Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

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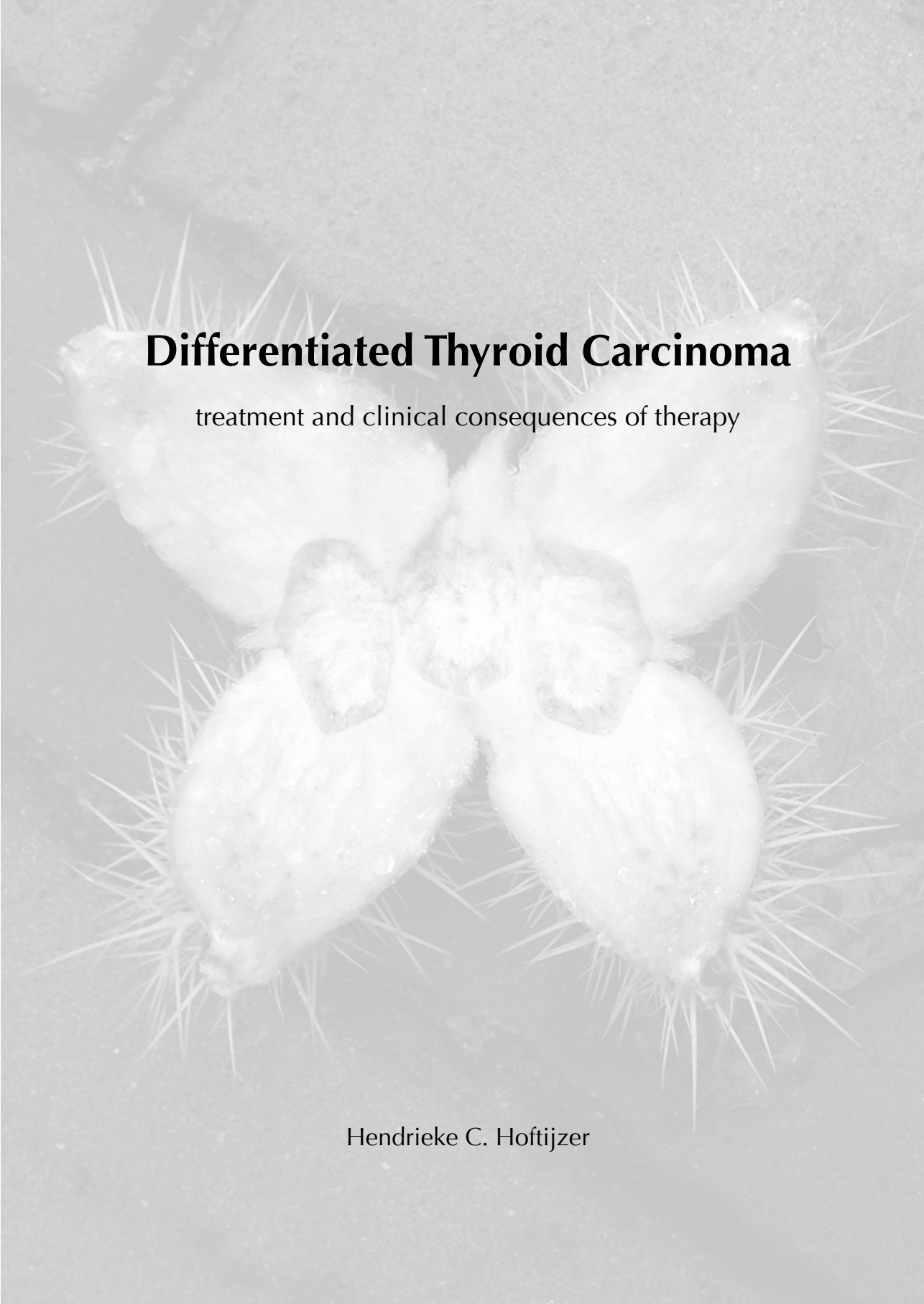

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Hendrieke C. Hoftijzer

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1

General introduction

Parts of this chapter will be published in Clinical Investigation volume 1, Issue 2 as the paper: Tyrosine kinase inhibitors in differentiated thyroid carcinoma: review of the clinical evidence

Hendrieke C. Hoftijzer, Ellen Kapiteijn, Tatiana Schneider, Guido C. Hovens, Hans Morreau, Hans Gelderblom, Johannes W.A. Smit

Contents

I. Introduction

Thyroid cancer is the most common endocrine malignancy; however it is still a low prevalent disease with an incidence of 2-10/100.000 persons per year. The incidence has increased during the last decades, and this trend appears to be continuing (1-3). In the Netherlands this represents approximately 320 new patients per year, with a 3 times higher incidence in women (4). However, the prevalence is higher because of a rather good prognosis (4).

Thyroid cancer is a heterogeneous disease which is classified into differentiated thyroid carcinoma (DTC), medullary thyroid carcinoma (MTC) and undifferentiated (anaplastic) thyroid carcinoma (ATC). Differentiated thyroid carcinoma is most common (95%) and includes papillary (80%), follicular (10-15%) and subtypes follicular variant of papillary thyroid carcinoma and Hürthle cell carcinoma. Mean age at diagnosis is between 45 and 50 year (5). The overall 10-year survival rates are about 90-95% (6). This is because of a combination of favorable biological (i.e. indolent) behavior of the tumor as well as the availability of effective therapy consisting of near-total thyroidectomy followed by radioactive iodine-131 (RaI) ablative therapy.

Once distant metastases have occurred, the prognosis of DTC becomes worse. This is the result of dedifferentiation of thyroid cancer cells. The subsequent loss of the ability to accumulate RaI leads to unresponsiveness to the only curative treatment option. Metastases are not immediately life threatening, but may impair quality of life considerably. In this situation, only palliative treatment options remain and include external beam radiation therapy or resection of symptomatic metastasis (7,8). Recently, increasing knowledge in tumor biology has led to the identification of potential targets and novel treatment options with tyrosine kinase inhibitors, including sorafenib and sunitinib (9,10). These multikinase inhibitors show promising results in patients with progressive metastatic DTC, refractory for RaI.

During routine follow-up for differentiated thyroid carcinoma patients are regularly withdrawn from thyroxine therapy to perform TSH stimulated whole body RaI scanning and measurement of thyroglobulin, a disease specific biomarker. This creates a state of controlled hypothyroidism. This and the fact that DTC patients are treated with TSH suppressive thyroxine replacement therapy makes DTC patients an interesting model to study the metabolic effects of thyroid hormone. There is no interference by endogenous thyroid hormone, because patients are treated with total thyroidectomy. Moreover there is no interfering effect from thyroid disease, like in patients treated with thyroxine for autoimmune thyroid disease.

In this introduction a general overview of DTC, the diagnosis, treatment and clinical consequences of therapy will be provided and the questions addressed in this thesis will be introduced.

II. Differentiated thyroid carcinoma

1. Pathogenesis

Human thyroid tumors originate from epithelial follicular cells or from parafollicular C-cells. Follicular cell-derived tumors represent benign adenomas, differentiated (follicular and papillary) and undifferentiated (anaplastic) carcinomas. Genetic alterations are involved in the pathogenesis of thyroid carcinoma. The identification of these alterations is important for the diagnosis of DTC and also for the understanding of the pathophysiology of thyroid disorders (11-17). Recent developments have provided a detailed map of these genetic alterations (**Figure 1**).

