-based, retrospective analysis of treatments and outcomes. Retrospective studies provide opportunities to learn and to improve future patient treatment. At the same time, they are prone to various biases which constrain the power of such studies. As a result, many questions regarding the treatment and the follow-up of patients with DTC are subject to debate even at the present time. Proponents and opponents of various regimens may produce similar studies with quite dissimilar results. The treatment and the follow-up of patients with DTC will keep evolving, as insight in the pathogenesis, the efficacy of various treatment modalities, and the prognosis of DTC grow.

Objectives

The research that is presented in this thesis was strongly inspired by questions arising from daily clinical practice. In an attempt to answer these questions, the following research objectives were laid out:

- What is presently known about the functioning of the normal thyroid, and what are the dilemmas in the state-of-the-art radioiodine treatment for DTC?
- Can a tumor size be defined above which there is an increased risk of advanced disease characteristics such as multifocal DTC, extra-thyroidal tumor invasion, lymph node metastases, or distant metastases?
- Can the optimal ^{131}I activity for radioiodine ablation treatment be assessed in patients with DTC?
- Can small amounts of ^{131}I, used for pre-ablative diagnostic scanning, cause stunning of thyroid cells?
- What is the course of disease after ^{131}I ablation in patients with papillary thyroid carcinoma who have clinical evidence of lymph node metastases at the time of diagnosis?
- Does successful ^{131}I ablation have prognostic significance for patients with DTC?
- What are the recurrence rates in patients with DTC after successful ^{131}I ablation?
- Are the differences in the prognosis of patients with papillary and follicular thyroid carcinoma intrinsic, or are they due to differences in disease stage at the time of presentation?
- What, with regard to our patient group, is the prognostic value of the many different staging systems for DTC?
- Which time point is the most suitable for measurement of thyroglobulin levels after the administration of recombinant human thyrotropin (rhTSH)?

- What problems are encountered in the measurement of thyroglobulin, and what alternative techniques may be clinically useful?
- How do heterophile antibodies interfere with thyroglobulin measurements?
- Can we, using the integrated findings from our research, optimize patient-tailored treatment and follow-up protocols for DTC? And can directions be pointed out for future research that may further improve the treatment and the follow-up of patients with DTC?

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Chapter Two

Use of radiopharmaceuticals for diagnosis, treatment and follow-up of differentiated thyroid carcinoma

Frederik A. Verburg, Bart de Keizer, Johannes W. van Isselt
Anticancer Agents Med Chem. 2007; 7: 399-409

Historical perspective

In 1942, Dr. Seidlin of the Memorial Hospital in New York was faced with a 51-year-old patient who had undergone a thyroidectomy in 1923.[1] At the time, the histologic diagnosis was a 'malignant adenoma' of the thyroid. In 1938 the patient returned with overt signs of thyroid hyperfunction (hyperthyroidism) and lower back pain. A metastasis was found in the lower spine, and surgically removed. Over the next years the patient remained hyperthyroid and developed more bone metastases. At the time of presentation to Dr. Seidlin, the patient was in an extremely poor condition: he was in severe pain, severely hyperthyroid, and severely underweight.

At this time radioiodine therapy had just reached the clinical arena. In 1937 Hertz, Roberts and Evans investigated the rabbit's thyroid function using ^{128}I.[2] Later they pursued therapeutic goals for e.g. Graves' disease using ^{130}I. They used dosages that we now know would have been merely diagnostic if it were not for a probable 10% ^{131}I contaminant.[3] Livingood and Seaborg identified ^{131}I as a separate isotope. In 1942 two groups independently reported on the successful treatment of hyperthyroidism with ^{131}I sodiumiodide.[4,5] Radioiodine was so rare that it was recovered from the urine, purified and re-administered to the patient.

The patient responded favorably to the radioiodine treatment, and he received several more courses of ^{131}I. Geiger counter examination of the patient revealed two previously unknown metastases, thereby indicating the diagnostic capabilities of radioiodine. The patient did very well on these courses: the hyperthyroidism subsided, the body weight increased from 38 to 53 kilograms, and the pains diminished.

This report of a potential cure for terminally ill patients fuelled the public imagination to a degree that it hit the political agenda. Effective on August 1, 1946, the Atomic Energy Act (AEA) made radioisotopes available for medical use in the USA. This date marks the beginning of 'atomic medicine', later named nuclear medicine.

Introduction

Thyroid carcinoma is the most common endocrine malignancy, even though it represents less than 1% of all cancer cases.[6] Its incidence varies throughout the world.[7] In about 80% of all cases it concerns a so called differentiated thyroid carcinoma (DTC): papillary (PTC) or follicular (FTC) thyroid carcinoma. These tumor types originate from follicular thyrocytes, and retain some of the properties that characterize these cells. The ratio of papillary:follicular carcinoma also varies, but generally papillary thyroid carcinoma is more prevalent.[8] About 10% of all cases are medullary thyroid carcinoma (MTC), originating from C-cells in the thyroid gland. The remaining

10% are anaplastic thyroid carcinoma (ATC), one of the most lethal human cancers. As PTC and FTC constitute by far the largest patient group, and the only one with a standard role for radionuclide imaging and therapy, this review focuses on these varieties. Knowledge of the normal thyroid function is paramount to the understanding of radioiodine imaging and treatment. The thyroid function will be discussed in the first part. The second part focuses on thyroid carcinoma and its treatment and follow-up, with special emphasis on nuclear medicine procedures.
MTC and ATC are more heterogeneous, and radionuclide imaging and therapy have no standard role in the management of these varieties. A full discussion of the diagnostic and therapeutic options for patients with MTC and ATC is beyond the scope of this review.

Normal follicular thyroid cell function and regulation

There are two types of thyroid cells: follicular cells and C-cells. Thyroid follicular cells are grouped in follicles; in the center of the follicle there is a large mass of colloidal fluid. The main function of the follicular thyroid cells is the production of thyroid hormone, the major regulator of metabolism in all mammalians.

Regulation of thyroid hormone synthesis and release

The most important factors regulating the synthesis and release of thyroid hormones are the levels of thyroid stimulating hormone (TSH, or thyrotropin) and iodine availability. TSH is produced by the pituitary. It affects every step in the production and release of thyroid hormones: the expression of the sodiumiodide symporter (NIS),[9] of thyroid peroxidase (TPO) and of thyroglobulin (Tg). TSH increases the production of H_2O_2 and the formation of triiodothyronine (T3) relative to thyroxine (T4); T3 and T4 are the thyroid hormones. TSH also stimulates the re-uptake of Tg from the thyroid follicles. All these steps are outlined in greater detail below. TSH levels in healthy persons are regulated via a negative feedback loop (figure 2.1). TSH increases the production and release of T3 and T4, which in turn have a negative effect on the secretion of TSH by the pituitary gland. The production of TSH is regulated by thyrotropin releasing hormone (TRH), which is secreted in the hypothalamus.
Iodine deficiency causes inadequate thyroid hormone synthesis, a condition leading to hypothyroidism, secondarily to elevated TSH levels, and eventually to thyroid hyperplasia (goiter). Iodine excess also inhibits thyroid hormone synthesis. This is known as the Wolff-Chaikoff effect:[10] inhibition of the production of H_2O_2 by high iodide concentrations.

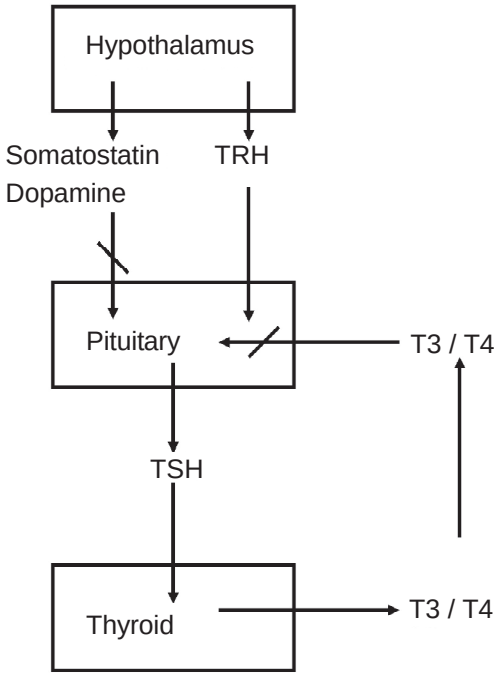

Figure 2.1 Schematic representation of the feedback mechanism of regulation of thyroid function.

TSH = thyroid stimulating hormone
TRH = thyrotropin releasing hormone
T3 = tri-iodothyronine
T4 = thyroxine
→ = stimulation
↛ = inhibition

Iodine

Iodine was discovered by Courtois in 1811 when he treated kelp (seaweed) with sulphuric acid to extract certain chemicals. He accidentally added too much sulphuric acid, and a violet-colored vapour formed. The name 'iodine' is derived from the Greek word *iodes*, meaning violet. In 1812 Lussac identified iodine as a chemical element. In 1895 Baumann established that iodine was present in the thyroid gland.[11]
Twenty years later Marine found that iodine was cleared from the blood by the thyroid.[12] This was shortly after Kendall succeeded in isolating in crystalline form 'the compound containing iodin, which occurs in the thyroid'. This was later called thyroxine, or thyroid hormone.[13]

Iodine uptake

Dietary iodine is reduced to iodide before being taken up from the gut and subsequently taken up from the plasma by the thyrocytes. The latter process is an active oxygen-dependent transport by the sodiumiodide symporter (NIS) at the basolateral plasma membrane of the thyrocytes. NIS is a 643-amino acid protein[14] which has 13 membrane spanning domains, with the carboxy terminus in the cytoplasm and the amino terminus located outside the cell.[15] In the model proposed by Eskandari a Na^+ ion first binds to the symporter. In the presence of iodine the symporter forms a complex that transfers iodide and two Na^+ ions to the cell interior.[16] Both the level of NIS expression at the membrane and its activity are controlled by TSH. The intracellular iodide concentration is about 20-50 times higher than the plasma concentrations.[17] Iodide is actively transported first through the cell and then through the apical plasma membrane into the follicular lumen. The protein responsible for the latter has not been identified, but there are two candidates: pendrin, and the apical iodide transporter.[18-20] Though some other tissues like the salivary or mammary glands also take up iodine, none of these do so in the concentrations and amounts comparable to the thyroid gland. A schematic representation of iodine intake, turnover and excretion is given in figure 2.2.

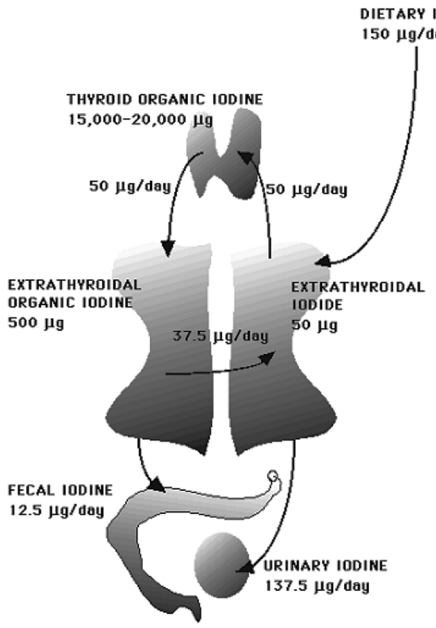

Figure 2.2 Iodine intake, turnover and excretion.

Organification

At the apical membrane in the follicle, the enzyme thyroid peroxidase (TPO) mediates the oxidization of free iodide. As TPO is a peroxidase, this process requires the presence of hydrogen peroxide. Thyroglobulin (Tg) is abundant in the thyroid follicle; it is produced by the follicular cells and then secreted into the follicle. This 660 kDa protein[21] contains a number of tyrosyl residues. Under the influence of TPO iodide groups are attached to the tyrosyl residues in Tg. The reaction by which this is achieved is not fully known.[22,23] To some tyrosyl residues only one iodide atom will be linked, to others two. In the final step, two iodinated tyrosyl residues are linked in a process catalyzed by TPO.[24-26] This results in the formation of thyroid hormone molecules containing either three (T3) or four (T4) iodine atoms. Also small quantities of a product called reverse-T3 are formed; this compound is biologically inactive. Structure formulas of T3, reverse-T3 and T4 are given in figure 2.3.

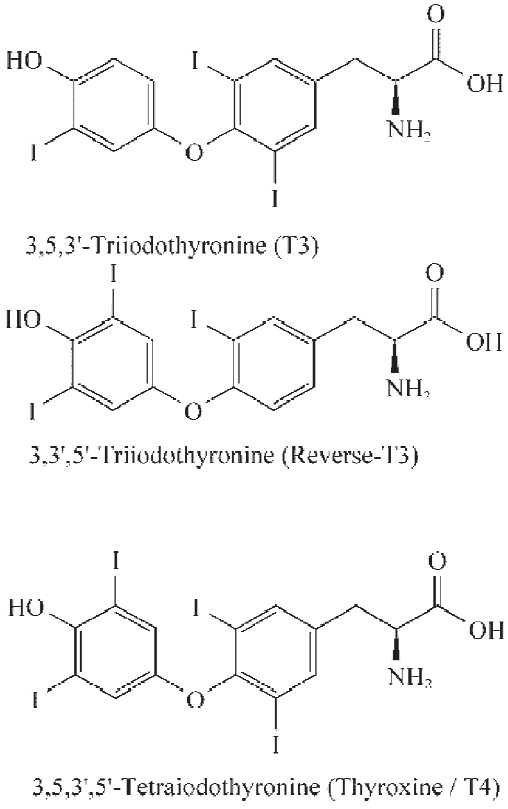

Figure 2.3 Structure formulas of T3, reverse-T3 and T4.

Thyroid hormone storage and excretion

The iodinated Tg molecules are accumulated in the follicles and reach concentrations of up to 0.3-0.5 mM. In healthy individuals the iodine pool is being turned over at a rate of about 1 percent per day.[27] The concentration of thyroid hormone in the follicles varies widely, depending on factors such as the iodide availability, the level of activity of catalyzing enzymes, and the Tg concentration. To release thyroid hormones, these molecules must first be freed from Tg. Mediated by TSH stimulation, Tg is re-ingested by the follicular thyroid cells in a process called micropinocytosis.[28] Subsequently Tg is conveyed to intracellular lysosome compartments, where it is broken down and the thyroid hormones are released. Any iodinated tyrosine residues that have not been conjugated into thyroid hormones are de-iodinated, and the iodide is returned to the intrathyroidal iodide pool. This mechanism provides about 3-5 times more iodide than enters the thyroid from outside.[27] T3 and T4 are then transported from the lysosome into the cytoplasm (most likely by lysosomal membrane transporters[29]), and released into the circulation. The estimated release of T3 and T4 is 10-22 and 94-110 μg per

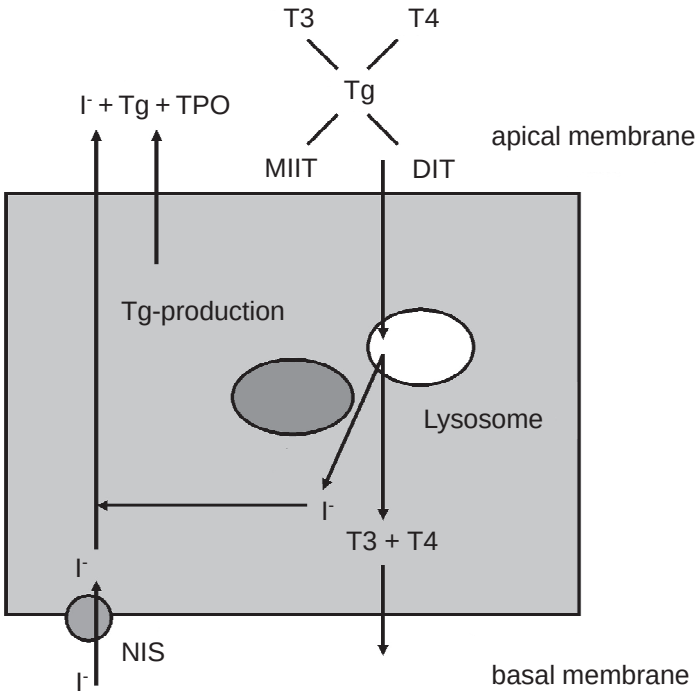

Figure 2.4 Schematic representation of the function of the thyrocyte.

day, respectively.[30] This indicates that far greater amounts of the biologically inactive T4 are released than of the biologically active T3. The hormonal potential of T4 is realized by its conversion into T3 in peripheral organs such as the liver. The thyroid gland releases not only thyroid hormones, but also small amounts of Tg into the circulation. A schematic representation of all steps involved in the organification of iodine can be found in figure 2.4.

Drugs influencing the thyroid function

The drugs most commonly used for inhibiting the thyroid function are the thionamides propylthiouracil and methimazole.[31] These drugs were pioneered in the 1940s.[32,33] In a rat model, they were shown to act by competing for oxidized iodine with tyrosyl residues of Tg.[34] Another drug that can be used to inhibit the thyroid function is sodiumiodide, as was discovered by Plummer.[35] The iodide excess temporarily inhibits thyroid iodine uptake and organification (Wolff-Chaikoff effect).[10] This intervention can be applied after accidental release of radioiodine in order to prevent harmful effects from radioiodine to the thyroid gland. Lithium is another inhibitor of the thyroid function. It prevents the release of organic iodine, thereby elevating the intrathyroidal iodine concentration. The mechanism of action involves a reduction of the rate of proteolysis of Tg.[36]

Differentiated thyroid carcinomas and their management

Incidence

Thyroid carcinoma is more frequent in females than in males (see also etiology, page 22), with reported incidences of 1.2-2.6 per 100,000 in males and 2.0-3.8 per 100,000 in females.[37] The incidence of thyroid carcinoma is increasing.[38] The mean age of onset is 45 years. Mortality varies from 0.2 to 1.2 per 100,000 in males and from 0.4 to 2.8 per 100,000 in females.[37] These gender differences are most prominent in the female reproductive period.

Prognosis

Survival

Generally, patients with differentiated thyroid cancer have a good prognosis. Nevertheless the overall survival is lower than in a reference population of the same age and sex.[39,40] The 10-year survival rate for patients with differentiated thyroid cancer is between 70 and 98 percent; patients with PTC do somewhat better than those with FTC.[39-42]

Prognostic factors

Many clinical researchers have tried to define prognostic factors at the time of diagnosis to predict outcome in patients.[39-50] The identification of patients at high risk of recurrent disease or of thyroid cancer death is important to establish the most appropriate treatment of individuals. Prominent prognostic factors are the patient's age at the time of diagnosis and the presence of distant metastases. This may partly explain why FTC has a slightly worse prognosis than PTC: at the time of diagnosis patients with FTC follicular carcinoma are older on average than patients with PTC.[51] Prognostic factors found in one study cannot be simply transferred to another. Furthermore, various analyses were based on populations from different parts of the world, and therefore with different ethnicity. This may lead to contradicting prognostic factors: in a study based on a North-American population, male sex was associated with poorer prognosis.[44] Others, in a study based on a Japanese population, reported a poorer prognosis for females.[50] The treatment of patients may vary between different centers. This empirical fact has a great influence on the prognosis. The prognosis of patients with papillary thyroid cancer is influenced significantly by the extent of surgery and by whether ^{131}I ablation was applied.[43,52] Different prognostically important variables are sometimes identified when identical methodologies from previous studies are applied to a new population.[53]

Prognostic systems

In attempts to further stratify the prognosis, many prognostic systems have been developed to categorize patients with regard to the risk of thyroid cancer related death.[39,41,43-45,47,48,50,52,54-58] There are several methods for comparing these systems.[59] A staging system does not merely serve to predict the outcome in individual patients, but also to compare different populations for initial disease characteristics. Therefore wide acceptation of a system is essential before it can be applied to a population. The International Union Against Cancer and the American Joint Committee on Cancer's TNM system (table 2.1) fulfills that requirement,[58] and has been shown to be as good as other systems.[59]

Table 2.1 TNM staging system for differentiated thyroid carcinoma (version 5, 1997).[58]

STAGE	age < 45 years	age ≥ 45 years
I	TxNxM0	T1N0M0
II	TxNxM1	T2-T3N0M0
III		T4N0M0 or TxN1M0
IV		TxNxM1

Etiology

The only clearly established external etiologic factor for thyroid cancer is irradiation of thyroid tissue,[60-64] especially at a younger age.[65] External electron beam irradiation for benign or malignant disorders in the neck region during childhood results in an elevated risk of thyroid cancer, occurring at least five years after radiation; the risk remains elevated even 40 years after exposure.[64] With increasing age at the time of exposure, this risk decreases significantly (little elevated risk if age > 20 years). For age < 15 years at the time of exposure a linear model best describes the dose response, even down to 0.1 Gy. At doses of > 10 Gy, the risk appears to level off or decrease.[64] Irradiation of the thyroid by exposure to high dosages of radioiodine also increases the risk of thyroid cancer,[8,63,65] as became apparent after the Chernobyl accident. Especially in children the incidence rose dramatically after the incident.[8,66] Iodine deficiency is a less certain etiologic factor, although different ratios of papillary, follicular and anaplastic thyroid carcinoma have been established in iodine deficient and iodine sufficient populations.[8,67,68] Estrogens most likely play a pivotal role in the different incidence between male and (younger) female patients. Estrogens increase the expression of the cyclin D1 protein, mitogen-activated protein (MAP) 1 and 2 kinases,[69] and the anti-apoptotic Bcl-xL protein.[70] Even though these are strong pointers to a cause of gender differences, estrogens most likely are not the only factor. In a case-control matched population study, limited support was found for the hypothesis that reproductive and hormonal exposures are responsible for the marked excess of thyroid cancer risk in adult females.[71] No other mechanisms are known for the gender bias in thyroid cancer incidence.

Genetic changes in differentiated thyroid cancer

Several genes have been implicated in the pathogenesis of thyroid cancer. The most commonly involved mutations in PTC are in the BRAF gene and in the RET gene; but several different mutations have been described.[72-84] At least 15 different known rearrangements of the RET gene are involved in papillary thyroid carcinoma, RET/PTC1 being the most common. The distribution and proportion of RET mutations differ between radiation-induced and sporadic papillary thyroid carcinoma.[75-78] Together, BRAF mutations and RET/PTC arrangements account for a maximum of 72 percent of PTCs.[85-87] Mutations of the NTRK1 (formerly trk) gene have been found in a proportion of papillary thyroid carcinomas,[88-91] as well as mutations of the MET gene.[23] RAS oncogene mutations have been encountered in benign as well as malignant thyroid nodules; these mutations may be an early stage in thyroid cell carcinogenesis. RAS mutations are especially found in follicular adenomas and

carcinomas.[8,76,90,92] PAX8-Pparγ rearrangements have also been described in a portion of FTCs.[93,94] Differentiated thyroid cancer seems to be associated with some familial tumor syndromes, such as familial adenomatous polyposis coli[90,92,95] including its subtype Gardner's syndrome, and Cowden disease (a hereditary hamartoma syndrome). These syndromes are caused by the APC gene (5q21) and the PTEN gene (10q23.3), respectively.[90] Evidence exists for a common mechanism for neuroblastoma and differentiated thyroid cancer.[96] Familial forms of differentiated thyroid cancer can also be found without association with other familial cancer syndromes. Mutations in the p53 tumor suppressor gene can be found in a fraction of differentiated and anaplastic thyroid carcinomas.[97-103] Different studies yield conflicting results about the induction, presence and role of p53 mutations in papillary thyroid carcinoma. Evidence exists both for and against induction of p53 mutations by thyroid irradiation.[100-103]

Histology

Papillary thyroid cancer

PTC is an unencapsulated tumor with papillary and follicular structures; it is characterized by overlapping cell nuclei that have a ground glass appearance and longitudinal grooves, with invaginations of cytoplasm into the nuclei.[104,105] Variations include encapsulated, follicular, tall cell, columnar cell, clear cell, diffuse sclerosing, solid or trabecular and oxyphilic forms of papillary thyroid cancer.[6,106] Papillary carcinomas are often multicentric. Many of these multicentric carcinomas are of different clonal origin; i.e., the various centers originate independently.[107] Metastasis occurs preferentially lymphogenous, first spreading to the cervical, and sometimes mediastinal, lymph nodes before spreading to the lungs.

Follicular thyroid cancer

FTC is characterized by follicular differentiation, without the nuclear changes characteristic of papillary thyroid carcinoma.[104,105] They are encapsulated masses, and are distinguished from follicular adenomas by the presence of invasion of the capsule and vessels. According to the pattern of invasion they can be divided into two categories: minimally invasive and widely invasive carcinoma. Follicular carcinomas are less often multicentric. A variety of the follicular carcinoma is the Hürthle cell carcinoma, which consists of at least 75% oxyphilic cells.[106] An important characteristic of Hürthle cell carcinomas is their poor or even absent iodine uptake, which renders them much harder to treat. Follicular thyroid carcinoma preferentially metastasizes hematogenously, and less frequently spreads to regional lymph nodes than PTC.

Presentation

In most cases the clinical presentation of a differentiated thyroid carcinoma is a solitary thyroid nodule. Occasionally metastases to lymph nodes, lungs or bones are the first sign of disease. Patients are usually euthyroid at the time of presentation. Sometimes thyroid carcinoma is encountered upon pathological examination of a thyroid specimen after surgery for benign indications (e.g. obstructive goiter).

Diagnostic procedures

Fine-needle aspiration biopsy

Approximately 10% of all solitary thyroid nodules are malignant. As palpable thyroid nodules are prevalent in 4-7% of the population, an accurate and quick diagnostic procedure is needed. Fine-needle aspiration (FNA) of cellular material from the nodule is presently the standard technique. The usefulness of this procedure was first demonstrated in the 1970s.[108,109] It has greatly reduced the number of thyroid surgeries.[110] Generally accepted criteria for adequate FNA sampling are a minimum of 5-6 clusters of well-preserved follicular epithelium, each containing 10-15 thyrocytes.[111,112] Ultrasound guided FNA reaches adequate sampling rates of 79-99% (mean 91%). It is definitely superior to non-ultrasound guided FNA,[113-117] because of the greater sensitivity and specificity and the lower rate of non-diagnostic outcomes. There are a number of confounding factors in the diagnosis of thyroid nodules by FNA,[118] which cause inconclusive diagnoses in approximately 20% of all cases. When an adequate sample is unable to provide a definitive diagnosis, surgical removal of the thyroid lesion (hemi-thyroidectomy) is recommended. However, only 20-37% of these cases show malignancies at histological examination after surgery.[119,120] Therefore in 63-80% hemi-thyroidectomy means overtreatment of a benign nodule and in 20-37% it means undertreatment of a malignant nodule.

Pertechnetate scintigraphy

It is desirable to know the functional nature of a thyroid nodule. Malignancy is highly unlikely in a nodule with normal or increased iodine uptake. If the function is less than in the surrounding normal thyroid tissue (‘cold nodule’, see figure 2.5), FNA is mandatory because of the increased risk of malignancy. The incidence of malignancy in cold nodules varies from 5 to 15%.[121,122] For functional imaging of nodular thyroid lesions ^{99m}Tc-pertechnetate is the procedure of choice. The physical properties of technetium-99m (^{99m}Tc) make it far superior to ^{131}I for imaging purposes (and with a much lower radiation burden). Alternatively the more expensive ^{123}I scintigraphy can be used for functional imaging of the thyroid.

Technetium was first discovered in 1937 by Segrè in a sample obtained from a

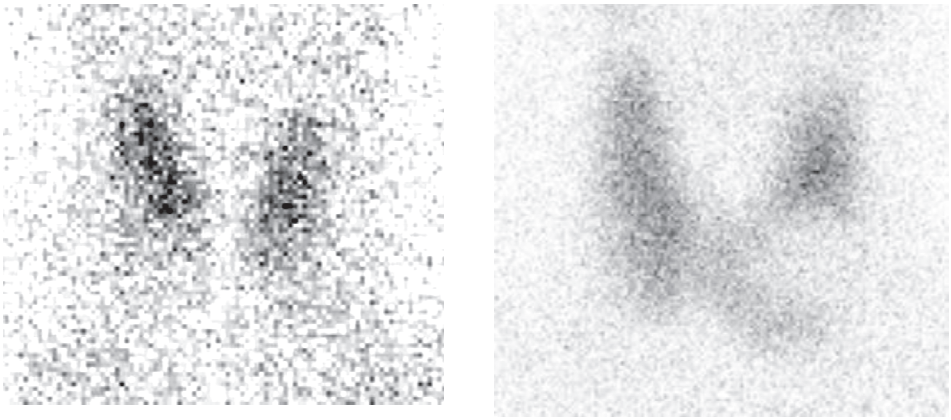

Figure 2.5 Pertechnetate scintigraphy of the thyroid. Left: normal thyroid scintigram. Right: area of low uptake in the left thyroid lobe, a so called 'cold nodule'.

cyclotron. The name of the element is derived from a greek word that means 'artificial'. Molybdenum-99 is the 'parent' of its metastable 'daughter' ^{99m}Tc. Technetium pertechnetate (^{99m}Tc-TcO_4^-) is the chemical form in which technetium is eluated from the molybdenum-technetium generator. Because its chemical properties are similar to iodide, pertechnetate can be used for thyroid imaging studies. By emission of a 141 keV photon ^{99m}Tc decays to ^{99}Tc with a half-life of 6.02 hours. For thyroid imaging purposes ^{99m}Tc-pertechnetate is administered by intravenous injection. It is taken up from the blood stream by the thyrocytes in the same way as iodine (i.e., by the NIS). As it cannot be organified, pertechnetate is discharged shortly after uptake.

Treatment

In the treatment of differentiated thyroid cancer multiple modalities are involved, each of which will be discussed separately.

Surgery

Primary surgery for differentiated thyroid carcinoma (DTC) consists of near-total thyroidectomy. Only for papillary microcarcinoma the optimal extent of surgery is still subject of discussion.[43,52,107,123-128] Very few authors found the therapy effect unrelated to the extent of surgery.[123] Relapse-free survival and thyroid cancer-specific survival are better after near-total thyroidectomy than after unilateral thyroid lobectomy. Moreover, in over 50 percent of all patients who have a completion thyroidectomy after initial unilateral lobectomy, malignant tissue is found in the thyroid remnant.[128] This is in agreement with the multifocal, multiclonal nature of papillary carcinoma.[107]

Arguments against total thyroidectomy are also available. Papillary thyroid carcinoma is often found in post-mortem studies without a single clinical symptom during life. This raises questions with regard to the clinical relevance of papillary microcarcinomas. The most serious complications include hypoparathyroidism and recurrent laryngeal nerve damage.
The final step in the treatment of DTC is the application of a high ('ablative') ^{131}I dosage, usually 3700 MBq, to destroy thyroid remnants after surgery. The prognosis of DTC and the effectiveness of the additional treatment with ^{131}I are so good that without at least cytologic proof a radical neck dissection (which is associated with significant morbidity) is unjustifiable. A modified radical neck dissection should be performed only after a diagnosis of lymph node metastases.[6]

Levothyroxine medication

After surgery patients are followed-up intensively by their endocrinologists. As by definition the production of endogenous thyroxine is discontinued by the surgical procedure, these patients require thyroid hormone (levothyroxine, LT4) replacement. Differentiated thyroid cancer cells still react to TSH stimulation; for this reason LT4 is usually administered in such high doses that TSH levels fall to immeasurably low levels (< 0.01 μU/ml). The ideal degree of TSH suppression, however, has not been established. Although complete TSH suppression seems to result in a longer relapse-free survival,[129] there is no effect of the degree of TSH suppression on relapse-free survival and cancer-specific mortality. Especially for low-risk patients TSH suppression is not generally advocated.[130,131] The exact dose of LT4 required to achieve TSH suppression varies from patient to patient, and depends in part on the body weight. In case of failure to suppress TSH by LT4 medication in doses well tolerated by the patient, administration of octreotide may be considered.[132]

Radioiodine (^{131}I) treatment

Iodine (atom number 53) belongs to the group of halogens. Because of its high reactivity it does not occur in a pure form in nature. In its natural state, iodine occurs as I_2. Of all 37 iodine isotopes (with atomic masses ranging from 108 to 144) ^{127}I is the only stable isotope. An overview of all iodine isotopes that are currently used in medical practice is given in table 2.2.
The therapeutically useful ^{131}I is produced by neutron bombardment of tellurium-131, and decays to xenon-131. ^{131}I emits beta-radiation (energy: 807 keV) with an average range (in soft tissue) of 1 mm, and a maximum range of about 3 mm. It also emits gamma rays (19 gammas ranging from 80 to 637 keV; most abundant (83%): 364 keV), which are suitable for imaging with a gamma camera.

Table 2.2 Isotopes of Iodine used for medical purposes.

isotope	$t_{1/2}$	emission	most abundant energy	medical use
^{123}I	13.2 h	γ	159 keV	diagnostic imaging (SPECT)
^{124}I	4.18 d	$\beta^+ \rightarrow \gamma$	603 keV (β^+) 511 keV (γ)	diagnostic imaging (PET)
^{125}I	59.41 d	γ	35 keV	brachytherapy laboratory procedures
^{131}I	8.04 d	β^-	807 keV	therapy
		γ	364 keV	diagnostic imaging (SPECT)

Iodine is administered as sodiumiodide, either orally or by intravenous injection. Iodine is excreted from the body both with urine and with faeces. It is also secreted in milk,[133] so breastfeeding will have to be discontinued at least one week before administering ^{131}I to a patient. This serves two objectives: (1) to prevent ^{131}I ingestion by the baby, and (2) to prevent unduly high radiation doses to the mother's breasts. Discontinuation of breastfeeding stops the milk production, and consequently also prevents iodine uptake from the blood by the mammary gland. Iodine-131-NaI closely approaches the ideal drug for oncologic purposes: it is specific for one type of cancer cell, rarely has side effects, emits therapeutically useful beta-radiation and gamma rays suitable for imaging the drug distribution. Following near-total thyroidectomy, patients with differentiated thyroid cancer are usually treated with ^{131}I ablation of residual normal and neoplastic thyroid tissue. This serves multiple purposes: firstly, it destroys remaining (normal or neoplastic) thyroid tissue, thereby increasing the specificity of thyroglobulin measurements during the follow-up. Secondly, ^{131}I may destroy occult microcarcinoma, thereby minimizing the incidence of recurrence. Thirdly, a high ^{131}I dose permits post-ablation ^{131}I whole-body scintigraphy (WBS) which can detect occult metastases.[6]

^{131}I ablation in addition to near-total thyroidectomy significantly reduces the risk of recurrence and cancer-specific mortality in patients with differentiated thyroid cancer.[43,123,134] The effect of ^{131}I ablation may be partially dependent on differences in surgical techniques.[135] The preferred ^{131}I dosage for remnant ablation is still a matter of debate.[136-142] Although good results may be obtained with a dosage of 1.1 GBq,[137,138] they seem to improve further with increasing dosages.[142] A plateauing of the dose-response curve of ^{131}I has been noted for dosages over 1.85 GBq.[136]

Diagnostic scintigraphy with ^{131}I preceding ablation is controversial; it may or may not cause 'stunning' of the remaining thyroid tissue (i.e., diminished ^{131}I uptake) and thus reduce the efficacy of the ^{131}I ablative dosage.[142-149] The benefit of pre-ablation scintigraphy is uncertain, as residual thyroid tissue is seen in virtually all patients.[142,146] Should pre-ablation scintigraphy nonetheless be performed then ^{123}I seems to be the safer choice to prevent a 'stunning' effect.[146,150,151]
Serious complications of ^{131}I therapy include radiation thyroiditis (especially in the case of large thyroid remnants), salivary gland problems ranging from sialoadenitis to complete xerostomia (occurring in a minority of patients), transient loss of taste or smell, and hematological abnormalities. The incidence of these complications depends on the administered dosage.[152,153] Recently evidence was found of earlier onset of menopause in women treated with ^{131}I for differentiated thyroid cancer.[154]

Chemotherapy

Chemotherapy is rarely indicated in differentiated thyroid carcinoma. Its use is contemplated only when a carcinoma has progressed extensively, has dedifferentiated, and has lost its capability to accumulate ^{131}I. However, also in these cases the experience with chemotherapy is both scattered and limited.

External beam radiation

Radiotherapy to thyroid remnants does not enhance the survival as effectively as ^{131}I.[43,126,155,156] It is indicated only in cases of incomplete or impossible surgical excision of tumors lacking ^{131}I uptake.[157] External beam radiation therapy in addition to ^{131}I is advocated in cases of microscopic residual disease after surgery.[158]

Follow-up

Contrary to most other cancer patients, thyroid carcinoma patients are never considered 'cured'. Even though most recurrences are observed in the first years after diagnosis and treatment, they may occur more than 30 years after the initial treatment.[39,43,135,159] Therefore, the follow-up of patients with differentiated thyroid cancer should be lifelong.

Thyroglobulin measurements

As thyroglobulin is produced only by (normal or neoplastic) thyroid follicular cells, detectable serum levels signal the presence of recurrent or metastatic disease. Thyroglobulin (Tg) is the best available tumor marker for PTC and FTC after a near-total thyroidectomy and subsequent radioiodine ablation of remaining thyroid tissue.[160-162] The methods in use for measuring Tg are either immuno(radio)metric assay (IMA/

IRMA) or radioimmunoassay (RIA). The former is often preferred as it allows for shorter incubation times and automation. There are, however, problems with the measurement of Tg.

- The presence of circulating auto-antibodies against Tg (TgAb) is a problem for the detection and the interpretation of serum thyroglobulin levels. TgAb can cause either over- or underestimation of Tg levels. Tg tests should therefore always be combined with TgAb tests; if TgAb test positive, Tg values are unreliable.[21,163,164] Tg antibodies themselves have been proposed as a tumor marker. Indeed, Tg antibodies react to the presence or absence of thyroid cells and of Tg.[165,166]
- Heterophilic antibodies can interfere with Tg measurements.[167]
- There is a significant inter-assay variation. Despite CRM-457 standardization, this variation supersedes within-person variability.[164] Most likely these differences reflect differences in assay specificity for circulating Tg isoforms.[168-170]
- The most sensitive Tg measurements are obtained during TSH stimulation.[171] On the other hand, high TSH levels also induce thyroid (cancer) cell proliferation.