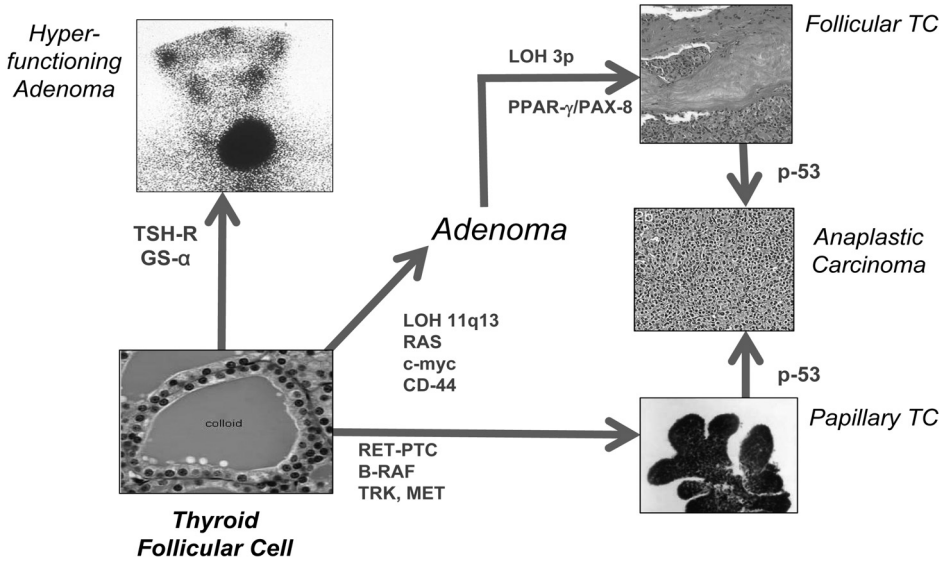

Figure 1
Pathogenesis of differentiated thyroid tumors

In DTC, genetic alterations that have been identified, and involve tyrosine kinase signaling pathways (12,15,16). In nearly all cases of papillary thyroid carcinoma (PTC), genetic defects involving the RET, RAS, and RAF protein kinase signaling cascade are identified. The BRAFV600E mutation has been found in 29–69% of PTC and has been associated with aggressive features including extrathyroidal extension and advanced stage (18). Translocations of RET observed in PTC result in a chimeric protein consisting of an activated RET tyrosine kinase domain (12,17,19-30).

Follicular thyroid carcinomas (FTC) frequently harbor mutations in one of the three RAS genes. The RET–RAS–RAF pathway is interconnected with the epithelial

growth factor receptor (EFGR) activated cascade that among others leads to vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) synthesis. Therefore, compounds targeting the activated RET–RAS–RAF pathway and beyond may be effective in non-RaI avid DTC. Other genetic alterations in FTC include PAX8-PPAR-gamma rearrangement. This is a unique combination of genes that traditionally are associated with thyroid development (PAX-8) and cell differentiation and metabolism (PPAR-gamma) (13, 31). This chimeric protein acts as a dominant negative competitor for PPAR-gamma and is associated with more aggressive growth and propensity for invasion (31). The follicular variant of PTC (FVPTC) shares some of the molecular features of follicular tumors, but less common also BRAF mutations are reported (11). Anaplastic carcinomas are frequently associated with mutations of the p-53 tumor suppressor gene (32).

The genetic alterations involved in the pathogenesis of DTC result in loss of thyroid specific gene expression and loss of thyroid specific protein expression. Thyroid peroxidase (TPO), which liberates iodide for addition onto tyrosine residues on thyroglobulin for the production of thyroxine (T_4) or triiodothyronine (T_3) is thought to diminish in an early phase. The Sodium-Iodide-Symporter (NIS) expression is subsequently lost. Herewith, the thyroid cell loses its ability to accumulate iodide, and thus becomes refractory for RaI therapy.

2. Diagnosis

Most differentiated thyroid carcinomas present as asymptomatic thyroid nodules. Carcinoma should be suspected if a hard irregular thyroid nodule is found, ipsilateral lymph nodes are enlarged and/or there is a history of progressive increase of the size of the nodule (5). Despite increasing standards of imaging techniques like ultrasound, fine needle aspiration (FNA) is still the procedure of choice in patients presenting with a thyroid nodule (33). The sensitivity of FNA for DTC is 90-95%. The specificity is lower (60-80%) when all patients with a non-benign FNA are referred for surgery (34). It is difficult to make the distinction between benign and malignant follicular tumors by FNA, since the essential criterion for FTC is capsular invasion, which cannot be determined by cytology. In addition, the distinction between a follicular adenoma and a follicular variant of a papillary thyroid carcinoma (FVPTC) is also difficult, because the crucial criterion here is the aspect of the nuclei. The consequence is that 70-80% of the patients with suspicious results from FNA, who undergo a hemithyroidectomy, have a benign tumor (35). The use of molecular markers (e.g. BRAF, RAS, RET/PTC, PAX8-PPARgamma or galectin-3) may be considered for patients with indeterminate cytology on FNA to help guide management (35-42).

After hemithyroidectomy the microscopical distinction between benign and malignant neoplastic thyroid nodules by conventional histology remains difficult as these lesions may share overlapping histological characteristics. Therefore, it is important to identify new markers to distinguish benign from malignant thyroid tumors. In recent years, several immunohistochemical markers have been studied to improve the differential diagnosis of thyroid lesions, using both candidate markers and unbiased approaches (43-54).

The expression of retinoid receptors may be interesting for the differentiation between benign and malignant thyroid tissues. Retinoids are important for growth, differentiation, and morphogenesis in vertebrates (55). Retinoids are derivatives of vitamin A (i.e. retinol). Retinoid receptors belong to the family of nuclear receptors and can be distinguished in retinoic acid receptors (RAR) and retinoid X receptors (RXR). According to the literature, retinoid receptors appear to be differentially expressed in benign and malignant thyroid tissues, the general picture being decreased expression of retinoid receptor subtypes in thyroid cancer (56-62), which may also have therapeutic implications (7,57,61,63-65) **(Chapter 2)**.

After primary diagnosis, the Tumor-Node-Metastases classification system (**Table 1 and Table 2**) is used for staging of the disease. This system is based on pathologic findings and divides patients into four stages (**Table 3 and Table 4**), with progressively poorer diagnosis and survival with increasing stage.

In studies in this thesis, the 5th edition of the TNM classification system is used. Recently, the 6th edition has become available (66). The most important difference with the 5th edition is the fact that the dimension of T1 has been extended to 2 cm and that tumors with limited extrathyroidal extension are designated T3 instead of T4, which

Table 1: TNM Classification system 5th edition

T0	No evidence of primary tumor
T1	Tumor 1 cm or less in greatest dimension limited to the thyroid
T2	Tumor > 1 cm, but not more than 4 cm in greatest dimension, limited to the thyroid
T3	Tumor more than 4 cm in greatest dimension limited to the thyroid
T4	Tumor of any size extending beyond the thyroid capsule
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases
N1a	Metastasis in ipsilateral cervical lymph node(s)
N1b	Metastasis in bilateral, midline, or contralateral cervical or mediastinal lymph node(s)
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Table 2: TNM classification 6th edition

T0	No evidence of primary tumor
T1a	Tumor 1 cm or less
T1b	Tumor more than 1 cm but not more than 2 cm
T2	Tumor > 2 cm, but not more than 4 cm, in greatest dimension limited to the thyroid
T3	Tumor > 4 cm in greatest dimension limited to the thyroid or any tumor with minimal extrathyroidal extension
T4a	Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
T4b	Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels. All anaplastic carcinomas are considered T4 tumors
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Regional lymph node metastases
N1a	Metastasis to Level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)
N1b	Metastasis to unilateral, bilateral, or contralateral cervical or superior mediastinal lymph nodes
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Table 3: TNM staging system 5th edition

Under 45 years			
Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1
45 years and older			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
	T3	N0	M0
Stage III	T4	N0	M0
	Any T	N1	M0
Stage IV	Any T	Any N	M1

has implications for the prognosis (67). Also the classification of regional lymph node metastases has changed. Some experts propagate to continue the use of the 5th edition.

3. Initial therapy

a. Surgery

Recent guidelines of the European Thyroid Association (ETA) and the American Thyroid Association (ATA) give an up-to-date overview of the treatment strategies

Table 4: TNM staging system 6th edition

Under 45 years			
Stage I	Any T	Any N	M0
Stage II	Any T	Any N	M1
45 years and older			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1, T2, T3	N1a	M0
Stage IVA	T1, T2, T3	N1b	M0
	T4a	N0, N1	M0
Stage IVB	T4b	Any N	M0
Stage IVC	Any T	Any N	M1

for DTC (33,68). Initial therapy consists of near-total thyroidectomy, followed by RaI ablative therapy of thyroid remnants. Although there is still some controversy about the extent of thyroid surgery, there are strong arguments in favor of total or near-total thyroidectomy in all patients (69), this to permit accurate long-term surveillance for disease recurrence. Only very low risk patients (PTC, T1N0M0, unifocal, intrathyroidal) may be treated with hemithyroidectomy. In tumor stages of T2 and higher a total thyroidectomy is indicated (70-72). In these patients near-total thyroidectomy results in lower recurrence rates because many papillary tumors are multifocal and bilateral (73,74). During follow-up both measurement of serum Tg as a tumor marker and RaI whole body scanning are explicitly affected by residual thyroid disease and warrant near-total or total thyroidectomy (33). The argument against total thyroidectomy is that it increases the risk of surgical complications such as recurrent laryngeal nerve injury (2%) and hypoparathyroidism. The latter occurs in 1/3 of the patients after total thyroidectomy, but persists only longer than 3 months in 2% of the patients (68). The experience of the surgeon plays an important role in determining the risk of complications (75-77).

b. Radioiodine-131 ablation therapy

Controversy also exists about the routine use of RaI ablative therapy for thyroid remnants. There are three reasons for post-surgical RaI therapy. First, as already stated above, ablative therapy destroys remaining normal thyroid tissue, which increases the specificity of detectable serum Tg and whole body scanning (72,78). Second, the use of high dose RaI permits post ablative scanning to detect persistent carcinoma or metastatic disease (79,80). The third reason is that RaI may destroy microscopic metastasis and carcinomas, thereby decreasing the risk of recurrence (72,81,82).

A number of large, retrospective studies show a significant reduction in the rates of disease recurrence and cause specific-mortality after RaI ablative therapy (71,83,84-86). However, other studies show no benefit, especially not in patients with small (<1.5 cm), intrathyroidal tumors (6,71,87-90). The beneficial effect is probably only present in patients with high risk or irradical surgery (91). Moreover, various papers reported a relation between RaI therapy and non-thyroidal carcinoma, so the use of it should be applied carefully (92-97). Thus RaI ablation is not indicated in T1N0M0 disease, but still the treatment of choice in patients with high risk of recurrence/mortality, gross extrathyroidal extension of the tumor, documented lymph node metastases, incomplete tumor resection and distance metastases as proposed by recent ATA and ETA guidelines (33,68).

The efficacy of RaI therapy depends on the radiation dose delivered to the thyroid remnant tumor or metastases and these are based on responsiveness of DTC to TSH. Therefore high TSH levels have to be established by either thyroid hormone withdrawal or by therapy with recombinant human TSH (rhTSH) (98,99). In addition, RaI uptake is increased by depletion of plasma inorganic iodide before RaI therapy through a low iodide diet (100-102).

In addition, efficacy of RaI ablative therapy of thyroid remnants is comparable between rhTSH and thyroxine withdrawal (68,103,104). In recent American Thyroid Association guidelines for the treatment of thyroid cancer it is recommended that ablation can be performed following either thyroxine withdrawal or rhTSH (33), and rhTSH is especially advised for patients unable to tolerate hypothyroidism.

c. TSH suppressive therapy

After initial therapy, all patients with DTC are treated with high doses of thyroxine aiming at suppressed TSH levels. The rationale for this approach is based on the potential harmful effects of TSH on tumor recurrence (83,105,106). Thyroid cancer cells express the TSH receptor on the cell membrane and respond to TSH stimulation by increasing the expression of thyroid cancer specific proteins and by increasing the rate of cell growth (107). A recent analysis by our institution indeed showed that TSH levels are positively associated with thyroid cancer associated death and relapse (108). Long-term TSH suppression however, may be associated with harmful effects on various systems including bone metabolism (109-111), glucose metabolism (112,113), cardiac function including higher risk of ischemic heart disease and increased risk of atrial fibrillation (114,115) and quality of life (116-118).

The European Consensus on thyroid cancer (119,120) recommends that not all patients with DTC should be indiscriminately treated with TSH suppressive therapy, because of these negative effects of subclinical hyperthyroidism. Recent ATA thyroid cancer guidelines recommend initial TSH suppression below 0.1 mU/L for high risk

and intermediate risk thyroid cancer, while maintenance of TSH at or slightly below the lower limit of normal (0.1-0.5 mU/L) is appropriate for low-risk patients (33). In the future, TSH suppression may be achieved with thyroid hormone analogues that suppress pituitary TSH secretion with less effect on the cardiovascular system and the bone (121-123).

4. Follow-up

The purpose of follow-up protocols in DTC is the early detection of tumor recurrence or metastatic disease in order to optimize additional treatment. Most patients during follow-up have been cured definitely and have a low pre-test probability for recurrent disease. Therefore, the sensitivity of the diagnostic tests must be adequate to detect the few patients with evident remnant carcinoma whereas specificity must also be high to avoid unnecessary treatment in patients without recurrent disease. Recurrence is usually detected during the early years of follow-up, but may be detected later. Therefore, in our center thyroid carcinoma patients are followed-up for 15 years after initial diagnosis, with tighter screening during the first years.

a. Thyroglobulin

Thyroglobulin (Tg) is a glycoprotein that is produced only by normal or neoplastic thyroid follicular cells and is stimulated by TSH. In patients treated with total thyroidectomy and RaI ablative therapy, Tg should be undetectable. The type of analysis (RIA or immunometric assay) affects the interpretation of serum Tg values (124). The clinical interpretation of Tg is hampered by analytical problems. First, there is lack of universal standardization, leading to significant inter-assay variability and a high intra-assay variability. Second, "hook" effects which primarily affect the immunometric assay method can lead to inappropriately low-or normal range Tg values (125). Dilution of the serum can detect this problem. Third, the presence of Tg auto-antibodies can lead to under- or overestimation of the serum total Tg concentration (126-129).

The Tg auto-antibody interference is the most serious diagnostic problem affecting serum Tg measurements. The incidence of serum Tg auto-antibodies in DTC is approximately 15-30%. Despite these analytical problems, Tg measurements are still the basis in the follow-up in DTC, because in the absence of antibody interference, serum Tg has a high degree of sensitivity and specificity to detect thyroid cancer, with highest degrees following thyroxine withdrawal or rhTSH stimulation (100). A single TSH stimulated serum Tg <0.5 ng/mL has an approximate 98-99.5% likelihood for the identification of patients completely free of tumor (130,131).