In recent years, Tg-mRNA in peripheral blood has emerged as a potential marker of recurrent or metastatic PTC and FTC. This technique does not suffer interference by antibodies, nor does it require TSH stimulation to obtain a sufficiently sensitive measurement. The Tg-mRNA technique seemed very promising at first,[172-174] but more recently the reliability and usefulness of this test have been questioned.[175-178] Doubt was cast by the phenomenon of 'illegitimate transcription': a low but measurable level of transcription of any gene in any cell. Illegitimate transcription of Tg-mRNA seems to occur in leukocytes.[179]

^{131}I whole-body scintigraphy

6-12 months after ^{131}I ablation, whole-body scintigraphy (WBS) is performed during TSH stimulation to evaluate whether the ablation was successful. If ^{131}I uptake is still observed, a second dosage of ^{131}I is administered to achieve complete ablation. Afterwards, different follow-up strategies coexist. At selected intervals TSH-stimulated ^{131}I WBS or ultrasound of the neck is used for the detection of recurrent or metastatic cancer. Either option should be combined with Tg measurements.

The ^{131}I dosage used for follow-up WBS ranges from 74 to 370 MBq.[142] Higher dosages increase the sensitivity of the test, but may also induce stunning of thyroid remnants and consequently lessen the efficacy of a therapeutic ^{131}I dosage.

With the advent of more sensitive Tg tests, diagnostic ^{131}I WBS has become controversial; negative ^{131}I WBS may be observed in the presence of detectable serum Tg levels. In most of these cases foci of iodine uptake can be observed after administration of a therapeutic ^{131}I dosage.[180-182] This is illustrated in figure 2.6. Positive ^{131}I WBS at

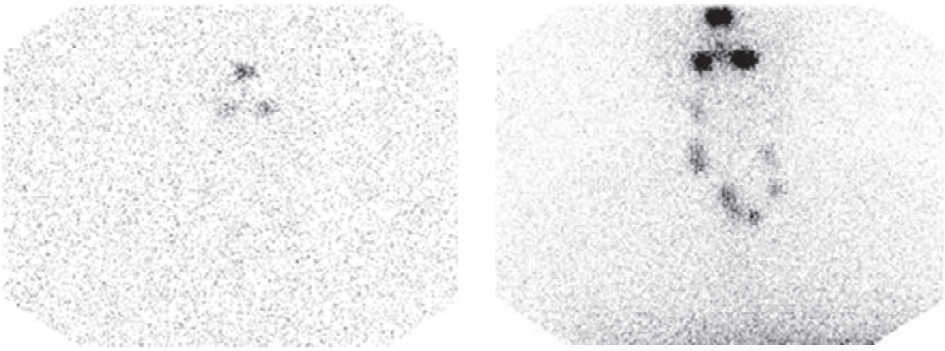

Figure 2.6 The case of patient V. Left: scintigram (370 MBq ^{131}I) of the thyroid region 1 year after ablation, showing only physiologic ^{131}I uptake in the mucous membranes of the nose and mouth. Tg level at the time of ^{131}I administration: 63 μg/l . Right: the subsequent post-therapy scintigram (7400 MBq ^{131}I) shows ^{131}I uptake in a number of cervical lymph node metastases.

undetectable serum Tg levels has become a rare observation. Furthermore, ultrasound of the neck is more sensitive than ^{131}I WBS for detecting cervical lymph node metastases.

^{131}I WBS during LT4 suppression medication is quite insensitive. To achieve adequate sensitivity TSH stimulation is required. Until recently this could only be realized by prolonged discontinuation of LT4 medication, but the ensuing hypothyroid state was poorly tolerated by many patients. This clinical problem can now be circumvented with recombinant human TSH (rhTSH), both in diagnostic and in therapeutic settings.[183-189] Similar sensitivity, specificity, positive and negative predictive figures are observed after LT4 withdrawal or administration of rhTSH.

It might become feasible to confine the follow-up to Tg measurements after rhTSH stimulation; only those patients with detectable serum Tg levels should then be subjected to further investigation with ^{131}I.[183,187]

Other imaging procedures during the follow-up of differentiated thyroid carcinoma

Ultrasound

The use of ultrasound (US) for the evaluation of thyroid nodules was first described in the early 1970s.[190] US was primarily used to distinguish between cystic and solid thyroid lesions. Over the years the spatial resolution of ultrasound imaging has progressively improved, and hence its clinical usefulness has expanded. As discussed previously, US guided fine-needle aspiration of thyroid nodules has a distinct role in the primary diagnostic process of thyroid carcinoma. Also during the follow-up ultrasound has a clear added value: ultrasound imaging is presently the most sensitive

imaging modality for the early detection of locoregional recurrence and/or metastases, especially cervical lymph node metastases. Size and location of cervical lymph nodes are the most important predictors of metastatic disease.[191] The US procedure may be easily combined with FNA biopsies from suspected lesions. US of the neck has been recommended as a standard procedure during follow-up of thyroid carcinoma.[131]

^{18}FDG-PET

2-deoxy-2-fluoro-d-glucose (FDG; see figure 2.7) was first described in 1972 as an agent to inhibit glycolysis in tumors.[192] FDG avid tumors are more metabolically active and more proliferative than normal cells.[193,194] In 1977 fluorine-18-2-deoxy-2-fluoro-d-glucose (^{18}FDG) was used for imaging studies of regional metabolism.[195] As glucose consumption is related to metabolic activity, malignancies take up large amounts of ^{18}FDG. FDG uptake is facilitated considerably by the increased expression of glucose transporters (GLUT) in the membranes of tumor cells. After intravenous injection ^{18}FDG is incorporated by the cell in the same way as glucose. Like glucose, it is phosphorylated by the enzyme hexokinase. Due to its chemical structure, the next step in the glycolysis (the transfer of a carbonyl group from the first to the second carbon atom in the ring) is not feasible; this reaction requires the presence of an oxygen atom at the second carbon atom, which is not the case for F-18-2-fluoro-2-deoxy-glucose. Consequently the FDG molecule is not metabolized, and is trapped in the cell. Eventually, ^{18}FDG is excreted from the body through renal clearance. Fluorine-18 ($t_{1/2}$ 1.83 hrs) emits β^+ particles that decay by annihilation, thus producing two 511 keV photons. ^{18}F is cyclotron-produced and therefore relatively expensive. Another disadvantage is the need for dedicated imaging equipment, i.e. a positron emission tomography (PET) scanner. Over the past decade the number of PET scanners in the world has rapidly increased, and ^{18}FDG has largely replaced other radiopharmaceuticals for tumor imaging.

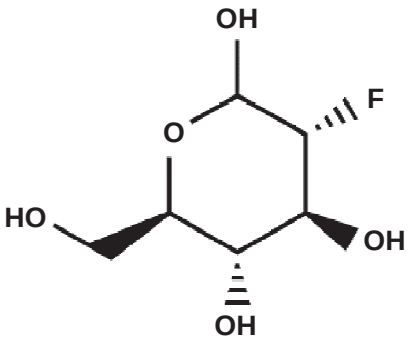

Figure 2.7 Structure formula of ^{18}FDG.

During the follow-up of PFTC, ^{18}FDG PET is used in patients with elevated serum Tg levels in the absence of ^{131}I

WBS or US abnormalities. Alternatively, ^{18}FDG PET can be used to study the disease activity of ^{131}I-negative lesions; in these circumstances ^{18}FDG PET is the most accurate imaging modality.

Thallium-201 SPECT and planar scintigraphy

Thallium-201 thallous chloride (^{201}Tl) scanning has no specific advantages in the follow-up of thyroid carcinoma. This aspecific tumor-seeking agent can be employed for the detection of ^{131}I-negative lesions if ^{18}FDG PET is unavailable. ^{201}Tl was successfully introduced for imaging of myocardial ischemia in the 1980s. Later on ^{201}Tl accumulation in tumor tissue was accidentally demonstrated. This accumulation is probably caused by the relatively large blood flow that most tumors require. At the cellular level ^{201}Tl is most likely handled as a potassium analogue, and primarily taken up through the Na-K-ATPase-pump. However, some evidence points to transport through the Na-K-pump, or even through membrane channels.
^{201}Tl ($t_{1/2}$ 73 hrs) emits several gamma rays, 98% of which have energies within the 68-84 keV range. The low gamma energy renders it a relatively unfavorable imaging agent from a technical point of view. With the advent of newer agents such as ^{18}FDG, with physical properties more suitable for imaging, the use of ^{201}Tl has declined.

Computed tomography

There is no standard role for computed tomography (CT) scans in the follow-up of patients with thyroid carcinoma; the use of this modality is at the discretion of the attending physician. CT scans can be done to visualize the anatomic substrate of a focus of ^{131}I uptake or ^{18}FDG uptake, or possibly to demonstrate metastases in Tg positive, ^{131}I negative patients.
An important issue in CT scanning is the use of contrast agents. These may serve to distinguish between structures with and without a large blood flow. However, the regular contrast agents contain substantial amounts of iodine, which will block the thyroidal iodide uptake for prolonged periods. Therefore non-iodine containing contrast agents should be used in patients who are to be treated with ^{131}I within 3 months after the CT scan.

Magnetic resonance imaging

There is no defined role for magnetic resonance imaging (MRI) in the treatment and follow-up of thyroid carcinoma. MRI can serve as an imaging modality for detection of metastases. As a result of the high protein (Tg) content of PTC and FTC, cervical lymph node metastases are sometimes identified by MRI. However, the low specificity will not allow its use as a screening technique in this area.[196]

Positive Tg with negative ^{131}I whole-body scan

Patients with negative ^{131}I WBS and detectable serum Tg during TSH stimulation pose a diagnostic problem. Often in these patients foci of ^{131}I uptake are discovered on WBS after administration of a 'blind' therapeutic ^{131}I dosage (7400 MBq). ^{131}I in high dosages has a much higher sensitivity than in diagnostic dosages (see figure 2.6). However, the Tg 'threshold' above which patients should receive a therapeutic ^{131}I dosage is still open to discussion. Some clinicians prefer further investigation with different imaging procedures before treating the patient.[197-199]

The management of recurrent and/or metastatic disease

The management of recurrent or metastatic disease depends on the histological differentiation and the localization. Metastatic lesions are sometimes found at the time of diagnosis or at post-ablation scintigraphy. In these cases case patients are treated with ^{131}I every 6-12 months, as long as metastatic deposits are present and continue to concentrate ^{131}I. Notably, a favorable response to previous therapy must have been demonstrated, such as decreasing Tg levels or decreasing tumor mass. Recurrence or metastases may also occur during the follow-up; they are often noticed through detectable serum Tg levels during TSH-suppression. Under these circumstances an activity of 7400 MBq ^{131}I is adequate for therapeutic purposes.[200]

If metastases develop in the cervical lymph nodes, a complete lymph node dissection is warranted, possibly combined with a therapeutic ^{131}I dosage. A selective dissection of the affected lymph nodes is sometimes preferred, using gamma probe guidance after ^{131}I administration.[201]

When detectable serum Tg levels (either during TSH suppression or during LT4 withdrawal) are not associated with ^{131}I uptake on the diagnostic or the post-therapy WBS, it must be assumed that metastatic cells have dedifferentiated and have lost their ability to take up iodine. Further investigations should then include one or more of the aforementioned imaging modalities. If metastases are found, only few therapeutic options remain, and the prognosis is grim. This is especially true for brain metastases.[202] Chemotherapy has little effect in patients with dedifferentiated thyroid cancer.[203,204] Radiotherapy can at best achieve partial remission in metastasized disease; it is reserved mostly for irradiation of bone metastases.

A possible future treatment option is induction of re-differentiation with retinoic acids.[203,205] In about 50% of patients with ^{131}I-negative metastatic thyroid cancer who were treated with retinoid acids, iodine uptake was restored.[205] However, the level of re-induced radioiodine uptake is often too low to deliver therapeutic radiation doses to the metastatic tissue. Therefore the search for effective re-differentiation agents is still on; in *in vitro* experiments thiazolidinediones have shown some promise.[206]

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Chapter Three

Morphologic primary tumor parameters as risk factors for advanced disease in differentiated thyroid carcinoma

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submitted

Abstract

Objectives: To study the relation between tumor size and the risk of multifocal carcinoma, locally invasive disease and lymph node or distant metastases.
Materials and methods: The files of 935 papillary (PTC) and 291 follicular thyroid carcinoma (FTC) patients treated in our hospital since 1978 were reviewed. Kaplan-Meier analyses and log rank tests were performed to assess the cumulative risk related to increasing tumor size.
Results: Accounting for primary tumor diameter, there were no significant differences in cumulative risk of multifocal carcinoma ($p = 0.12$) or distant metastases ($p = 0.49$) between PTC and FTC. PTC showed a higher cumulative risk of lymph node metastases ($p < 0.0001$) and extra-thyroidal tumor growth ($p < 0.0001$).
Tumor multifocality increases with a cumulative risk of 5 percent per cm of tumor diameter, whereas the increase in cumulative risk shows an exponential trend with regard to locally invasive disease, nodular metastases and distant metastases. The anchoring point on the x-axis of the curve for the risk of extra-thyroidal growth, lymph node metastases in PTC and distant metastases was located at a threshold tumor diameter of 10 mm. In FTC, lymph node metastases are caused almost exclusively by tumors that show extra-thyroidal growth.
Conclusions: Increasing tumor size is associated with an exponentially increasing risk of extra-thyroidal tumor growth, lymph node and distant metastases. A tumor diameter ≥ 1 cm is associated with a sharply increasing risk of such adverse findings. One can therefore question the current standard of classifying all carcinomas < 2 cm as T1.

Introduction

Differentiated thyroid carcinoma (DTC), consisting of papillary (PTC) and follicular thyroid carcinoma (FTC), is the most common endocrine malignancy. Even though both PTC and FTC derive from the same progenitor cells, they show some marked differences in clinical behavior. For instance, PTC generally is discovered at a smaller tumor diameter than FTC[1,2] and conventionally it is assumed that PTC primarily metastasizes via the lymphatic pathway, whereas FTC primarily metastasizes via the bloodstream and will only rarely show lymph node metastases.[3]
Many risk factors have been described as determinants for outcome in DTC. One of these is the diameter of the primary tumor. Even though the exact limits for what

constitutes a low-risk or a high-risk tumor size differs slightly among authors, there is consensus in the literature that a larger tumor diameter carries a higher risk for death of thyroid cancer.[1,4-8] It has also been suggested that a larger tumor diameter is related to the occurrence of other prognostically adverse phenomena such as multifocality, extra-thyroidal growth, lymph node metastases, or distant metastases.[9] The aim of this study was to study the risks of these phenomena occurring as a function of the primary tumor diameter.

Subjects and Methods

Database

The Würzburg thyroid cancer database was established primarily to monitor the quality of patient treatment. Secondarily this database allows for retrospective scientific population studies. Data was recorded for each visit, starting with the first visit after the diagnosis of thyroid carcinoma had been established. Data recorded at initial treatment include histology, primary tumor diameter, pTNM-staging (according to version 5 of the TNM system,[7] multicentricity of the tumor and whether or not there was extra-thyroidal invasion.

Patients

Retrospectively we reviewed 1593 patients (1122 females, 471 males, mean age 47.5 y, range: 5-87 y) with differentiated thyroid carcinoma who were treated in our hospital from 1978 onwards (earliest available data). In 1232 patients sufficient information on the diameter of the primary tumor was available.

Due to incomplete information 283 patients were classified as Nx and 56 patients as Mx. These patients were excluded from analysis involving the risk of lymph node metastases and distant metastases, respectively.

Initial treatment

All patients except those with papillary microcarcinoma underwent total thyroidectomy in one or two sessions according to the standards at the time. Lymph node dissection was not performed in all patients. All patients except those with papillary microcarcinomas subsequently received ablative ^{131}I treatment.

Pathological analysis and staging

Surgical specimens were analyzed according to the standard at the time of initial treatment, and classified as PTC or FTC according to prevailing guidelines at the time. Data were processed as stated in the original pathology report. Patients with one

or more insular or anaplastic foci were regarded as having undifferentiated thyroid carcinoma and were excluded from the present study. The diameter of the primary tumor was determined on the basis of macroscopic analysis of the surgical specimen, when possible. In the case of multifocal tumors the diameter of the largest tumor focus was taken for the primary tumor diameter. In order to be classified as free of lymph nodes, patients should have at least undergone a lymph node dissection; otherwise they were classified as Nx and excluded from analysis concerning lymph node metastases. Being classified as having lymph node metastases, or having multifocal or extra-thyroidal disease required histologic confirmation; for the classification as having distant metastases other evidence, such as a positive post-therapy ^{131}I whole-body scan, CT scan or MRI, was deemed sufficient.

Analysis

Statistical analysis was performed using SPSS 12.0 for Windows. P-levels < 0.05 (two-tailed) were considered statistically significant. The method of Kaplan-Meier was originally designed to compensate for missing observations due to censoring in the assessment of survival over time. However, this technique can also be used to compensate for forms of censoring other than loss-to-follow-up or end-of-follow-up before an event occurs. In this study we regarded tumor diameter as a representation of time (tumors grow over time, and larger tumors will thus have had more time to produce an event). The moment of initial treatment, i.e. the operative removal of the tumor, can be considered as the moment that the tumor is lost to follow-up. The Kaplan-Meier method of analysis can give us an indication of the tumor size adjusted cumulative risk.[10] Differences between survival curves were examined using the log rank test. Differences between groups were assessed using the Mann-Whitney test.

Results

Patient and tumor characteristics

938 PTC and 294 FTC patients were included in the study. Mean diameter of the primary tumor was 18.9 mm for PTC and 31.7 mm for FTC ($p < 0.001$).

Comparisons between papillary and follicular carcinoma

As a first step, PTC and FTC were analyzed for differences in tumor size adjusted risk. Log rank tests on the Kaplan-Meier curves revealed that accounting for primary tumor diameter, there were no significant differences in cumulative risk of multifocal carcinoma ($p = 0.12$) (figure 3.1) or distant metastases ($p = 0.49$) (figure 3.2) between

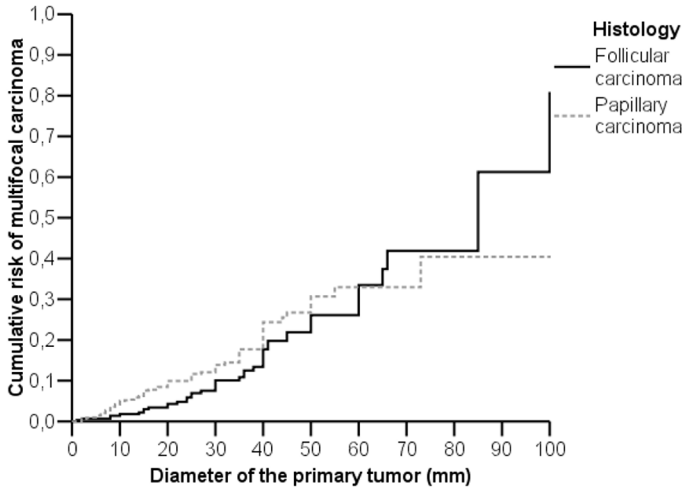

Figure 3.1 Tumor size and cumulative risk of multifocal carcinoma.

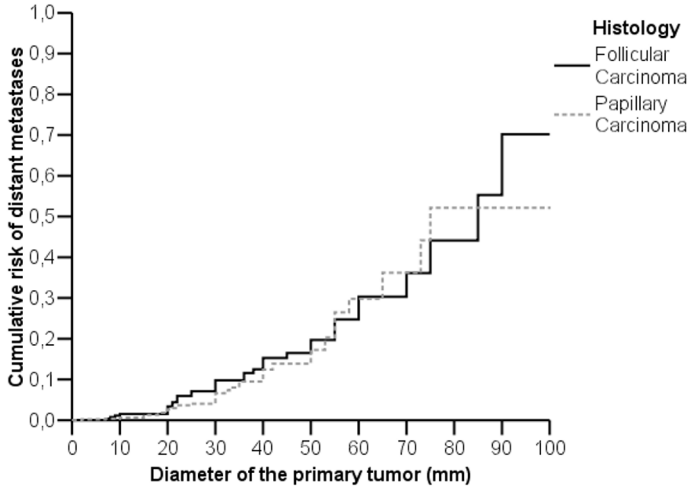

Figure 3.2 Tumor size and the cumulative risk of distant metastasis.

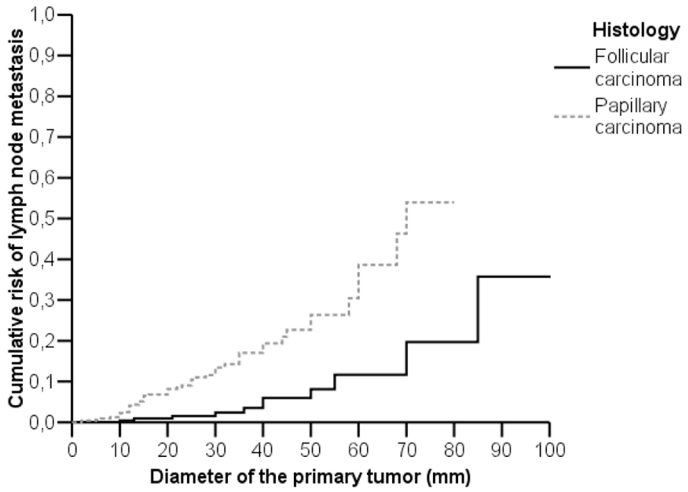

Figure 3.3 Tumor size and the cumulative risk of lymph node metastasis in papillary and follicular thyroid carcinoma.

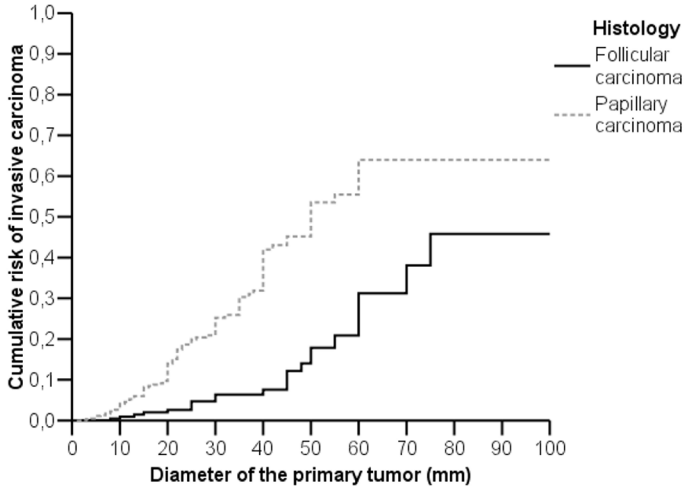

Figure 3.4 Tumor size and the cumulative risk of extra-thyroidal growth.

PTC and FTC, but that PTC showed a higher cumulative risk of distant lymph node metastases ($p < 0.0001$) (figure 3.3) and extra-thyroidal tumor growth ($p < 0.0001$) (figure 3.4).

Cumulative tumor size adjusted risk

As there were no significant differences between PTC and FTC in tumor size adjusted risk of multifocality or distant metastases, we assessed the cumulative risk for the group of DTC patients as a whole. For the risk of extra-thyroidal growth or distant metastases PTC and FTC were assessed separately.
The risk of tumor multifocality seems to be linearly increasing with a cumulative risk of 5 percent per cm tumor growth, whereas the increase in cumulative risk shows an exponential trend with regard to locally invasive disease, nodular and distant metastases. The anchoring point on the x-axis of the curve for the risk of extra-thyroidal growth, lymph node metastases in PTC, and distant metastases is located at a tumor diameter of about 10 mm.

Tumor size adjusted risk in low- and high-risk primary tumors

Multifocal carcinoma and extra-thyroidal invasion are considered prognostically adverse findings. We assessed whether there were differences in tumor size adjusted risks for patients with or without any of these histopathologic tumor characteristics. Between patients with and without multifocal carcinomas, there was no significant

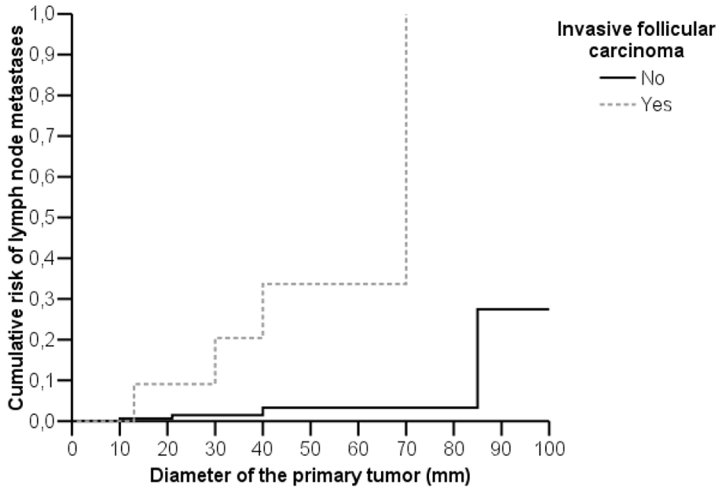

Figure 3.5 Tumor size and the cumulative risk of lymph node metastases in FTC patients with and without extra-thyroidal invasion.

difference in tumor adjusted risk of lymph node metastases ($p = 0.52$), but there was a slightly increased risk of distant metastases for those with multifocal carcinoma ($p = 0.005$). Patients with extra-thyroidal invasion had a higher risk of lymph node metastases ($p = 0.001$) and distant metastases ($p < 0.001$) than those without.
As patients with PTC and FTC showed a clear difference in the prevalence of lymph node metastases, we repeated the above risk assessment for lymph node metastases for each histology separately. In PTC there was no difference in the tumor size adjusted risk of lymph node metastases between patients with or without multifocal disease ($p = 0.11$), or between patients with or without extra-thyroidal growth ($p = 0.18$). In FTC, however, there was no difference with regard to the risk of developing lymph node metastases between patients with or without multifocal disease ($p = 0.94$). There was a clear difference between patients with or without extra-thyroidal invasion ($p < 0.001$, see also figure 3.5). Patients without extra-thyroidal invasion have a very low risk of lymph node metastases even when they have large tumor diameters, whereas in patients with extra-thyroidal invasion the Kaplan-Meier curve has its anchoring point at a tumor diameter of about 10 mm. Interestingly, there was no difference in tumor size adjusted risk of developing lymph node metastases between invasive PTC and invasive FTC ($p = 0.68$).

Discussion

In the current study we found a clear relation between the primary tumor diameter and the development of more advanced disease. When tumor diameter is taken into account, FTC actually shows a more indolent behavior than PTC. The tumor size adjusted risk of multifocal carcinoma and distant metastases does not differ between the two differentiated thyroid carcinoma entities, whereas in PTC the risk of developing lymph node metastases and extra-thyroidal tumor growth occurs at considerably smaller tumor size than in PTC.
The identification of primary tumor size as a risk factor in itself is not new. Many prognostic scoring systems have embraced this parameter.[1,4-8] The findings from the present study are largely in agreement with those of Machens *et al.*[9] However, Machens *et al* – regardless of histologic entity – found an increased risk of distant metastases for tumors larger than 20 mm, versus 10 mm in the present study. Our findings could explain the observation of Passler *et al*, who found that patients with a tumor diameter over 1 cm had a poorer survival than those under 1 cm.[11]
This could have major consequences. Machens *et al* suggest to pursue aggressive diagnostic and therapeutic measures to rule out malignancy in any nodule exceeding

20 mm in diameter; these constitute about one-third of all thyroid nodules.[12] Pursuing this strategy in all nodules over 10 mm would involve a far greater part of the population, and would therefore incur much greater costs. The present results, however, indicate that from a diameter of about 1 cm onwards, thyroid nodules deserve appropriate specialist evaluation in order to detect carcinomas in a curable stage. This is also advocated in the 2006 European consensus on the management of thyroid nodules.[13]
The results of the current study also contribute to the discussion whether the TNM system should define tumors smaller than 10 mm as T1, as the 5th edition did,[7] or whether the current criterion of 20 mm from the 6th edition[8] is more appropriate. An important criterion for classifying tumors is risk. Risk, however, is a wide-ranging concept and can be specified for many parameters. As patients with differentiated thyroid carcinoma (especially the ones with lower tumor stages) have a long-term survival of > 95%,[14] additional parameters for defining risk are useful to assist in determining the classification of tumors. The occurrence of distant metastases, which is generally considered to be a severe adverse event in the course of disease progression, can be such a criterion. In the present study patients with tumor diameters over 10 mm run an increasing risk of distant metastases. Therefore we postulate that classifying tumors larger than 10 mm as T1 (as the current version of the TNM system does) is questionable.
An interesting observation in the present study is the non-trivial percentage of FTC patients who develop lymph node metastases. This is somewhat contradictory to the supposedly primarily hematogenic spread of FTC.[3] However, these lymph node metastases are almost exclusively caused by carcinomas with extra-thyroidal growth. It is conceivable that tumor cells that spread outside the thyroid can also be transported via the lymphatic pathways. Support for this idea can be derived from the lack of difference in the tumor size adjusted cumulative risk of lymph node metastases between extra-thyroidal growing PTC and FTC. This has practical consequences: FTC patients with extra-thyroidal tumor growth should probably undergo a central compartment neck dissection in order to ascertain their lymph node status.
As can be seen from the curves of tumor size adjusted cumulative risks, some tumors start metastasizing at considerably smaller diameters than others. Should one want to make a more accurate analysis of the risk of metastases (whether in addition to, or as a replacement of existing morphologic classification) genetic analysis is unavoidable. In the past few years several mutations have been found which increase the risk of metastases. The most important one seems to be the BRAF(V600E) mutation in PTC, which is associated with greater tumor size, multicentricity, extra-thyroidal invasion and development of lymph node metastases, as well as progression to anaplastic

carcinoma.[15-19] For FTC no mutations have as yet been identified that are associated with a less favorable behavior. Such tumors can be characterized by a variety of biological markers: regions of PTC invading other tissues show an overexpression of TGF-beta, NFKB, as well as overexpressing Vimentin which has been associated with nodal metastases.[20] Other reports showed a lowered expression of p27kip in lymph node metastases of PTC,[18] or an overexpression of LIMD2 and PTPRC (CD45).[21] The clinical usefulness of these parameters is still undecided. More research is needed to establish the most sensitive markers and the diagnostic criteria that they should fulfill. Until such biological markers reach the clinical arena, morphological characteristics such as tumor diameter remain a useful tool in the initial risk stratification of PTC and FTC.

Conclusion

Increasing tumor size is associated with an exponentially increasing risk of extrathyroidal tumor growth, lymph node metastases and distant metastases. A tumor diameter $\geq$ 1 cm seems to be related to a sharply increasing risk for such adverse findings. In FTC, lymph node metastases are largely caused by tumors demonstrating extrathyroidal tumor growth.

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Chapter Four

The success rate of ^{131}I ablation in differentiated thyroid cancer: comparison of uptake-related and fixed-dose strategies

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Abstract

Introduction: The aim of the present study was to compare the success rate of an uptake-related ablation protocol in which the dosage depends on an ^{131}I 24-h neck uptake measurement and a fixed-dosage ablation protocol in which the dosage depends on tumor stage.

Methods: All differentiated thyroid carcinoma patients with M0 disease who had undergone near-total thyroidectomy followed by ^{131}I ablation were included. In the uptake-related ablation protocol, 1100 MBq (uptake > 10%), 1850 MBq (uptake 5-10%) and 2800 MBq (uptake < 5%) were used. In the fixed-dosage ablation strategy, 3700 MBq (T1-3, N0 stage) and 5550 MBq (N1 and/or T4 stage) were applied. We used ^{131}I uptake on whole-body scintigraphy and thyroglobulin (Tg)-off values to evaluate the ablation 6-12 months after treatment.

Results: In the uptake-related ablation protocol, 60 out of 139 (43%) patients were successfully treated, versus 111 out of 199 for the fixed dose ablation protocol (56%) ($p = 0.022$). The differences were not statistically significant for patients with T4 ($p = 0.581$) and/or N1 ($p = 0.08$) disease or for patients with T4N1 tumor stage ($p = 0.937$).

Conclusion: The fixed-dosage ^{131}I ablation protocol is more effective in ablation of the thyroid remnant in differentiated thyroid carcinoma patients than an uptake-related ablation protocol. This difference is not observed in patients with N1 and/or T4 tumor stages.

Introduction

The therapy of choice in patients suffering from differentiated thyroid cancer (DTC), subdivided into papillary and follicular thyroid carcinoma, is near-total thyroidectomy. This is routinely followed by the administration of radioiodine-131 (^{131}I) to destroy any remaining benign or malignant thyroid tissue, so called ablation. There are several reasons for routine ablation after surgery[1,2]: (1) to be able to detect a carcinoma recurrence by radioiodine scanning; (2) radioiodine can destroy microscopic foci of carcinoma in the thyroid remnant; (3) possible carcinoma outside the thyroid bed may be detected and treated by radioiodine; (4) in order to use thyroglobulin (Tg) as tumor marker of recurrent carcinoma, all normal thyroid tissue has to be destroyed. Although ^{131}I has been used for many years to ablate thyroid remnants following thyroid surgery, a single optimal ablation strategy is still not established. Reports on the amount of ^{131}I required to achieve successful ablation show a considerable range.[3-7]

At the Leiden University Medical Center (LUMC) and the University Medical Center Utrecht (UMCU), two academic hospitals in the Netherlands, different ablation strategies have been used over the years. At the LUMC, a relatively low-dosage uptake-related ablation strategy was applied until June 2002,[8] whereas in the UMCU a fixed-dosage strategy with relatively high administered ^{131}I activities is used since January 1990.[9,10] The aim of this study was to compare the success rates of ablation according to these two protocols.

Methods

Study population

Patients were selected who received ablation treatment with ^{131}I at the LUMC or UMCU from January 1990, as this date marks the start of the fixed-dosage protocol in the UMCU. Both subgroups consisted of patients in whom surgery had been performed, either at the university medical centers or at referring non-university hospitals. All DTC patients with M0 disease who had undergone near-total thyroidectomy followed by ^{131}I ablation were included. Tumor staging was scored according to the criteria of the 5th edition of the TNM atlas. In all patients with N1 disease, a neck dissection had been performed in addition to the near-total thyroidectomy and prior to ablation. So, in patients with N1 disease, this stage was established prior to ^{131}I ablation. In order to avoid a bias, all patients with M1 disease were excluded. M1 disease as exclusion criterion was based on post-ablation whole-body scans using high-dosage ^{131}I, CT scanning, and/or chest x-rays in patients with T4 and/or N1 disease. Finally, additional inclusion criteria were: (1) ablation has been performed in accordance with either protocol; (2) 6-12 months after ablation patients returned for follow-up studying consisting of Tg-off measurements and/or ^{131}I whole-body scintigraphy.

Uptake-related ablation protocol

This treatment strategy was used at the LUMC until June 2002. All patients had undergone near-total thyroidectomy, followed by ^{131}I ablation 4-6 weeks after surgery. During this interval, no suppressive treatment with L-thyroxine was initiated. Furthermore, low-iodine diets were prescribed to optimize the therapeutic outcome.[11,12] The 24-h pretreatment radioiodine uptake percentage in the neck region was measured using standard techniques: 40 MBq ^{131}I was given orally, followed by planar scintigraphy of the neck region 24 h later. A standard of 40 MBq ^{131}I that was calibrated on the day of admission and measured in a neck phantom after 24 h, was used as a reference. An uptake of less than 5%, of between 5% and 10% and of more than 10%

was followed by 2800, 1850 and 1110 MBq of ^{131}I, respectively. The rationale of this quantitative approach was to avoid unnecessary exposure[13] and local side effects from radioiodine.[14,15] In this regimen, no other adjustments were made in the case of cervical lymph node metastases.

Fixed dose ablation protocol

This strategy was used in the UMCU from 1990 onward. All patients had undergone near-total thyroidectomy, followed by ^{131}I ablation 4-6 weeks after surgery. A standard activity of 3700 MBq was administrated in cases without any (known) metastases. In case of pre- or peri-operatively detected lymph node involvement or T4 tumor stage an activity of 5550 MBq was given. Between surgery and ablation patients did not receive L-thyroxine medication, and they had been instructed to keep a low-iodine diet for approximately one week.[11,12] No pre-ablative diagnostic scintigraphy was performed.

Follow-up strategy

Between 6-12 months after ^{131}I ablation, patients were evaluated by the measurement of Tg-off values, i.e. Tg values under TSH stimulation. For this purpose, hormonal medication was withdrawn for 4 weeks, whereas in a minority of the UMCU cohort recombinant human TSH was applied. In the UMCU study group, all patients underwent ^{131}I WBS, whereas in the LUMC cohort ^{131}I WBS was performed in case of Tg < 1 μg/l. In both hospitals, scintigraphy was performed at least 3 days after administration of ^{131}I. In case of increased Tg levels or abnormal WBS, additional treatment was given followed by WBS within 7 days after the administration of a therapeutic dose. Follow-up results 6-12 months after this treatment were not included in the present analysis. At the starting point of inclusion for this study, ultrasonography was not yet routinely used in the follow-up of DTC patients; therefore, this technology was not included in the present study.

Laboratory analysis

From 1990 onwards, various kits were used for the measurement of thyroglobulin (Tg) and Tg antibodies in both hospitals. In the presence of Tg antibodies, IRMA assay test results for Tg are not reliable.[16,17] Therefore, Tg values below the cut-off level were excluded from analysis in the presence of measurable Tg antibodies. As results of Tg measurements are not interchangeable between kits,[18] Tg values in any patient were considered undetectable if they were below the lower detection limit of the kit used (i.e. cut-off level). Until 1997 serum Tg was measured by using an immunoradiometric assay (IRMA), the Dynotest TG (Brahms Diagnostica GmBH,

Germany), with a detection limit of 1 μg/l. From 1997 onwards the Dynotest TG-s (Brahms Diagnostica GmBH) was used, with a detection limit of 0.5 μg/l. Recurrent disease was defined as Tg levels > 1 μg/l. TSH levels were measured by means of an immunofluorometric assay (IFMA) with the Delfia® (Wallac, Turku, Finland) until 1997. Thereafter, an immunoluminometric assay (ILMA) was used with the Elecsys® (Boehringer Mannheim, Germany). Serum Tg-abs were determined by the Ab-HTGK-3 IRMA test (DiaSorin Biomedics, Italy).

Criteria for successful ablation

Ablation was considered successful if 6-12 months after ablation patients fulfilled all of the following criteria:
- Tg-off values below the cut-off level of the assay used;
- negative ^{131}I whole-body scintigraphy.

Statistics

For statistical analysis, we used SPSS version 12.0.1 for Windows (SPSS inc.). The quantitative data (continuous parameters) were analyzed using the Mann-Whitney U-test. For categorical data, the Chi-squared test was used. A statistically significant difference was defined as $p < 0.05$.

Results

A total of 359 patients were included in this study. According to the uptake-related ablation protocol, 153 patients were treated and 206 patients according to the fixed-dosage ablation protocol. In table 4.1, the patient characteristics and results of tests for differences between the two groups are displayed.
Papillary microcarcinomas were not observed in the groups studied. In the uptake-related protocol, 20% of patients were treated with 1110 MBq ^{131}I, 19% with 1850 MBq, and 61% with 2800 MBq. The mean 24-h ^{131}I uptake value in this group was 6.86% (range: 0.03-12.0%). In the fixed-dosage protocol, 69% of the patients were treated with 3700 MBq ^{131}I and 31% with 5550 MBq. In this protocol, the 24-h uptake values were not routinely measured.
According to the evaluation with ^{131}I whole-body scintigraphy as the single tool (see table 4.2), 89 out of 153 (58%) patients in the uptake-related ablation protocol had no radioiodine uptake in the neck at the first follow-up scintigraphy. In 174 out of 206 patients (84%) treated according to the fixed-dosage ablation, scintigraphy did not reveal radioiodine uptake in the neck. This difference was statistically significant ($p < 0.001$). The scintigraphic ablation results in various subgroups as well as the

Table 4.1 Differences in population characteristics for the ablation protocols studied. N-stage = lymph node stage (N0 = without clinical evidence of lymph node metastases; N1 = with histopathologically proven lymph node metastases); T-stage = primary tumor stage.

	uptake-related	**fixed dosage**	**p-value**
Total number of patients	153	206	
Mean age (y) (range)	42.6 (15-87)	43.1 (19-82.0)	0.675
Gender			0.07
male (%)	33 (22)	62 (30)	
female (%)	120 (78)	144 (70)	
Histology			0.294
papillary (%)	123 (80)	156 (76)	
follicular (%)	30 (20)	50 (24)	
24-h ^{131}I-uptake (%)	6.86% (0.03-12)	NA	
N-stage			0.008
N0 (%)	125 (82)	140 (68)	
N1 (%)	27 (18)	66 (32)	
NX (%)	1 (< 1)	0 (0)	
T-stage			0.005
T1-3 (%)	131 (86)	194 (94)	
T4 (%)	22 (14)	12 (6)	

differences between the protocols (displayed in table 4.2) demonstrate significant differences between the different protocols in all subgroups with the exception of T4 tumor stages.

Patients with Tg antibodies and a negative diagnostic ^{131}I WBS were excluded in order to avoid a bias; this left 338 patients available for analysis. With the uptake-related ablation protocol 60 out of 139 (43%) patients were successfully treated. The fixed dose ablation protocol was successful in 111 out of 199 (56%) patients. Again, this difference was statistically significant ($p = 0.022$). The results of ablation in various subgroups and tests for differences between the protocols are also displayed in table 4.2. We found significant differences between the protocols for almost all subgroups defined. However, differences were not statistically significant for patients with papillary thyroid cancer ($p = 0.23$) and for patients with N1 ($p = 0.08$) and/or T4 ($p = 0.581$) disease. In addition, 10 patients in the uptake-related dosage group and 9 patients in the fixed-dosage group had T4N1 disease ($p = 0.006$). In both subgroups, only 1 patient had a successful ablation ($p = 0.937$) revealing a high failure rate for these tumor stages. All patients with radioiodine uptake in the neck and/or elevated

Table 4.2 Successful ablation results in the entire population and various subgroups according to ^{131}I WBS and ^{131}I WBS with Tg measurements. 21 patients with positive Tg antibodies were excluded from the evaluation using ^{131}I WBS and Tg measurements. PTC = papillary thyroid carcinoma; FTC = follicular thyroid carcinoma; N = lymph node stage; T = tumor stage.

Group	^{131}I whole-body scan			^{131}I whole-body scan and Tg		
	Uptake-related (n = 153) (%)	Fixed dosage (n = 206) (%)	p-value	Uptake-related (n = 139) (%)	Fixed dosage (n = 199) (%)	p-value
All patients	89 (58)	174 (84)	< 0.001	60 (43)	111 (56)	0.022
PTC	73 (59)	133 (85)	< 0.001	47 (43)	75 (50)	0.230
FTC	16 (53)	41 (82)	0.006	13 (45)	36 (72)	0.016
N0	76 (61)	119 (85)	< 0.001	55 (48)	86 (63)	0.017
N1	12 (44)	55 (83)	< 0.001	5 (20)	25 (40)	0.080
T1-3N0	70 (62)	117 (85)	< 0.001	53 (51)	86 (65)	0.034
T4	10 (45)	12 (75)	0.236	3 (15)	1 (8)	0.581

Tg levels 6-12 months after ablation, the so called failures, underwent subsequent treatment with high therapeutic ^{131}I activities. An analysis of the post-therapy scans was not part of the present study, but all patients with uptake on the diagnostic follow-up WBS also demonstrated uptake on the post-therapy scan. The post-therapy scans of patients with increased Tg levels were not evaluated.

Discussion

In the present study, two fundamentally different ablation strategies were compared. The rationale of the quantitative uptake-related protocol was to avoid unnecessary exposure[13] and minimize local radioiodine side effects,[14,15] whereas the fixed-dosage ablation protocol was designed to maximize the chance of successful ablation after one ^{131}I treatment. The present study showed that the fixed-dosage ablation protocol had a significantly higher rate of successful ablation compared to the uptake-related ablation strategy. However, of all subgroups, the fixed-dosage ablation protocol failed to show a significant advantage in patients with extra-thyroidal invasion of the primary tumor (T4 tumors) and/or lymph node involvement (N1 stage). We also demonstrated the low sensitivity of ^{131}I WBS compared to the Tg measurements in the follow-up of

thyroid cancer patients. This finding concurs with the recommendation presented in the European guidelines; in low-risk patients Tg measurements are the first step in the follow-up and WBS is no longer recommended.

Several studies have shown that higher ^{131}I activities lead to higher ablation rates, although estimations of the optimal activity vary between authors. A randomized trial reported by Bal *et al* [19] revealed no differences in remnant ablation for activities over 925 MBq (25 mCi). Successful ablation (defined as a follow-up scintigram of the neck with less than 0.2% uptake and Tg < 10 μg/l) was achieved in 81.6% of the patients using activities of 925 MBq or more. The absence of activity-related differences in success rates of ablation in the study by Bal *et al* is in sharp contrast with the differences encountered in the present study between patients treated with lower activities (with a minimum of 1110 MBq) and those treated with higher activities (minimum 3700 MBq). The findings of Bal *et al* are also in contrast with the results described in their earlier study,[5] in which an activity of 1850 MBq (50 mCi) or more performed significantly better than an activity of 1110 MBq.

Also in contrast with our results are the findings of Johansen *et al* [4] who found a success rate of 81% with 1073 MBq and 84% with 3700 MBq. However, they analyzed only 63 patients in total, whereas their reported success rates were based on scintigrams performed 3-4 months after ablation. They also reported that the elevated Tg values in the ablated subjects were not statistically significant. However, their lower detection level for Tg was 5 μg/l, which is higher than our cut-off levels (0.2-1.0 μg/l). The similar ablation results with 1073 MBq and 3700 MBq may thus be caused by less sensitive follow-up procedures (short follow-up period after ablation and higher cut-off levels for Tg).

An interesting comparison can be made with the uptake-related protocol published by Zidan *et al* [19] who reported results of an uptake-related protocol using activities varying from 3145 MBq (85 mCi) for the patients with the lowest uptake to 1110 MBq (30 mCi) for those with the highest uptake. Despite the fact that their definition of a successful ablation was based solely on a diagnostic ^{131}I WBS, they reported a higher overall success rate (94%). However, compared to the uptake-related protocol described in the present study, Zidan *et al* used higher ^{131}I activities (approximately 1100 MBq more) for uptake values between 6 and 15%.

In two recently published articles, systematic meta-analysis were presented on the ^{131}I activity for remnant ablation in patients with differentiated thyroid cancer. In the analysis by Hackshaw *et al* [21] 41 case notes, 12 prospective cohorts and 6 randomized trials were used to compare the outcome in patients treated with 30 mCi with those treated with 100 mCi. The pooled ablation success rates in the observational studies

were 10% lower for 30 mCi in comparison with the higher dose. The meta-analysis of the randomized trials gave equivocal results. Despite these findings and because of the small number of randomized trials, these authors concluded that it is not possible to reliably determine whether ablation success rates using low activities are similar to using high activities. This statement was in contrast with the analysis presented by Doi *et al*.[22] In line with their previous report they stated that the available data favor higher dosages of radioiodine (ranging from 2775 MBq to 3700 MBq) for remnant ablation, especially after near-total thyroidectomy. The use of high dosages in these patients results in about one-third less risk of non-ablation than low dosages. Our data support the use of higher ablation dosages.

Regarding the results in the present study and the data published in the literature, it is important to stress the difference between the ^{131}I activity administered and the absorbed dose of radiation in thyroid tissue. The absorbed dose causes the ablation effect. This dose depends on several factors, such as uptake of ^{131}I and retention time in the remnants, the mass of the thyroid remnant, different TSH levels, the initial activity given, and patient preparation. Despite the fact that standard amounts of radioactivity were used in the present study protocols, these may result in different absorbed radiation doses.

In both academic hospitals the aim of surgery was the optimal resection of malignant tissue, which is a combination of a near-total thyroidectomy combined with a neck dissection in case of lymph node metastases. In T4 tumors there is a high risk of residual malignant cells in the thyroid remnants. Likewise for N1 disease, occult lymph node micrometastases cannot be excluded after a modified neck dissection. Due to the lower expression of the sodiumiodine symporter in thyroid carcinoma cells, the uptake and processing of iodine is less efficient than in normal thyroid cells.[20-22] In a recently published study on prognostic parameters in thyroid cancer, both N1 and T4 tumors stages were significantly associated with local tumor recurrences.[26] Consequently, minimal residual disease might explain the lack of statistically significant differences between the two ablation protocols in the present study for patients with T4 and/or N1 tumors. Even a mean activity of ^{131}I up to 5000 MBq as used in the fixed-dosage ablation dose, which is twice as much as applied in the uptake-related strategy, fails to achieve a complete response in 60% and 92% of the patients with N1 and T4 tumor stages, respectively. Moreover, successful ablation was achieved in only 1/10 and 1/9 patients with T4N1 disease in the uptake-related and fixed-dosage strategies, respectively. These findings are in agreement with data published by Rosaria *et al*,[27] who studied 274 patients with differentiated thyroid cancer and found a relation between ablation failures and the presence of metastases and tumors larger

than 4 cm in diameter. However, also thyroid remnants with an uptake > 5% resulted in a higher failure rate.

Two factors could have influenced the results in the present study. First, the two centers used different follow-up strategies. An endocrinologist of the LUMC performed the clinical follow-up of patients who underwent ablation in the LUMC. The clinical follow-up of a part of the UMCU-patients was conducted in their own (referring) hospitals by means of ^{131}I diagnostic scintigraphy and Tg measurements. This subgroup of UMCU-patients only returned to the UMCU if additional radioiodine treatment was required (i.e., in case of unsuccessful ablation). This effectively may have created a bias, as a number of patients with successful ablation were not included in the present study. However, inclusion of these patients would have further increased the superior success rate in the fixed-dosage group. Second, it has been shown that relatively low diagnostic ^{131}I activities may lead to impaired ability of remnant thyroid tissue to concentrate the subsequent ablative dose of ^{131}I (the so called stunning effect) and may thereby reduce the therapeutic efficacy.[23-26] Although this phenomenon has been acknowledged for some time, the precise time interval and the ^{131}I activity after which it occurs is still subject of discussion. If 40 MBq ^{131}I induces a lower concentration of ^{131}I in the thyroid remnant, part of the effect seen in this study could have been attributed to the stunning effect with a probable bias against the uptake-related protocol. Data on stunning effects caused by such low doses have not been reported yet.

In the present study we found a significant difference in short-term outcome (at 6-12 months after ablation). Data on long-term recurrence rates are currently not available. Studies evaluating differences in long-term outcome in patients treated with high or low ^{131}I doses are scarce. Chow *et al* demonstrated the influence of radioiodine after surgery on the 5-, 10- and 15-year local relapse rate.[32] In patients not treated with radioiodine after surgery, the cumulative relapse rate after 15 years follow-up was 20.9% compared to 9.2% in patients treated with 3400 MBq radioiodine. In another study we found that successful ablation itself is an important prognostic factor for the long-term outcome; 10 years after treatment, 87% of patients with a successful ablation were still free of disease versus only 50% of the patients with an unsuccessful ablation ($p < 0.001$).[33] Therefore, higher recurrence rates may be expected for the uptake-related protocol than for the fixed-dosage protocol. However, this assumption is not supported by data recently published by Rosario *et al*.[34] They treated 82 patients with 3700 MBq ^{131}I and 44 patients with 1100 MBq. At the end of a 5-year follow-up period, the recurrence rate was 3.6% in patients who had received the high dosage and 3.4% in those treated with the low dosage (p = NS). As the criteria used for

patient selection and risk factors were not reported by these authors, a selection bias cannot be ruled out.
More randomized trials are required to assess the short-term and long-term outcomes in relation to the ^{131}I ablation dosage used in patients with differentiated thyroid cancer.

Conclusion

The fixed-dosage ablation protocol, using relatively high ^{131}I activities, was generally more effective for thyroid remnant ablation than a 24-h ^{131}I uptake-related ablation protocol using relatively low ^{131}I activities. This difference, however, was not observed in patients with T4 and/or N1 tumor stages; this could be caused by minimal residual malignant disease.
Follow-up studies are necessary to decide whether the short-term differences between the two dosage protocols are indeed indicative of the long-term outcome.

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Chapter Five

Success of ^{131}I ablation in thyroid cancer patients is significantly lower after a pre-ablative diagnostic activity of 40 MBq ^{131}I

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Abstract

Objective: Dosimetric studies have shown that activities of ^{131}I as small as 10-20 MBq may cause a stunning effect. Stunning may result in a lower success rate of the ablative ^{131}I therapy for differentiated thyroid carcinoma (DTC). The aim of this study was to determine whether pre-therapeutic dosimetry with 40 MBq ^{131}I causes a lower success rate of ablation.
Methods: In two hospitals the ablation protocols differed in one respect only: in the one hospital no pre-therapeutic ^{131}I was applied (group 1), whereas in the other hospital the ablation treatment was preceded by a 24-h uptake measurement with 40 MBq ^{131}I (group 2). Data from both groups were reviewed retrospectively. All DTC patients without distant metastases were included who had undergone ^{131}I ablation between July 2002 and December 2005, and who had returned for ^{131}I follow-up. Ablation was considered successful in the case of absence of ^{131}I uptake on whole-body scintigraphy and undetectable Tg levels under TSH stimulation.
Results: A total of 99 patients were included in group 1 (n = 48) and group 2 (n = 51). Ablation was successful in 31/48 patients (65%) in group 1 and in 17/51 patients (33%) in group 2 ($p = 0.002$). Multivariate analysis showed that pre-therapeutic uptake measurement using 40 MBq ^{131}I was an independent negative predictor determinant for success of ablation ($p = 0.002$).
Conclusions: After pre-therapeutic dosimetry using 40 MBq ^{131}I the success of ablation is practically halved. Consequently, the routine application of ^{131}I for diagnostic scintigraphy or uptake measurement prior to ^{131}I ablation is best avoided.

Introduction

The therapy of choice in patients suffering from differentiated thyroid carcinoma (DTC) is near-total thyroidectomy. This is routinely followed by the administration of high activities of ^{131}I, with the intent to ablate remnant thyroid tissue.
In many centers this ^{131}I ablation is preceded by pre-therapeutic dosimetry using smaller activities of ^{131}I.[1,2] A potential disadvantage of such measurements is the presumed stunning of thyroid remnants,[3,4] i.e. a diminished uptake of ablative ^{131}I activity after administration of a diagnostic ^{131}I activity. This stunning effect may be noticed either by a lower-than-expected ^{131}I uptake on a post-ablation scintigram, or as a higher failure rate of ablation. Whereas not all authors agree that this phenomenon occurs,[5-7] it has been demonstrated by others.[3,8-11]

Dosimetry studies have shown that activities of ^{131}I as small as 10-20 MBq may deliver a significant dosage to thyroid cells,[3,12] suggesting that the stunning effect may be due to direct radiation damage to thyrocytes. Evidence was presented of downregulation of the sodiumiodine symporter (NIS) expression in reaction to diagnostic activities,[13] thus reducing the uptake of ^{131}I.[14]

At the University Medical Center Utrecht (group 1) and at the Leiden University Medical Center (group 2), two academic hospitals in the Netherlands with geographically partially overlapping patient populations, comparable fixed activity ablation protocols have been used since July 2002.[15] There is, however, one distinctive difference: in group 1 no pre-therapeutic dosimetry is performed, whereas in group 2 a pre-ablative 24-h uptake measurement is performed using 40 MBq ^{131}I. The aim of the present study is to determine whether the pre-therapeutic diagnostic procedure with 40 MBq ^{131}I causes a lower success rate of ablative ^{131}I therapy in post-operative DTC patients.

Subjects and methods

Study population

All DTC patients without distant metastases who received ^{131}I ablation treatment in either of the centers after thyroidectomy between July 2002 and December 2005, were included in a retrospective study. Further inclusion criteria were: (1) ablation had been performed in accordance with the protocol; (2) 6-12 months after ablation, patients had returned for diagnostic scintigraphy or additional treatment with ^{131}I and for measurements of thyroglobulin (Tg) levels during TSH stimulation. Staging in both centers was registered in accordance with TNM version 5.[16]

Pre-ablative 24-h ^{131}I uptake measurement

In group 1 the ablative activity was administered without prior diagnostic scintigraphy. In group 2 pre-ablative 24-h ^{131}I uptake measurements were performed in order to assess the percentage of ^{131}I taken up by the thyroid remnant using standard techniques: a capsule with 40 MBq ^{131}I was given orally, followed by planar scintigraphy of the neck region 24 h later. A standard of 40 MBq ^{131}I, calibrated on the day of administration and measured in a neck phantom after 24 h, was used as a reference. The ablative ^{131}I activity was administered on the day after the uptake measurement. Patients with ^{131}I uptake > 15% would be referred to the surgical department for evaluation of possible additional surgical treatment; in the population under study such additional surgery was never performed.

Fixed activity ablation protocol

A fixed activity ablation protocol was used in the University Medical Center Utrecht since January 1990, and at the Leiden University Medical Center since July 2002. All patients had ^{131}I ablation treatment 4-6 weeks after near-total thyroidectomy. Patients did not receive LT4 medication between surgery and ablation. In both centers TSH levels > 30 mU/l were required before ablation. As The Netherlands is an iodine-sufficient country, in both centers patients had been instructed to keep a low-iodine diet for one week prior to ablation.[17,18] The ^{131}I ablation activity was 3700 MBq for patients without (known) metastases, and 5550 MBq for patients with nodular involvement (detected either pre- or peri-operatively). Node negative patients with extensive extra-thyroidal tumor growth (n = 8) or Hürthle carcinomas (n = 6) also received 5550 MBq.

Follow-up

6-12 months after ablation, patients returned to their respective hospitals for follow-up. At the UMCU this was performed using 370 MBq ^{131}I after rhTSH stimulation; at the LUMC 185 MBq ^{131}I was administered after levothyroxin withdrawal for 4 weeks. In all patients the TSH levels were > 30 mU/l before ^{131}I administration. TSH-stimulated serum Tg levels were also quantified. In both centers, whole-body scintigraphy and separate planar acquisitions of the cervical region were performed with a large-field-of-view camera with high-energy collimators.

Laboratory analysis

For the measurement of Tg levels and levels of Tg antibodies, the BRAHMS Dynotest Tg-pluS kit with a lower detection limit of 0.2 µg/l (BRAHMS Diagnostica GmbH, Berlin, Germany) was used in group 1. In group 2 the BRAHMS Dynotest Tg-S kit, with a lower detection limit of 0.5 µg/l (BRAHMS Diagnostica GmbH, Berlin, Germany) was used.

In the presence of antibodies, test results for Tg are not reliable.[19,20] As the assays used in both hospitals were IRMA assays, interference from antibodies against Tg generally would have resulted in underestimation of Tg levels. Hence, 8 patients with Tg test results below the cut-off level and with negative whole-body scintigraphy were excluded from analysis because Tg antibodies were present in their serum.

Criteria for successful ablation

Ablation was considered successful if 6-12 months after the initial ^{131}I therapy patients fulfilled all of the following criteria:

- no additional therapy of any kind for thyroid cancer needed between ^{131}I ablation and first TSH-stimulated follow-up;
- TSH-stimulated Tg and Tg-Ab levels below the detection limits of the assays;
- Absence of pathologic ^{131}I accumulation on whole-body scintigraphy, including absence of visually discernable uptake foci in the thyroid bed (as rated by the nuclear medicine physician at the time).

Statistics

For statistical analysis we used SPSS version 12.0 for Windows (SPSS inc., Chicago, Illinois, USA). Statistical significance was defined as $p < 0.05$. The quantitative data (continuous parameters) were analyzed using the Mann-Whitney U-test. For categorical data the Chi-squared test was used. Multivariate analysis was performed using binary logistic regression with a forward selection method based on likelihood ratios.

Results

Study population

The 48 patients in group 1 received ^{131}I ablation treatment without pre-ablative ^{131}I uptake measurement; the 51 patients in group 2 underwent a 24-h ^{131}I-uptake measurement prior to the ablation treatment. In table 5.1 the patient characteristics and the differences in base line characteristics between the two groups are displayed; none of these differences were statistically significant.

Overall, the ablation was successful in 31/48 patients (65%) of group 1, and in 17/51 patients (33%) of group 2. The difference is statistically significant ($p = 0.002$).

The analysis of various subgroups can be found in table 5.2. For most subgroups there were significant differences between group 1 and group 2. For some subgroups (e.g., male patients, or patients with follicular thyroid carcinoma) the size was insufficient to show a significant difference; the distributions of successful versus unsuccessful ablation approximated those of the total group. Remarkable was the lack of a significant difference between group 1 and group 2 for those patients who received 5550 MBq ^{131}I.

In order to avoid bias resulting from differences in tumor size, we compared all 30 node-negative patients without extra-thyroidal tumor invasion from group 1 (no uptake data available) with all 18 patients from group 2 who had a ^{131}I uptake < 5% (reflecting smaller thyroid remnants). In this analysis, too, group 1 did significantly better ($p = 0.024$). Also, in group 2, we compared 6 node-negative patients with extra-thyroidal tumor invasion with an uptake $\geq$ 8% with 11 patients who had an uptake

Table 5.1 Base line characteristics of patients treated without (group 1) and with (group 2) pre-ablative diagnostic ^{131}I scintigraphy and differences between the two protocols.

	group 1	group 2	p-value
Number of patients	48	51	
Mean age (y) (range)	45.2 (19-80)	43.8 (13-79)	0.57
Gender			0.98
male (%)	14 (29%)	15 (29%)	
female (%)	34 (71%)	36 (71%)	
Histology			0.40
papillary carcinoma	40 (83%)	39 (76%)	
follicular carcinoma	8 (17%)	12 (24%)	
Extra-thyroidal invansion			0.57
not present	44 (92%)	45 (88%)	
present	4 (8%)	6 (12%)	
Lymph node metastases			0.10
not present	30 (59%)	40 (78%)	
present	18 (41%)	10 (20%)	
unknown	0	1 (2%)	
Administered activity			0.40
3700 MBq	34 (71%)	32 (63%)	
5550 MBq	14 (29%)	19 (37%)	

Table 5.2 Comparison of various subgroups in group 1 and group 2.

	group 1 successful ablation	**group 2** successful ablation	**p-value**
Total group	31/48 (65%)	17/51 (33%)	0.002
Males	9/14 (64%)	5/15 (33%)	0.10
Females	22/34 (65%)	12/36 (33%)	0.009
Papillary carcinoma	25/40 (63%)	11/39 (28%)	0.002
Follicular carcinoma	6/8 (75%)	6/12 (50%)	0.26
No extra-thyroidal invasion and node negative	23/30 (77%)	15/37 (41%)	0.003
Extra-thyroidal tumor invasion and/or node positive	8/18 (44%)	2/13 (15%)	0.09
3700 MBq	25/34 (74%)	11/32 (34%)	0.001
5550 MBq	6/14 (43%)	6/19 (32%)	0.51

≤ 2%; these two subgroups represent the highest and lowest registered uptake percentages, respectively. The difference between these two subgroups was not significant ($p = 0.62$).
For groups 1 and 2 we also compared the results for the patients receiving 3700 MBq with those for the patients receiving 5550 MBq. The differences were marginally significant ($p = 0.047$) for group 1, but not significant ($p = 0.83$) for group 2.
Multivariate analysis showed that the factor most significantly influencing the success of ablation was having undergone pre-ablative scintigraphy or not. The only other significant factor was extra-thyroidal tumor growth ($p = 0.007$).

Discussion

The present study shows substantial differences in efficacy of ^{131}I ablation, correlated with pre-therapeutic administration of 40 MBq ^{131}I: the success rate in the group without pre-ablative scintigraphy is nearly double that of the group who underwent pre-therapeutic ^{131}I dosimetry.
Thyroid stunning has been a controversial issue. Jeevanram *et al*[8] were the first to report a 25-75% decrease in uptake of therapeutic ^{131}I activities after diagnostic scanning with 111-185 MBq ^{131}I. Subsequently, several authors have reported various degrees of stunning of thyroid remnants after the administration of ^{131}I activities ranging from 74 MBq,[3] to 111 MBq,[9] 185 MBq,[10,21] and 370 MBq,[11] all resulting in a less successful outcome than a control group that was scanned either with a much lower (37 MBq) ^{131}I activity,[9] with ^{123}I,[10,11] or without any pre-therapeutic ^{131}I before ablation.[21]
On the other hand, McDougall *et al*[5] and Cholewinski *et al*[22] reported no visually apparent stunning effects after diagnostic activities of 74 MBq and 185 MBq ^{131}I, respectively. However, in neither of the latter studies the ablation success rates were reported. Dam *et al*[23] reported that even though visually apparent stunning was encountered in a part of their patient population, there were no differences in the ablation success rates between those patients who did and those who did not show stunning on pre-ablation or post-ablation scintigraphy. Sisson *et al* even argued that visually apparent stunning may not be attributed to a diagnostic activity, but rather to early effects from the subsequent ablation activity;[24] they did, however, not make a comparison with patients who had not received diagnostic ^{131}I activities. Silberstein[7] reported no different ablation success rates between patients receiving 14.8 MBq ^{123}I or 74 MBq ^{131}I for pre-therapeutic dosimetry.
The activity of 40 MBq ^{131}I used in the present study is lower than usually reported in

the literature. Thus far there was only scant evidence as to whether or not stunning may be caused by such low ^{131}I activities. Medvedec[12] performed a meta-analysis by fitting a regression model on results reported in 4 studies, and concluded that thyroid remnant stunning might already occur after ^{131}I activities as low as 10-20 MBq.

Whether there is a time point at which a pre-therapeutic diagnostic activity does not influence the outcome of the following ablative activity is questionable, and should be the subject of further study. Few data exist in the literature; in the present study success of ablation is diminished even if the diagnostic ^{131}I activity is given only 24 h before the ablative ^{131}I activity.

The success of ablation treatment is influenced by the size of the thyroid remnant.[25] In the present study there may have been a number of patients in group 1 with relatively large thyroid remnants; however, patients from group 2 did still significantly worse even when only the patients with the smallest remnants were selected.

From the results of this study it can be deducted that patients with a favorable prognosis suffer most from performing pre-therapeutic ^{131}I dosimetry: there was a difference between patients in group 1 and group 2 receiving 3700 MBq (i.e., patients with low-risk tumors), but no difference between those receiving 5550 MBq (i.e., patients with higher risk tumors). In addition in group 1 there is a large difference in ablation success between patients receiving 3700 and 5550 MBq ^{131}I; this difference is absent in group 2.

Other conditions being equal, it is highly likely that the lower ablation success rate for group 2 was caused by stunning from the pre-therapeutic dosimetry procedure with 40 MBq ^{131}I. Consequently, in order to maximize the success rate of ^{131}I ablation, 24-h uptake measurements and diagnostic scintigraphy are best avoided in patients with differentiated thyroid cancer. Legal requirements, or a suspicion of a large post-operative thyroid remnant, may necessitate pre-ablative diagnostic scintigraphy. In such cases ^{123}I scintigraphy may provide a valuable alternative with only little stunning of thyroid remnants.[26-28]

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Chapter Six

Persistent disease in patients with papillary thyroid carcinoma and lymph node metastases after surgery and ^{131}I ablation

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Abstract

Aim: to asses the efficacy of treatment of patients with papillary thyroid carcinoma (PTC) with lymph node metastases at the time of diagnosis, and its influence on the course of disease.

Patients and methods: retrospective review of all 51 patients with PTC and histologically proven lymph node metastases, treated with ^{131}I ablation in our center between January 1990 and January 2003.

Patients were considered disease-free if during follow-up thyroglobulin levels were undetectable and scintigraphy with 370 MBq ^{131}I was negative during TSH stimulation. Staging of patients was in accordance with the 5th edition of the TNM system.

Results: After a median follow-up of 84 months, 33 (65%) patients were never free of detectable disease, of whom 3 had died of PTC. 22 patients still showed persistent activity in the neck outside the thyroid bed, suspect for cervical lymph node metastases, on the post-ablation scintigram; this was neither related to the initial clinical presentation (lymph node metastasis or a thyroid nodule without evidence of metastatic disease), nor to the extent of surgery. 34 patients required additional treatment. Patients presenting with clinically overt lymph node metastases had a significantly ($p = 0.022$) lower rate of becoming disease-free than those in whom microscopic lymph node involvement was unexpectedly found upon pathologic examination. There was no significant association of the eventual outcome neither with the extent of surgery, nor with the TNM stage, or the patient's age.

Conclusions: Patients with lymph node metastases are considerably less likely to become disease-free. If the initial treatment does not result in a disease-free status, chances are low that additional treatment will succeed in achieving this.

Introduction

Papillary thyroid carcinoma (PTC) accounts for 80% of all cases of thyroid carcinoma,[1] and metastasizes primarily to the cervical lymph nodes. The presence of lymph node metastases is significantly correlated with the persistence and with the recurrence of the disease.[2] Lymph node involvement occurs in 15-80% of the patients, depending on the extent of lymph node dissection and the meticulousness of pathologic examination.[3,4]

If lymph node metastases are detected there are several therapeutic options. The surgical removal of all cancerous tissue is the treatment of choice.[3,5] Subsequently, treatment

with one or more therapeutic dosages of ^{131}I can be applied. An additional possibility is probe-guided surgery after ^{131}I therapy to remove remaining metastases.[6]
The aim of this study was to assess the efficacy of treatment of patients with PTC with nodular metastases at the time of diagnosis, and its influence on the course of disease.

Patients, materials and methods

Patients

All 235 patients who were referred to our center for ^{131}I ablation of thyroid carcinoma between January 1, 1990 and January 1, 2003, were reviewed.
For this retrospective study all patients were included who had histologically proven node-positive (T0-4, N1, any M) PTC, and a known outcome one year after the initial ^{131}I ablation. 51 patients met these criteria (details in table 6.1). Included patients had presented either with clinically overt metastatic lymphadenopathy, or with lymph node metastases discovered upon pathological examination of the surgical specimen. Clinical staging was determined according to the 5th edition of the TNM staging system.[7]

Table 6.1 Patient characteristics.

number of patients	51
gender	
male	17 (33%)
female	34 (67%)
median age in years (range)	38 (19-78)

Initial treatment

Patients received treatment according to the known clinical extent of disease at the time of diagnosis. All patients underwent a near-total thyroidectomy, with lymphadenectomy if deemed necessary, and received an ablative activity of 5550 MBq or 7400 MBq ^{131}I (depending on the extent of disease) 4-6 weeks after thyroidectomy.[8]

Laboratory analysis

On the day of administration of the ablative ^{131}I activity, blood samples were drawn for measurement of serum thyroid stimulating hormone (TSH) levels, total serum

concentrations of thyroid hormone, thyroglobulin levels and antibodies against thyroglobulin.

Follow-up after ablation

Patients treated for differentiated thyroid carcinoma were routinely evaluated one year after administration of the ablative dosage. At this point, serum TSH levels were elevated either by withdrawal of levothyroxin or administration of recombinant human TSH. During TSH stimulation, thyroglobulin levels were measured and whole-body scintigraphy using 370 MBq ^{131}I was performed. This was repeated at 4 years post-ablation, and then repeated every 5 years if no signs of disease were encountered. Indications for additional treatment consisting of surgery and/or a (blind) therapeutic dosage of ^{131}I[9] were based on the findings during follow-up. Some changes in management have occurred over time. E.g., in earlier years a policy of 'watchful waiting' was deemed the wisest course of action for patients showing lymph node metastases on ^{131}I scintigraphy. Later, such patients were referred for additional surgery, or sometimes for probe-guided surgery shortly after ^{131}I therapy.[6] Furthermore, the use of ultrasound for the localization of persistent and/or metastatic disease has increased over the years.

Measures of outcome

For this study, three measures of outcome were identified: (1) becoming free of disease, (2) recurrence of disease, and (3) thyroid cancer related mortality.

Patients were considered disease-free if during follow-up the physical examination was negative, thyroglobulin levels were undetectable and scintigraphy with 370 MBq ^{131}I was negative during TSH stimulation, and (if applicable) ultrasound of the neck was negative.

A recurrence was defined as any of the following happening:

- cytological/histological evidence of newly developed disease;
- detectable Tg levels;
- positive ^{131}I scintigraphy.

Analysis

For statistical analysis we used SPSS for Windows (SPSS inc.). Statistical significance was defined as $p < 0.05$.

Subgroups of patients were compared using Pearson's Chi-square test. The comparison of two groups for a continuous variable was done using the Mann-Whitney test. Prognostic significance of variables was assessed using univariate Cox' regression analysis.

Results

Median follow-up was 84 months (range: 9-179). 33/51 patients (65%) did not become free of disease of whom 3 patients (6%) died of PTC (details in table 6.2) while another 33/51 patients (65%) did not became free of disease. Of the 18/51 (35%) patients who became free of disease, none had recurrent disease at any time during follow-up.

Initial presentation and initial treatment are summarized in table 6.3. In one patient no record of the initial presentation was found. 7/38 patients presenting with lymph node metastases did not undergo cervical lymph node dissection. In 1 patient it was unclear whether the patient had a primary tumor in a cyst of the thyroglossic duct or a lymph node metastasis. In 2 patients only palpable lymph nodes were resected. In the remaining 4 patients it was not recorded explicitly why surgery had not been more extensive.

Table 6.2 Characteristics at diagnosis of the 3 patients who later died of DTC. Time to death is the time measured from ^{131}I ablation. TNM staging is given according to the 5th edition.

Number	age	sex	TNM-stage	extent of lymph node dissection	time to death
1	75	male	T1N1M0	bilateral	24 months
2	70	male	T4N1M0	unilateral	17 months
3	76	female	T4N1M0	bilateral	9 months

Table 6.3 Number of patients, their clinical presentations, and the extent of initial surgery.

	thyroidectomy	thyroidectomy + unilateral cervical lymph node dissection	thyroidectomy + bilateral cervical lymph node dissection
thyroid nodule	9	-	-
lymph node metastasis	7	31	2
treatment of other disease	-	-	1
unknown	1	-	-

Table 6.4 No influence of initial presentation or extent of surgery on the presence of cervical lymph nodes on post-ablation scintigraphy.

Initial presentation	no lymph node metastases on scan	lymph node metastases on scan	test
thyroid nodule	5	3	Chi-square = 1.41 (p = 0.49)
lymph node metastasis	23	18	
treatment of other disease	-	1	
unknown	1	-	
Extent of surgery			
thyroidectomy	11	6	Chi-square = 1.16 (p = 0.56)
thyroidectomy + unilateral cervical lymph node dissection	17	14	
thyroidectomy + bilateral cervical lymph node dissection	1	2	

Table 6.5 Number of patients with ^{131}I uptake outside the thyroid bed on post-ablation scintigraphy, suspicious for lymph node metastases.
Rows: extent of cervical lymph node dissection (CLND). Columns: side of visible pathologic ^{131}I uptake outside the thyroid bed in relation to the side of the CLND.

	unilateral uptake on the side of CLND	**unilateral uptake opposite the side of CLND**	**uptake in median line**	**bilateral uptake**
no CLND	4	-	1	1
one-sided CLND	6	3	3	2
two-sided CLND	2	-	-	-

All patients received an ablative ^{131}I dosage. 22/51 patients had foci of ^{131}I uptake outside the thyroid bed on the post-ablation scintigram, suspicious for lymph node metastases. Table 6.4 shows that this was neither related to the initial presentation nor to the extent of surgery.

In 14 patients, there were lymph nodes visible on the side of the neck where no lymph node dissection had taken place: in 8 patients after a unilateral lymph node dissection and in 6 patients who had had no lymph node dissection. In another 8 patients metastatic lymph nodes remained visible even after lymph node dissection on the involved side of the neck (table 6.5).

15/51 (29%) patients became disease-free after initial treatment. Three of the patients

receiving additional surgery and ^{131}I eventually became disease-free. During the course of follow-up a total of 34 patients received additional treatment: 21 patients received additional ^{131}I therapy (7400 MBq), 2 patients received additional surgery to remove affected cervical lymph nodes, and 11 patients received both. Three out of these 34 patients also required external beam radiotherapy on metastases that failed to accumulate ^{131}I. At the end of follow-up, in 26/33 patients persistent disease was established solely on the basis of positive Tg levels. In table 6.6 the specific criteria for diagnosing persistent disease are detailed by TNM stage.
After univariate analysis of prognostic factors only the initial presentation had prognostic significance. Patients who presented with a lymph node metastasis had a significantly lower chance of becoming free of disease than those who turned out to have positive lymph nodes at surgery ($p = 0.022$). The treatment outcome was significantly related neither to the extent of surgery ($p = 0.23$), nor to lymph node metastases on the post-ablation scintigram ($p = 0.74$). We were unable to show a statistically significant relationship between the TNM stage and the prognosis ($p = 0.11$) or the patient's age ($p = 0.11$). None of the variables analyzed was significantly associated with thyroid cancer-related mortality.