Several studies have been performed to determine the diagnostic value of Tg measurements. The application of receiver operator (ROC) curves is essential, since

a chosen cut-off level is a subjective choice based on the balance between a desired percentage of missed recurrences *versus* unnecessary therapies. Therefore, in the European Consensus paper, it was recommended to define institutional Tg cut-off levels (119). High sensitive Tg assays lead to a higher sensitivity at the cost of a lower specificity (132). It is generally believed that high sensitive Tg assays cannot replace the need for TSH stimulated Tg measurements.

b. RaI whole body scanning, ultrasound and FDG-PET

The result of iodine-131 whole body scanning (WBS) depends upon the presence and the ability of thyroid-cancer tissue to take up iodine-131 in the presence of a high serum TSH, which is achieved by withdrawal of thyroxine supplementation for 4 weeks or by recombinant human TSH stimulation. WBS is most useful during follow-up when there is little or no remaining normal thyroid tissue. It may be of value in high or intermediate risk patients, but has no use in low-risk patients with an undetectable Tg and a negative ultrasound (120,133,134). Diagnostic RaI WBS has a much lower sensitivity than ultrasound and Tg measurement. Therefore the routine use of RaI scintigraphy in the diagnostic follow-up of low-risk DTC patients is no longer recommended (33,135).

Ultrasound combined with FNA has the highest sensitivity (94%) for local recurrence and lymph node metastases (134,136) and thus, ultrasound has an important role in the follow-up of DTC.

Fluorodeoxyglucose-positron emission tomography (FDG-PET) may be indicated in patients with elevated serum Tg levels, in whom no RaI uptake is observed after diagnostic or therapeutic whole body scanning, especially in patients with invasive or metastatic Hürthle cell carcinoma (137-139). ATA guidelines recommend FDG-PET as a prognostic tool for staging in high risk patients with metastatic disease or at risk for rapid disease progression (33).

The sensitivity of FDG-PET may be marginally increased with TSH stimulation, but the clinical benefit of identifying additional small foci is yet to be proven (138). However, high FDG-PET uptake in large tumor masses may have an unfavorable prognostic significance (140).

c. Thyroxine withdrawal and recombinant human TSH

Serum Tg measurements, RaI ablation and RaI diagnostic whole body scanning are based on the responsiveness of DTC to TSH. TSH stimulated Tg measurements have superior diagnostic value in DTC compared to Tg measurements on thyroxine replacement therapy (100). High serum TSH levels can be achieved either by thyroxine withdrawal or intramuscular injection of rhTSH. In the first option, 4 weeks thyroxine withdrawal results in a controlled state of profound hypothyroidism with low FT4 and

high TSH. This is poorly tolerated by patients and has negative effects on multiple organs and systems: it exacerbates neuropsychiatric illness and cardiovascular disease, negatively influences lipid metabolism, increases atherosclerotic risks and thus negatively influences quality of life (141). There is also a theoretical disadvantage in thyroxine withdrawal, which states that high TSH levels may be unfavorable with respect to enhancement of tumor proliferation (105). High TSH level as a result of injections of rhTSH has less impact on quality of life (142). RhTSH is an adequate method to detect recurrence or metastases (98,99,143). Tg measurements during rhTSH have comparable accuracy with thyroxine withdrawal (144). The sensitivity and negative predictive value of Tg after rhTSH injections combined with ultrasound are 96.3% and 99.5% respectively (135). Also whole body scans performed after rhTSH injections have a similar sensitivity and negative predictive value compared to thyroxine withdrawal (98,99,143).

5. Therapeutic strategies for metastatic disease

a. Conventional treatment options

Distant metastases, usually in the bones and lungs, occur in approximately 10-15% of patients with DTC. Lung metastases are most frequent in young patients with papillary carcinomas. In general, bone metastases are more common in older patients and in those with follicular carcinomas. Metastatic thyroid cancer that has become inoperable or refractory to RaI therapy is associated with a poor 10 year survival of 5-10%. Because of the indolent character of DTC, metastases are not immediately life threatening, but may impair quality of life considerably (145).

In case of residual disease or metastases, surgery can be attempted when the lesion is accessible. In other cases high dose RaI therapy will be given in patients that accumulate RaI. The remission rate in pulmonary metastases treated with RaI is ~50%, varying from 90% in patients with microscopic metastases to only 10% in macronodular disease. The remission rates of bone metastases are worse, varying between 7 and 20% (5,146). The major problem in this category of patients is the dedifferentiation of thyroid cancer and with that the diminished or lost ability to accumulate RaI. Only palliative treatment options remain. These include external beam radiation therapy and chemotherapy, which have limited success (147).

External beam radiation can be used as palliative therapy in locally advanced disease and can reduce pain of bone metastases or dyspnea in case of airway obstruction. Many conventional chemotherapeutic protocols have been tried in progressive thyroid carcinoma, with overall disappointing results. The most frequently tested agent is doxorubicin. Doxorubicin alone or in combination with cisplatin and bleomycin may induce temporary remissions or stationary disease in about 30-50% of the

patients (147,148). Furthermore, responses are typically short-lived and associated with a high degree of toxicity (149,150).

b. Experimental additional treatments

Lithium has been associated with increased trapping of iodine by the thyroid gland, without impairing iodine uptake, thus enhancing RaI retention. However, despite an increase in RaI uptake in tumor deposits, there are no data that demonstrate a better outcome of patients treated with lithium as an adjunct to RaI therapy (151-152).

PPAR-gamma agonists have been introduced as anti-diabetic agents. Their proposed mechanism is the differentiation of pre-adipocytes, thereby increasing the fatty-acid storing capacity of adipose tissue. Altered expression of PPAR-gamma and *in-vitro* beneficial effects of PPAR-gamma agonists have been described in a number of malignancies. Although PPAR-gamma agonists showed promising results in pre-clinical models for thyroid cancer, they do not result in clinically benificial response (153).

Retinoids are derivates of vitamin A, and are important for growth, differentiation and morphogenesis in vertebrates (55). Beneficial effects of retinoids have been reported *in-vitro* in thyroid carcinoma (63,154-156) including increased NIS mRNA expression and iodine uptake in some thyroid cancer cell lines (154). Interestingly, the promoter of the NIS gene has a retinoic acid responsive element (157). A limited number of human studies have been performed on the effects of retinoids on RaI-131 uptake and reported variable results (64,65,158,159). The main conclusion however is that unfortunately retinoids are not able to restore susceptibility to RaI therapy (7).

c. Future treatment options

As a result of the increasing knowledge of the biologic mechanisms of thyroid cancer pathogenesis and progression, therapeutic agents that could target these mechanisms have been identified. **Table 5** gives an overview of the literature addressing the results of studies on tyrosine kinase inhibitors and DTC.

The anti-EGFR compound gefitinib was not successful in 27 patients with DTC, medullary and anaplastic thyroid carcinoma (160). In a phase II study in 60 thyroid carcinoma patients with various histologies, the VEGFR inhibitor axitinib showed a partial response of 30% (median progression free survival (PFS) 18 months) (161). Recently, 3 phase II studies have been published, using multi-kinase inhibitors (9,10,162). Motesanib diphosphate induced a partial response in 14% of 93 DTC patients (median PFS 40 weeks) (162). Sunitinib, an inhibitor of RET, VEGFR and PDGFR induced a response rate of 13% and 32 % in DTC and MTC patients (163, 164).