Table 6.6 Initial staging at presentation (TNM version 5), the number of patients with persistent disease per staging category, and the primary reason for classifying the patient as persistent disease (PD).

TNM stage	No. of patients in category	No. of PD patients	PD Tg positive	PD scintigraphy positive	PD histologic proof of metastasis
I	31	17	16	1	-
II	0	-	-	-	-
III	18	14	9	1	4
IV	2	2	1	-	1

Discussion

Patients presenting with cervical lymph node metastases from papillary thyroid carcinoma pose a clinical problem. In this study, after a median follow-up of 7 years 65 percent of patients still had detectable disease. This percentage is much higher than in the general population of thyroid carcinoma patients.[10] These results are largely in line with the literature: the presence of lymph node metastases is associated with a higher rate of persistent disease,[11-14] and with an increased risk of recurrences in the

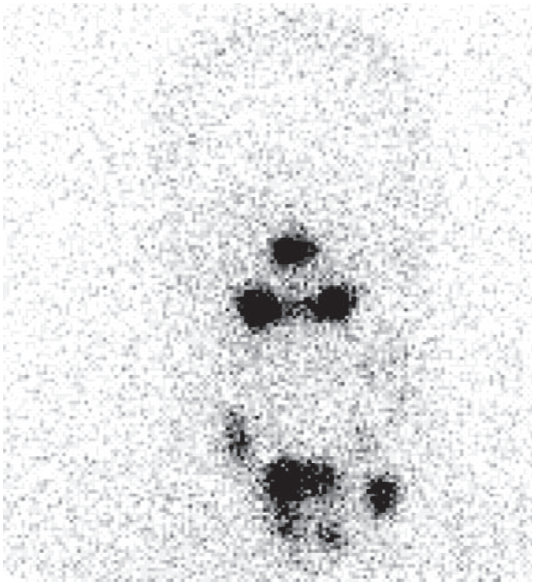

Figure 6.1 Post-ablation scintigraphy of a 44-year-old male after thyroidectomy and left-sided unilateral cervical lymph node dissection. Clearly, there are cervical lymph node metastases both on the non-operated right side and on the operated left side.

neck,[15-17] but not with higher mortality.[12,18-20] The low recurrence rate in the present study may be a matter of definition, as our study used very strict criteria for being disease-free. Our rate of persistently detectable Tg levels was 65% (all patients with persistent disease). This is appreciably higher than Leboulleux and co-workers who found detectable Tg levels in only 24% after surgery and ^{131}I.[21] Our finding that nodal metastases at presentation (corresponding with larger metastases) have a worse prognosis, is in agreement with a study by Sugitani who found a worse prognosis for patients with lymph node metastases with diameters $\geq$ 3 cm.[22]

Persistent disease does not appear to have a clear influence on survival. In concurrence with the study by Leboulleux *et al*,[21] we found a high survival rate of patients with persistent disease.

We cannot explain why the extent of surgery is not related to the treatment result. As the extent of the surgery is in all cases patient-tailored, it could be argued that surgeons have chosen the adequate treatment in each individual case. On the other hand, it could be argued that some patients should have had more than unilateral cervical lymph node dissection. This is illustrated in figure 6.1 (see also table 6.4).

Although no significant influence of the extent of initial surgery on outcome was shown in the present study, it nonetheless seems that a patient has the best chance of becoming disease-free during initial therapy: if initial treatment is unsuccessful in rendering a patient disease-free, there is only a low success rate at a later time. This would warrant a relatively aggressive approach at the initial treatment of PTC.
Patients with metastases discovered by chance in the fat surrounding the thyroid (i.e. level VI) had a higher chance of becoming disease-free than those presenting with lymph node metastases in other cervical regions. Therefore the present study supports the limiting of stage N1a metastases in the 6th edition[23] of the TNM system (metastases in the pre-tracheal compartment, level VI) compared to the 5th edition (ipsilateral lymph node metastases) (table 6.6).
Additional surgery is only recommended when there is a clear focus for surgery, such as a lymph node metastasis visible on ultrasound or on ^{131}I scintigraphy. Good results have been reported with the combination of radioiodine therapy and subsequent probe-guided surgery for lymph node metastases visualized on whole-body scintigraphy.[6,24,25] However, additional and/or more extensive surgical intervention carries a higher risk of complications such as recurrent laryngeal nerve damage,[26] in patients who on average have a significant number of years left to live with the consequences of these complications. Alternatively or additionally, high dose ^{131}I therapy can result in good local control and a significant decrease of Tg levels.[9,27,28] However, also radioiodine treatment should be weighed against its possible complications, such as salivary or lacrimal gland dysfunction.[29,30]

Conclusion

The presence of lymph node metastases in patients with papillary thyroid carcinoma is associated with a much lesser chance of becoming disease-free. The significance of persistent disease with regard to overall survival, however, seems to be limited. The precise prognostic impact of the extent of surgery remains unclear.

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Chapter Seven

Prognostic importance of successful ablation of differentiated thyroid cancer patients

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Abstract

Objectives: Currently little is known about the prognostic significance of achieving successful ablation with the first dosage of ^{131}I in patients with differentiated thyroid cancer.
The aims of this study were to assess (1) whether successful or unsuccessful ablation at post-ablation follow-up has prognostic consequences, and (2) possible factors predicting success of ablation in a patient.
Methods: In order to do this, we retrospectively studied 180 patients with a median follow-up of 55 months. Ablation was considered to be successful if 1 year after the initial dosage of ^{131}I patients fullfill all of the following criteria: not dead from thyroid cancer, undetectable thyroglobulin (Tg) levels under TSH stimulation, and negative ^{131}I scintigraphy. P-values were calculated by Mann-Whitney U-test and Chi-square test, respectively.
Results: Tg levels at the time of ablation ($p < 0.001$), nodular metastases ($p = 0.04$) and distant metastases ($p < 0.001$) have a significant influence on the success of ablation. Patients with successful ablation had a better prognosis than those with unsuccessful ablation: disease-free survival was 87% versus 49% after ten years; additionally, thyroid cancer related survival was 93% versus 78%.
Conclusion: We conclude that the extent of the remaining normal or neoplastic thyroid tissue influences the outcome of ablation, and that successful ablation leads to a better prognosis. It seems that it is very important to reach complete ablation as soon as possible in order to ensure the best possible prognosis for a patient.

Introduction

Treatment of differentiated (papillary or follicular) thyroid cancer consists of thyroidectomy and an ablative dosage of Iodine-131.[1] The addition of ^{131}I ablation after surgery, leads to a significantly improved prognosis, especially in patients who are at high risk for its recurrence or death by thyroid cancer.[2-6] Unfortunately, the first dosage of ^{131}I is not always sufficient to achieve complete ablation of thyroid remnants.[7-13]
The aims of this study were to assess (1) whether successful or unsuccessful ablation at post-ablation follow-up has prognostic consequences, and (2) possible factors predicting success of ablation in a patient.

Patients, materials and methods

Patients

All patients with differentiated thyroid carcinoma, who were referred to our center for their first, ablative dosage of ^{131}I after January 1, 1990, were reviewed retrospectively. To be included in this study, at least one of the following criteria had to apply:

- the patient returned for diagnostic scintigraphy and measurement of thyroglobulin levels during TSH stimulation (Tg-off) one year after ablation;
- the patient has received an additional therapeutic dosage of ^{131}I within the first year following the ablative dosage of ^{131}I;
- the patient has died of thyroid cancer within the first year following the ablative dosage of ^{131}I.

Out of 192 eligible patients 180 met at least one of these criteria. The patient characteristics are given in table 7.1.

Table 7.1 Patient characteristics.

N	180
mean age in years (range)	46 (14-84)
female/male ratio	126/54
PTC/FTC ratio	123/57

Initial treatment

All patients underwent a near-total thyroidectomy, and received an ablative dosage of ^{131}I four to six weeks after the thyroidectomy. Between surgery and ablation patients did not receive Levothyroxine (LT4) supplementation, and they were instructed to have a low-iodine diet for one week (5 days before and 2 after ^{131}I administration).[14] To prevent stunning of thyroid remnants,[15-17] no diagnostic scintigraphy was performed before the administration of the ablative dosage of ^{131}I. Patients were treated with a fixed dosage: 3700 MBq of ^{131}I in cases without known lymph node or distant metastases, 5550 MBq of ^{131}I in cases of pre- or peri-operatively detected nodular involvement, or 7400 MBq of ^{131}I in cases of known distant metastases.

Follow-up after ablation

In our department, patients that are treated for differentiated thyroid carcinoma are usually evaluated by the measuring of Tg levels and whole-body scintigraphy using 370 MBq ^{131}I one year after administration of the ablative dosage, which happens after LT4 withdrawal for four weeks. In all but 4 patients this resulted in TSH levels of > 30 mU/l, with a maximum of 469 mU/l.

Ablation was considered to be successful if, one year after the initial dosage of ^{131}I, patients fulfilled all of the following criteria:

- not dead from thyroid cancer;
- no additional therapy of any kind needed for thyroid cancer within the first year;
- had undetectable Tg-off levels;
- had negative ^{131}I scintigraphy.

Tg levels were not used as a criterion for success of ablation in the cases when a patient tested positive for the presence of Tg antibodies since test results for Tg cannot be considered reliable in these patients.[18,19]

Disease-free interval and recurrence

A disease-free patient was considered to have a recurrence if one or more of the following conditions developed:

- detectable Tg levels;
- pathologic evidence of disease, e.g. by fine-needle aspiration biopsy;
- positive ^{131}I scintigraphy.

In our analysis, the disease-free interval started at the time of the first completely negative follow-up.

This could be at the time of the 1-year post-ablation scintigram (as in the group with successful ablation) or at a later follow-up scintigram if a patient required additional treatment to become free of disease (as in the group with unsuccessful ablation).

Statistical analysis

For statistical analysis we used SPSS version 10.1 for Windows (SPSS inc.). Statistical significance was shown with $p < 0.05$. For testing between different groups of patients the Mann-Whitney test (Z-approximation) was used if one of the variables involved was continuous. The Chi-squared test was used in case both variables involved were categorical data. Survival times were analyzed using the method of Kaplan-Meier. Additionally, the difference between survival curves was examined using a log rank test.

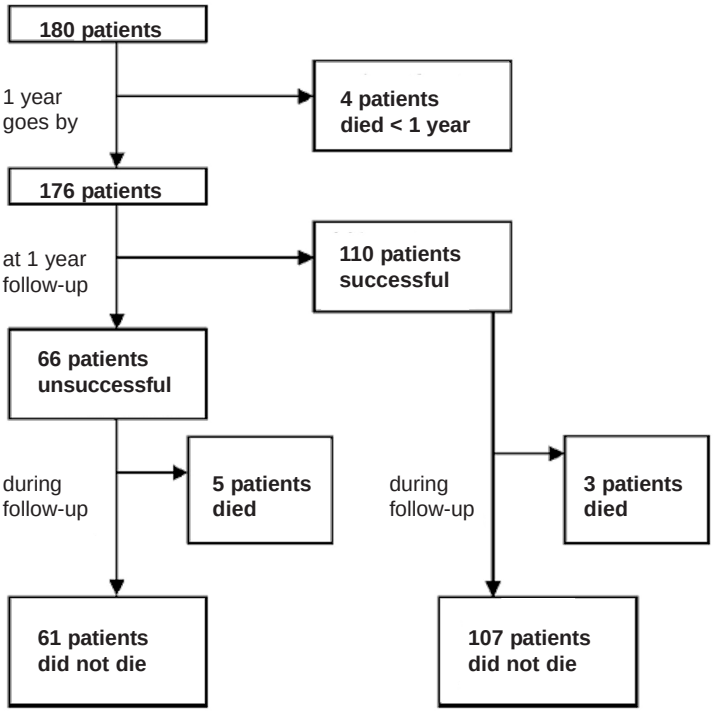

Figure 7.1 Status of patients, one year after administration of the ablative ^{131}I dosage. Die: to die of differentiated thyroid carcinoma before the end of follow-up.

Results

Success of ablation

According to the criteria used in this study, ablation turned out to be successful in 110 out of 180 patients (61%) (figure 7.1).

Median follow-up time was 55 months. 12 patients died of thyroid cancer, of which 4 had died within one year of initial treatment. 16 patients had recurrence of thyroid cancer. 29 patients never became free of the disease during follow-up, including 6 out of the 8 patients who died. 7 of those 29 patients had a short follow-up of less than four years, so they might not have received the maximum treatment yet. 2 patients died of recurrent disease.

Of the patients with successful ablation 87% (SE 4.8%) were still free of the disease ten years later. Of the patients with unsuccessful ablation who eventually did become free of disease, only 50% (SE 14%) were still free of the disease after ten years (see

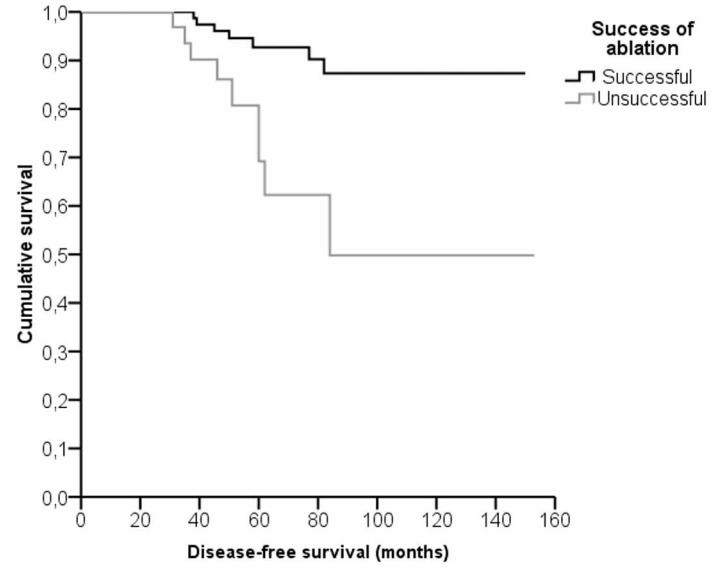

Figure 7.2 Kaplan-Meier plot of the duration of the disease-free interval in those with successful and those with unsuccessful ablation.

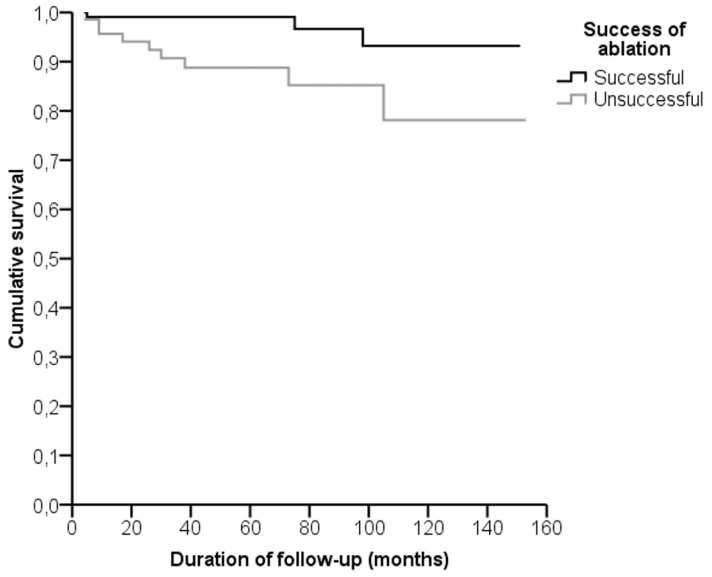

Figure 7.3 Kaplan-Meier plot of thyroid cancer-specific survival in those with successful and those with unsuccessful ablation.

figure 7.2). The difference between the two Kaplan-Meier curves was significant (log rank = 11.1, $p < 0.001$). At five years, disease-free survival was 93% versus 62%.
In patients who were alive at the one-year follow-up, those with successful ablation had a thyroid cancer-specific survival of 100% for five years after ablation and 95% (SE 4,1%) for ten years after ablation; whereas patients with unsuccessful ablation had a thyroid cancer-specific survival of 89% (SE 4.0%) for five years after ablation and of 78% (SE 8.3%) for ten years after ablation (see figure 7.3). The difference between these two survival curves was also statistically significant (log rank = 7.16, $p = 0.007$).
We analyzed factors at the time of ablation that could possibly influence the outcome of the first dosage of ^{131}I. Results of these analyses are displayed in table 7.2. Patients with a higher serum Tg level at the time of ablation ($p < 0.001$), patients with lymph node metastases discovered pre- or peri-operatively ($p = 0.04$) and patients with distant metastases ($p < 0.001$) turned out to have less chance of achieving successful ablation with the initial dosage of ^{131}I.

Table 7.2 P-values of factors influencing the success of ablation.

Possible prognostic variable	p
age	0.09
TSH levels	0.19
total T4 levels	0.17
Tg levels	< 0.001
sex	0.07
papillary/follicular carcinoma	1.00
tumor diameter	0.15
nodular metastases	0.04
distant metastases	< 0.001

Discussion

Our study shows that the success of ablation is a prognostic factor for disease-free interval and survival in differentiated thyroid cancer patients. Unsuccessful ablation carries with it a considerably higher risk of recurrence; more importantly, it is not even certain that a patient can be made free of detectable disease. Our results support previous studies on the beneficial effects of radioiodine ablation for prognosis of patients with differentiated thyroid cancer.[2,5,20] In these studies, however, success of ablation as determined by Tg levels and scintigraphy was not reported as a prognostic factor. Some factors can influence the success of ablation. These factors include Tg

levels at the time of ablation, presence of pathological lymph nodes and distant metastasis. Since Tg is produced by normal or neoplastic thyrocytes, one can assume that higher Tg levels indicate a larger mass of functioning thyroid cells. Therefore, the chance of successful ablation is determined by the mass of normal and neoplastic thyroid tissue remaining after surgery. What could be of further importance is the grade of differentiation of the thyroid cancer cells. In some patients, the carcinoma dedifferentiates in the course of the disease.[21,22] One of the mechanisms affected by this dedifferentiation is the sodiumiodine symporter, which is essential for the uptake of ^{131}I by thyroid cancer cells. Cells that do not take up ^{131}I are much more likely to be persistent, but cannot be seen or measured scintigraphically. It is therefore impossible to say how much our results are influenced by this phenomenon.

Finding that a patient has an unsuccessful ablation adversely affects prognosis: a considerable number of patients in whom initial ablation was unsuccessful will never, or only after a number of additional therapeutic dosages of ^{131}I, become free of detectable disease. Furthermore, even if they eventually do become free of disease, there is an increased chance that the thyroid cancer will return sooner or later.

Lymph node or distant metastasis is a well-known adverse prognostic variable for thyroid cancer.[2,20,23-26] Surprisingly, however, the age at the time of ablation had no statistically significant influence on the outcome of the first dosage of ^{131}I in our study population. Normally, age is considered to be one of the more important prognostic factors in thyroid cancer. Several studies have reported that high Tg levels at the time of ablation can be related to initial metastasis or recurrence.[27-32] Muratet *et al*[13] also found a significant relationship between Tg levels and the chance of successful ablation. Interestingly, they observed that a rise in Tg levels shortly after administration of ^{131}I is correlated with a lower chance of successful ablation. A similar report came from the group of Baudin *et al*[33] who reported that the slope of rise or fall of Tg levels measured in high TSH conditions, compared with Tg levels at the time of ablation, had a higher value for prediction of recurrences.

Further research into the significance of Tg levels at the time of ablation seems warranted, especially with regard to the success of ablation. This could possibly lead to improved dosage schemes for individual patients. In that area, it would be interesting to conduct further research into the significance of measuring Tg levels after the ablative dosage: what is the earliest moment that they need to be undetectable during TSH stimulation, if there is to be a chance of achieving complete ablation? This could possibly lead to earlier additional therapy. In order to answer this question, we may want to turn to ^{131}I therapy for benign conditions. In patients treated for benign thyroid diseases, no further effect of ^{131}I therapy can be measured from 3 months after

administration onward.[34,35] This is considered to be so, even though 3 months after ablation would be much earlier than recent recommendations of 6-12 months.[36] Perhaps that would be a good point in time for post-ablation follow-up, instead of the current follow-up at 1 year. This means that any thyroid cancer cell not killed in the first blast will have 9 months less to proliferate and spread.
Unsuccessful ablation is an adverse event in the follow-up of differentiated thyroid carcinoma. Patients in whom this is found should be followed much more intensively than patients in whom ablation was successful: they may never become free of disease, and even if they do, there is a high risk of recurrence. Therefore, it seems important to treat thyroid cancer as early and as intensively as possible, in order to achieve the best possible prognosis for patients.

In conclusion, unsuccessful ablation dramatically lowers the prognosis of disease-free survival in patients; they should be followed much more intensively than those in whom ablation was successful with one dosage. The extent of the remaining normal or neoplastic thyroid tissue contributes to a higher risk of unsuccessful ablation after the first dosage of ^{131}I for the treatment of thyroid cancer.

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Chapter Eight

Absence of late recurrence in patients after successful initial treatment with surgery and radioiodine

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Submitted

Abstract

Objectives: (1) to investigate whether the stratification in high risk and low risk according to initial pTNM stage still applies in cured patients and (2) to study the modalities by which recurrences are discovered as well as to determine risk factors for recurrence after successful ablation.

Methods: Retrospective data from three university hospitals for differentiated thyroid carcinoma were pooled. Out of 1993 patients, 526 cured patients were included. Curation was defined as a negative TSH-stimulated Tg levels and a negative ^{131}I whole-body scan (WBS) at the first follow-up after ablation. All patients received at least one more TSH-stimulated WBS and Tg measurement within 5 years after initial treatment.

Results: 12 patients (2.1%) developed a recurrence after an average interval of 35 months (range: 12-59 months) following administration ^{131}I ablation. Overall disease-free survival according to the method of Kaplan-Meier was 96.6%. There was no difference in disease-free survival between high- and low-risk patients ($p = 0.61$). Recurrence was first discovered by Tg measurement during levothyroxin therapy in 7 patients, and by TSH-stimulated Tg measurement in 5 patients. ^{131}I WBS did not contribute to the detection of recurrences. Multivariate analysis showed that age, TNM stage ($p = 0.015$) and histology ($p = 0.032$) were independent predictors of disease-free survival.

Conclusion: Recurrence is a rare event in patients with DTC who received total thyroidectomy with subsequent ^{131}I ablation, and who had a negative first follow-up TSH-stimulated ^{131}I WBS and negative concurrent Tg. In the study population there were no recurrences after more than 5 years follow-up.

Introduction

Many retrospective studies have presented the course of differentiated thyroid carcinoma (DTC) in large series of patients followed over a long period of time.[1-5] These studies showed that at least half of recurrences of differentiated thyroid carcinoma occur within 5 years of diagnosis. Also they have shown that a first recurrence could still occur as late as 40 years after the initial diagnosis of DTC, and that therefore DTC patients can never be considered ‘cured’, and should be monitored throughout their lifetime.[6] Successful ablation has been shown to be an indicator of favorable prognosis.[7] The American Thyroid Association guidelines[8] and several consensus statements[9-11] state that after ^{131}I ablation in patients with a favorable prog-

nosis, a negative first TSH-stimulated measurement of thyroglobulin (Tg), possibly combined with ^{131}I whole-body scintigraphy, TSH-stimulated follow-up can be omitted. Further follow-up in these patients should consist of at least annual Tg measurement during TSH-suppressive therapy, preferably combined with ultrasound of the neck. For patients with high-risk carcinomas a more intensive and stringent follow-up is recommended. Unfortunately there exists no uniform definition of what constitutes a high-risk differentiated thyroid carcinoma patient.

The aim of this study is to investigate whether the stratification in high risk and low risk according to initial pTNM stage still applies in cured patients (and if consequently follow-up recommendations should differ between the two groups). Secondary objectives are to study the modalities by which recurrences are discovered as well as determine risk factors for recurrence after successful ablation.

Patients, materials and methods

Hospitals

In this study three university hospitals participated: the University Clinic Würzburg (UKW) Germany, the Leiden University Medical Center (LUMC) and the University Medical Center Utrecht (UMCU), both in the Netherlands. All three hospitals are referral centers for post-surgical ^{131}I treatment of DTC patients.

Patients

Out of a total of 1993 patients we included 526 patients with DTC who had undergone near-total thyroidectomy and subsequently received their initial ^{131}I treatment, and in whom TSH-stimulated Tg measurement and ^{131}I whole-body scintigraphy (WBS) after initial treatment were completely negative. Data were reviewed retrospectively.

Initial staging and treatment

Patients were included independent of the ablation protocol used, as inclusion would only follow after a successful ablative treatment. Depending from the protocol, the histology, the remnant size and the tumor stage, administered activities ranged from 1100 MBq ^{131}I in patients with large thyroid remnants to 7400 MBq in patients with extensive locally invasive or metastatic disease.[12,13]

Laboratory analysis

Test results for Tg may not be considered reliable in the presence of antibodies,[14,15] the presence of which was shown either through direct measurement or through determination of Tg recovery rates. Patients were excluded from analysis in the

presence of measurable Tg antibodies or insufficient Tg recovery. For the purpose of this study Tg levels in any patient were considered undetectable if they were below institutional cut-off values.

Follow-up after ablation

Within the first year after ^{131}I ablation, patients returned to our hospitals for TSH-stimulated follow-up. High TSH levels were induced either by LT4 withdrawal or by intramuscular injection of recombinant human TSH. During TSH-stimulation Tg levels were measured and a diagnostic WBS using 185-370 MBq ^{131}I was acquired. All patients received at least one more TSH-stimulated follow-up within 5 years of ablation.

Definitions

The TNM stage of all patients was determined according to the 5th edition of the UICC/AJCC TNM staging system.[16] High-risk patients were defined along the lines of the 2006 European consensus,[11] in which all patients with T3 or T4 tumors, as well as patients with N1 or M1 disease, were considered at high risk for recurrence. By definition, all patients included in this study were considered disease-free. Recurrence was defined as any of the following occurring after a documented disease-free period:

- cytologic/histologic evidence of disease;
- detectable Tg levels;
- positive ^{131}I scintigraphy.

Analysis

Statistical significance was defined as $p < 0.05$. Survival times were assessed using the method of Kaplan-Meier. Differences in survival times were assessed with a log rank test. Multivariate analysis was performed using a Cox-regression on any variable that had $p \leq 0.20$ in univariate Cox-regression, using a forward selection method based on likelihood ratios.

Results

Out of 1,993 patients with differentiated thyroid carcinoma seen in our hospitals, 526 patients fulfilled the inclusion criteria and were included in the present study: 312 from the UKW (start of inclusion: 1980), 102 from the UMCU (start of inclusion: 1990) and 112 from the LUMC (start of inclusion: 1990). Mean follow-up was 79.6 months after ablation (range: 4-306 months). No patient who presented with distant metastases was free of disease after initial treatment. For patient characteristics see table 8.1. 12 patients (2.3%) developed a recurrence after a mean interval of 35 months (range: 12-59 months) following administration of the ablative activity of ^{131}I, of

Table 8.1 Basic patient characteristics of the patients included in this study.

Mean age (years)	44.1
no. patients aged < 45 years	283
no. patients aged > 45 years	243
Histology	
papillary thyroid carcinoma	381
follicular thyroid carcinoma	142
unknown histology	3
TNM stage (5th edition)	
I	309
II	160
III	49
unknown	8
Nodular metastases	
node negative	449
node positive	76
unknown	1
Low / high risk	
low risk	329
high risk	177
unknown	20
Extra-thyroidal tumour invasion	
no invasion present	473
invasion present	53

whom 2 eventually died of DTC. Recurrence was first discovered by Tg measurement during suppression in 7/12 patients, and by TSH-stimulated Tg measurement in 5/12 patients. Only one patient showed a concurrent positive ^{131}I whole-body scan; in none of the patients was a recurrence first detected only by means of a ^{131}I WBS.
In 6/12 patients an anatomical substrate for recurrence was found: 4 patients had distant metastases, and two patients had local recurrence.
In the remaining patients we were unable to identify a focal source for the elevated Tg levels: one patient died of cardiac causes before a diagnosis could be made, one patient received a blind therapeutic dosage and Tg levels were undetectable ever since, and in four patients no anatomical correlate for the elevated Tg levels has been found thus far.
The overall 5-year and 10-year disease-free survival calculated according to the method of Kaplan-Meier both were 96.6 $\pm$ 1.0% (figure 8.1). No recurrences occurred at more than 60 months after ablation. There were no differences in survival times between patients with different T-stages ($p = 0.73$) or between patients with or without lymph node metastases ($p = 0.53$). Neither did they differ between male and female

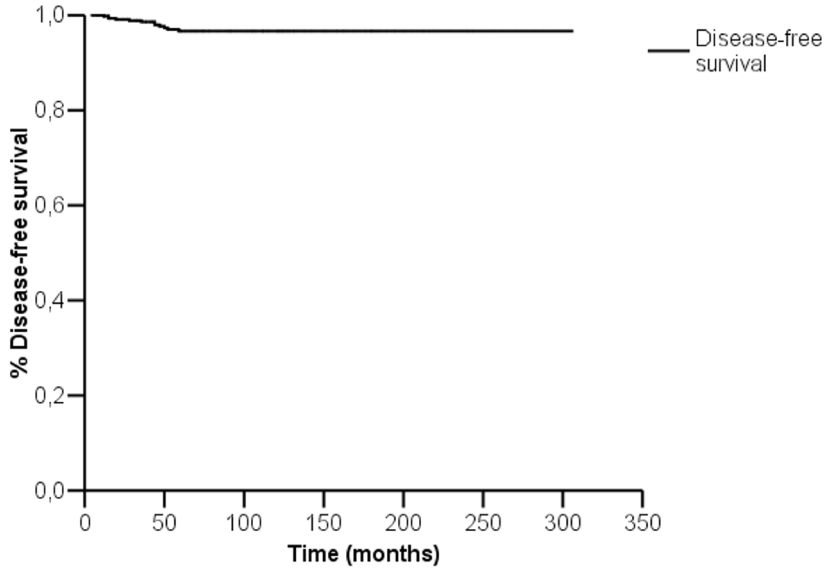

Figure 8.1 Overall disease-free survival.

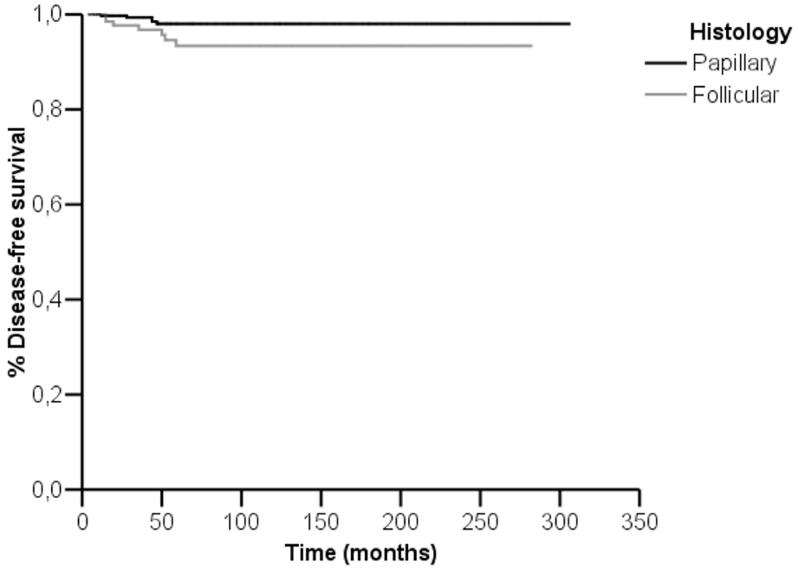

Figure 8.2 Disease-free survival for patients with papillary or follicular thyroid carcinoma. The difference is statistically significant (p = 0.03).

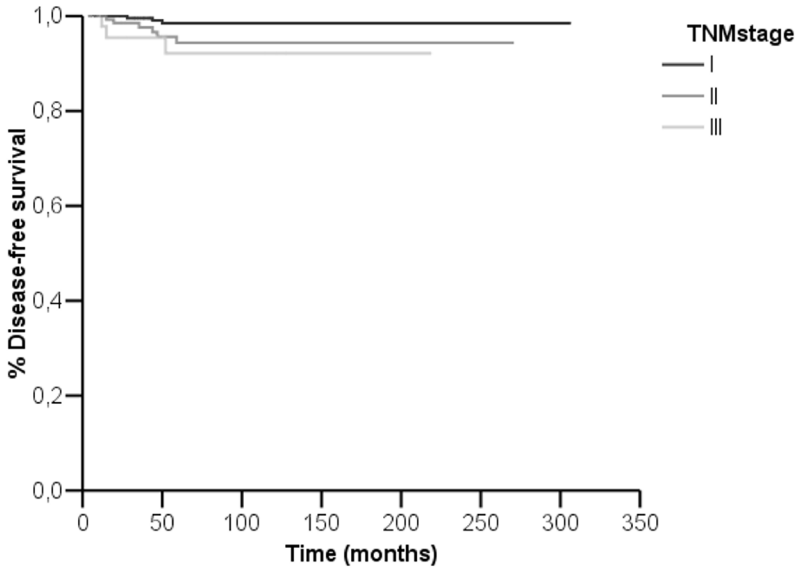

Figure 8.3 Disease-free survival of patients per TNM stage. The difference between the three curves is statistically significant (p = 0.03)

patients (p = 0.33). Neither the presence of lymph node metastases (p = 0.53) nor infiltrative growth outside the thyroid (p = 0.18) at initial staging led to a significantly lower disease-free survival rate. Neither were there differences in disease-free survival between the participating centers (p = 0.31). High-risk patients did not have a higher recurrence rate than low-risk patients (p = 0.61): the long-term survival-adjusted risk of recurrence was 3.4% $\pm$ 1.3% in low-risk patients, and 3.7% $\pm$ 1.7% in high-risk patients. Patients aged $\geq$ 45 years at the time of ablation had a slightly, but significantly lower 5-year disease-free survival than patients aged < 45 years (94.4 $\pm$ 1.8%, versus 98.4 $\pm$ 0.9%; p = 0.03).

Patients with papillary thyroid cancer did significantly better than those with follicular thyroid carcinoma (p = 0.03) (figure 8.2). The various stages according to the TNM system showed a significant difference with regard to disease-free survival (p = 0.03) (figure 8.3). Multivariate analysis showed that TNM stage (p = 0.015) and histology (p = 0.032) independently influenced the disease-free survival.

Discussion

The advent of highly sensitive Tg measurements has not only allowed strict follow-up of DTC and a close monitoring of therapy response; it has also opened the possibility

for re-stratification of risk based on this monitoring and follow-up. This adaptation of risk stratification is also propagated in the guidelines of the American Thyroid Association (ATA) as well as three consensus statements,[8-11] which all recommend reducing the intensity of follow-up for patients with negative TSH-stimulated Tg levels after ^{131}I ablation. The results of the present study support such a policy of re-stratification, but go further. The guidelines of the ATA and the various consensus statements have limited the relaxation of follow-up only to patients who can be classified, with different criteria, as 'low risk' at the time of initial treatment. This study shows that some patients who were initially classified as 'high risk' (e.g., patients with lymph node metastases) can be downgraded to 'low risk' for recurrence. Furthermore, even for those patients who have one or more risk factors for recurrence as identified in this study (i.e. follicular carcinoma, or higher TNM stage) a long-term disease-free survival rate of at least 92 percent may be expected.

The results of this study are in line with findings by Kloos and Mazzaferri.[17] Their study with a relatively small group of patients with a negative first TSH-stimulated follow-up (n = 68), who were treated in a single center according to a single protocol, showed a recurrence rate of ~2% in patients with rhTSH-stimulated Tg levels < 0.5 ng/ml. A limitation of the latter study was the limited follow-up of 3-5 years. The results in the present larger, multicenter, bi-national, long-term study of DTC patients also show that a negative TSH-stimulated serum Tg level and a negative ^{131}I WBS predict a very favorable prognosis with an overall survival-adjusted recurrence rate of ~3.4 percent.

A study by Pacini *et al*,[18] later confirmed in another study by the same group,[19] concluded that the combination of clinical examination, TSH-stimulated Tg measurement and ultrasound of the neck would be sufficient to follow thyroid cancer patients, and that ^{131}I whole-body scintigraphy did not reveal any additional findings except persistent uptake in the thyroid bed. In that study the long-term disease-free remission rate (89.5%) in patients with a negative first stimulated Tg measurement was considerably lower than in the present study. A possible explanation for this discrepancy may lie in the inclusion criteria: Pacini *et al* included all patients with negative Tg, regardless of the outcome of the ^{131}I WBS, whereas in the present study patients had to have a negative ^{131}I WBS as well. Robbins *et al*[20] largely concurred with this opinion, but only for patients who had had at least one prior negative WBS: in these cases further follow-up WBS did not yield significant information. The contrast between the results of the present study and the one by Pacini *et al* highlights the value of ^{131}I WBS for stratifying patients more accurately according to prognosis after initial treatment. Using ^{131}I WBS in addition to Tg measurement our study

population showed a recurrence rate of only one-third of that of the population studied by Pacini *et al*. In concurrence with Pacini *et al* and Robbins *et al*, our study shows that a follow-up ^{131}I WBS does not contribute any clinically relevant information in patients with a prior negative ^{131}I WBS. Despite the recommendations in recent guidelines to omit ^{131}I WBS, this procedure may be useful to reclassify risk status after initial treatment. Further follow-up should then consist of Tg measurement and ultrasound.[19] Whether TSH stimulation (either with recombinant human TSH or after withdrawal) is necessary for accurate Tg measurements is still undecided: in our study only 5 extra recurrences were found with TSH-stimulated follow-up; to detect one additional recurrence, 100 patients need to be withdrawn from levothyroxin medication, or have to be administered rhTSH. This incurs considerable costs and/or burden to the patient.[21-24] Similar to these findings, Castagna *et al* reported one patient with a positive Tg measurement after rhTSH stimulation in a group of 67 patients with a prior negative 'Tg-on'.[25]

Conclusion

After successful ^{131}I ablation, established by negative Tg levels and negative ^{131}I scintigraphy, disease-free survival rates in patients with differentiated thyroid carcinoma are high, even in some patients who were initially classified as high-risk. ^{131}I WBS is useful for the re-stratification of risk. If the initial ^{131}I WBS is negative, follow-up scanning should be omitted as it yields no additional diagnostic information.

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Chapter Nine

Histology does not influence tumor-specific survival in differentiated thyroid carcinoma when tumor diameter, invasive growth and metastases are accounted for

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Submitted

Abstract

Objective: Patients with papillary (PTC) and follicular (FTC) thyroid carcinoma show considerable differences in staging at initial presentation. The aim of this study was therefore to investigate whether there are differences in tumor-specific survival if initial staging is accounted for.

Methods: Retrospectively we reviewed 1,225 patients (856 females, 369 males, mean age 47.8 years, range: 5-87 years) with differentiated thyroid carcinoma (875 papillary, 350 follicular carcinoma) who were treated in our hospital and received initial treatment from 1978 (earliest available data) up to and including 2002. All patients received total thyroidectomy with subsequent ^{131}I ablation except for those patients with a solitary papillary microcarcinoma.

Results: FTC patients on average are older and are more likely to be male, to present with larger tumors, and they more frequently have multifocal carcinomas and distant metastases than PTC patients, whereas they present less frequently with extra-thyroidal invasion or lymph node metastases. 20-year tumor-specific survival in PTC was 90.6% and in FTC 73.7% ($p < 0.001$). In a multivariate analysis the presence of distant metastases ($p < 0.001$), age ($p < 0.001$), tumor size ($p = 0.001$) and the presence of extra-thyroidal invasion ($p = 0.007$) were explanatory variables for tumor-specific survival.

Conclusion: There are no differences in tumor-specific survival between patients with PTC and FTC when accounting for the presence of metastases, the patient's age, the tumor size and the presence of extra-thyroidal invasion.

Introduction

Papillary (PTC) and follicular (FTC) thyroid carcinoma, derived from the same follicular thyroid cell lineage, are often grouped together as a single entity which is referred to as differentiated thyroid carcinoma. This grouping together suggests that the clinical behavior of these cancer types should be virtually similar. However, there are important differences, especially with regard to tumor-specific survival. In several studies it has been shown that FTC has considerably shorter long-term survival than PTC.[1,2] Whereas some prognostic scoring systems do not differ between PTC and FTC,[3-5] others do regard FTC as an adverse prognostic factor.[6,7]

As far as the clinical presentation is concerned, PTC and FTC also differ markedly. FTC patients probably present after a longer diagnostic delay,[8] with a larger diameter

of the primary tumor, and they more often have distant metastases at the time of initial presentation.[1,2,9,10] All of these are considered indicators of poor prognosis with regard to tumor-free survival. On the other hand FTC, contrary to PTC, infrequently shows lymph node metastases.[2,8]

Others have shown that in fact when the tumor diameter is compensated for, PTC and FTC show the same rate of distant metastasis.[8] FTC develops lymph node metastases or extra-thyroidal growth at a much greater tumor diameter than PTC. Taking these findings from the literature together, it is conceivable that FTC merely *seems* to have a poorer prognosis because it is discovered later in the course of the disease. The aim of this study was to investigate whether there are differences in tumor-specific survival between PTC and FTC if initial staging is accounted for.

Patients and methods

Database

The Würzburg thyroid cancer registry was established primarily to monitor the quality of patient treatment. Secondarily this registry allows for retrospective scientific population studies. Data are recorded for each visit, starting with the first visit after the diagnosis of thyroid carcinoma has been established. Data recorded at initial visit included histology, pTNM staging (according to version 5 of the TNM system), multicentricity of the tumor, whether or not there was extra-thyroidal invasion (but unfortunately not the precise extent of this invasion).

For patients who are no longer attending our clinic for follow-up the registry is regularly updated through inquiries at the referring physicians.

Patients

Retrospectively we reviewed 1,225 patients (856 females, 369 males, mean age 47.8 y, range: 5-87 y) with differentiated thyroid carcinoma (875 papillary, 350 follicular carcinoma) who were treated in our hospital and received initial treatment from 1978 (earliest available data) up to and including 2002. The latter cut-off date was chosen to allow for a minimum follow-up of 5 years.

Treatment

All patients received total thyroidectomy with subsequent ^{131}I ablation except those patients with a solitary papillary microcarcinoma, who usually had a hemi-thyroidectomy. ^{131}I ablation was performed with 1500-3500 MBq ^{131}I, depending on the size of the thyroid remnant. 6-12 months after initial treatment patients returned for ^{131}I whole-body scintigraphy and Tg measurement after withdrawal of levothyroxine or,

in later years, after the administration of recombinant human TSH (rhTSH). If any signs of persistent disease were encountered, an additional activity of 7000 MBq ^{131}I was administered. If the patient was disease-free, at least one more follow-up scan after thyroid hormone withdrawal or rhTSH injection was performed within the first 5 years of follow-up. For the first 5 years patients were furthermore followed at 6-month intervals and thereafter at yearly intervals by means of thyroglobulin measurement and neck ultrasound. X-rays, CT scans or MRI scans were performed on indication.

Pathological analysis and staging

Surgical specimens were analyzed according to the standard at the time of initial treatment, and classified as PTC or FTC according to the guidelines at the time. Data were processed as stated in the original pathology report. Patients with one or more anaplastic foci were excluded from the present study. The primary tumor diameter was determined from macroscopic analysis of the surgical specimen, when possible. In the case of multifocal tumors the diameter of the largest tumor was taken for the primary tumor diameter. Histologic grading was not determined, because this was not recommended by the WHO. For a classification of N0 patients should have at least undergone a lymph node dissection of the central compartment; otherwise they were classified as Nx. A classification of lymph node metastases, multifocal disease or extra-thyroidal disease required histologic confirmation; for a classification of distant metastases, other evidence (such as a positive post-therapy ^{131}I whole-body scan, CT scan or MRI) was deemed sufficient.

Analysis

Statistical analysis was performed using SPSS 12.0 for Windows. P-levels < 0.05 (two-tailed) were considered to be statistically significant. For comparing two groups of categorical data a Chi-squared test was used. For comparing two groups for a continuous variable the Mann-Whitney test was used. Survival was analyzed using the method of Kaplan-Meier. Differences between survival curves were examined using the log rank test. Multivariate analysis was performed using a Cox regression employing a step-wise backward selection method based on likelihood ratios.

Results

The median follow-up was 9.9 years. There was no significant difference in the length of follow-up between PTC and FTC ($p = 0.49$). Comparisons of patient characteristics between PTC and FTC patients can be found in table 9.1. FTC patients on average were older, they were more likely male, more likely to present with a larger tumor,

and they more frequently had multifocal carcinomas and distant metastases than PTC patients, whereas they presented less frequently with extra-thyroidal invasion or lymph node metastases.

The difference in tumor-specific mortality between PTC and FTC was considerable, with 20 year survival rates in PTC being at 90.6% but for FTC only at 73.7% ($p < 0.001$) (figure 9.1). In patients without distant metastases, long-term survival in FTC patients (80.2%) was significantly lower than in PTC patients (93.1%, $p = 0.0001$).

For a multivariate analysis we entered the histologic data as well as all the factors in which PTC and FTC patients differed from each other into the model. In the Cox regression, sex was removed from the model in the first step, tumor multifocality in the second step, histology in the third step and the presence of lymph node metastases in the fourth step. The presence of distant metastases ($p < 0.001$), age ($p < 0.001$), tumor size ($p = 0.001$) and the presence of extra-thyroidal invasion ($p = 0.007$) remained as explanatory variables for survival. Compensating for these factors should theoretically remove the survival differences between the two types of carcinomas. As an example two curves from opposite ends of the severity spectrum were analyzed – in figure 9.2, patients aged < 45 years with a unifocal, non-metastasized tumor with a diameter $\leq$ 1 cm without extra-thyroidal invasion (microcarcinoma); and in figure 9.3, patients aged 45 years or over with distant metastases (stage IV according to the TNM system). In both cases the curves do not show significant differences between PTC and FTC (figure 9.2: $p = 0.81$, figure 9.3: $p = 0.52$).

Table 9.1 Comparison of characteristics between PTC and FTC patients.

	PTC	FTC	p-value
no. of patients	875	350	
no. of male / female patients	247/628	122/228	0.02
mean age (range) in years	46.1 (5-87)	52.2 (8-81)	< 0.001
mean follow-up in years	10.8	10.9	0.49
mean tumor diameter in mm	19.2 $\pm$ 0.6	31.7 $\pm$ 1.3	< 0.001
no. of patients with multifocal carcinoma	92 (10.5%)	51 (14.5%)	0.047
no. of patients with extra-thyroidal invasion	184 (21.0%)	48 (13.7%)	0.006
no. of patients with lymph node metastases	78 (8.9%)	18 (5.1)	0.02
no. of patients classified as Nx	199 (22.7%)	74 (21.1%)	0.54
no. of patients with distant metastases	64 (7.3%)	55 (15.7%)	< 0.001

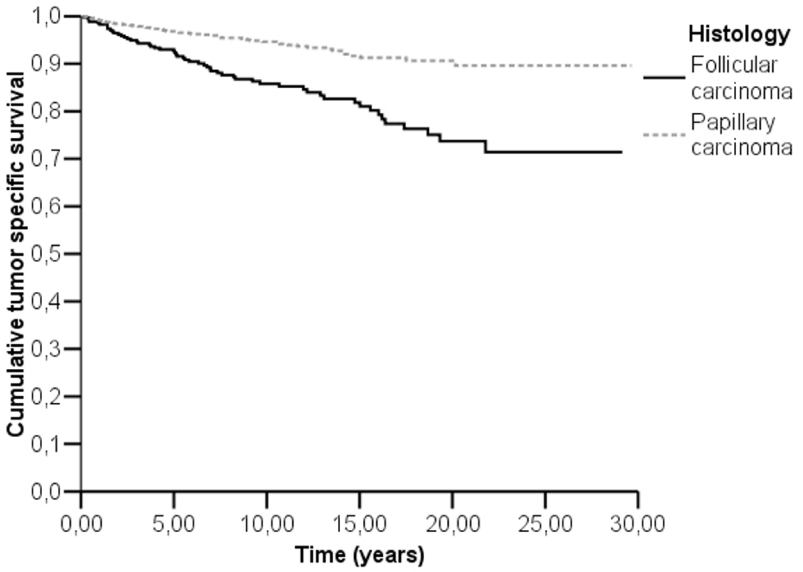

Figure 9.1 Tumor-specific survival in patients with papillary and follicular carcinoma. The difference is statistically significant ($p < 0.001$).

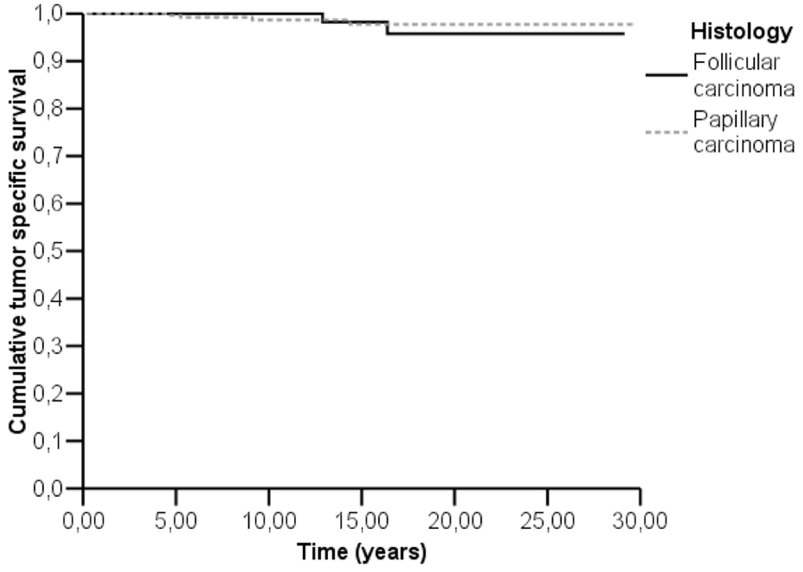

Figure 9.2 Tumor-specific survival in patients with papillary and follicular thyroid carcinoma with a diameter of the primary tumor $\leq$ 1 cm. The difference is not significant ($p = 0.81$).

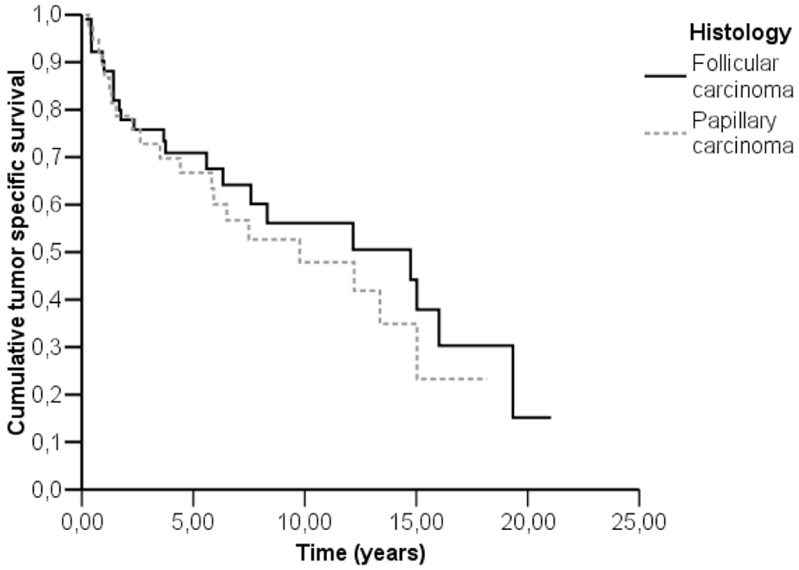

Figure 9.3 Tumor-specific survival in patients aged ≥ 45 years with papillary and follicular thyroid carcinoma with distant metastases. The difference is not significant (p = 0.52).

Discussion

The results of the current study show that PTC and FTC show a clinically equal behavior: when compensating for differences in stage at the time of presentation, differences in tumor-specific survival between the two histologic entities of differentiated thyroid carcinoma disappear. Many studies have been published detailing the risk factors in DTC, of which histology may be one. Shaha[6] introduced a staging system in which FTC patients were classified in a higher risk category than PTC patients with the same stage of disease. The same is proposed by the National Thyroid Cancer Cooperative Treatment Study group staging system.[11] The AMES[5] and various versions of the TNM prognostic scoring systems[3,4] do not include histology as an independent risk factor in their analysis. In various other studies in which multivariate analysis were performed on populations of differentiated thyroid carcinoma, in some studies histology does,[12] and in others it does not[13] emerge as an independent prognostic factor for survival in DTC. DeGroot *et al*[14] report a contrast in the clinical behavior of FTC and PTC with FTC doing considerably worse; they do not however report what happens when differences at the time of presentation are compensated for. Contrary to our observations, Mazzaferri and Jhiang report that there are no survival differences between FTC and PTC if patients with distant metastases are left out.[1] The differences

between PTC and FTC at initial presentation, as well as the survival differences reported in the current study are in agreement with a similar study by Chow *et al*.[2]

The present study is hampered by imperfect data. Especially the lymph node status of patients has often not been assessed. This resulted in a high proportion of Nx patients, and a low proportion of N1 patients. This is mostly explained by the large number of patients who had been scheduled for a hemi- or total thyroidectomy for a seemingly benign goiter, and in whom a thyroid carcinoma was diagnosed on pathologic examination of the thyroid. In these cases either there was not enough thyroid tissue left to justify a re-operation or there was so much scar tissue that a central lymph node dissection would be associated with a high risk of recurrent laryngeal nerve damage.

As PTC and FTC are caused by different gene mutations invoking distinctly different cellular signalling pathways, with PTC mainly being caused by mutations involving the BRAF and RET genes while in a large number of FTC cases PAX8-PPARγ1-rearrangements or RAS mutations play a role, this lack of difference in survival rates requires further explanation.[15-17] There is some evidence that various mutations can imply different degrees of malignancy, even when working along the same pathways.[15,18,19] There is also some evidence that, especially in FTC, thyroid carcinomas can start out as benign goiters with a RAS-mutation or PAX8-PPARγ1 rearrangement and will gradually undergo malignant transformation,[15] making a malignancy impossible to diagnose on the basis of a mutation alone. Further study might allow us to stratify carcinomas according to genetic risk instead of histologic morphology. The prognostic data in the present study may have several practical consequences. First of all, the prognosis of all DTC patients is independent from its histology; it is the disease stage at the time of diagnosis that matters. In clinical practice, patients with FTC present with more advanced disease than patients with PTC. Diagnosis later in the course of the disease, which would also explain the somewhat higher age at diagnosis, may be one of the causes. Unlike PTC, FTC cannot be distinguished on the basis of morphologic aspects on fine-needle aspiration biopsy.[20,21] The diagnosis of FTC requires histologic confirmation, and as thyroid surgery is not without risk in many patients the careful weighing of risk of cancer versus risk of surgery may lead to a postponement of surgery. In order to overcome the disparity in early diagnosis rates, new techniques are needed. MicroRNAs or messenger RNA expression profile analysis as well as mutation detection on fine-needle aspirates currently show the most promise.[22-25] These techniques are still in the experimental stage and are not yet ready for clinical use.

A second clinical question is what to do with follicular microcarcinomas. In figure 9.2 it can be seen that the prognosis for patients with follicular microcarcinomas is

identical to that for patients with papillary microcarcinomas. For papillary microcarcinomas it has been shown that total thyroidectomy and ^{131}I ablation does not lead to any marked benefit. Some have postulated that total thyroidectomy and ^{131}I ablation lead to improved treatment results for the follicular microcarcinoma; however, such assumptions are not based on experimental or even observational work. On the contrary, a study on microcarcinomas by Baudin *et al*[26] showed no differences in survival or recurrence rates between papillary and follicular microcarcinoma. The current study cannot answer this question, as FTC microcarcinoma patients and PTC microcarcinoma patients had different treatments. Further study seems warranted in order to determine whether total thyroidectomy and ^{131}I ablation bring a survival benefit in follicular microcarcinoma or can be left out, as in papillary microcarcinoma.

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Chapter Ten

A comparison of prognostic scoring systems for differentiated thyroid carcinoma

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Submitted

Abstract

Objectives: To identify and compare prognostic scoring systems based on basic tumor characteristics that were developed for differentiated thyroid carcinoma (DTC).
Methods: The literature was studied using PubMed. Fifteen different prognostic scoring systems were identified, of which seven were developed or validated for DTC patients and were based on basic tumor characteristics. These systems were applied to 1,225 DTC patients treated at the University Clinic Würzburg between 1978 and 2002.
Results: Log rank analysis of Kaplan-Meier cancer-specific survival curves showed that the curve of the TNM system had the greatest discriminatory power (log rank Chi-square = 366, $p < 0.001$). Cox regression analysis showed that the TNM system was the most powerful determinant of cancer-specific survival curves. Proportion of variance explained (PVE) analysis showed that the TNM system had the highest PVE.
Conclusion: Of the prognostic scoring systems analyzed in this study, the TNM system performs best in all three analyses, and is therefore the most suitable for predicting outcome in DTC patients.

Introduction

Thyroid cancer is a relatively rare form of cancer with an incidence ranging from 0.5 to 10 cases per 100,000 throughout the world.[1] In about 70-80% of cases it concerns the well-differentiated papillary or follicular varieties. Mortality in differentiated thyroid cancer (DTC) is much lower than its incidence. Still, the mortality among DTC patients is higher than in the general population.[2]
Over the years many authors have investigated which factors have a prognostic significance in the follow-up of thyroid cancer. Careful analysis and identification of risk factors will facilitate the identification of patients with a high mortality risk and, to a lesser extent, those with high risk of cancer recurrence. Better risk assessment facilitates appropriate treatment choices. After multivariate analysis of combined prognostic factors, many prognostic scoring systems have been developed to distinguish between patients at low and high risk of recurrent disease or death related to thyroid cancer (see table 10.1).
The aim of this study was (1) to review existing prognostic scoring systems for thyroid cancer, and (2) to determine whether they can be applied to patients with DTC who were treated in our center according to our current protocol. Applicable systems were compared to patient outcomes.

Patients, materials and methods

Database

The Würzburg thyroid cancer registry was established primarily to monitor the quality of patient treatment. Secondarily this registry allows for retrospective scientific population studies. Data are recorded for each visit, starting with the first visit after the diagnosis of thyroid carcinoma has been established. Data recorded at initial visit included histology, pTNM staging (according to version 5 of the TNM system), multicentricity of the tumor, whether or not there was extra-thyroidal invasion (but unfortunately not the precise extent of this invasion, making the use of TNM v. 6 impossible).

For patients who no longer attend our clinic for follow-up, the registry is regularly updated through inquiries at the referring physicians.

Patients

Retrospectively we reviewed 1,225 patients (856 female, 369 male, mean age 47.8 y, range: 5-87 y) with differentiated thyroid carcinoma (875 papillary, 350 follicular carcinoma) who were treated in our hospital and received initial treatment from 1978 (earliest available data) up to and including 2002. The latter cut-off date was chosen to allow for a minimum follow-up of 5 years.

Treatment

All patients received total thyroidectomy with subsequent ^{131}I ablation except those patients with a solitary papillary microcarcinoma, who usually had a hemi-thyroidectomy. ^{131}I ablation was performed with 1500-3500 MBq ^{131}I, depending on the thyroid remnant. 6-12 months after initial treatment patients returned for ^{131}I whole-body scintigraphy and Tg measurement after withdrawal of levothyroxine or, in later years, after the administration of recombinant human TSH (rhTSH). If any signs of persistent disease were encountered, an additional activity of 7000 MBq ^{131}I was administered. If the patient was disease-free, at least one more follow-up scan after thyroid hormone withdrawal or rhTSH injection was performed within the first 5 years of follow-up. During the first five years patients were furthermore followed at 6 month intervals and thereafter at yearly intervals by means of thyroglobulin measurement and neck ultrasound. X-rays, CT scans or MRI scans were performed on indication.

Pathological analysis and staging

Surgical specimens were analyzed according to the standard at the time of initial

treatment, and classified as PTC or FTC according to the guidelines at the time. Data were processed as stated in the original pathology report. Patients with one or more anaplastic foci were excluded from the present study. The diameter of the primary tumor was determined from macroscopic analysis of the surgical specimen, when possible. In the case of multifocal tumors the diameter of the largest tumor was taken for the primary tumor diameter. Histologic grading was not determined, as this was not recommended by the WHO. For a classification of N0, patients should at least have undergone a lymph node dissection of the central compartment; otherwise they were classified as Nx. A classification of lymph node metastases, multifocal disease, or extra-thyroidal disease required histologic confirmation; for a classification of distant metastases, other evidence (such as a positive post-therapy ^{131}I whole-body scan, CT scan or MRI) was deemed sufficient.

Prognostic systems

A literature search was performed for prognostic scoring systems for thyroid cancer, using PubMed (http://www.pubmed.org/) and the existing literature. Fifteen prognostic scoring systems for thyroid carcinoma were identified. Table 10.1 displays an overview of the criteria used in each system. For the final analysis we limited ourselves to those systems that had been developed or validated for both papillary and follicular carcinoma. Only basic patient characteristics and tumor characteristics were considered, as histologic grading had not been consistently determined throughout the inclusion period (as it is not recommended by the WHO) and no extensive analysis such as DNA ploidy studies had been performed on tumor material.

Analysis

Statistical analysis was performed using SPSS 12.0 for Windows. P-levels < 0.05 (two-tailed) were considered statistically significant. Survival was analyzed using the method of Kaplan-Meier. Differences between survival curves were examined using the log rank test. The system with the highest Chi-squared value in the log rank test is assumed to have the greatest discriminatory power between stages. Cox regression analysis were used to assess which prognostic system was most explanatory for patient outcome; each prognostic scoring system was entered as a categorical value with the lowest risk category serving as the reference category for each prognostic scoring system. As a third way of comparing prognostic scoring systems we calculated the proportion of variance explained (PVE) for each prognostic scoring system according to the method described by Schemper and Henderson.[3] The better the PVE, the better the prognostic scoring system is assumed to be.

Table 10.1 An overview of prognostic scoring systems and their criteria.

AGES (8): For papillary thyroid carcinoma

0.05 x age in years (if age ≥ 40) or +0 (if age < 40)
+ 1 (if grade 2) or +3 (if grade 3 or 4)
+ 1 (if extra-thyroidal tumor invasion)
+ 3 (if distant metastases present)
+ 0.2 x tumor size (max. diameter in cm)

Group 1: AGES score < 4.00
Group 2: AGES score = 4.00 - 4.99
Group 3: AGES score = 5.00 - 5.99
Group 4: AGES score ≥ 6.00

AMES (10): For differentiated thyroid carcinoma

Low risk
A. Age at diagnosis < 41 years for men, < 51 years for women, without metastases
B. All older patients with
   1. intrathyroidal papillary carcinoma *or* minor tumor capsular involvement follicular carcinoma *and*
   2. primary tumor < 5 cm in diameter *and*
   3. no distant metastases

High risk
A. All patients with distant metastases
B. All older patients with:
   1. extra-thyroidal papillary carcinoma or major tumor capsular involvement follicular carcinoma and
   2. primary carcinoma tumor 5 cm in diameter or larger regardless of extent of disease.

Clinical Class (17): For differentiated thyroid carcinoma

Class I: tumors confined to the thyroid gland
Class II: patients with cervical nodular metastases
Class III: patients with tumor extending beyond the thyroid gland or with incompletely resected nodular disease
Class IV: patients with distant metastases

DAMES (18): For papillary thyroid carcinoma

Low risk: patients who are AMES low risk with euploid tumors
Intermediate risk: patients who are AMES high risk with euploid tumors
High risk: patients who are AMES high risk with aneuploid tumors

EORTC (19): For all thyroid carcinomas

Age at diagnosis in years
+ 12 if male
+ 10 if medullary thyroid carcinoma
+ 10 if poorly differentiated, follicular cell derived thyroid carcinoma but not anaplastic
+ 45 if anaplastic carcinoma
+ 10 if extension beyond the thyroid capsule
+ 15 if single distant metastasis present
+ 30 if multiple distant metastases present.

Group 1: EORTC score < 50
Group 2: EORTC score = 50-65
Group 3: EORTC score = 66-83
Group 4: EORTC score = 84-108
Group 5: EORTC score ≥ 109

MACIS (20): For papillary thyroid carcinoma

3.1 (if age ≤ 39 years) or 0.08 x age (if age ≥ 40)
+ 0.3 x tumor size (max. diameter in cm)
+ 1 if incompletely resected
+ 1 if locally invasive
+ 3 if distant metastases present

Group 1: Macis score < 6.00
Group 2: Macis score = 6.00 - 6.99
Group 3: Macis score = 7.00 - 7.99
Group 4: Macis score ≥ 8.00

MSK (21): For differentiated thyroid carcinoma

	low risk	**intermediate risk**		**high risk**
Age (y)	< 45	< 45	> 45	> 45
Distant metastases	M0	M1	M0	M1
Tumor size	< 4 cm	> 4 cm	< 4 cm	> 4 cm
Histology and grade	papillary	follicular and/or high grade	papillary	follicular and/or high grade

Noguchi (11): For papillary thyroid carcinoma

	Males	**Females**
Excellent prognosis	all patients age ≤ 45 and ≤ 60 years without gross nodal metastases	all patients age ≤ 50, age 50-55 without metastases
Intermediate prognosis	all patients age > 60, age 45-55 with gross nodal metastases	all patients age 50-55 without or with gross nodal metastases, age > 65 with primary tumor < 30 mm in max. diameter
Poor prognosis	age > 55 with gross nodal metastases	all remaining females

NTCTCS (22): For all thyroid carcinomas

	TUMOR TYPE			
	Papillary carcinoma		**Follicular carcinoma**	
	age < 45 y	age ≥ 45 y	age < 45 y	age ≥ 45 y
Primary tumor size (cm)				
< 1	I	I	I	II
1 - 4	I	II	I	III
> 4	II	III	II	III
Primary tumor description				
Microscopic multifocal	I	II	I	III
Macroscopic multifocal	I	II	II	III
Macroscopic capsule invasion	I	II	II	III
Microscopic extraglandular	I	II	I	III
Macroscopic extraglandular	II	III	II	III
Poor differentiation	-	-	III	IV
Metastases				
Cervical lymph nodes	I	III	I	III
Extracervical lymph nodes	III	IV	III	IV

Medullary carcinoma	
C-cell hyperplasia	I
Tumor size < 1 cm	II
Tumor size ≥ 1 cm or positive cervical lymph nodes	III
Extraglandular invasion or extracervical metastases	IV

Ohio State University (23): For differentiated thyroid carcinoma

STAGE	**tumor size (cm)**	**cervical metastases**	**multiple thyroid tumors (> 3) any size**	**local tumor invasion**	**distant metastases**
I	< 1.5	no	no	no	no
II	1.5-4.4	yes	yes	no	no
III	≥ 4.5	any	any	yes	no
IV	any size	any	any	any	yes

SAG (24): For papillary thyroid carcinoma

+ 1 if male sex
+ 1 if age ≥ 70
+ 1 if VAN histologic grade = 2

Group I: SAG score = 0
Group II: SAG score = 1
Group III:: SAG score = 2 - 3

TNM, version 5 (25): For differentiated thyroid carcinoma

T0: no evidence for primary tumor
T1: primary tumor ≤ 1 cm, confined to the thyroid gland
T2: primary tumor > 1 cm and ≤ 4 cm, confined to the thyroid gland
T3: primary tumor > 4 cm, confined to the thyroid gland
T4: primary tumor of any size, extending beyond the thyroid capsule
N0: no lymph node metastases, N1a: metastasis in ipsilateral cervical lymph nodes, N1b: metastasis in bilateral, middle line, contralateral, mediastinal lymph nodes
M0: no distant metastases, M1: distant metastases present

STAGE	stage < 45 years	age ≥ 45 years
I	M0	T1
II	M1	T2-3
III		T4 or N1
IV		M1

TNM, Version 6 (26): For differentiated thyroid carcinoma

T0: no evidence for primary tumor.
T1: primary tumor ≤ 2 cm, confined to the thyroid gland
T2: primary tumor > 2 cm and ≤ 4 cm, confined to the thyroid gland
T3: primary tumor > 4 cm, confined to the thyroid gland or any tumor with minimal extra-thyroid extension
T4a: primary tumor with invasion of subcutaneous soft tissues, larynx, trachea, esophagus, recurrent laryngeal nerve
T4b: primary tumor with invasion of prevertebral fascia, mediastinal vessels, encasing of carotid artery

N0: no lymph node metastases, N1a: metastasis in level VI (pre-and paratracheal) N1b: metastasis in lymph nodes outside of level VI, including mediastinal lymph nodes.
M0: no distant metastases, M1: distant metastases present

STAGE	age < 45 years	age ≥ 45 years
I	M0	T1
II	M1	T2
III		T3 or N1a
Iva		T1-3N1bM0, T4aN0-1M0
Ivb		T4bNxM0
Ivc		M1

University of Alabama-Birmingham & M.D. Anderson Cancer Center (27): For differentiated thyroid carcinoma

Low risk: patients < 50 years without distant metastases
Intermediate risk: patients ≥ 50 years without distant metastases
High risk: patients of any age with distant metastases

University of Münster (28): For differentiated thyroid carcinoma

Low risk: all patients without extra-thyroid tumor invasion or distant metastases
High risk: patients with extra-thyroidal tumor invasion and/or distant metastases

Results

Follow-up data

Total follow-up was 13,282 patient-years. Mean and median follow-up were 10.9 and 9.9 years, respectively. 108 patients (8.8%) died of thyroid cancer in the course of the follow-up.

Prognostic systems

Based on the available data in our database and the selection criteria described above we were able to classify patients according to the following prognostic scoring systems: AMES, Clinical Class, Memorial Sloan-Kettering (MSK), Ohio State University, TNM version 5, University of Alabama, University of Münster.

A Kaplan-Meier analysis with log rank test was performed for each of the prognostic scoring systems. Results from the log rank tests are in table 10.2; Kaplan-Meier survival curves are displayed in the figures 10.1-10.7. It can be seen both graphically and numerically that in our population the TNM system has the greatest discriminatory power between stages.

Subsequently a Cox regression analysis was performed using a forward selection procedure based on likelihood ratio tests, in which all prognostic scoring systems were entered as categorical explanatory variables using the lowest stage as a reference category.

In this analysis, the TNM prognostic scoring system was the most significant prognostic scoring system ($p < 0.001$). Only the combination with the AMES system added slightly, but significantly ($p = 0.04$) to the predictive power of the regression model.

Table 10.2 Prognostic scoring systems compared for results of a log rank test.

System	Chi-squared	p-value
AMES	195.22	< 0.001
Clinical Class	223.47	< 0.001
MSK	114.47	< 0.001
Ohio State University	214.03	< 0.001
TNM	366.02	< 0.001
University of Alabama	248.13	< 0.001
University of Münster	126.88	< 0.001

Table 10.3 Proportion of variance explained (PVE) for each prognostic scoring system.

System	PVE
AMES	0.170
Clinical Class	0.119
MSK	0.176
Ohio State University	0.108
TNM	0.236
University of Alabama	0.225
University of Münster	0.083

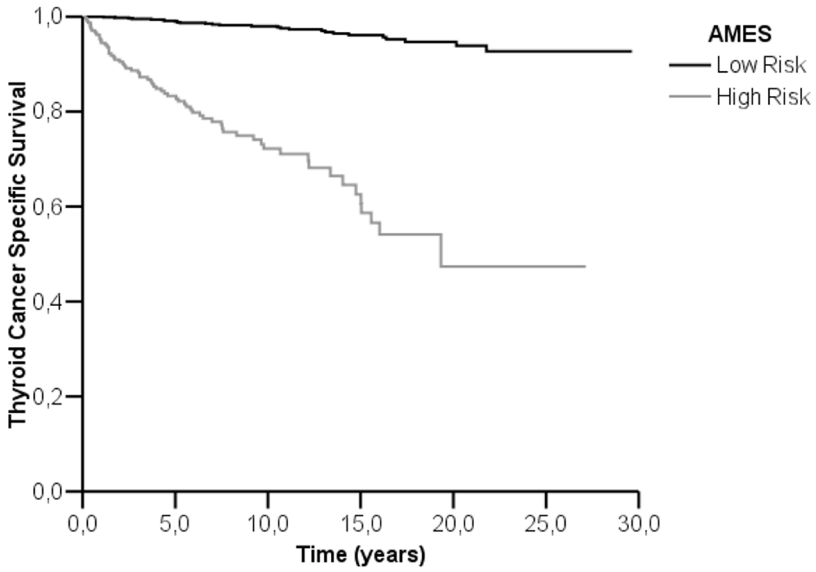

Figure 10.1 Survival curve of patients staged according to the AMES system.

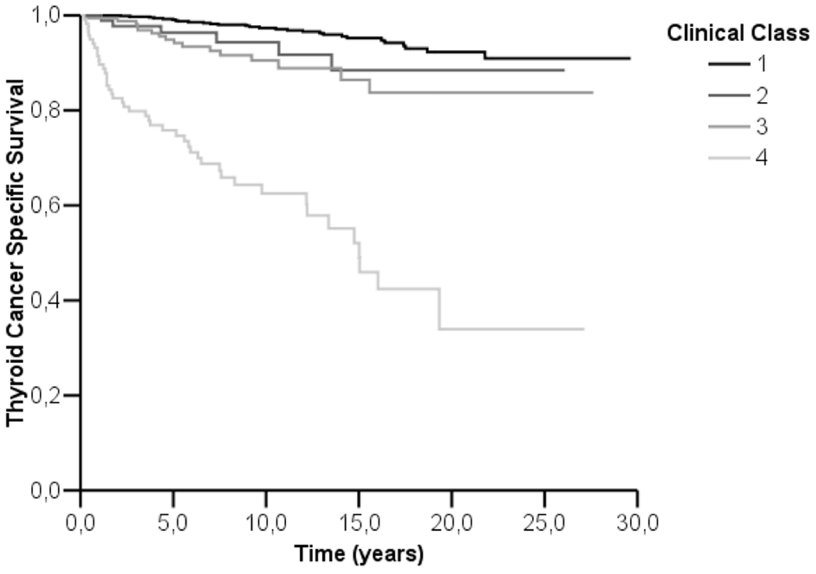

Figure 10.2 Survival curve of patients staged according to the Clinical Class system.

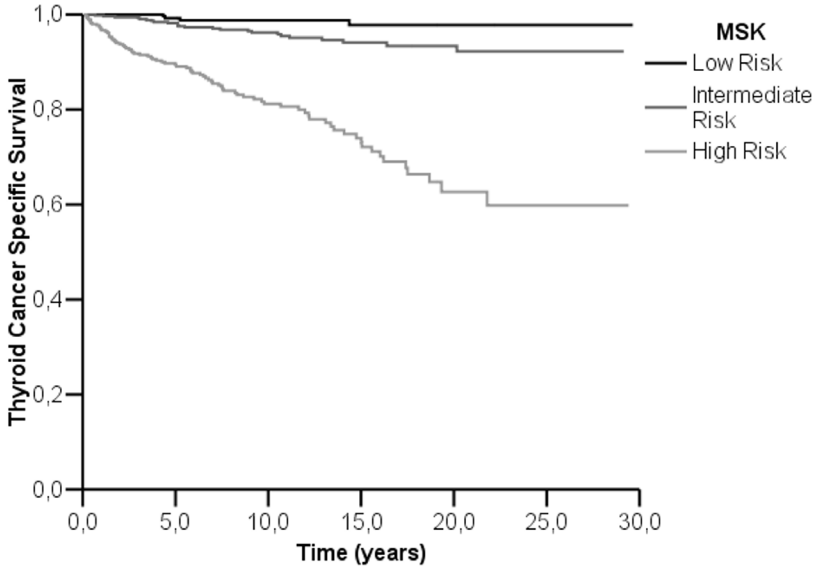

Figure 10.3 Survival curve of patients staged according to the Memorial Sloan Kettering system.