Two studies have been published using sorafenib. Sorafenib (BAY 43-9006) is an inhibitor of RET, C-RAF, wild-type and mutant (V600E) BRAF, VEGFR1, -2, -3, Flt3, and

Table 5: Literature on tyrosine kinase inhibitors in DTC

Drug	Target	Tumor type	Number of patients	Response Rate (%)	Stable Disease (%)	Progression free survival	Reference
Gefitinib	EGFR-RET	DTC + MTC	18 + 4	0%	24% (>24 weeks)	16 weeks	Pennell et al. (160)
Axitinib	VEGFR	DTC	45	31 %	42 %	18 months	Cohen et al. (161)
Motesanib	RET- PDGF -VEGFR-KIT	DTC	93	14 %	35% (>24 weeks)	40 weeks	Sherman et al. (162)
Sunitinib	RET, VEGFR, PDGFR	DTC	37	13 %	68 %	-	Cohen et al. (163)
		DTC + MTC	26 + 7	32 % (7 CR)	-	-	Carr et al. (164)
Pazopanib	VEGFR, PDGFR	DTC	26	32%	65 %	12 months	Bible et al. (167)
Sorafenib	RET-RAS-RAF-VEGF-VEGFR-PDGF-cKIT	DTC	41	15 %	56% (>24 weeks)	15 months	Kloos et al. (10)
			30	23 %	53 %	79 weeks	Gupta et al. (9)

c-KIT. In the first study, including DTC, anaplastic and medullary thyroid carcinoma patients, sorafenib induced a partial response in 23% (median PFS 79 weeks) (9). In the second study, including patients with DTC and anaplastic thyroid carcinoma, a partial response was observed in 11% (median PFS 4.5–16 weeks) (10). As bone metastases of DTC are poorly responsive to RaI, the response of bone metastases to the above-mentioned therapies is of interest. However, there are no separate analyses for patients with bone metastases in the above-mentioned studies.

In DTC, a relationship has been identified between genetic alterations in the RET, RAS, RAF cascade and loss of NIS expression (165,166). Interestingly, in an *in-vitro* study, sunitinib was able to reinduce NIS expression in RET/PTC transformed thyroid cells (168). In addition, sunitinib also increased RaI uptake in FRTL-5 cells. We therefore hypothesized that treatment with a multiple kinase inhibitor not only reduces tumor progression, but may also restore RaI uptake in non-RaI avid DTC. Therefore, we performed a single arm phase II trial to determine the efficacy of sorafenib in patients with iodine refractory metastatic DTC, focusing on the reinduction of RaI uptake and the efficacy in bone metastases **(Chapter 3)**.

Therapy with tyrosine kinase inhibitors is associated with alterations in thyroid hormone parameters. Sunitinib has been associated with hypothyroidism in 14–85% of the patients (169-174) and in some patients it induced hyperthyroidism (174-176). Both sunitinib induced hypothyroidism and hyperthyroidism may be caused by destructive thyroiditis, but other mechanisms, such as interference of sunitinib with thyroid peroxidase (177) or inhibition of thyroidal vascularization leading to thyroid

atrophy (178,179), have been proposed. Likewise, sorafenib was associated with TSH elevation in 18% of patients with metastatic renal cell carcinoma (180).

All these studies are performed in patients with intact thyroid function. However, thyroid function abnormalities have also been observed during therapy with imatinib (181,182), motesanib (162), and sorafenib (9) in athyreotic patients treated for medullary thyroid carcinoma and non-medullary thyroid carcinoma. Because an increased need of thyroxine is observed in athyreotic patients who are treated with tyrosine kinase inhibitors, the mechanisms of hypothyroidism may include alterations in thyroid hormone metabolism. Stepwise deiodination is the major route of thyroid hormone degradation and is mediated by iodothyronine deiodinases (D1, D2, and D3) (183) and by hepatic conjugating enzymes (184). To address this subject, we studied the effect of sorafenib on peripheral thyroid hormone metabolism in athyreotic humans **(Chapter 4)**.

III. Consequences of treatment of thyroid carcinoma

Patients with DTC who are treated with total thyroidectomy and RaI ablative therapy become completely dependent on exogenous thyroid replacement therapy. Because of the favorable effects on tumor recurrence, patients used to be treated with TSH suppressive doses of thyroxine (see *TSH suppressive therapy)* for approximately 15 years. Whereas this long-term TSH suppression is associated with an overall better prognosis (108,185), this subclinical hyperthyroid state is associated with deleterious effects on the cardiovascular system (114,115,186,187), carbohydrate metabolism (112,188) and psychological well-being (116,118,189,190).

During follow-up, DTC patients are regularly withdrawn from thyroxine therapy to evaluate recurrence and disease state with TSH stimulated whole body scintigraphy and Tg measurement. This creates a state of controlled hypothyroidism, which can exacerbate neuropsychiatric illness and cardiovascular disease, negatively influences lipid metabolism, and increases atherosclerotic risks and also has an impact on quality of life. The long-term subclinical hyperthyroidism combined with episodes of short-term hypothyroidism make DTC patients an interesting model to study the metabolic effects of thyroid hormone. Also, there is no interference by endogenous thyroid hormone, because patients are treated with total thyroidectomy. Moreover there is no inference from thyroid disease, like in patients treated with thyroxine for autoimmune thyroid disease.

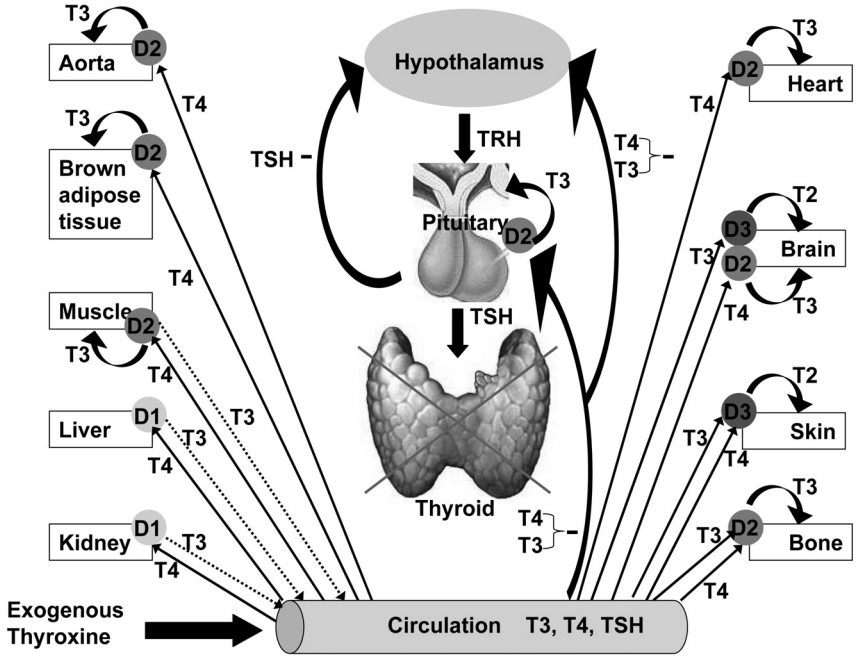

Figure 2
Thyroid hormone production and homeostasis in thyroidectomized patients

1. Thyroid physiology

The production of thyroid hormones is regulated by the hypothalamus-pituitary-thyroid axis (**Figure 2**). Thyrotropin releasing hormone (TRH), which is produced by the hypothalamus, stimulates the secretion of thyrotropin (TSH) by the anterior pituitary. TSH promotes the thyroid to synthesize the prohormone thyroxine (T4). Iodine is actively taken up by the thyroid gland by the sodium-iodide-symporter NIS. The expression and activity of NIS is controlled by TSH. Thyroglobulin, which is synthesized by the follicular cells, is then iodinated with one or two iodides to form monoiodotyrosine (MIT) or diiodotyrosine (DIT). This process is catalyzed by the enzyme thyroid peroxidase (TPO). Two DIT molecules are then coupled to form T4 and one DIT and one MIT are coupled to form T3. The thyroid secretes 90% T4 and 10% T3. T3 is the active form of thyroid hormone. The majority of T3 is formed after conversion of T4 in T3 in peripheral tissues like liver, kidney and muscle. T4 and T3 have a negative feedback mechanism on TRH secretion by the hypothalamus and the TSH production by the pituitary.