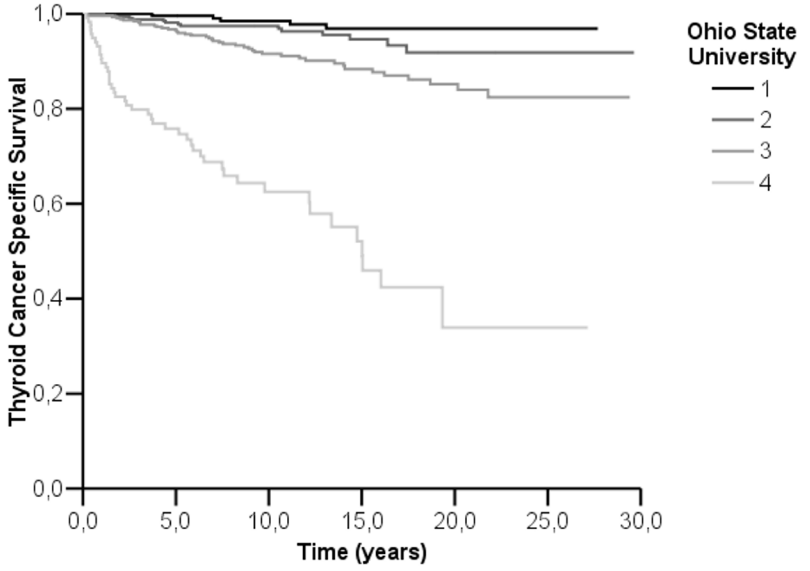

Figure 10.4 Survival curve of patients staged according to the Ohio State University system.

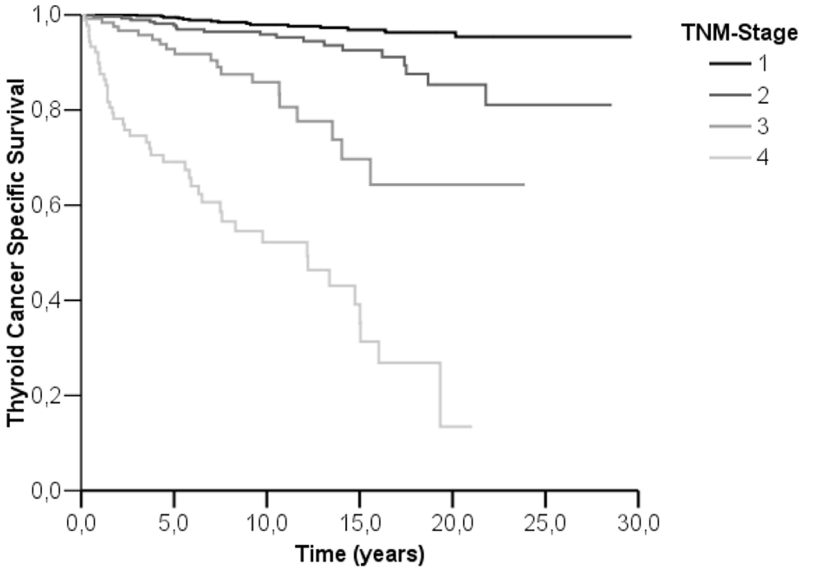

Figure 10.5 Survival curve of patients staged according to the TNM system.

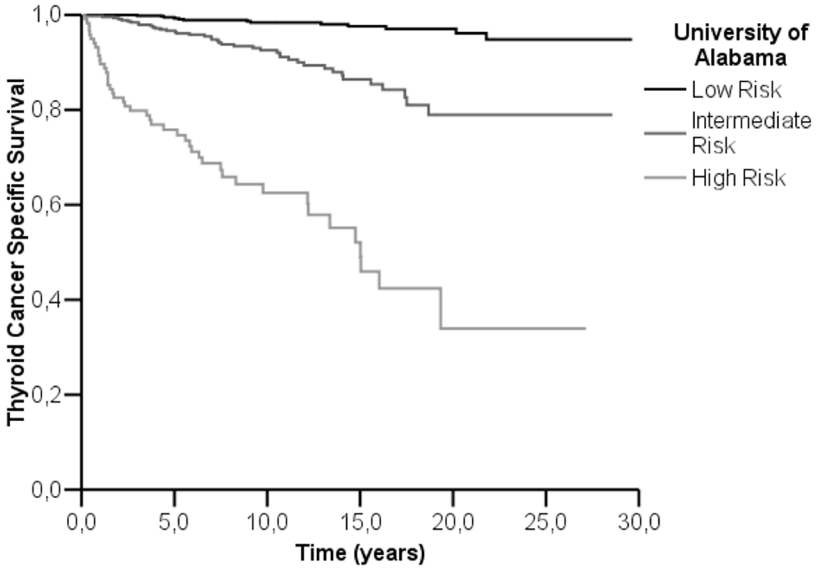

Figure 10.6 Survival curve of patients staged according to the University of Alabama system.

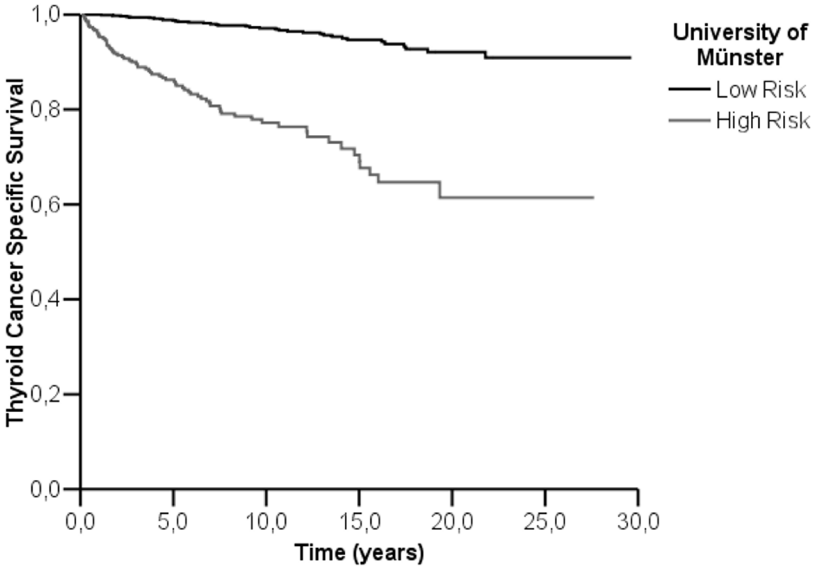

Figure 10.7 Survival curve of patients staged according to the University of Münster system.

Proportion of variance explained

Calculation of the PVE for each prognostic scoring system (table 10.3) revealed that the TNM prognostic scoring system had the best prognostic accuracy for DTC, followed by the UAB-MDACC score and the MSK system.

Discussion

There is no methodological consensus for comparing prognostic scoring systems for cancer. The TNM system, a largely empirically derived prognostic scoring system that was not based on the statistical analysis of a particular patient population, performed best in the present study.

Several authors have compared prognostic scoring systems for differentiated thyroid carcinoma. Examples of such studies are those by Brierley *et al* and Passler *et al*;[4,5] both confirmed the superiority of version 5 of the UICC/AJCC TNM system. The value for the PVE in our study (TNM: 23.6%) was higher than in the study by Passler *et al* (TNM: 14.0%), but lower than in the study by Brierley *et al* (TNM: 28.3%). The explanation for this difference most likely lies in the use of different methods for calculating the PVE.[6,7] In the past two decades multiple methods of calculating the

PVE have been described by various authors, but as yet none has been generally accepted.[3,7]

In contrast to the present study, both these studies also compared prognostic scoring systems that had been developed and/or validated for one variant of differentiated thyroid cancer only, or to include all varieties of thyroid carcinoma.

Others demonstrated a superiority of the AGES and AMES systems over the TNM and EORTC systems in a population of patients with papillary thyroid cancer,[8] or of the AGES and EORTC score over the AMES system in a population of patients with follicular carcinoma.[9]

Problems in determining the optimal prognostic scoring system arise also from variations in base population and treatment protocols: various prognostic scoring systems have been developed in different centers, with clinicians treating patients of distinctive ethnicities and habits in varying manners, causing differences in survival. Systems based on populations in different parts of the world may even cause contradicting risk factors. In a North-American population study, the male sex was associated with poorer prognosis,[10] whereas in a Japanese study a poorer prognosis was reported for females.[11]

One specific problem with the UICC/AJCC TNM system is that multiple versions exist. The most recent version is by definition the recommended one (in this case the 6th version, published in 2002). However, not all researchers agree that the changes made to the 1997 version were fully justified.[12,13] This dispute hinders the universal acceptation of the TNM system.

Hannequin *et al*[14] reproduced the exact analysis used to construct several prognostic systems on their own patient population and found different prognostic variables as a result. It should perhaps be recommended for every center to perform such an analysis, to compensate for ethnic differences.

In the present study the TNM system performed best in every statistical analysis. Prognostic scoring systems that rely on the extent of the disease at the time of initial treatment – such as the ones studied here – are seemingly imperfect for the classification of patients according to prognosis, as they do not take into consideration indicators of tumor aggressiveness. Histologic grading or DNA ploidy may introduce ways of including such biologic behavior in the prognostic scoring process, but such methods require intensive (and costly) processing. Since the advent of sensitive thyroglobulin measurements, risk stratification according to treatment result has emerged. The recommended follow-up procedure both in Europe[15] and America[16] is no longer based on the initial stage alone, but also on the result of treatment. Such methods, however, are unable to determine the extent of the required initial treatment. Easy-to-use

prognostic scoring systems based on the outcome in large populations will therefore remain a valuable source of inspiration.

In this comparison of prognostic scoring systems for differentiated thyroid carcinoma, based on the extent of the disease at the start of treatment, the UICC/AJCC TNM system version 5 had the best performance for our specific patient population.

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Chapter Eleven

Value of diagnostic radioiodine scintigraphy and thyroglobulin measurements at 2 time points after rhTSH injection

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Nuclear Medicine / NuclearMedizin, in press

Abstract

Objective: Measurements of thyroglobulin (Tg) levels 72 h after administration of recombinant human thyrotropin (rhTSH) are recommended by the manufacturer in the follow-up of patients with differentiated thyroid carcinoma (DTC). In our department, Tg measurements are performed both 24 h and 72 h after administration of rhTSH, together with 72h post rhTSH ^{131}I whole-body scintigraphy (WBS). The objective of this study is to compare the diagnostic usefulness of Tg measurements 24 and 72 h after rhTSH administration, and ^{131}I WBS.

Patients and Methods: 181 patients were included who had been referred to our Nuclear Medicine Department for follow-up after ^{131}I ablation of DTC. Tg measurements 24 h (Tg24) and 72 h (Tg72) after rhTSH, and ^{131}I WBS, were done in all patients. The lower detection limit of Tg was 0,2 µg/l.

Results: 47 patients (26%) had detectable Tg levels: in 4/47 cases (8%) only Tg24 was detectable (but always < 1 µg/l), and in 6/47 cases (11%), only Tg72 was detectable. In 10/47 patients with detectable Tg levels, Tg24 and Tg72 tested equally. In 27/47 cases, Tg24 was lower, and in 10/47 higher, than Tg72. Two patients with one or two positive Tg test results also had a positive ^{131}I WBS. In 8 patients (14%) only the ^{131}I WBS was positive; an anatomical substrate for such a Tg negative positive WBS was confirmed in only 2 patients.

Conclusion: Tg measurement 72 hours after rhTSH injection reveals all clinically relevant detectable Tg levels. Diagnostic ^{131}I scintigraphy may be omitted, even in high-risk patients.

Introduction

Treatment of differentiated thyroid carcinoma (DTC) typically consists of near-total thyroidectomy followed by ^{131}I ablation. In patients without evidence of metastases, follow-up of DTC after ^{131}I ablation consists of levothyroxine (LT4) medication in TSH-suppressive dosages, regular measurements of thyroglobulin (Tg) levels in blood during suppression of TSH, and periodic measurements of Tg levels in blood, combined with ^{123}I or ^{131}I whole-body scintigraphy (WBS) at high TSH levels. Elevated TSH levels can be obtained either by prolonged withdrawal of LT4 (often associated with disabling hypothyroidism) or by intramuscular injection of 0.9 mg recombinant human Thyrotropin (rhTSH; Thyrogen, Genzyme corp., Cambridge, MA, USA) on two consecutive days. Patient preparation with rhTSH provides an effective,[2,3,9-12] repro-

ducible[7] and cost-effective[4,6] follow-up. Furthermore, the rhTSH approach offers superior quality of life, because of the near-absence of side effects resulting from hypothyroidism.[14] Use of rhTSH also avoids potential morbidity that may be associated with hypothyroidism.

Based on the results from an international trial,[2] the manufacturer's recommendation is to perform serum Tg measurements 72 hours after the second rhTSH injection. Some authors have indicated that this procedure alone may suffice for the follow-up of DTC.[10]

The aim of the present study is to compare the diagnostic value of two strategies, with regard to the detection of signs of persistent or recurrent disease in the follow-up of DTC patients: (1) Tg measurement at 72 hours after rhTSH, and (2) Tg measurements at both 24 h and 72 h, combined with ^{131}I WBS at 72 h after rhTSH.

Subjects and Methods

Patients

The first rhTSH-stimulated follow-up procedure from 181 consecutive patients who were referred to the Department of Nuclear Medicine for follow-up after total thyroidectomy and ^{131}I ablation of DTC after January 1, 2003 (the start of the present follow-up protocol) and in whom Tg measurements both 24 h and 72 h after rhTSH was performed, were reviewed retrospectively. The median time elapsed since diagnosis was 4 years (1-30 years).

rhTSH protocol

Patients received intramuscular injections of 0.9 mg rhTSH on two consecutive days, followed by the oral administration of 370 MBq (10 mCi) ^{131}I on the third day. Blood samples were taken for measurement of serum Tg levels and anti-thyroglobulin antibody titers on day 3 (Tg24) and day 5 (Tg72). Serum TSH levels were measured only on day 3. On day 5, diagnostic ^{131}I WBS was performed with a dual-head gamma camera (MCD, Philips Medical Systems, Best, Netherlands) fitted with high-energy collimators. Patients with Tg > 1 µg/l were generally given a blind therapeutic activity after ca. 3 months.

Diagnostic criteria

^{131}I WBS was considered positive if ^{131}I uptake was observed outside of regions of physiologic uptake (salivary glands, oral, nasal, or gastric mucosa or urogenital regions).

For overall analysis, a follow-up examination was considered positive (i.e. indicative of persistent or recurrent disease) if either Tg24 or Tg72 was detectable, or if ^{131}I WBS was considered positive.
In the absence of a follow-up modality that can be used as a gold standard, any negative test result was considered false-negative if in the same patient a positive result was found with another one of the three tests regarded in this study. In choosing this approach, we accept that this method is more sensitive rather than specific.
Ultrasound of the neck was not yet routinely performed in all patients, and could therefore not be used as diagnostic criterion.

Laboratory analysis

Tg and Tg antibody levels were measured using the Brahms DYNOtest Tg-pluS (Brahms Diagnostica GmbH, Berlin, Germany). The functional sensitivity (defined here as the lowest Tg level that can be measured with a variation of less than plus or minus 20%) for this assay is 0.2 μg/l. At this low level the coefficient of variation was 12%. Tg antibody concentrations above 60 mU/l were considered positive. Differences between Tg24 and Tg72 were considered significant when Tg72 was lower or higher than Tg24 by more than 2.77 times the standard deviation of the Tg assay for the Tg level in question.

Results

Patient characteristics

Patient characteristics are shown in table 11.1. The median TSH level at 24 h after rhTSH was 120 mU/l (range: 25-299 mU/l). In 55/181 patients (30%) either Tg24, Tg72, ^{131}I WBS, or any combination of these was positive.
All patients with negative Tg24, Tg72 and ^{131}I WBS remained free of disease during their follow-up.

Tg measurements

At least one detectable Tg measurement was registered in 47/181 (26%) patients. Three patients with detectable Tg levels and three patients with undetectable Tg levels tested positive for the presence of Tg antibodies.
In the 41 patients who had detectable Tg24 levels, the median Tg24 level was 0.9 μg/l (range: 0.2 - 5300 μg/l). In the 43 patients with detectable Tg72-levels, the median Tg72 level was 1.0 μg/l (range: 0.2 - 4700 μg/l). In 10/47 patients (21%) the difference between Tg24 and Tg72 was within the limits of the 95% confidence interval for the assay; therefore Tg24 and Tg72 were considered equal in these patients. In 27/47

Table 11.1 Patient characteristics.

Number of patients	181
Gender	
female	134 (74%)
male	47 (26%)
Histology	
papillary carcinoma	142 (78%)
follicular carcinoma	38 (21%)
histology not specified	1 (1%)
High-risk tumor characteristics at diagnosis:	
extra-thyroidal growth	8 (4%)
lymph node metastases	39 (22%)
distant metastases	2 (1%)
Mean age (y) (range)	49 (11-87)
Median time elapsed since diagnosis (y) (range)	4 (1-30)

patients (57%), Tg24 was significantly lower and in 10/47 (21%) patients significantly higher than Tg72. In no patient in whom Tg72 was under 1 μg/l was Tg24 over 1 μg/l. In the entire patient population, Tg24 was significantly lower than Tg72 (Wilcoxon signed ranks test, p = 0.001). The relationship between Tg24 and Tg72 is shown in figure 11.1.

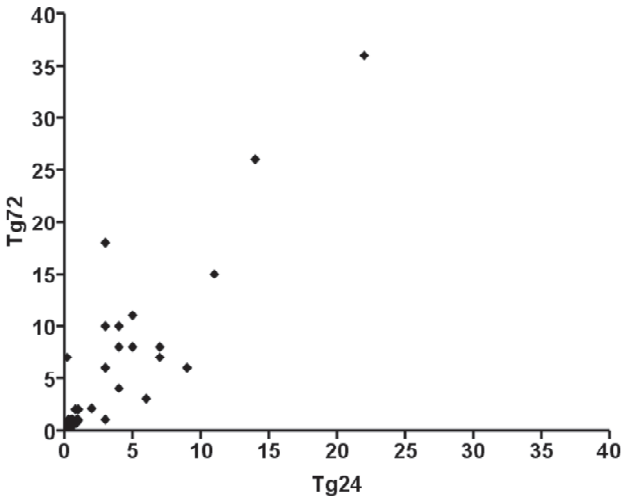

Figure 11.1 Scatter plot showing the relation between Tg24 and Tg72. One data point of a patient with a Tg24 of 5300 μg/l and Tg72 of 4700 μg/l was omitted.

Table 11.2 Distribution of detectable and undetectable Tg and WBS test results.

	^{131}I WBS negative		^{131}I WBS positive		Total
	Tg72 pos	Tg72 neg	Tg72 pos	Tg72 neg	
Tg24 pos	36 (20%)	4 (2%)	1 (0.5%)	0	41
Tg24 neg	5 (3%)	126 (70%)	1 (0.5%)	8 (4%)	140
Total	41	130	2	8	181

In 4/47 patients with detectable Tg levels Tg72 was undetectable while Tg24 was detectable in low levels (0.2 µg/l in 3 patients and 0.4 µg/l in one). In 6/47 patients Tg24 was undetectable while Tg72 was detectable (but < 1 µg/l in all cases).
The distribution of detectable versus undetectable Tg levels and the results of WBS is presented in table 11.2.

Diagnostic whole-body scintigraphy

10/181 patients (6%) showed pathologic accumulation of ^{131}I on the WBS. Only 2/47 patients (4%) with detectable Tg levels also had a positive ^{131}I WBS. In 8/10 patients (80%) with a positive ^{131}I WBS, both Tg24 and Tg72 were undetectable. In 2 patients it concerned thyroid bed uptake, in 6 patients it concerned a suspicion of metastastic tissue. Ultrasound showed an anatomic substrate in only 1/2 patient with positive scintigraphy and detectable Tg levels and 2/8 of the patients with positive scintigraphy and negative Tg levels. In one of these patients surgery was carried out and a metastatic lesion was confirmed histologically. None of the patients who tested positive for Tg antibodies had pathological ^{131}I accumulation on WBS.

High risk patients

In 21/44 high-risk patients follow-up was considered positive. In the 19 patients in whom Tg was positive both Tg24 and Tg72 were positive; Tg24 did not contribute additional clinically useful information. In two patients only scintigraphy was positive, in one of whom this was confirmed to be a metastasis by other modalities and eventual surgical removal; in both latter patients Tg was negative.

Discussion

The question of when best to measure serum Tg levels after rhTSH administration was addressed in an international trial reported by Haugen *et al*.[2] They found that after two dosages of 0.9 mg rhTSH on two consecutive days, Tg levels reached a maximum 3 days after the second rhTSH administration; however the report of this trial was sparse on details of the course of Tg levels in the included patients. Based on this trial, the recommendation was made to perform Tg measurements 72 h after the second rhTSH dose. It remained however unclear whether additional clinical information could be had from measuring rhTSH-stimulated Tg at multiple time points after injection. Pacini *et al*[10] performed Tg measurements on multiple days after rhTSH in 72 patients, of whom 31 had detectable Tg levels. Whereas some patients with detectable Tg levels showed a peak at 24 h (2/31 patients) or 48 h (8/31) after rhTSH injection, most patients (21/31) showed peak Tg levels at 72 h after rhTSH. The authors did not report on how many patients had undetectable Tg levels at one point, and detectable Tg levels at another. The present study concurs with the one by Pacini *et al* in that in some patients the Tg levels were higher at 24 h after rhTSH than at 72 h. In our population there were even some patients who had detectable Tg levels only at 24 h. However, in all these patients it concerned very low levels (all well < 0,5 µg l). A point of discussion here is the clinical value of such low thyroglobulin levels (< 1.0/µg/l) measured by the assay used in this study. The true clinical meaning of such low Tg levels has yet to be established in clinical research. What constitutes a positive Tg level may vary between institutions and between assays used. Several guidelines and consensus statements suggest using a cut-off of 1 µg/l, or alternatively using institutional cut-offs, for considering a Tg level positive.[1,5,13] In clinical practice in our hospital patients were only given an additional therapeutic dosage if Tg exceeded 1 µg/l[13] or if ^{131}I WBS was positive. In light of this clinical perspective measurement of Tg24 neither in high-risk patients nor in the general patient population produced any clinically relevant additional results and can therefore be omitted.

Robbins *et al* reported a negative predictive value of 97% for the combination of serum Tg levels 72 h after rhTSH and ^{131}I WBS 48 and 72 h after rhTSH.[12] Pacini *et al* stated that serum Tg measurement after rhTSH administration suffices.[10] This was partly contradicted by Robbins *et al*,[11] who reported that Tg measurement after rhTSH was not sufficient for the detection of residual thyroid tissue in high-risk patients. The results of the present study seem to agree with the conclusions of the latter study by Robbins *et al* in that ^{131}I WBS and Tg measurement do not show a perfect overlap.

An interesting alternative for the combination of rhTSH-stimulated serum Tg meas-

urement and ^{131}I WBS was proposed by Pacini *et al* They reported that by combining serum Tg measurement after rhTSH with ultrasound of the neck sensitivity and a negative predictive value both exceeding 95% could be achieved.[9] The clinical meaning of a positive ^{131}I WBS can therefore be discussed; especially in the case of thyroid bed uptake where exists some compelling evidence to refrain from further ^{131}I therapy.[8] It is possible that the ^{131}I WBS will show lesions suspected for lymph node metastases, with an ultrasound showing no anatomical correlate for this finding, as was the case in several of our patients. In 7/8 patients with positive WBS and negative Tg, the positive ^{131}I WBS findings remain unconfirmed by means of histology, and in only one of these 7 patients ultrasound showed an anatomical correlate, which makes it possible that in 6 patients (3% of a total of 183 patients) with a positive follow-up it concerned a 'false-positive' case, reducing the number of true-positive cases found by ^{131}I WBS alone to 2 (1% of a total of 181 patients); both these cases were detected by ultrasound, too. Should these numbers hold true, this would mean that scintigraphy would (at least in the cervical region) yield no positive results that cannot be obtained by other means, whereas it introduces a number of false-positive results. These numbers lead us to question the usefulness of ^{131}I WBS in the locoregional follow-up of DTC. Most existing consensus statements and guidelines[1,5,13] establish clear guidelines for so called low-risk patients (even though these publications use different definitions of what exactly constitutes low risk) in recommending a less intensive follow-up after a negative stimulated Tg test. For low-risk patients scintigraphy is abandoned as a standard procedure in the follow-up, especially when it comes to further follow-up after confirmation of successful ablation. For high-risk patients the picture is less clear, and often it is recommended not to rely on stimulated Tg measurement alone. The current study shows that follow-up scintigraphy shows little clear benefit from scintigraphy in any patient, even those who were high risk initially. Pacini *et al*[10] showed that ultrasound by itself might suffice to follow patients, without further scintigraphy. Later on Pacini *et al* showed that the combination of ultrasound and rhTSH-stimulated Tg might even be superior to the combination of ultrasound and WBS.[9]

In conclusion, the present study shows that there is no clinical benefit from measuring Tg levels 24 h after ablation in addition to measuring Tg 72 h after ablation. Also it shows that even in high-risk patients there is very little clinically verifiable information to be obtained from ^{131}I WBS.

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Chapter Twelve

Detection of circulating Tg-mRNA in the follow-up of papillary and follicular thyroid cancer: how useful is it?

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Abstract

To investigate the usefulness of thyroglobulin mRNA (Tg-mRNA) detection in peripheral blood in the follow-up of papillary and follicular (differentiated) thyroid cancer, a literature study was performed. Both evidence for and evidence against the usefulness of Tg-mRNA detection was found. Also, evidence for the expression of Tg-mRNA in other cells than normal or neoplastic thyroid follicular cells was found. It is concluded that currently Tg-mRNA detection is not a useful tool in the follow-up of differentiated thyroid carcinoma, but that the concept of using RT-PCR measurements during follow-up still warrants further research.

Introduction

Differentiated thyroid cancer is among the most manageable of cancers; with a 10-year survival of 80-95 percent,[1] its prognosis is good. However, patients with differentiated thyroid cancer can never be considered 'cured'; recurrences do occur, sometimes as late as 30 years after the original diagnosis.[1,2] It is therefore imperative to regularly check these patients during a lifelong follow-up.

Checking patients for the presence of persistent or recurrent disease can be done by regular measurement of serum thyroglobulin (Tg) levels, both during suppressed TSH and during stimulation with high TSH levels, achieved either through levothyroxine (LT4) withdrawal or administration of recombinant human TSH, and periodic radioiodine whole-body scintigraphy (WBS) after withdrawing LT4 supplementation. However, both these methods do have drawbacks. It often happens that ^{131}I WBS is negative, but serum thyroglobulin is readily detectable.[3-5] One should keep in mind that measurement of Tg levels cannot be considered reliable if antibodies against thyroglobulin are detected.[6-8]

The limitations of current techniques have prompted research into new ways of detecting persistent or recurrent thyroid cancer. One of these techniques is detection of circulating thyroid cells by the measurement of thyroglobulin messenger RNA (Tg-mRNA) in peripheral blood, first reported by Ditkoff *et al*, 1996. This technique seems to be quite promising according to some authors[9-13] but results of other studies contradict its usefulness.[14-19]

The goal of this study is to review the pros and cons of thyroglobulin mRNA detection in peripheral blood and determine whether it can currently be considered useful in the follow-up of differentiated thyroid cancer.

Tg-mRNA

Tg-mRNA is the 8.7 kilobase (kb) transcript of the thyroglobulin gene, which covers at least 300 kb of DNA.[20] It codes for thyroglobulin, a 660 kilodalton (kDa) glycoprotein that serves as a prohormone for thyroid hormone production.
With 2.6% of the total transcription products of thyrocytes, Tg-mRNA is the most highly expressed mRNA transcript in normal thyrocytes.[21] In neoplastic thyroid tissue, however, its expression is considerably lower.[22-25]

Technique of detection of Tg-mRNA in peripheral blood

After a blood sample is drawn from a patient, total RNA is extracted from the sample, either directly from the blood or from the mono-nuclear layer after separation by using commercially available kits. The method varies only in details between the studies reviewed here.
Subsequently an aliquot, usually 1 µg, of total RNA is reverse-transcribed using various commercially available kits containing viral reverse-transcriptase (RT). The cDNA acquired from this reaction is then amplified using a Polymerase Chain Reaction (PCR). Quantification of these reactions can be performed with one of several methods. The amplified DNA sample is then analyzed for content of Tg-mRNA. This can be done using a gel electrophoresis of the sample with Tg-mRNA negative and positive controls, or by other methods, based on detection of a specific sequence of RNA.
Sensitivity of assays can vary: Bojunga *et al*,[14] reported using two different assays, one of which had a lower limit of detection of 50-100 Tg-mRNA producing cells per ml blood, the other one had a lower limit of detection of 10-20 Tg-mRNA producing cells per ml. Ringel *et al* report being able to detect 10 Tg-mRNA producing cells per ml.[12]

Significance of Tg-mRNA detection in peripheral blood

Investigation of the applicability of the RT-PCR reaction for the detection of Tg-mRNA was based on the assumption that patients with adequately treated thyroid cancer should not have circulating Tg-mRNA-producing cells, nor should individuals without thyroid cancer. Although the first studies showed promising results, later reports pointed to some problems with this technique and were sceptic concerning its usefulness.
Ditkoff *et al*,[9] first investigated the possibility of detecting circulating malignant thyroid

cells using an RT-PCR reaction for the detection of Tg-mRNA; this could serve as an indicator of post-operatively present metastatic thyroid cancer.
This investigation was based on the assumption that patients with adequately treated thyroid cancer should not have circulating Tg-mRNA-producing cells; nor should individuals without thyroid cancer.

Pro

Ditkoff *et al*,[9] were the first to investigate the possibility of detecting circulating malignant thyroid cells using RT-PCR. They found that Tg-mRNA could be detected in all 9 patients with known metastatic thyroid cancer. Seven out of 78 patients with no currently known metastases, of whom 5 had a history of surgically treated metastases, also showed detectable Tg-mRNA in peripheral blood samples. No Tg-mRNA was detected in 6 patients with benign thyroid disorders or in 7 healthy subjects. The study of Ditkoff *et al* therefore clearly demonstrated the usefulness of Tg-mRNA detection in the follow-up of thyroid cancer patients by having positive cases and only negative controls
Two studies by Ringel *et al*[11,12] showed that Tg-mRNA detection could be useful in the follow-up of differentiated thyroid cancer. In the first study, 33 patients had either thyroid bed uptake (n = 19) or metastatic iodine-avid tissue (n = 14) on the most recent withdrawal scan.[11] In 12 out of 19 patients with thyroid remnants on the most recent follow-up scintigram Tg-mRNA could be detected in their blood. In addition, all 14 patients with metastatic disease showing on the most recent follow-up scintigram had detectable Tg-mRNA in their peripheral blood. Serum Tg levels were detectable in only 12 of these 33 patients. 7 out of 35 patients with negative scintigrams were positive for Tg-mRNA too. All 10 healthy control subjects turned out to be positive for Tg-mRNA in peripheral blood due to the improved sensitivity of the RT-PCR.
In the second study of Ringel *et al*,[12] a quantitative method of Tg-mRNA detection was used. Using a threshold of 3 pg Tg Eq/μg thyroid RNA for the detection of Tg-mRNA, analysis was positive in 38% of patients with a negative follow-up scintigram, 75% of patients with thyroid bed uptake, 84% of patients with cervical/regional disease and 94% of patients with distant metastases. Thyroglobulin antibodies were shown not to affect the result of the analysis.

Contra

Bojunga *et al*,[14] reported that using a 'normal sensitivity' (30 cycles of PCR) resulted in detection of Tg-mRNA in 9 out of 13 patients with thyroid cancer and known metastases, 63 out of 137 patients with a history of thyroid cancer without known

metastases, 21 out of 85 patients with benign thyroid disorders and in 9 out of 50 control subjects. Using a 'high sensitivity' (40 cycles of PCR), however, resulted in detection of Tg-mRNA in peripheral blood of 11 out of 13 patients with thyroid cancer and known metastases, 111 out of 137 patients with a history of thyroid cancer without known metastases and also in 61 out of 85 patients with benign thyroid disorders, and 41 out of 50 control subjects.

Takano *et al*[16] reported in their study that Tg-mRNA could be detected in samples from all patients who have had a thyroidectomy. Additionally, no statistically significant difference in expression levels could be found between patients with and patients without metastases in a quantitative analysis.

Illegitimate transcription of Tg-mRNA

Tissue specificity of Tg gene expression by detection of Tg-mRNA in various human tissues obtained through routine surgery was investigated by Bojunga *et al*.[14] Using a 'normal sensitivity' they found Tg-mRNA expression to be specific for thyroid tissue. Using 'high sensitivity' on the other hand resulted in the detection of Tg-mRNA transcripts not only in thyroid tissue, but also in various other tissues.

This confirms the findings of Tallini *et al*,[26] who, when studying 10 non-thyroid malignant human cell lines and 11 control subjects (including one patient who had had a total laringectomy for squamous cell carcinoma with a complete thyroidectomy), found no detectable expression of Tg-mRNA after 30 cycles of PCR, but found detectable Tg-mRNA expression in all samples after 40 cycles of PCR.

On top of that, Bugalho *et al*[15] reported in a study of healthy individuals and patients who have had a thyroidectomy for other reasons than thyroid cancer, that expression of Tg-mRNA was detectable in all subjects, and that quantitative analysis revealed no significant difference between those with and those without thyroid glands. Furthermore they found that, when separating the mononuclear and polymorphonuclear layer of the blood samples for analysis, both layers showed expression of Tg-mRNA, thereby suggesting that Tg-mRNA transcription also takes place in circulating white blood cells.

The observations regarding the detection of Tg-mRNA in patients and tissues where it should not be detected can be attributed to a phenomenon called 'illegitimate transcription':[27] any gene is expressed in any cell at very low, but detectable, levels.

Filtering out illegitimate transcription

Savagner *et al*[13] tried to circumvent this problem by using Prostate Specific Antigen (PSA) mRNA to determine the level of illegitimate transcription. Any patient expres-

sing Tg-mRNA in higher quantities than PSA mRNA was expected to have circulating follicular thyroid cells. Thus, they tried to filter out the non-thyroid expression of Tg-mRNA. Using this method, Savagner *et al* found that serum Tg-mRNA detection has a better sensitivity for detecting recurrent thyroid cancer than serum Tg measurement, especially during LT4 suppression therapy. Instead of prostate Specific Antigen, Eszlinger *et al*[18] and Span *et al*[19] used beta-actin mRNA transcription levels for correcting the illegitimate transcription. However, their results showed no statistical differences between patients and controls with respect to corrected Tg-mRNA expression levels.

Table

Results of various studies are summarized in table 12.1. For comparison of the different studies, a patient was considered to be positive for the presence of thyroid (cancer) tissue if he/she had proven thyroid cancer, and was showing either detectable serum Tg levels or showed scintigraphic evidence for the presence of disease when blood for Tg-mRNA detection was drawn.

Table 12.1 Summary of results of various studies into the usefulness of Tg-mRNA detection.

	quanti-tative?	false positive	false negative	true positive	true negative	positive controls	negative controls
Ditkoff[9]	no	7/78	0/9	9/9	71/78	0/15	15/15
Biscolla[10]	no	3/19	5/14	10/15	16/19	--	--
Ringel (1999)[11]	yes	13/33	13/74	61/74	20/33	--	--
Savagner[13]	yes	3/15	6/25	19/25	12/15	--	--
Bojunga[14] Method1	no	63/137	4/13	9/13	74/137	30/135	105/135
Bojunga[14] Method2	no	111/137	2/13	11/13	26/137	102/135	33/135
Elisei[28]	yes	22/29	2/17	15/17	7/29	17/20	3/20
Takano[16]	yes	All patients and controls are positive					
Bugalho[15]	semi	No difference in expression levels between individuals with and without thyroid glands					
Bellantone[17]	no	Varies according to histology					
Eszlinger[18]	yes	No difference between patients with and without metastases or other thyroid diseases					
Span[19]	yes	No difference between patients and controls					

Discussion

The question we addressed here was whether Tg-mRNA detection in peripheral blood can be used for follow-up in differentiated thyroid cancer. Tg-mRNA detection certainly does not turn out to be specific for the presence of metastatic thyroid cancer: Tg-mRNA is detected in peripheral blood samples of patients with benign thyroid disorders and even in samples from healthy subjects.[12,13,16,26,28] This suggests that Tg-mRNA producing cells are present in blood even in patients without thyroid cancer, which could be attributed to illegitimate transcription of Tg-mRNA, or could mean that cell shedding is a physiologic rather than pathologic process, taking place even in normal thyroids. Especially remarkable is the finding of Bojunga *et al*[14] that changing the number of PCR cycles in analysis completely changes the specificity of Tg-mRNA detection. This suggests that a relatively low level of expression of Tg-mRNA transcription also takes place in many other cells in the body, which, when sufficiently enhanced, will also be detected when analyzing with PCR for the presence of circulating thyroid cells. This study certainly provides compelling evidence for the occurrence of illegitimate transcription of Tg-mRNA. This hypothesis is certainly supported by the finding of Sellitti *et al*,[29] who reported that human kidney cells respond to TSH stimulation with the production of Tg-mRNA. Additionally, when using an immunofluorescent staining with a monoclonal anti-Tg antibody, positive staining can be identified in the cytoplasm of mesangial cells.

Possible enhancements to Tg-mRNA detection

Under the hypothesis that Tg-mRNA is expressed at low levels in various human cell lines, and at higher levels in normal or neoplastic thyroid cells, one could consider using a quantitative RT-PCR reaction with a clearly defined threshold for detecting Tg-mRNA. However, this might not be the solution. No significant differences in expression levels between patients with thyroid cancer and subjects without thyroid disorders or with benign thyroid conditions could be detected by Takano *et al*[16] and Fenton *et al*.[30]

Determining the level of illegitimate transcription and correcting for it, like Savagner *et al*,[13] Eszlinger *et al*[18] and Span *et al*[19] did, is also still open to further research: using PSA-mRNA should at least be further validated. Accordingly, one can consider investigating other mRNA molecules than PSA-mRNA or Beta-actin-mRNA for correction for illegitimate transcription.

Another option is to further experiment with varying sensitivities of the RT-PCR procedure; e.g. 30 cycles of PCR instead of 40 when using the same technique as Bojunga *et al.*[14] However, this option is also still open to research.

Ringel[31] also suggested some interesting possibilities for further research using RT-PCR in the follow-up of thyroid carcinoma.

Alternatives to Tg-mRNA

As an alternative to the detection of Tg-mRNA, several groups have investigated the possibility of using measurement of mRNA of other thyroid-specific proteins in the follow-up of differentiated thyroid carcinoma. Biscolla *et al*,[10] in their study also investigated the possibility of detecting sodiumiodine symporter (NIS) mRNA. Their results however showed no benefit from these measurements; NIS-mRNA detection was inferior to Tg-mRNA detection. Tallini *et al*[26] correlated in their study the detection of thyroid peroxidase (TPO) mRNA and Tg-mRNA. TPO-mRNA was detected in the same samples of peripheral blood as Tg-mRNA and TPO-mRNA was not found in samples in which Tg-mRNA was also lacking. Finally Roddiger *et al*,[32] investigated the use of TPO-mRNA exclusively. TPO-mRNA expression in peripheral blood was detected in significantly more patients with thyroid disease than in control patients. It is also correlated with the presence of metastases, and in patients without known metastases, it is correlated significantly with grade, lymph node stage at the time of diagnosis and Tg levels. Based on this data, further investigation of TPO-mRNA seems warranted.

Pauws *et al*[21] have already analyzed the entire spectrum of transcripts from thyrocytes, using a method called 'serial analysis of gene expression' (SAGE). More on this method can be found at the URL www.sagenet.org. It should be analyzed which of these transcripts in the thyrocyte gene profile are unique to thyroid epithelial cells. Any thyroid-specific mRNA transcript would also be a candidate for use in follow-up. Perhaps the next step could even be to analyze each patients' thyroid carcinoma for its specific gene profile and monitor the patient by detecting circulating cells matching the profile at key points. Such key points would have to be determined in future research.

Using RT-PCR

RT-PCR for Tg-mRNA has so far mainly been investigated for use in the regular follow-up of all patients with differentiated thyroid carcinoma, using only one measuring point. Even if Tg-mRNA RT-PCR eventually does not turn out to be useful in the regular follow-up of differentiated thyroid cancer, there might be specific subsets of patients in whom this technique could be used. In patients with medullar thyroid carcinoma, non-quantitative RT-PCR for calcitonin mRNA turns out to correlate with the presence, extent, and aggressiveness of metastatic disease.[33] In this study by Saller

et al, it was also found that in patients who had a clinically significant response to chemotherapy, calcitonin mRNA had become undetectable.
Perhaps RT-PCR for Tg-mRNA could be used in an analogous way for the monitoring of response to therapy in patients with known metastatic thyroid cancer, especially in those patients in whom normal Tg measurements are not reliable due to the presence of Tg antibodies. Especially monitoring the progression of Tg-mRNA expression levels over time might prove useful.
A single positive sample at any level might not be considered irrefutable proof for the persistence or recurrence of disease, due to phenomena such as illegitimate transcription, but a Tg-mRNA level rising over time will reflect increasing activity of normal or neoplastic thyrocytes. RT-PCR could possibly detect recurring or persisting disease much earlier than Tg measurements could, especially during LT4-suppressive therapy.

Conclusion

Based on a the various studies reviewed in this paper, it can be concluded that at this time there are still too many contradicting results on the usefulness of Tg-mRNA detection in peripheral blood; this technique can therefore not yet be considered a proven and reliable method of following patients after treatment for differentiated thyroid carcinoma.
Further research, e.g. whether different levels of sensitivity of arrays could produce a useful level of specificity, or the usefulness of other thyroid-specific gene transcripts, such as TPO-mRNA, should however be performed; further thought should also be given to the use of complete gene profiles of the tumor in the follow-up of differentiated thyroid carcinoma.

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Chapter Thirteen

Heterophile antibodies significantly influence the measurement of thyroglobulin and thyroglobulin antibodies in differentiated thyroid cancer patients

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Submitted

Abstract

Objective: to determine the impact of heterophile antibodies (HAB) on the measurement of serum thyroglobulin (Tg) and Tg antibody (TgAb) levels.
Materials and methods: we studied serum samples of 100 individual patients that were followed in our hospital for differentiated thyroid carcinoma. Samples were split and half were treated by incubating the sample for 1 h in HAB-blocking tubes, the remainder was left untreated. Subsequently Tg and TgAb levels were measured in both the blocked and untreated samples with two methods. A difference between the two samples was considered significant if the blocked sample deviated from the untreated one by more than 2.77 times the standard deviation for the method.
Results: In 45 control subjects and 1 control buffer no changes were observed in Tg values after blocking treatment. In 59 patients no significant change in either Tg or TgAb-levels was observed. 30 patients (30%) showed a change in Tg levels; 22 patients showed a change in both assays and 8 in only one assay. 11 patients showed a change in TgAb levels; 6 patients in both assays and 5 in only one assay.
Patient management would have been changed in at least 9 (9%) patients due to HAB-blocking treatment of samples; in the most extreme case Tg levels changed from undetectable to 580 μg/l.
Conclusion: There is a significant influence of HAB on the measurement of Tg and TgAb, which may affect management in a non-trivial number of thyroid cancer patients. The influence may vary across assays.

Introduction

The measurement of thyroglobulin (Tg) and antibodies against thyroglobulin (TgAb) is an important diagnostic tool in the follow-up of differentiated thyroid carcinoma (DTC).[1-5]
In the presence of TgAb, results of Tg measurements cannot be considered reliable.[6,7] Besides TgAb other factors may interfere with the reliability of the measurement of Tg. One of these factors is the so called heterophile antibodies (HAB). HAB are antibodies that can bind to animal antigens, e.g. antibodies (goat, rabbit or mouse) used in the immunoassays. In immunometric assays, they can interfere with measurements by forming a bridge between capture and detection antibody. This may result in a falsely higher or even a false-positive result in case of an absent analyte. Alternatively, HAB may bind to the capture antibody in such a way that binding of the analyte and/

or the detection antibody is hindered, thereby causing false-negative or falsely low results.

Most modern immunometric assays contain additives to reduce HAB interference but have been unable to eliminate this interference completely.[8]

Physicians who are involved in thyroid cancer treatment will at some point be confronted with a patient who has signs of metastasized disease (e.g., positive ^{131}I scintigraphy or positive neck ultrasound), but no detectable Tg or TgAb.

As a possible explanation for at least some of these cases the presence of HAB in these patients' blood should be considered. A study by Preissner *et al*[9] already described the possibility of HAB interfering with Tg measurement; however, this study included only cases with *a priori* positive Tg levels and showed that mostly HAB interference caused falsely elevated serum Tg levels. The aim of this study was therefore to study the impact of HAB on Tg measurements in thyroid carcinoma patients regardless of known Tg values.

Materials and methods

Samples

Stored serum samples from 100 consecutive individual patients with differentiated thyroid carcinoma where selected. No further selection was applied with regard to results of prior Tg and TgAb measurements. Samples were anonimyzed before use. As controls 10 samples from healthy control subjects and one sample of assay dilution buffer (Tg-free serum) were included.

Procedures

After thawing, samples were centrifuged for 5 min at 3000 rpm. Subsequently the samples were split: 0.5 ml of the sample was left standing untreated at room temperature for 1 h and 0.5 ml serum was incubated for 1 h at room temperature in HAB-blocking tubes (HABBT; Scantibodies Laborat. Inc. Santee, Ca, USA) which contain antigen designed to block any HAB antibodies. After incubation the samples were again centrifuged for 5 min at 3000 rpm. Tg and TgAb levels were measured in each of the HABBT-treated and untreated samples with two different methods per sample.

To test for possible interference from the blocking tubes in the Tg and TgAb assays we measured Tg and TgAb in a dilution buffer which was supplied with the BRAHMS kit and 45 samples from patients, not suffering from thyroid disease.

Blocked and untreated samples were always measured together in the same run or kit.

Assays

Tg and TgAb were measured using conventional hand-based assays (Tg-PluS (immunoradiometric method) and anti-Tg_n (radioimmunoassay): BRAHMS diagnostica GmbH, Henningsdorf, Germany; method1) and automated assays (Modular Analytics E170 Tg- and TgAb-assay (electro-chemiluminescence immunoassay): Roche GmbH, Mannheim, Germany; method2). Both methods for Tg measurement were standardized against CRM-457; in addition method1 uses a scaling factor of 0.5 to arrive at the result of measurements.

According to the manufacturer's information inserts included with the respective kits, both method1 and method2 contain additives to reduce interference from human anti-mouse antibodies.

Lower detection limits were 0.2 µg/l for method1 and 0.1 µg/l for method2. TgAb were considered positive if ≥ 60 mU/l for method1 and ≥ 150 mU/l for method2. The within-run coefficients of variation (CV) for the two methods as determined in our own laboratory can be found in table 13.1. Knowing the CVs we chose to analyze and report any measured value that was equal to or above the lower detection limit of the assays used.

Table 13.1 Mean within-run analytical variation estimated in the laboratory (coefficients of variation) for the Tg and TGAb assays from Brahms and Roche.

	Brahms		Modular E170	
	concentration	**CV%**	**concentration**	**CV%**
Tg	0.55 µg/l	3.2	0.64 µg/l	4.9
	6.9 µg/l	0.9	3.9 µg/l	1.0
	116 µg/l	0.64	93 µg/l	0.95
TgAb	62 U/mL	8.4	35 U/mL	4.2
	290 U/mL	3.7	115 U/mL	2.0
	1640 U/mL	3.4	680 U/mL	1.3

Criteria

A difference was considered significant if the HABBT-treated sample differed from the untreated sample by more than 2.77 times the within-run standard deviation for the assay used.

Additionally, for antibody measurement a change in levels was only counted if at least one of the two samples presented with positive TgAb; this criterion was chosen due to the relatively high coefficient of variation in the range of TgAb values that are considered negative.

Patient management

In our center the routine follow-up of DTC-patients consists of Tg measurement (with method1) during levothyroxine suppression, with periodic levothyroxin withdrawal or after rhTSH stimulation. If a patient shows a Tg level of ≥ 1 µg/l with method1, additional diagnostic or therapeutic measures are taken which means a change in the patient management. In the presence of positive TgAb, Tg levels are considered unreliable and thus patients are subjected to scintigraphy more often. Various guidelines and consensus reports recommend considering Tg values of 1 µg/l or higher pathologic; alternatively institutional cut-off values can be used.[2-5] For the purpose of this study, it is assumed that a change of management would occur if a thyroglobulin level changes across the value of 1 µg/l (method1) or 2 µg/l (method2) (the difference between the two methods lies in the scaling factor of 0.5 used in the calibration of method1 against CRM457), or if the TgAb levels crossed the limit of what is considered positive in the respective assays. In the results a change was deemed clinically significant if any of these criteria were met for at least one of the two assays.

Results

Using method2, one blocked Tg measurement failed; there was not enough material to repeat the test. In two other patients both methods showed Tg levels above 100,000 µg/l, making a reliable Tg or TgAb level measurement impossible. These patients were therefore excluded from further analysis.

No changes were observed in the control samples with either method1 or method2 as a result of the HABBT treatment.

In 59 patients no significant change in either Tg levels or TgAb levels was observed. The results of 39 patients in whom a change was observed are displayed in table 13.2. Tg measurements showed a change in 30 patients (30%). 22 patients showed a change in Tg levels with both assays; in only one of these 22 patients were the changes in opposite directions. An additional 8 patients showed a change in only one assay.

TgAb measurements showed a change in 11 patients, of which 6 patients with both methods. In all these patients the direction of the change was concurrent between methods. 5 patients showed a change in only one assay.

11 patients showed a clinically relevant change of Tg and/or TgAb levels: in 4 patients Tg levels changed from undetectable to ≥ 1 µg/l (reaching as high as 95 µg/l or 580 µg/l with the respective assays in one patient). In 2 additional patients Tg levels rose from < 1 µg/l to ≥ 1 µg/l. In 2 patients, TgAb levels changed from positive to negative and in 3 patients TgAb levels changed from negative to positive in the respective

Table 13.2 Results of Tg and TgAb measurements with method1 (Tg/TgAb1.y) and method2 (Tg/TgAb2.y) before (x.1) and after (x.2) treatment in the HABBT. Change = possible management change.

No.	Tg1.1	Tg1.2	Tg2.1	Tg2.2	TgAb1.1	TgAb1.2	TgAb2.1	TgAb2.2	Change
Tg difference only in Method 1									
1	0.4	1	2.8	2.6	< 20	< 20	17	16	YES
2	< 0.2	0.5	1.5	1.1	< 20	< 20	30	17	
3	0.3	0.7	1.1	FAIL	< 20	< 20	17	38	
4	< 0.2	0.4	0.4	0.5	< 20	< 20	41	59	
Tg difference only in Method 2									
5	4	5	9	11	< 20	< 20	12	< 10	
6	< 0.2	0.3	< 0.1	0.4	< 20	24	33	34	
7	0.4	0.4	2.3	1.3	< 20	< 20	11	< 10	
Tg difference in Method 1 and Method 2									
8	82	75	230	147	< 20	< 20	43	29	
9	1	2	5	7.3	< 20	< 20	23	43	
10	9	12	33	25	< 20	< 20	30	23	
11	1650	2175	4790	4380	28	39	< 10	< 10	
12	250	362	553	710	< 20	25	< 10	17	
13	580	808	1220	1665	22	27	21	29	
14	< 0.2	95	< 0.1	580	< 20	< 20	13	19	YES
15	63	193	175	393	< 20	< 20	< 10	42	
16	0.7	3	4.1	6	< 20	< 20	17	26	YES
17	33	65	87	133	< 20	< 20	14	33	
18	5	13	16	31	< 20	< 20	< 10	25	
19	34	137	216	277	< 20	41	81	114	
20	61	111	169	240	< 20	< 20	< 10	18	
21	3	7	11	18	< 20	< 20	< 10	22	
22	38	67	109	130	< 20	< 20	12	18	
23	6	12	19	26	< 20	< 20	< 10	14	
24	< 0.2	0.4	< 0.1	0.3	< 20	28	40	81	
25	162	186	347	374	< 20	< 20	< 10	15	
26	35	97	144	231	< 20	< 20	15	18	
27	< 0.2	21	< 0.1	49	< 20	< 20	14	25	YES
28	< 0.2	5	< 0.1	12	< 20	< 20	18	27	YES
Differences in Tg and TgAb									
29	0.3	0.8	2.1	1.7	< 20	57	158	108	YES
30	< 0.2	1	0.1	2.3	44	53	273	175	YES
TgAb difference only in Method 1									
31	< 0.2	< 0.2	< 0.1	< 0.1	176	105	16	16	
TgAb difference only in Method 2									
32	< 0.2	< 0.2	< 0.1	< 0.1	34	53	163	306	
33	< 0.2	< 0.2	< 0.1	< 0.1	21	25	185	125	YES
TgAb difference in Method1 and Method 2									
34	< 0.2	< 0.2	< 0.1	< 0.1	< 20	88	140	246	YES
35	< 0.2	< 0.2	< 0.1	< 0.1	45	77	393	453	YES
36	< 0.2	< 0.2	< 0.1	< 0.1	114	193	520	693	
37	< 0.2	< 0.2	< 0.1	< 0.1	270	440	663	862	
38	< 0.2	< 0.2	< 0.1	< 0.1	240	440	929	1440	
39	< 0.2	< 0.2	< 0.1	< 0.1	48	85	84	168	YES

assays. The clinical history of these 11 patients was analyzed: in 2 patients the sample was taken at the time of ^{131}I ablation. One patient suffered from a recurrent hypopharyngeal carcinoma. One patient showed a slight ^{131}I uptake in the thyroid bed, another patient showed dubious pathological uptake in the cervical region. In yet another patient, a parietal bone metastasis was demonstrated on ^{131}I whole-body scintigraphy. In 5 patients the follow-up data were unremarkable. As thyroglobulin levels at the time of ablation do not readily affect the patient management in our clinic, the management of disease would have been changed in 9 patients.

Discussion

In a study of 100 patients, we detected HAB interference resulting in mostly lower or even false-negative results for either Tg or TgAb measurement in a surprisingly large portion of samples (nearly 40%); two-thirds of these interferences were observed independently in two assays.

This interference is much more frequent than has been reported in the literature. Preissner *et al*[9] published a large study of repeated Tg measurements in which the over-all interference in 1106 Tg positive samples was 3%. In 6/48 (13%) known thyroid cancer patients a possible HAB interference was encountered. Our study showed a possible HAB interference in Tg measurements in 30% of the patients. In contradiction to the study by Preissner *et al*, our results were significantly higher after HAB-blocking. Preissner *et al* had excluded samples which had shown a result < 1 µg/l in regular measurements, so in their study false-negative sera were unlikely to show.

Persoon *et al* encountered HAB-interference in Tg measurements in only 1/127 patients – but these authors did not use any form of HAB-blocking and identified the HAB-influence through an elevated Tg recovery.[10] Giovanella and Ghelfo described a patient with undetectable Tg levels in whom Tg recovery was pathologic, and who was subsequently discovered to have a false-negative Tg level after HABBT-treatment.[11]

In literature there is no agreement on the frequency of HAB-interference, with reported ranges from less than 1% to as much as 80%.[8] The percentage of interference may also depend on the type of assay.[8] In a recent study by Preissner *et al*, assays for several antigens were assessed. HAB interference ranged from 0.2% for an alpha-fetoprotein assay to 3.7% for a calcitonin assay.[12] Bjerner *et al*[13] reported 4.0% interference in an assay for carcinoembryonic antigen. In comparison with these data, it seems that for reasons yet unknown thyroid cancer patients show a far larger degree of HAB interference in Tg assays than the general population across assays. Many explanations are possible. It has been suggested[9] that thyroid carcinoma patients have

a higher tendency to develop HAB. As the manufacturers point out in their information inserts, patients who have undergone prior treatment or diagnostic testing with monoclonal mouse antibodies may show erroneous results in these assays. Even though the assays all contained additives to minimize effects from human anti-mouse-antibodies (HAMAs), there was no mention of additives to minimize the results of other types of heterophile antibodies, e.g. against goat or rabbit. In contrast the HAB-blocking tubes were specifically developed to block a broader range of antibodies than HAMAs alone. It could be considered that the changes seen in patient samples after HABBT treatment are due to undesired interference from this HABBT treatment. However, in neither method an influence of HABBT treatment was found on Tg and TgAb levels in control subjects and a buffer sample, which makes interference from HABBT treatment highly unlikely.

In 8 patients a significant change in Tg levels was observed in only one assay; the same was seen for TgAb levels in 5 patients. The differences between assays could be due to differences in (proprietary) reagents or differences in the antibodies used in the immunoassays; further experiments are required to identify the precise cause of these differences. Due to the differences between antibodies used in the assays the 13 patients in whom Tg or TgAb measurement produced discordant results between assays do not necessarily reflect false-positive results. Rather such discordant results reflect the extremely heterogeneous nature of heterophile antibodies and the non-exchangeability of results between Tg/TgAb assays which has been firmly established in literature.[14,15] The consequences of HAB-interference in the measurement of Tg and TgAb are evident: in 9 patients (9%) management could or would have been changed.

Conclusion

There is a significant influence of heterophile antibodies on the measurement of Tg and TgAb in thyroid cancer patients. The influence may cause inappropriate patient management in a non-trivial percentage of patients. The precise extent of such interference is however assay-dependent.

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Chapter Fourteen

General discussion: proposed treatment and follow-up protocols, and future perspectives

General discussion

Since the advent of radioiodine as an adjuvant treatment for patients with differentiated thyroid carcinoma (DTC) in the 1940s and the availability of synthetic thyroid hormones in the same era, the prognosis of DTC has dramatically improved. Despite this long-standing history, and ample study by authoritative researchers from top referral centers, there is still no complete consensus with regard to the classification, the optimal treatment, and follow-up schemes for patients with DTC. The generally slow progression and good prognosis of DTC makes it difficult to conduct prospective randomized controlled studies, due to continually changing insights in treatment of patients over the course of their long follow-up. Consequently it is difficult to assess the value of any such protocol changes.
Generally, patients with DTC have a good prognosis. Nevertheless the overall survival is lower than in a reference population of the same age and sex.[1,2] The 10-year survival for patients with DTC is between 70 and 98 percent; patients with PTC do somewhat better than those with FTC.[1-4] Clinical researchers have tried to define prognostic factors at the time of diagnosis of DTC to predict the outcome of the disease.[1-12] To establish the most appropriate treatment of individuals it is necessary to identify patients at high risk of recurrent disease or of thyroid cancer-related death. Important prognostic factors are the patient's age at the time of diagnosis, and the presence of distant metastases. This may be the explanation for the slightly worse prognosis of FTC in comparison with PTC in some studies: on average at the time of diagnosis patients with FTC are older than patients with PTC.[13] Prognostic factors found in one study cannot be simply transferred to another. Furthermore, various analyses were based on populations from different parts of the world, and therefore with different ethnicities. This may lead to contradicting prognostic factors: in a study based on a North-American population, male sex was associated with poorer prognosis.[6] Others, in a study based on a Japanese population, reported a poorer prognosis for females.[12] The treatment of patients with DTC may vary between different centers. These variations may influence prognosis. The prognosis of patients with PTC is influenced significantly by the extent of surgery and to some extent by ^{131}I ablation.[5,14] Sometimes different prognostically important variables are found when identical methodologies from previous studies are applied to a new population.[15]

Some issues are undisputed. Patients with PTC, especially those without clinical signs of metastatic disease, have a highly favorable prognosis. Of all patients with DTC, those who are diagnosed with distant metastases have the worst prognosis. The

definition of low-risk and high-risk patients, however, has varied over time and throughout the world; in recent guidelines and consensus papers, issued over the past two years by recognized professional bodies, different definitions are being used.[16-18] This problem is reflected in the many coexisting staging systems. In a comparison of seven staging systems for DTC, applied to our patient population, the TNM system version 5[19] outperformed all others in terms of prognostic power (chapter 10).

No treatment modality can effectively replace the surgical removal of cancerous thyroid tissue. The required extent of surgery is not much debated. Hemi-thyroidectomy is generally recommended for patients with isolated PTC with diameters up to 1 cm. There are no differences in survival between patients who had a total thyroidectomy and those who had a hemi-thyroidectomy. Total thyroidectomy is the standard therapy for patients with FTC with tumor diameters < 1 cm. A survival benefit of total thyroidectomy over hemi-thyroidectomy has never been proven conclusively for this patient group, and without further study it is impossible to establish the best surgical approach towards follicular microcarcinoma. In our patient population we found no difference in disease-specific survival between patients with PTC and patients with FTC (all with tumor diameters < 1 cm), where PTC had been treated with hemi-thyroidectomy and FTC with total thyroidectomy (chapter 9). We therefore conclude that for follicular microcarcinoma a hemi-thyroidectomy may suffice.
Lymph node dissection is the only way to cure large lymph node metastases. Primary surgery should be preceded by an ultrasound evaluation of the neck and FNAB of suspicious lymph nodes.[20] In patients without known cervical lymph node involvement the extent of the lymph node dissection remains controversial. Usually, recommendations involve minimally the *en block* resection of the central compartment (level VI), as this also permits staging of the disease.[17] There is, however, no evidence that this procedure results in longer disease-free survival or longer tumor-specific survival.[17] Also a more extensive exploration of the cervical region carries a higher risk of complications such as recurrent laryngeal nerve damage as well as complicating a possible future re-exploration in case of recurrent disease.

The likelihood of developing advanced tumor characteristics such as multifocal disease, extra-thyroidal tumor invasion, lymph node metastases, and distant metastases, is a function of the primary tumor diameter.[21] We demonstrated that this likelihood is increased greatly for tumor diameters exceeding 10 mm. The limit of a primary tumor diameter up to 2 cm diameter constituting the lowest risk group as accepted in the TNM version 6[22] is therefore disputable. Because of this we advice to change the T1

classification of the TNM system to include only those tumors with a diameter ≤ 1 cm.

There is no consensus on the preferred activity of radioiodine for ablation treatment. Some researchers apply higher activities than others, sometimes weighing the histological tumor characteristics.[23] Some take the size of thyroid remnants into account, whereas others do not.[23,24] In two similar groups of patients with DTC, we compared different ablation protocols (chapter 4). In the first protocol we used an algorithm for calculating the ablation activity (described in chapter 4) that accounts for radioiodine uptake in thyroid remnants (as determined by pre-ablative diagnostic scintigraphy). Consequently, patients received lower ^{131}I activities if the thyroid remnants were larger. In the second protocol, the ablative activity was determined solely by the primary tumor characteristics at the time of diagnosis; patients with more advanced disease (e.g. lymph node or distant metastases or unfavorable histology) received higher activities. The ablation of thyroid remnants and residual disease was significantly more successful in the latter protocol. We conclude that the fixed activity protocol in which fixed (high) activities are administered regardless of thyroid remnant size is more advisable for ^{131}I ablation.

The efficacy of ^{131}I ablation protocols may further be negatively affected by thyroid stunning, the very existence of which has been disputed by various authors.[25-27] It is as yet unclear whether the phenomenon of 'stunning' may be depending on the time elapsed since administration of the diagnostic activity of radioiodine.
We demonstrated that thyroid stunning occurred after relatively small activities of ^{131}I; pre-ablative diagnostic scintigraphy with 40 MBq effectively reduced the success of radioiodine ablation by half (chapter 5). It is therefore recommended to use either ^{123}I or ^{131}I activities not exceeding 10 MBq[28] for pre-therapeutic dosimetry.

Lymph node metastases at the time of diagnosis are associated with an unfavorable outcome in patients with PTC. We found that two-thirds of the patients with nodal metastases were not free of disease after a median follow-up of 7 years, irrespective of the extent of surgery (chapter 6). The prognosis was worse for patients who were presented with clinically overt lymph node metastases than for patients who were diagnosed with subclinical lymph node metastases during surgery and subsequent pathological examination.

The goals of radioiodine ablation after total thyroidectomy are twofold: first to destroy remaining potentially malignant thyroid cells, and second to facilitate the follow-up of these patients by the elimination of all thyroidal cells (including non-malignant thyrocytes) capable of producing Tg and/or taking up radioiodine. The determinants and the prognostic significance of successful ^{131}I ablation were examined in chapter 7. The height of thyroglobulin levels at the time of ablation and the presence of nodular or distant metastases at diagnosis appeared to be determinants of success of ablation. After successful ablation (defined as the combination of undetectable thyroglobulin levels and a negative ^{131}I whole-body scan under TSH stimulation) the disease-free survival and the thyroid cancer-specific survival were longer.

In additional studies the course of the disease after successful ablation was examined in a larger group of 526 patients with DTC from three university centers (chapter 8). In a Kaplan-Meier analysis the long-term disease-free survival was 96.6 percent. All recurrences occurred within 5 years after initial treatment. Remarkably, there was no difference in disease-free survival between patients initially classified as low risk and high risk. In the follow-up after successful ablation diagnostic ^{131}I scintigraphies did not have additional diagnostic value; in these cases, the procedure seems to be redundant.

According to the literature, patients with PTC and FTC have a different prognosis.[5,29] In our patient population the considerable difference in thyroid cancer-specific survival between PTC and FTC patients was explained by the more advanced disease and higher age at presentation (both well-established poor prognostic factors) in the latter (chapter 9). This more advanced disease at presentation may be due to delays in diagnosis caused by difficulties in the interpretation of cytology. After correction for the differences in presentation, differences in thyroid cancer-specific survival between PTC and FTC could no longer be demonstrated. As a consequence, there is no rationale for a different therapeutic approach towards PTC and FTC patients if the tumor size does not exceed 1 cm.

Thyroglobulin (Tg) measurements are extremely useful in the follow-up of patients with DTC. The sensitivity of Tg measurements during suppressive levothyroxine medication, however, is inferior to those during elevated thyroid stimulating hormone (TSH) levels. With the FDA approval of recombinant human TSH (rhTSH) for diagnostic purposes, there is no longer a need for a 4-week withdrawal of levothyroxine and the concurrent prolonged periods of hypothyroidism.[30] There has been some debate

over the optimal time point for Tg measurements after rhTSH preparation. Following the manufacturer's recommendation, which is based on the results of an international trial,[31] Tg should be measured 72 hours after the second (and last) intramuscular injection of rhTSH. The present study confirms the validity of this recommendation. Tg measurement 24 hours after rhTSH does not add clinically relevant information; neither does ^{131}I whole-body scintigraphy (chapter 11). However, Tg measurements suffer from inherent technical difficulties. The main problem in this domain is the illegitimate transcription of Tg-mRNA occurring in white blood cells. This Tg-mRNA may interfere, as circulating thyroid carcinoma cells are found in the white blood cell fraction of centrifuged blood. In the past decade the determination of Tg mRNA (a means of detecting circulation thyroid cancer cells) has emerged as an important candidate to replace Tg measurements.[32-36] Arguments both for and against the use of Tg-mRNA were found in a meta-analysis of the literature (chapter 12). However, the majority of studies show that the mRNA technique does not yet have sufficient sensitivity and specificity for instant clinical use. The main problem in this domain is the illegitimate transcription of Tg-mRNA occurring in white blood cells. This Tg-mRNA may interfere, as circulating thyroid carcinoma cells are found in the white blood cell fraction of centrifuged blood.

Another problem in the measurement of Tg is the presence of heterophile antibodies (not to be confused with human anti-mouse antibodies). These antibodies may cause inordinately high or low (even false-positive or false-negative) results of Tg and Tg antibody measurements. In a group of 100 patients with DTC, we established interference in nearly 40% of all patients, with potential clinical relevance in 11% of the total (chapter 13).

Proposed treatment protocol and future perspectives

Several unresolved issues related to the diagnosis, treatment and follow-up of differentiated thyroid carcinoma are discussed hereafter. From our present investigations several new insights have emerged that should lead to adjustments of currently accepted treatment and follow-up protocols such as the 2004 and 2006 European consensus statements and various guidelines.[16-18] An alternative protocol is proposed in figure 14.1.

Diagnosis

There is a considerable degree of uncertainty involved in the diagnosis of DTC, mainly originating from non-diagnostic fine-needle aspiration biopsy (FNAB) results.[37-47]

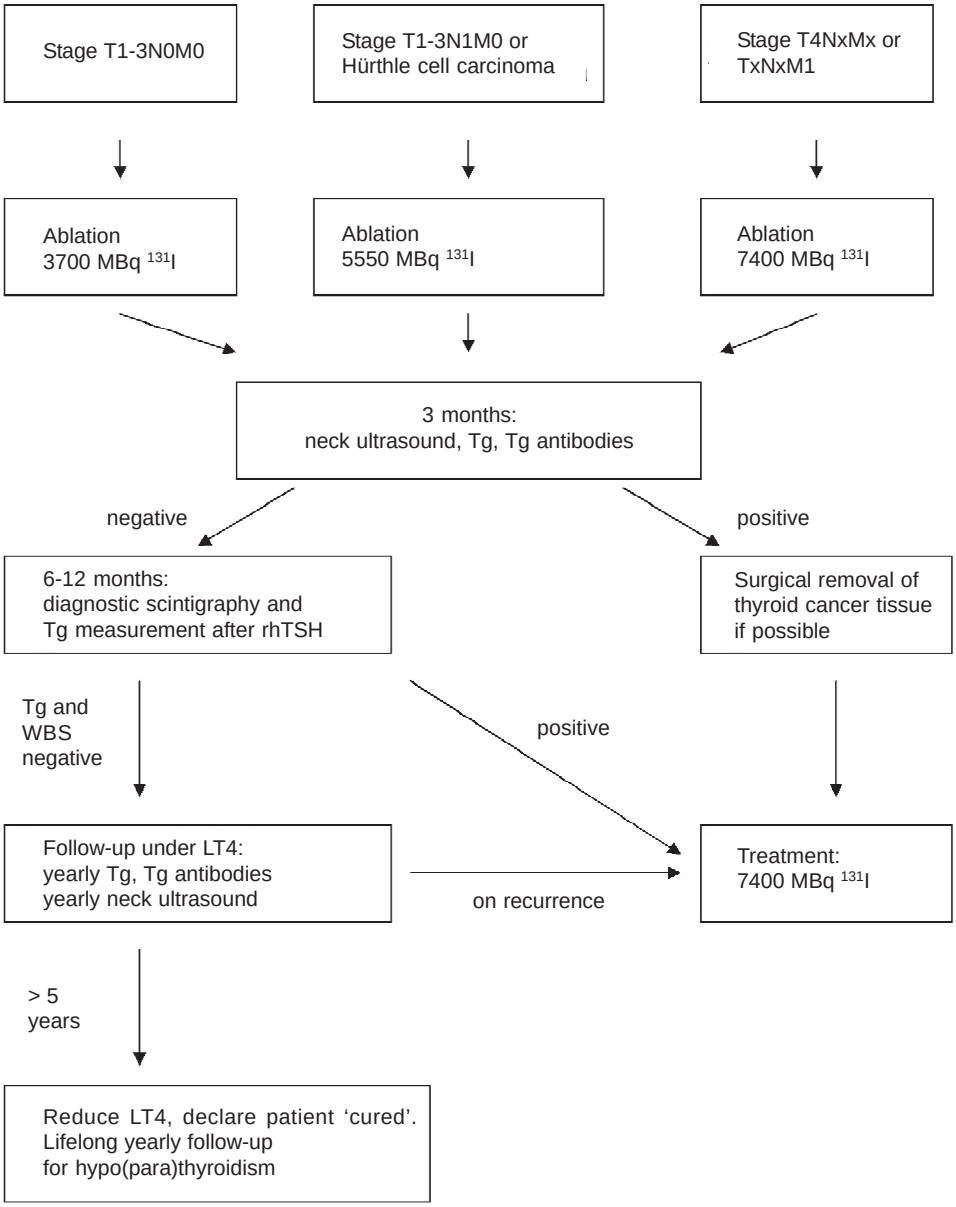

Figure 14.1 Proposed treatment and follow-up algorithm for DTC patients, staged according to UICC/AJCC TNM system, v. 5 (19). Tg = thyroglobulin, WBS = ^{131}I whole-body scan, LT4 = levothyroxin therapy.

For FTC this situation is even more pronounced. Contrary to PTC, where cells lying in finger-like groups, clear cell nuclei, nuclear pseudo-inclusions, psammoma-bodies and nuclear grooves provide clear hallmarks for a cytological diagnosis of malignancy, cellular morphology does not allow to distinguish between follicular adenomas and follicular carcinomas. Histological examination of an entire nodule is therefore required. This leads to many unnecessary hemi-thyroidectomies for simple adenomas and to additional completion thyroidectomies (which are technically more complicated) in patients with carcinoma. Also, false-negative interpretation may lead to delayed recognition of the disease, and consequently to more advanced age and more advanced disease at the time of diagnosis.[21,48] Improvements in FNAB results are currently being sought in the determination of protein markers, genetic alterations,[49,50] mRNA expression profiles,[51-53] or microRNA expression profiles[54] in FNAB specimens. However, most of these methods have mainly been tested on surgical specimens. A major problem with FNAB is the aspiration of large volumes of blood along with a limited number of thyrocytes. Even in the application of methods for the isolation of DNA and mRNA from the mononuclear layer of centrifuged samples only, an excess of blood cells may severely compromise the efforts of determining the expression profiles, much like it does with the determination of Tg-mRNA (chapter 11). This problem might be overcome by using magnetically labeled antibodies for the isolation of thyrocytes from the mononuclear layer. This method has been successfully applied for the isolation of cells from several other carcinomas in peripheral blood.[55-59]

Surgery

No treatment modality can effectively replace the surgical removal of cancerous thyroid tissue in the foreseeable future. Surgical methodology is however under continuous development. Improvements in surgery may be found in minimally invasive procedures for thyroidectomy,[60-62] and sentinel lymph node biopsy.[63,64] At present, neither technique is sufficiently established for routine clinical use in DTC.

^{131}I ablation

^{131}I treatment is recommended for all patients after total thyroidectomy for DTC. This additional treatment is given for three reasons. First, it destroys any remaining normal thyroid tissue, thereby increasing the specificity of detectable serum Tg and positive whole-body scintigraphy as markers for persistent or recurrent tumor.[65-67] Second, iodine-131 therapy may destroy occult microscopic carcinomas, thereby decreasing the long-term risk of recurrent disease.[1,5,66,68] Third, the use of a large activity of iodine-131 for therapy permits post ablative scanning, a test for detecting persistent carcinoma.[69,70]

Survival benefits of ^{131}I ablation have not been unequivocally demonstrated for low-risk patients (TNM stage T2N0M0), and the measured effects on the prevention of recurrent disease differ between studies.[71,72] In patients with non-invasive primary tumor < 2 cm in diameter no significant difference can be found in remission rates between those who did and did not receive ^{131}I ablation.[73] On the other hand, beneficial effects of ^{131}I therapy have been shown in patients with a high risk or irradical surgery.[5,74-76]

Concerns about the potential harmful effects of high ^{131}I activities have changed the view on the indication for ^{131}I ablation for all patients with DTC. An excess degree of secondary malignancies can be observed after ^{131}I treatment for DTC,[77-79] as ^{131}I treatment will lead to a higher mutation frequency. Whether the ^{131}I treatment is the sole causative factor of these secondary malignancies is open to debate.[80] Nonetheless the concerns about the safety of ^{131}I therapy have changed the definite indications for ^{131}I ablation to high-risk patients only.[72]

As was established by ourselves (chapter 5) and by several other researchers,[81-85] pre-ablative scintigraphy with ^{131}I can severely reduce the success of ^{131}I treatment. A meta-analysis indicates that an activity lower than 10 MBq ^{131}I may not influence the efficacy of ^{131}I ablation,[28] but this has yet to be confirmed in patient data. The elapsed time between the diagnostic activity and the administration of ^{131}I therapy may play a role in the occurrence of stunning. Furthermore it may be possible that the use of rhTSH for ^{131}I ablation may influence the occurrence of stunning although informative studies on this subject are still lacking in literature.