Peripheral thyroid metabolism is mainly regulated by the iodothyronin deiodinases D1, D2, and D3 (183,191) **(Figure 2**). D1 is present in the thyroid, liver and kidneys. It converts T4 to T3, and is involved in the serum T3 production. In addition, it plays a role in the breakdown of rT3 (192,193). D2 catalyzes local T3 production

in various tissues. It is present in brain, skeletal muscle, thyroid, pituitary, brown adipose tissue, aortic smooth muscle cells, osteoblasts and the heart (194-196). In the brain, D2 is expressed mainly in astrocytes in the cerebral cortex, hippocampus and amygdala (197). D2 in skeletal muscle may also contribute to plasma T3 production. D3 inactivates T3 and T4 and thus regulates the clearance of T3 and T4. It is present in the brain, skin, placenta and fetal tissues. It is thought that it contributes to thyroid hormone metabolism by protecting tissues from excess thyroid hormone. The deiodinases adjust the thyroid hormone levels of individual tissues in response to various conditions.

Several polymorphisms in D1 and D2 have been described, of which some are associated with circulating levels of T4, T3 and TSH (198-200), with most studies investigating the consequences of the D2-Thr92Ala polymorphism (rs225014). Two polymorphisms in the D1 gene, D1-C785T (rs11206244) and D1-A1814G (rs12095080), have been associated with changes in the balance of thyroid hormones in the serum, with raised T3, low T4 and low rT3 levels, but these changes have not been associated with differences in TSH. This implies that the net effect is perceived by the hypothalamus and pituitary as "neutral" (192,201,220).

Controversy exists about the functional implications of the D2-Thr92Ala polymorphism, which has been associated with a decreased D2 activity in some *in-vitro* experiments (198) but not in others (199,200). So far no associations are found between the D2-Thr92Ala polymorphism and serum thyroid hormone levels in studies in healthy subjects (192,198-200).

Torlontano *et al.* reported in thyroidectomized differentiated thyroid carcinoma patients that homozygous carriers of the D2-Ala92 allele need higher dosages of T4 (202). This difference was most prominently observed in the group with near-suppressed TSH (TSH values between 0.1 and 0.5 mU/l). This study has various limitations, because actual values of serum TSH levels for wild type and homozygous groups within the near-suppressed TSH group are not given. It is, therefore unclear whether TSH levels in both groups are indeed identical, which would be a key finding to ascribe the slight differences in thyroxine dose to the polymorphism. We therefore performed a study to reconfirm the findings of Torlontano *et al.* We studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and T4 dosage in 154 patients treated for DTC and 141 patients substituted with T4 for Hashimoto thyroiditis **(Chapter 5)**.

In healthy blood donors the D2-ORFa-Asp variant (rs12885300) was associated with lower levels of serum T4, fT4 and rT3, but not with plasma T3 and TSH levels (203). This suggests that the D2-ORFa-Asp polymorphism leads to higher activity of D2 at the pituitary level. However, this association was not seen in elderly men (203).

Intraindividual variation in serum T4, T3 and TSH is narrow; however there is a considerable interindividual variability (204). A large body of evidence suggests that every individual has a unique thyroid function setpoint, compatible with a genetic influence on the regulation of the pituitary-thyroid axis (204-206). We hypothesized that polymorphisms in D1 and D2 could influence the setpoint of the hypothalamus-pituitary-thyroid axis. We tested this hypothesis in 151 patients treated for differentiated thyroid carcinoma (DTC). During follow-up of DTC, patients are treated with thyroxine substitution therapy in a dose intended to suppress TSH level, which results in a constant and precisely measurable supply of T4. During this period they regularly come for routine measurements of TSH and FT4, moreover they are regularly withdrawn from thyroxine to perform TSH stimulated radioactive iodine-131 whole body imaging, which leads to a wide individual range of combined measurements of TSH and FT4. These patients are therefore an ideal group to assess the relationship between polymorphisms in deiodinases and the setpoint of the hypothalamus-pituitary-thyroid axis **(Chapter 6)**.

2. Bone metabolism

The involvement of thyroid hormone in bone metabolism has been well documented clinically, ranging from decreased skeletal development in childhood hypothyroidism (207-209), accelerated growth in childhood hyperthyroidism (210) to an increased risk for osteoporosis in overt and subclinical hyperthyroidism (109,211-213).

Although clinical observations suggest a clear involvement of thyroid hormone in bone metabolism, the molecular mechanisms by which thyroid hormone acts on bone is so far only been partially uncovered. T3 promotes osteoblastic proliferation, differentiation and apoptosis and, by induction of interleukin 6 (IL-6), prostaglandins, and RANKL, probably also promotes osteoclast formation and activation. This suggests that osteoblasts are the primary target cells for T3 in the regulation of bone remodeling (208,209,213-217). A functional role of TSH on skeletal development and metabolism has been proposed on the basis of data obtained in animal studies (218-220) and in humans as well (221). This was however disputed by data obtained in thyroid hormone receptor (TR) deficient mice, which indicated that bone remodeling was predominantly mediated by T3 via TRalpha (222,223). It has also been reported recently that in humans there is a significant association between bone mineral density (BMD) and serum thyroid hormone concentrations rather than TSH (224).

Designing a study to address the potential role of deiodinases, including D2 on skeletal metabolism is difficult in humans. The study of the effects of functional D2 polymorphisms on BMD and bone turnover in humans may shed light on this role. Interestingly, a study by Canani *et al*. reported (198) that the maximal velocity of D2

was decreased by 3 to 10-fold in thyroid and skeletal muscle of carriers of the D2-Thr92Ala polymorphism. This effect was observed in the absence of differences in D2 mRNA level or in the biochemical protein properties of the 92Ala allele. Therefore, it was suggested that either a functionally relevant single nucleotide polymorphism occurs in linkage disequilibrium with the Thr92Ala polymorphism or the 92Ala allele affects protein translation or stability.

We therefore performed a study in an attempt to elucidate a potential role for D2 in skeletal metabolism and BMD by evaluating the relationship between the D2 Thr92Ala polymorphism, BMD and bone turnover markers in cured thyroidectomized differentiated thyroid carcinoma patients receiving thyroid hormone substitution. This human model has the advantage of having strictly regulated serum thyroid hormone levels which are kept in a relatively narrow range **(Chapter 7)**.

We have also designed a study in an attempt to discriminate between potential effects mediated by decreased thyroid hormone levels from those mediated by increased TSH levels in a human model in which the reciprocal relationship between thyroid hormones and TSH was interrupted. To this effect, we studied parameters of bone metabolism after parenteral administration of recombinant human TSH (rhTSH) resulting in exogenously increased TSH levels while preserving normal thyroid hormone levels by uninterrupted thyroid hormone substitution in athyroid differentiated thyroid carcinoma (DTC) patients. We studied the same parameters in age-, gender- and BMI matched athyroid DTC patients during short-term thyroxine withdrawal, resulting in decreased thyroid hormone levels and endogenously increased TSH levels, and after reestablishment of thyroid hormone substitution **(Chapter 8)**.

3. Cardiac function

Thyroid hormone has profound effects on the cardiovascular system. Hyperthyroidism induces cardiac arrhythmias, left ventricular (LV) hypertrophy and diastolic dysfunction, and enhances systolic function (225-227). Subclinical hyperthyroidism resulting from TSH suppressive thyroxine therapy, is associated with increased heart rate and supraventricular arrhythmias, including atrial fibrillation, increased LV mass (LVM) with a slightly enhanced systolic function, and diastolic dysfunction. Diastolic dysfunction is at least partly reversible after restoration of euthyroidism and is associated with an increase in mortality (114,228-230). Conversely, hypothyroidism is associated with bradycardia, mild hypertension, increased peripheral cardiovascular resistance, heart failure (225,227,231), decreased cardiac output and diastolic dysfunction (225,227,232-234). Long-standing hypothyroidism can even result in asymmetrical septal hypertrophy (235) and pericardial effusion (230). Hypothyroidism is also associated with coronary artery disease, presumably because of associated hyper-

cholesterolaemia, hypertriglyceridaemia and hypertension (225,227,236). Thyroxine substitution reverses most cardiovascular alterations associated with hypothyroidism (227,230,231,237).

The consequences of acute hypothyroidism during thyroxine withdrawal on cardiac function have been investigated in only a few studies. The results of those studies have been inconclusive, varying from mainly decreased diastolic function to mainly altered systolic function (238-246). In these studies, without control groups, these parameters are measured by different techniques (echocardiography, radionuclide imaging), without blinding the observers with regards to treatment modalities. Therefore, we performed a prospective study in a homogeneous group of athyreotic DTC patients to assess the impact of overt hypothyroidism induced by short-term thyroxine withdrawal on cardiac function measured by a new sophisticated echocardiography technique: tissue Doppler imaging (TDI). Cardiac parameters of the patients are compared to a matched group of controls without cardiovascular co-morbidities **(Chapter 9)**.

4. Quality of life

Differentiated thyroid carcinoma is associated with an excellent medical prognosis, with 10-year survival rates reaching 90-95 %. The excellent prognosis and the moderate invasiveness of the initial therapy may implicate that quality of life in cured DTC patients may be relatively normal. On the other hand, the awareness of having a malignant disease and the TSH suppressive thyroxine replacement therapy may lead to a decreased quality of life (114,228,247). Only a few studies have evaluated quality of life in cured DTC patients (117,118,189,190,248). These studies are limited by small patient numbers (118,189), a limited number of quality of life parameters (117,248), and/or the absence of a healthy control group (117,189,190). Studies that focused on the relation between the level of TSH suppression and quality of life in DTC patients are inconclusive because of small patient numbers, selection of patients with symptoms of hyperthyroidism or selection of patients with a long duration of cure (100,228). For that reason, we investigated quality of life in a large cohort of cured DTC patients, compared to healthy controls matched for age, gender and socioeconomic status. In addition, determinants of quality of life, including TSH level were investigated **(Chapter 10)**.

IV. Outline of this thesis

In **Chapter 2** we evaluate the diagnostic value of the expression of retinoic acid receptors (RAR) and retinoid X receptors (RXR) expression for the differential diagnosis

of thyroid neoplasms. To improve especially the differentiation between benign and malignant thyroid tissues we defined optimal semi quantitative cut-off levels using ROC analysis and hierarchical cluster analysis.
In **Chapter 3** we describe a single arm phase II trial to determine the efficacy of sorafenib treatment in patients with iodine refractory metastatic DTC, focusing on the reinduction of RaI uptake and the efficacy in bone metastases.
Chapter 4 focuses on the observation of changes in thyroid hormone levels during treatment with sorafenib (described in chapter 3). We did this, in order to elucidate the mechanism behind the tyrosine kinase induced thyroid function alterations.

The remainder of this thesis describes the clinical consequences of exogenous subclinical hyperthyroidism during TSH suppressive therapy and of short term hypothyroidism during thyroxine withdrawal in order to study the metabolic effects of thyroid hormone.
Deiodinase type 2 catalyzes local T3 production in various tissues. Controversy exists about the functional implications of the D2-Thr92Ala polymorphism. In **Chapter 5**, we studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and thyroxine dose in 154 patients treated for DTC and 141 patients substituted with T4 for Hashimoto thyroiditis.
It is suggested that every individual has a unique thyroid function setpoint, compatible with a genetic influence on the regulation of the pituitary-thyroid axis. **Chapter 6** studies the influence of polymorphisms in D1 and D2 on the setpoint of the hypothalamus-pituitary-thyroid axis.
Chapter 7 investigates the relationship between the D2-Thr92Ala polymorphism and bone mineral density and bone turnover parameters.
Controversy exists about whether high TSH or low FT4 influences bone metabolism in hypothyroidism. In **Chapter 8**, we describe a prospective study which investigates the effects of high TSH levels, either achieved by hypothyroidism or by recombinant human TSH on bone metabolism. Therefore we will be able to discriminate between potential effects mediated by decreased thyroid hormone levels *versus* those mediated by increased TSH.
In **Chapter 9**, we show the effects of short term overt hypothyroidism, 4 weeks after thyroxine withdrawal on cardiac function.
Chapter 10 describes quality of life in a large cohort of cured DTC patients compared to age, gender and socioeconomic status.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, and Thun MJ. Cancer statistics, 2009. CA: A Cancer Journal for Clinicians 2009 59 225-249
2. Davies L and Welch HG. Thyroid cancer survival in the United States: observational data from 1973 to 2005. Archives of Otolaryngology - Head&Neck Surgery 2010 136 440-444
3. Davies L and Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA 2006 295 2164-2167
4. Oncoline 2010
5. Schlumberger MJ. Papillary and follicular thyroid carcinoma. New England Journal of Medicine 1998 338 297-306
6. Hundahl SA, Fleming ID, Fremgen AM, and Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995. Cancer 1998 83 2638-2648
7. Liu YY, Stokkel MP, Morreau HA, Pereira AM, Romijn JA, and Smit JW. Radioiodine therapy after pretreatment with bexarotene for metastases of differentiated thyroid carcinoma. Clinical Endocrinolology (Oxf) 2008 68 605-609
8. Philips JC, Petite C, Willi JP, Buchegger F, and Meier CA. Effect of peroxisome proliferator-activated receptor gamma agonist, rosiglitazone, on dedifferentiated thyroid cancers. Nuclear Medicine Communications 2004 25 1183-1186
9. Gupta-Abramson V, Troxel AB, Nellore A, Puttaswamy K, Redlinger M, Ransone K, Mandel SJ, Flaherty KT, Loevner LA, O'Dwyer PJ, and Brose MS. Phase II trial of sorafenib in advanced thyroid cancer. Journal of Clinical Oncology 2008 26 4714-4719
10. Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, Liang J, Wakely PE, Jr., Vasko VV, Saji M, Rittenberry J, Wei L, Arbogast D, Collamore M, Wright JJ, Grever M, and Shah MH. Phase II trial of sorafenib in metastatic thyroid cancer. Journal of Clinical Oncology 2009 27 1675-1684
11. Couto JP, Prazeres H, Castro P, Lima J, Maximo V, Soares P, and Sobrinho-Simoes M. How molecular pathology is changing and will change the therapeutics of patients with follicular cell-derived thyroid cancer. Journal of Clinical Pathology 2009 62 414-421
12. Fagin JA. How thyroid tumors start and why it matters: kinase mutants as targets for solid cancer pharmacotherapy. Journal of Endocrinology 2004 183 249-256
13. Kroll TG, Sarraf P, Pecciarini L, Chen CJ, Mueller E, Spiegelman BM, and Fletcher JA. PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected]. Science 2000 289 1357-1360
14. Nikiforov YE. Thyroid carcinoma: molecular pathways and therapeutic targets. Modern Pathology 2008 21 Suppl 2 S37-S43
15. Soares P and Sobrinho-Simoes M. Recent advances in cytometry, cytogenetics and molecular genetics of thyroid tumours and tumour-like lesions. Pathology Research and Practice 1995 191 304-317
16. Sobrinho-Simoes M, Preto A, Rocha AS, Castro P, Maximo V, Fonseca E, and Soares P. Molecular pathology of well-differentiated thyroid carcinomas. Virchows Archives 2005 447 787-793
17. Tallini G. Molecular pathobiology of thyroid neoplasms. Endocrine Pathology 2002 13 271-288
18. Riesco-Eizaguirre G, Gutierrez-Martinez P, Garcia-Cabezas MA, Nistal M, and Santisteban P. The oncogene BRAF V600E is associated with a high risk of recurrence and less differenti-

ated papillary thyroid carcinoma due to the impairment of Na+/I- targeting to the membrane. Endocrine Related Cancer 2006 13 257-269