If legal requirements or the frequent occurrence of large thyroid remnants should necessitate routine pre-ablative scintigraphy, this is best performed using ^{123}I. There is no consensus on the amount of ^{131}I to be applied for thyroid remnant ablation. A fixed-dosage strategy had better results than an uptake-related protocol in which larger remnants received lower ^{131}I activities (chapter 4). Nonetheless it seems too early to recommend a standardized, high activity of ^{131}I for remnant ablation. There is evidence in literature that there is little difference in efficacy between activities of 1100, 1850 MBq ^{131}I or more.[86] Older data already suggest that it is not so much the administered activity, but the dose of radiation delivered to the thyroid remnant that determines the success of ablation,[87] even though these two are related. The latter study would indicate that dosimetry should best be performed before ^{131}I ablation in all patients in order to observe the ALARA (as low as reasonably achievable) principle. Further studies should be performed in order to determine what the best strategy for ^{131}I ablation is. Currently both in Great Britain (HiLo trial) and France (NCT0043585) prospective randomized trials have been initiated in which ^{131}I ablation with low and high activity are being

compared. On the basis of our clinical experience with the fixed-dosage protocol (chapter 4), for the time being we advocate the application of 3700 MBq ^{131}I for patients with T1-3N0M0 carcinoma, 5550 MBq for those with lymph node metastases or Hürthle-cell carcinoma, and 7400 MBq for those with distant metastasis or extra-thyroidal tumor growth. In elderly patients some caution is advisable, as such high dosages may exceed the tolerated blood dose of 2 Gy.[88]

Follow-up

The follow-up of DTC-patients should be based on proper risk stratification. In compliance with various consensus statements and guidelines,[16-18] the classification of patients as high risk or low risk is based on the initial staging. Risk stratification has to be redefined with regard to recurrence-free survival. After successful ablation, we found no difference in recurrence-free survival between patients with initially high-risk and low-risk profiles (chapter 8). There are considerable differences, both in recurrence-free survival and thyroid cancer-specific survival, between patients with successful and those with unsuccessful ablation (chapter 7). Hence we propose a stratification of low-risk and high-risk patients based on the first TSH-stimulated follow-up. Patients with undetectable Tg levels and negative ^{131}I whole-body scintigraphy (WBS) are classified as low risk; when Tg is positive or ^{131}I WBS is positive (or both), patients are classified as high risk. With regard to WBS the administered amount of ^{131}I does not seem to influence the scintigraphic results;[89] the ALARA principle should probably govern here. An ongoing discussion is the identification of Tg cut-off levels, especially in patients with negative ^{131}I WBS. This problem has become even more prominent with the introduction of highly sensitive Tg tests. It could be argued that any detectable Tg level constitutes a positive Tg measurement. However, in various treatment guidelines and consensus statements a cut-off level of 1 ng/l or 'institutional cut-off values' are recommended (even though Tg levels are not readily comparable between assays).[90,91] Even if Tg levels just above the cut-off value are found it may be appropriate to temporize additional ^{131}I treatment for several years as spontaneous reduction of low levels of serum Tg to undetectable has been observed during the 2-5 years following RAI therapy in 38% of patients followed by Bachelot *et al*,[92] 68% of patients in one series of Pacini *et al*[93] and 26% of patients in the experience of Kloos and Mazzaferri.[94]

For low-risk patients the recent literature[95] and the data presented in this thesis support the policies advocated by the two European consensus statements.[16,17] After successful ablation it is not useful to perform additional TSH-stimulated follow-up in low-risk patients – neither for obtaining ^{131}I WBS, nor for Tg measurement – if TSH

stimulated Tg levels are below institutional cut-off levels and if ultrasound of the neck is negative.[95] Guidelines and consensus statements have indicated ultrasound examination of the neck as the most sensitive procedure in the follow-up of DTC patients. In experienced hands this is indeed a quick and harmless procedure; it should be noted, however, that the recommendation was based on the experiences reported by a few highly specialized experts.[96-98] The high-sensitivity levels reported by these authors may not be attained if the procedure is performed by physicians who are less experienced in cervical ultrasonography.

After successful ^{131}I ablation, the follow-up may be limited to yearly Tg measurements under levothyroxine medication; it goes without saying that in case of iatrogenic hypothyroidism or iatrogenic hypoparathyroidism lifelong specialist care is required. Impediments in the measurement of Tg are positive Tg antibodies or heterophile antibodies. Both types of antibodies interfere significantly with Tg measurements.[90,99-101] The detection of circulating thyroid cancer cells has been proposed as an alternative to Tg measurements. This can be realized by RT-PCR analysis for any thyroid-specific mRNA (usually Tg-mRNA) from the mononuclear layer in centrifuged venous blood samples.[32-36] This technique is still lacking sufficient diagnostic accuracy. In the presence of large amounts of lymphocytes illegitimate transcription may interfere with the detection of Tg-mRNA: the expression of any mRNA transcript in very low levels occurs in every human cell[102] (chapter 12). The separation of thyroid (cancer) cells from blood cells by means of magnetically labeled antibodies might also improve this method.[55-59]

rhTSH

The advent of recombinant human TSH (rhTSH) has simplified the follow-up of patients with differentiated thyroid carcinoma. The withdrawal of thyroid hormones for several weeks is no longer needed. Disabling symptoms of hypothyroidism can be avoided,[103] which enhances the patients' quality of life.[104,105] Through a substantial reduction of loss of labor during each follow-up period, rhTSH pre-treatment may also reduce the societal cost of thyroid cancer follow-up,[106] as patients have lower comorbidity and can return to work sooner. rhTSH has now also been registered for the ablation of thyroid remnants after surgery. Whether rhTSH may influence the occurrence of thyroid remnant stunning is as yet unclear. The drug has not yet been approved as an adjuvant to therapeutic radioiodine treatment of patients with metastatic disease.[107,108]

Several issues concerning the use of rhTSH are unresolved. The initial registration studies have indicated the optimal time point for Tg measurement at 72 hours after

injection of rhTSH;[31] data on the exact time-response curve in large patient groups are lacking. Although rhTSH has by now proven its worth in the follow-up of DTC, large-scale data with long-term follow-up on the efficacy of rhTSH-stimulated ablation are still lacking in literature.

Diagnosis and treatment of advanced disease

In patients with metastatic disease the treatment and follow-up of DTC are more complicated. Often the first sign of recurrent and/or metastatic disease is a rise in serum Tg levels[65] (chapter 8). It is not always possible to find an anatomical substrate to corroborate the suspicion of recurrent malignancy because lesions may be too small, or they may have lost the capacity to concentrate iodine.[109,110] Also ^{18}FDG-PET sometimes fails to demonstrate abnormalities; in such cases imaging with radiolabeled somatostatin analogues may be an alternative.[111]

An alternative to the rather insensitive imaging with diagnostic activities of ^{131}I[112-114] could be ^{124}I, a positron emitter and therefore suitable for PET maging, which is now being evaluated in the follow-up of DTC in various centers around the world. Preliminary data suggest that the extent of lesions may be demonstrated better with ^{124}I than with ^{131}I.[115] Combined PET/CT imaging may be used even more effectively to identify metastases of thyroid carcinoma.[116]

When recurrent and/or metastatic DTC has been identified, the best curative approach is the surgical removal of all cancerous tissue; this may, however, not be feasible in case of multiple metastases or extensive bone metastasis. Repeated high-activity ^{131}I treatment may in any case provide a chance at ablation of (part of) the malignant tissue, provided the tumor has retained its iodine-avid properties. The amount of ^{131}I to be administered in these cases is a matter of some debate. Most investigators apply no more than 7400 MBq (200 mCi) ^{131}I in cases of metastasized DTC, but this may not always be efficacious. Using pre-therapeutic blood-based ^{131}I dosimetry, greater amounts of ^{131}I could probably be administered without excess hematologic toxicity. This method, for which the EANM has recently published a standard operational procedure,[117] is currently being tested in a clinical environment.

Lesion-based dosimetry could be another practicable approach. ^{124}I PET seems to be ideally suited for this purpose;[118,119] when administered simultaneously with ^{131}I it may facilitate dosimetric studies during therapy[120] and normal-organ-based dosimetry.[121]

Iodine-avid thyroid cancer can be treated with high success rates even in patients with distant metastases. Nonetheless, in some patients less differentiated, non-iodine avid local recurrences or metastases are developed. For these patients few (if any) viable

curative treatment options are currently available. Efforts have been made to reverse dedifferentiation and to restore iodine-avidity. Research has focused on drugs belonging to the class of retinoids.[122,123] Thus far, the restoration of iodine uptake in patients who were treated with retinoids was never sufficient to deliver an effective radiation dose to the tumors.[110,122]
Other attempts to restore iodine uptake, such as transfecting thyroid cancer cells with a functioning sodiumiodine symporter gene,[124] have not yet been tested *in vivo*. Recent developments in the identification of treatment targets, however, offer promising perspectives for new treatment options.

Future perspectives

The future of thyroid cancer research may well be in basic science – focusing on genetic alterations and the effect of such changes on cellular function. Advanced knowledge of these processes may provide new targets for new therapies, and the key to better staging and stratification of the disease. Many believe that the theory of the multistep nature of cancer[125] may be the starting point for reaching this goal. According to this theory cancer is not the result of one mutation, but the result of a series of consecutive mutations, each one causing a further shift towards malignant transformation. Hanahan and Weinberg[126] state that all malignant cells in the course of this transformation have to acquire six essential changes in cell physiology:

- insensitivity to anti-growth signals;
- self-sufficiency in growth signals;
- evasion of apoptosis;
- sustained angiogenesis;
- limitless replicative potential;
- tissue invasion and metastasis.

The order of acquisition of these traits may differ between different types of cancer. For medullary thyroid carcinoma the mechanism and order of occurrence have been more or less elucidated,[127] but the picture of multistep carcinogenesis is not yet clear for DTC. The existence of multistep carcinogenesis in DTC may be deduced from the fact that both RAS-, PAX8-PPARγ1 and RET-PTC genetic alterations are found both in benign thyroid adenomas and in thyroid carcinomas.[128,129] In a multistep carcinogenesis of thyroid carcinoma the self-sufficiency in growth signals seems to be the initiating step.
PTC and FTC are caused by different mutations. PTC is caused mainly by genetic alterations involving the BRAF and RET genes (chromosomal rearrangements in RET

are called RET/PTC).[130] Although there are many different RET/PTC rearrangements which each represent fusion of RET with entirely different genes, there is no record of differences in clinical behavior between the various rearrangements;[130,131] in contrast BRAF mutations are associated with more aggressive disease.[132,133] The frequency of RET/PTC rearrangements occurring in adult PTC patients without prior childhood neck irradiation varies between 2.5% and 35%. In these tumors, the frequencies of RET/PTC1 and RET/PTC3 were similar and that of RET/PTC2 was lower. The RET/PTC rearrangements were more frequently found (in 60% to 80% of cases) in PTC cases occurring either in children even in the absence of radiation exposure or in subjects of any age after radiation exposure during childhood, either external irradiation or contamination after the Chernobyl accident.[134,135] RET/PTC3 was more frequently found in aggressive tumors that occurred early after the accident and RET/PTC1 in less aggressive tumors that occurred later. The finding of RET/PTC rearrangement in micropapillary thyroid carcinomas suggests that it constitutes an early event in thyroid carcinogenesis.

Several additional oncogenes may occasionally be involved in PTC, including NTRK1 (also named TRKA), which codes for a neural growth factor receptor with a tyrosine kinase domain and which is activated by rearrangement in about 10% of PTCs. An activating point mutation of the RAS genes is found in about 10% of PTCs, mostly in the follicular variant.[136] More recently, a single activating point mutation of the BRAF gene at codon 600 has been found in 40% (range, 29% to 69%) of PTCs occurring in adults in the absence of neck exposure to radiation during childhood.[137] Its presence did not overlap with RET/PTC and it was rarely found in PTC occurring in children or following neck exposure to radiation.[138] BRAF mutation is more frequently found in aggressive PTC variants, but it has not been found in other thyroid tumor types. Finally, an intrachromosomal rearrangement of the BRAF gene with the AKAP9 gene has recently been found in PTC occurring after the Chernobyl accident.[139]

In PTC mutations in the RAS gene are sometimes encountered.[140] In patients with FTC PAX8-PPARγ1 rearrangements or RAS mutations often play a role.

There is evidence that (at least in the tumor igenesis of PTC) the RET/PTC, RAS and BRAF genes lead to similar downstream activation[141] although differences in downstream effects of RET/PTC and RAF mutations have also been documented. Pathways involved in the tumor igenesis of FTC remain to be elucidated further; as RAS-mutations are involved in at least part of FTC cases,[128,142] it is possible that FTC and PTC share common pathways. On the other hand, RAS-mediated activation of the phosphatidylinositol 3-kinase (PI3K) pathway and mutations in PI3K have also been implicated[143] in the pathogenesis of especially advanced thyroid carcinoma.

Methylation of PTEN, which is a tumor suppressor gene acting downstream of PI3K, is also observed in advanced thyroid carcinoma. PPARγ stimulates PTEN activity. Downregulation of PPAR gamma, as is the case in the PAX8-PPARγ1 rearrangement in FTC therefore indirectly activates the PI3K signal transduction route.[144,145]

There is some evidence that the various genetic alterations involved in PTC and FTC are associated with different degrees of tumor aggressiveness, even if they are working along the same pathway.[128,132,133] It was learned from animal experiments that constitutive activation of this pathway either by RET/PTC mutation or BRAF mutation is the initiating step.[146,147] Via MEK and ERK (the so called MAPK signalling cascade), mutations in these genes induce a continuous upregulation of the transcription of genes involved in cell differentiation, proliferation, and survival (see also figure 14.2).

This is an indication that the self-sufficiency in growth signals is the initial step in the multistep carcinogenesis for DTC; which of the other six steps follows is still unclear. For medullary thyroid carcinoma (which is derived from the thyroid's c-cells, and is also caused by RET mutations) this mechanism has been elucidated further. It is likely that DTC follows the same path. It has been postulated that the next step in MTC genesis is insensitivity to growth-inhibitory signals, caused (among others) by p18 mutations.[127] For DTC no indications have been found yet that mutations in this gene play a key role. To elicit mitogenesis in MTC, the simultaneous downregulation of p18 and p27kip is mandatory.[148] Lymph node metastases of PTC demonstrated a decreased p27kip expression;[149] possibly, the simultaneous downregulation of p18 may also have to take place.

As stated before, the sequence of carcinogenesis is unclear. It seems that later in the carcinogenesis of thyroid tumors p53 mutations may occur; they are strongly associated with anaplastic thyroid carcinomas,[150] and therefore convey a poor prognosis.[151]

These genetic changes mentioned before are also reflected in the expression of various mRNA transcripts in the cell. When compared to normal thyrocytes, changes in mRNA or microRNA expression profiles may be indicative of aggressive behavior. Tg-mRNA is downregulated and ets1 (a transcription factor involved in signal transduction, cell cycle progression, and differentiation) is upregulated[152] in differentiated thyroid carcinoma cells. Regions of PTC invading other tissues overexpress TGF-beta and NFKB, as well as Vimentin, which was also associated with nodal metastases.[153] Combinations of a limited number of such transcripts may facilitate the identification of DTC.[154]

Based on the knowledge of the mechanisms along which thyroid carcinomas develop, new drugs could be developed with are specifically targeted (mostly meaning: block)

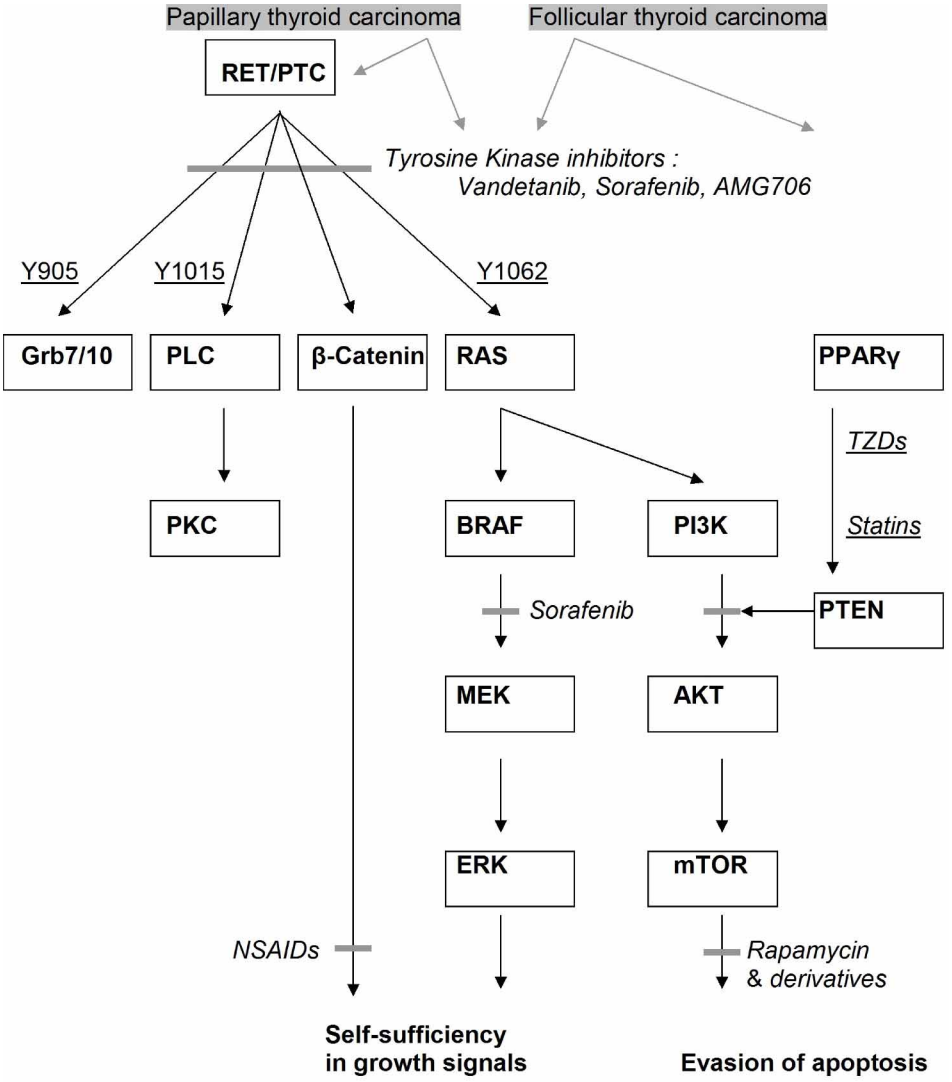

Figure 14.2 Putative pathways of tumor igenesis and possible medical interventions in differentiated thyroid carcinoma. Adapted from Fagin,[130] Riesco-Eizaguirre and Santisteban,[161] Gujral *et al*,[162] van Veelen[127] and Lips CJM (personal communication).

ERK: Indicates a step activated in a pathway
Y1062: Tyrosine phosphorylation site inducing the specific pathway
— Sorafenib: Indicates that the compound named blocks the specific step in the pathway
Rosiglitazone: Indicates that the compound stimulates the specific pathway

at a certain step in the cascade leading to tumor igenesis. In the case of thyroid carcinoma the target could be a specific step in the MAPK pathway, or a specific mutation. Some tumor targeted therapies are already being tested. In an *in vitro* experiment cells with a BRAF(V600E) mutation appeared to be sensitive to several MEK inhibitors,[155] whereas cells without the mutation were not. Some novel drugs belonging to the class of tyrosine kinase inhibitors preferentially target RET.[156] Some of these tyrosine kinase inhibitors have already been used with some success in small groups of patients with advanced thyroid carcinoma.[157] Other drugs, the so called multi-kinase-inhibitors, hit multiple targets at the same time.[158] In PTC with activating RET/PTC rearrangements one might try and influence β-Catenin – which in MTC is directly stimulated by RET, and increases the transcription of genes. Aspirin and other NSAIDs increase the degradation of β-Catenin.[159]

For follicular neoplasms inhibition of the PI3K pathway seems feasible; *in vitro* studies have suggested that rapamycin and its derivatives (a class of potent immunosuppressants) such as everolimus do so. Therefore this class of drugs warrants further investigation in the treatment of follicular, and possibly also less-differentiated, thyroid carcinomas. For FTC an alternative approach would be to stimulate the PParγ-mediated PTEN expression; through inhibition of the PI3K-pathway, both the anti-diabetic drugs belonging to the class of thiazolidinediones (TZDs) and several cholesterol-lowering statins stimulate the PParγ-mediated PTEN expression.[145,160] In figure 14.2 we present an overview of these potential drugs and of their mechanisms of action in the signaling cascades involved in thyroid carcinogenesis.

An outlook on the future differentiated thyroid carcinoma treatment.

Most of what was written in this chapter has yet to be tested clinically. However, it seems likely that we shall soon see a radical change in the treatment of DTC, where treatment will be both patient-tailored and tumor-specified.

A patient presenting with a thyroid nodule may undergo an ultrasound-guided fine-needle aspiration biopsy from a thyroid nodule or an enlarged lymph node. Not only an expression profile (though whether this will concern an mRNA, micro-RNA or protein expression profile still remains an open question) will be determined from the cells in the FNAB specimen, but also the mutation involved. Based on the expression profile and the causative mutation, a diagnosis of benign adenoma or thyroid cancer will be made. In case of a malignancy the risk of metastases will be determined and proper treatment can be provided. Possibly in the elderly low-risk patients one tablet that attacks the causative mutation may suffice. Probably in a young patient with confirmed metastases surgery and ^{131}I ablation will still retain their roles, possibly

accompanied by a course of drugs specifically targeting the causative mutation or pathway.

Today, most patients with differentiated thyroid carcinoma have an excellent prognosis. If we are willing to commit ourselves to a better understanding of what this disease is really about, maybe tomorrow the same can be said for all.

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Summary

In **chapter 1** the aims of this thesis have been outlined.

In **chapter 2** an overview is presented of the literature involving the treatment and follow-up of differentiated thyroid carcinoma, outlining what is presently known about the functioning of the normal thyroid, and what the dilemmas are in the state-of-the-art radioiodine treatment for DTC.

Chapter 3 emphasizes the relation between tumor size and the risk of multifocal carcinoma, locally invasive disease and lymph node or distant metastases. There were no significant differences in tumor size adjusted cumulative risk of multifocal carcinoma or distant metastases between PTC and FTC, but PTC showed a higher cumulative risk of lymph node metastases and extra-thyroidal tumor growth. It was established that tumor multifocality increased with a cumulative risk of 5 percent per cm of tumor diameter, whereas the increase in cumulative risk showed an exponential trend with regard to locally invasive disease, nodular and distant metastases. The anchoring point on the x-axis of the curve for the risk of extra-thyroidal growth, lymph node metastases in PTC and distant metastases was located at a threshold tumor diameter of 10 mm. Interestingly a non-trivial percentage of FTC patients also developed lymph node metastases, but these were caused almost exclusively by follicular tumors that already showed extra-thyroidal growth.

In **chapter 4** two ablation protocols were compared. In the first protocol the activity of ^{131}I was determined according to an algorithm including thyroid remnant size as determined by pre-ablative diagnostic scintigraphy, with activities inversely related to remnant size. In the second protocol, the activity of ^{131}I was determined by the prognostic characteristics of the primary tumor at the time of diagnosis: a higher activity was administered in high-risk patients. It was shown that the second protocol (success rate of ablation: 56%) was more effective for ^{131}I ablation of thyroid remnants and/or residual disease than the first (success rate of ablation: 43%), but only for patients with low-risk tumors. In patients with high-risk tumors (T4 and/or N1) no significant differences could be shown between the two protocols.

Chapter 5 aimed to determine whether pre-therapeutic dosimetry with 40 MBq ^{131}I influenced the radioiodine ablation success rate. Success of ablation was defined as

negative TSH-stimulated Tg levels and absent visual uptake on whole-body scintigraphy. In a group of patients who did not receive 40 MBq ^{131}I the success rate of ablation was 65%. Another group from a second hospital (using the same ablation protocol, but administering 40 MBq ^{131}I before ablation) had a success rate of 33%. As there were no significant differences in baseline characteristics between the two groups, stunning of thyroid remnants is the most likely cause of this difference in success rate of ablation.

In **chapter 6** the prognosis of patients with lymph node metastases at the time of diagnosis of papillary thyroid carcinoma was examined. It was found that lymph node metastases are a prognostic factor for unfavorable outcome: after a median follow-up of 7 years, two-thirds of all patients with lymph node metastases at the time of primary therapy were still not free of disease, irrespective of the extent of primary surgery. A worse prognosis was found in patients who were presented with clinically overt lymph node metastases than in patients in whom lymph node metastases were discovered during surgery and subsequent pathological examination.

In **chapter 7** the determinants of successful ^{131}I ablation of thyroid remnants and the prognostic significance of successful ablation were investigated. The presence of lymph node or distant metastases at the time of diagnosis appeared to be determinants of successful ablation. Successful ablation was defined as serum thyroglobulin levels being below the detection limit in combination with a negative diagnostic ^{131}I whole-body scintigraphy during TSH stimulation. Both the disease-free survival and the thyroid cancer-specific survival were longer after successful ablation than in patients who had no successful ablation.

In **chapter 8** the course of the disease in DTC patients after successful ablation was further examined in a larger group of 526 patients from three university thyroid clinics. The long-term disease-free survival was 96.6 percent, and no recurrences occurred more than 5 years after initial treatment. After curation, no difference in disease-free survival was found between patients who had initially been staged as low-risk and those who had initially been staged as high-risk. Once ablation was successful, further ^{131}I scintigraphy did not assist in revealing recurrent disease; as a consequence it can be omitted.

In **chapter 9** the difference in prognosis between papillary and follicular thyroid carcinoma patients was examined. It was found that even though there was a con-

siderable difference in thyroid cancer-specific survival between papillary and follicular thyroid carcinoma patients, this is due to the more advanced disease and more advanced age (well-established adverse prognostic factors) at the time of presentation for follicular thyroid cancer patients. After correction for these differences, disease-specific survival was comparable for papillary and follicular thyroid carcinoma. The implication may be that the initial treatment of papillary and follicular thyroid carcinoma should be similar in all stages, including tumors that are unifocal and < 1 cm in diameter at the time of diagnosis.

In **chapter 10** fifteen prognostic scoring systems for differentiated thyroid carcinoma were analyzed. Seven of these prognostic scoring systems, all of which had been developed and/or validated for both PTC and FTC and were applicable to our patient population, were studied and compared using three different methods: Kaplan-Meier survival estimation with log rank analysis, Cox regression, and a comparison of the proportion of variance explained. In all analyses the TNM system (version 5) best predicted the prognosis of our patients with differentiated thyroid carcinoma.

In **chapters 11, 12**, and **13** several aspects of serum thyroglobulin (Tg) measurements in the follow-up of DTC were examined. In **chapter 11** the measurement of Tg after stimulation with recombinant human TSH (rhTSH) was examined. The manufacturer's recommendation to measure Tg 72 hours after the last injection of rhTSH was supported by our data. Tg measurement 24 hours after rhTSH did not provide clinically relevant information; neither did concurrent ^{131}I scintigraphy. However, given the many pre-analytical and analytical determinants influencing the accuracy of Tg measurements, alternatives are being sought. In the past decade an important candidate marker for tumor presence has been the determination of Tg-mRNA as a means of detecting circulating thyroid cancer cells. In **chapter 12** the literature was studied and a meta-analysis was performed. We concluded that the diagnostic accuracy of Tg-mRNA is not yet sufficient for clinical use. The main problem seemed to be the illegitimate transcription of Tg-mRNA occurring in white blood cells. As circulating thyroid cancer cells are mixed into the white blood cells, it is often impossible to distinguish between samples with or without circulating thyroid cancer cells.

In **chapter 13** one of the analytical problems interfering with the accuracy of Tg measurements was studied. The presence of heterophile antibodies, which include but are not limited to human anti-mouse-antibodies, may cause inaccurately high or low results of Tg or Tg antibody measurements. In a group of 100 DTC patients, we

found interference from heterophile antibodies in nearly 40% of patients. This interference would have led to a change of management in 11% of all patients.

In **chapter 14** the implications of the various findings in this thesis are discussed. We propose that patients should not be characterized as low-risk or high-risk before ^{131}I ablation, but rather after the first TSH-stimulated follow-up. After successful first ablative ^{131}I treatment (defined as a negative whole-body scintigraphy in combination with undetectable Tg levels at the first TSH-stimulated follow-up) further TSH-stimulation for follow-up Tg measurements is not essential. Furthermore, an oversight of the molecular pathogenesis of thyroid carcinoma was given, as further research in the field of differentiated thyroid carcinoma shall rely heavily on knowledge of these genetics, genomics and proteomics.

Samenvatting

Het gedifferentieerde schildkliercarcinoom is de meest voorkomende vorm van kanker van het hormoonstelsel. Er zijn twee vormen van gedifferentieerd schildkliercarcinoom: papillair schildkliercarcinoom en folliculair schildkliercarcinoom. De behandeling bestaat in het algemeen uit het operatief verwijderen van (een deel van) de schildklier (en wanneer van toepassing ook van lymfeklieren in de hals). Omdat het bijna nooit mogelijk is de gehele schildklier te verwijderen zonder de zenuwen van de stembanden onherstelbaar te beschadigen, volgt meestal nog een behandeling met radioactief jodium (^{131}I), de zogenoemde ablatie. Daarna moet een patiënt zijn leven lang nazorg ondergaan, want schildklierkanker kan zelfs na 40 jaar nog terugkomen. In dit proefschrift werd een aantal aspecten van de eerste behandeling en de nazorg van het gedifferentieerde schildkliercarcinoom onderzocht.

In **hoofdstuk 1** worden de vraagstellingen vermeld die in dit proefschrift aan bod komen.

In **hoofdstuk 2** wordt een overzicht gegeven van de resultaten van het onderzoek naar gedifferentieerd schildkliercarcinoom tot nu toe.

In **hoofdstuk 3** wordt gekeken of een groter schildkliercarcinoom een hoger risico geeft op het hebben van uitzaaiingen binnen de schildklier, naar de lymfeklieren in de hals, en naar andere plaatsen in het lichaam. Het blijkt dat bij gelijke grootte een papillair schildkliercarcinoom een hoger risico heeft op uitzaaiingen naar de lymfeklieren dan een folliculair schildkliercarcinoom, maar dat het risico op uitzaaiingen binnen de schildklier en elders in het lichaam gelijk is. Het risico op uitzaaiingen binnen de schildklier neemt per centimeter tumordoorsnee met ongeveer 5% toe. Het risico op invasie van weefsel rondom de schildklier, uitzaaiingen naar de lymfeklieren en naar andere plaatsen in het lichaam neemt met toenemende tumordoorsnede exponentieel toe, vanaf een tumordoorsnee van ongeveer 1 cm.

In **hoofdstuk 4** zijn twee protocollen voor ^{131}I ablatie met elkaar vergeleken. In het ene protocol krijgen patiënten met grotere schildklierresten een lagere dosis om eventuele bijwerkingen van de ^{131}I-ablatie te voorkomen. In het andere protocol krijgen patiënten met een verder voortgeschreden ziekte (onafhankelijk van de grootte van de schildklierrest) een hogere dosis. Het blijkt dat het laatste protocol (56% succes) een hoger succespercentage heeft dan het eerste (43% succes), en dus beter is. Dit

hogere succespercentage kan echter alleen worden aangetoond bij patiënten zonder invasie van omliggende weefsels of uitzaaiingen naar de lymfeklieren. Is van één of beide van deze kenmerken sprake, dan kan geen verschil in succes worden aangetoond tussen beide behandelprotocollen.

In **hoofdstuk 5** wordt gekeken of het geven van een kleine diagnostische dosis van 40 megabecquerel ^{131}I (die een dag vóór de ablatie wordt toegediend om de grootte van de schildklierrest te bepalen) invloed heeft op het succes van de behandeling. Om dit te bepalen werden de behandelresultaten van het Universitair Medisch Centrum Utrecht (waar voor de ablatie geen diagnostische dosis werd gegeven) vergeleken met die van het Leids Universitatair Medisch Centrum (hier kregen patiënten 40 MBq ^{131}I als diagnostische dosis voor de ablatie). Het succespercentage in het Universitair Medisch Centrum Utrecht was 65%; het succespercentage in het Leids Universitair Medisch Centrum was 33%. Omdat de patiëntengroepen van de twee centra geen statistisch significante verschillen in kenmerken van patiënten vertoonden, wordt het verschil in succespercentage waarschijnlijk veroorzaakt door een negatief effect van de diagnostische dosis op de jodiumopname door de schildkliercellen. Het wordt dan ook aangeraden geen diagnostische dosis ^{131}I te geven voorafgaand aan de ^{131}I ablatie bij patiënten met een gedifferentieerd schildkliercarcinoom.

In **hoofdstuk 6** wordt gekeken naar het ziekteverloop bij patiënten die uitzaaiingen naar de lymfeklieren hebben ten tijde van de diagnose van het gedifferentieerde schildkliercarcinoom. Zelfs lang na de eerste behandeling is ongeveer tweederde van deze patiënten nog niet ziektevrij. Het blijkt dat patiënten bij wie uitzaaiing naar de lymfeklieren het eerste symptoom van de ziekte was een kleinere kans hebben om ziektevrij te worden dan patiënten bij wie de lymfekliermetastasen pas tijdens of na de operatie worden ontdekt. Bij patiënten die na de eerste behandeling nog lymfeklieruitzaaiingen hebben, kan alleen een adequate chirurgische behandeling ervoor zorgen dat patiënten alsnog ziektevrij worden.

In **hoofdstuk 7** wordt de betekenis van een succesvolle ^{131}I-ablatie onderzocht. Het blijkt dat patiënten met een succesvolle ablatie een betere prognose hebben (ze leven langer en de ziekte komt minder vaak terug) dan mensen bij wie de eerste ^{131}I-behandeling niet slaagt.

In **hoofdstuk 8** wordt het resultaat van hoofstuk 7 in een veel grotere groep van 526 patiënten uit 3 ziekenhuizen bevestigd. Bij al deze patiënten was de eerste ^{131}I-

ablatiebehandeling succesvol. Het ziektevrije overleven in deze groep bedroeg op lange termijn 96,6%. In dit ziektevrije overleven bestond er geen verschil tussen patiënten die oorspronkelijk als 'hoog-risico' ingeschat werden, en 'laag-risico' patiënten. Het blijkt tevens dat, indien bij de eerste nazorg het totaallichaamsscintigram geen ^{131}I opname laat zien, het zinloos is deze procedure te herhalen omdat verdere scintigrammen geen nieuwe informatie opleveren.

In **hoofdstuk 9** wordt het verschil in prognose tussen papillair en folliculair schildkliercarcinoom onderzocht. Patiënten met een folliculair schildkliercarcinoom hebben een slechtere prognose dan patiënten met een papillair schildkliercarcinoom. Dit komt omdat folliculaire schildkliercarcinomen vaak pas in een verder gevorderd stadium worden ontdekt. Als alleen gekeken wordt naar papillaire en folliculaire schildkliercarcinomen die bij diagnose in hetzelfde ziektestadium verkeren, is er namelijk geen verschil in prognose tussen de twee soorten schildkliercarcinoom. Daarbij doet zich de vraag voor of de huidige verschillen in behandeling tussen papillair en folliculair schildkliercarcinoom, vooral bij tumoren kleiner dan 1 cm, gerechtvaardigd zijn.

In **hoofdstuk 10** worden de vele prognostische systemen voor gedifferentieerd schildkliercarcinoom met elkaar vergeleken. Het TNM-systeem (versie 5) bleek het krachtigst voorspellende systeem te zijn.

In **hoofdstuk 11** wordt gekeken naar de controles met behulp van recombinant humaan TSH (rhTSH). Vooral wordt gekeken of de tumormarker thyreoglobuline frequenter dan alleen op het aanbevolen tijdpunt van 3 dagen na injectie van rhTSH zou moeten worden gemeten, en of ^{131}I-totaallichaamsscintigrafie zinnig is. De conclusie van het onderzoek was dat een meting van thyreoglobuline 72 uur na injectie van rhTSH voldoende was, en dat ^{131}I-scintigrafie relatief veel fout-positieve resultaten opleverde.

In **hoofdstuk 12** wordt gekeken naar een alternatief voor de meting van thyreoglobuline, namelijk het meten van thyreoglobuline-mRNA in bloed. Alle op dit moment bekende wetenschappelijke onderzoeken op dit gebied werden bekeken, en uiteindelijk luidde de conclusie dat de methode nog niet bruikbaar was voor de nazorg van patiënten met een gedifferentieerd schildkliercarcinoom.

In **hoofdstuk 13** werd de invloed bepaald van heterofiele antilichaampjes (dit zijn antilichaampjes tegen materiaal van schapen, geiten, konijnen, muizen, etc. – materi-

aal van al deze dieren wordt met enige regelmaat gebruikt bij de behandeling van patiënten) op de meting van thyreoglobuline bepaald. In dit onderzoek werden monsters van 100 patiënten behandeld in buisjes gevuld met een middel dat deze heterofiele antilichaampjes blokkeert. Het bleek dat in bijna 40% van de schildkliercarcinoompatiënten, maar in geen van de gezonde controlepersonen, er een invloed van deze heterofiele antilichaampjes te vinden was; bij 11% van de patiënten zou blokkade van de heterofiele antilichaampjes tot een andere behandeling geleid hebben.

In **hoofdstuk 14** worden de bevindingen van bovenstaand onderzoek en de implicaties daarvan voor de behandeling van patiënten met een gedifferentieerd schildkliercarcinoom bediscussieerd. De belangrijkste aanbevelingen zijn dat patiënten met een gedifferentieerd schildkliercarcinoom pas na het evalueren van de eerste ^{131}I-behandeling als hoog-risico of laag-risico kunnen worden geclassificeerd, en dat wanneer de eerste ^{131}I-behandeling een succes blijkt, tijdens TSH-gestimuleerde follow-up een verdere follow-up geen verdere klinisch relevante informatie oplevert en dus niet zinnig is. Verder wordt een overzicht gegeven van het belang van de laatste ontwikkelingen rondom het gedifferentieerde schildkliercarcinoom in de moleculaire wetenschappen en worden lijnen uitgezet voor toekomstige research. Het is waarschijnlijk dat onderzoek in de toekomst onmogelijk zal zijn zonder gebruik te maken van de wetenschappen die mutaties in het genetische materiaal van cellen, de veranderingen in het tot uitdrukking komen van genen en de veranderingen in de aanmaak van eiwitten in kankercellen bestuderen.

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Curriculum Vitae

Erik Verburg was born on March 1, 1980 in Breda, the Netherlands. After obtaining his Gymnasium diploma in 1997 at the Sint Oelbert Gymnasium in Oosterhout (NB), The Netherlands, he commenced his medical studies at the Katholieke Universiteit Leuven in Belgium. After a year he continued his studies at Utrecht University in Utrecht, The Netherlands, where he obtained his medical degree in 2004. During his studies in Utrecht at the end of 2000 he started the research program which eventually culminated in this thesis.

After his graduation from medical school he worked as a physician in the emergency room of the Amphia Ziekenhuis in Breda and Oosterhout (NB), The Netherlands, after which – in June 2005 – he took on the position of research physician at the departments of nuclear medicine of the UMC Utrecht and the St. Antonius Ziekenhuis in Nieuwegein, The Netherlands.

Next to the project described in this thesis he also spent part of his time working on a project in the field of nuclear cardiology. During this period contacts were made with the department of nuclear medicine of the Universitätsklinikum Würzburg, Germany, where he commenced his training as a nuclear medicine physician on March 1, 2007, next to continuing his research in the field of differentiated thyroid carcinoma.