19. Cinti R, Yin L, Ilc K, Berger N, Basolo F, Cuccato S, Giannini R, Torre G, Miccoli P, Amati P, Romeo G, and Corvi R. RET rearrangements in papillary thyroid carcinomas and adenomas detected by interphase FISH. Cytogenetics and Cell Genetics 2000 88 56-61
20. Corvi R, Berger N, Balczon R, and Romeo G. RET/PCM-1: a novel fusion gene in papillary thyroid carcinoma. Oncogene 2000 19 4236-4242
21. Greco A, Pierotti MA, Bongarzone I, Pagliardini S, Lanzi C, and Della PG. TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. Oncogene 1992 7 237-242
22. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della PG, Fusco A, and Vecchio G. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 1990 60 557-563
23. Klugbauer S, Jauch A, Lengfelder E, Demidchik E, and Rabes HM. A novel type of RET rearrangement (PTC8) in childhood papillary thyroid carcinomas and characterization of the involved gene (RFG8). Cancer Research 2000 60 7028-7032
24. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, and Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Research 1997 57 1690-1694
25. Puxeddu E, Moretti S, Elisei R, Romei C, Pascucci R, Martinelli M, Marino C, Avenia N, Rossi ED, Fadda G, Cavaliere A, Ribacchi R, Falorni A, Pontecorvi A, Pacini F, Pinchera A, and Santeusanio F. BRAF(V599E) mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. Journal of Clinical Endocrinology and Metabolism 2004 89 2414-2420
26. Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G, and Fusco A. Molecular characterization of RET/PTC3; a novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma. Oncogene 1994 9 509-516
27. Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, Manzo G, Picone A, Portella G, Santelli G, Vecchio G, and Fusco A. Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. Oncogene 1996 12 1821-1826
28. Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, Maximo V, Botelho T, Seruca R, and Sobrinho-Simoes M. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene 2003 22 4578-4580
29. Viglietto G, Chiappetta G, Martinez-Tello FJ, Fukunaga FH, Tallini G, Rigopoulou D, Visconti R, Mastro A, Santoro M, and Fusco A. RET/PTC oncogene activation is an early event in thyroid carcinogenesis. Oncogene 1995 11 1207-1210
30. Xing M. BRAF mutation in thyroid cancer. Endocrine Related Cancer 2005 12 245-262
31. Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, and Nikiforov YE. PAX8-PPARgamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. American Journal of Surgical Pathology 2002 26 1016-1023
32. Vecchio G and Santoro M. Oncogenes and thyroid cancer. Clinical Chemistry and Laboratory Medicine 2000 38 113-116
33. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, and Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009 19 1167-1214

34. Ravetto C, Colombo L, and Dottorini ME. Usefulness of fine-needle aspiration in the diagnosis of thyroid carcinoma: a retrospective study in 37,895 patients. Cancer 2000 90 357-363
35. Haugen BR, Woodmansee WW, and McDermott MT. Towards improving the utility of fine-needle aspiration biopsy for the diagnosis of thyroid tumours. Clinical Endocrinology (Oxf) 2002 56 281-290
36. Bartolazzi A, Orlandi F, Saggiorato E, Volante M, Arecco F, Rossetto R, Palestini N, Ghigo E, Papotti M, Bussolati G, Martegani MP, Pantellini F, Carpi A, Giovagnoli MR, Monti S, Toscano V, Sciacchitano S, Pennelli GM, Mian C, Pelizzo MR, Rugge M, Troncone G, Palombini L, Chiappetta G, Botti G, Vecchione A, and Bellocco R. Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study. Lancet Oncology 2008 9 543-549
37. Franco C, Martinez V, Allamand JP, Medina F, Glasinovic A, Osorio M, and Schachter D. Molecular markers in thyroid fine-needle aspiration biopsy: a prospective study. Applied Immunohistochemical Molecular Morphology 2009 17 211-215
38. Kim SW, Lee JI, Kim JW, Ki CS, Oh YL, Choi YL, Shin JH, Kim HK, Jang HW, and Chung JH. BRAFV600E Mutation Analysis in Fine-Needle Aspiration Cytology Specimens for Evaluation of Thyroid Nodule: A Large Series in a BRAFV600E-Prevalent Population. Journal of Clinical Endocrinology and Metabolism 2010 95 3693-3700
39. Kwak JY, Kim EK, Kim JK, Han JH, Hong SW, Park TS, and Choi JR. Dual priming oligonucleotide-based multiplex PCR analysis for detection of BRAFV600E mutation in FNAB samples of thyroid nodules in BRAFV600E mutation-prevalent area. Head&Neck 2010 32 490-498
40. Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, and Nikiforova MN. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. Journal of Clinical Endocrinology and Metabolism 2009 94 2092-2098
41. Sapio MR, Posca D, Raggioli A, Guerra A, Marotta V, Deandrea M, Motta M, Limone PP, Troncone G, Caleo A, Rossi G, Fenzi G, and Vitale M. Detection of RET/PTC, TRK and BRAF mutations in preoperative diagnosis of thyroid nodules with indeterminate cytological findings. Clinical Endocrinology (Oxf) 2007 66 678-683
42. Segev DL, Clark DP, Zeiger MA, and Umbricht C. Beyond the suspicious thyroid fine needle aspirate. A review. Acta Cytology 2003 47 709-722
43. Zhao J, Leonard C, Brunner E, Gemsenjager E, Heitz PU, and Odermatt B. Molecular characterization of well-differentiated human thyroid carcinomas by cDNA arrays. International Journal of Oncology 2006 29 1041-1051
44. Zhao J, Leonard C, Gemsenjager E, Heitz PU, Moch H, and Odermatt B. Differentiation of human follicular thyroid adenomas from carcinomas by gene expression profiling. Oncology Reports 2008 19 329-337
45. Wasenius VM, Hemmer S, Kettunen E, Knuutila S, Franssila K, and Joensuu H. Hepatocyte growth factor receptor, matrix metalloproteinase-11, tissue inhibitor of metalloproteinase-1, and fibronectin are up-regulated in papillary thyroid carcinoma: a cDNA and tissue microarray study. Clinical Cancer Research 2003 9 68-75
46. Stolf BS, Abreu CM, Mahler-Araujo MB, Dellamano M, Martins WK, de Carvalho MB, Curado MP, Diaz JP, Fabri A, Brentani H, Carvalho AF, Soares FA, Kowalski LP, Hirata R, Jr., and Reis LF. Expression profile of malignant and non-malignant diseases of the thyroid gland reveals altered expression of a common set of genes in goiter and papillary carcinomas. Cancer Letters 2005 227 59-73

47. Rosen J, He M, Umbricht C, Alexander HR, Dackiw AP, Zeiger MA, and Libutti SK. A six-gene model for differentiating benign from malignant thyroid tumors on the basis of gene expression. Surgery 2005 138 1050-1056
48. Lubitz CC and Fahey TJ, III. Gene expression profiling of thyroid tumors--clinical applicability. Nature Clinical Practice Endocrinology & Metabolism 2006 2 472-473
49. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, and Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED-1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor-gamma, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. European Journal of Endocrinology 2008 158 375-384
50. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, and de la CA. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. Proceedings of the National Academy of Science U S A 2001 98 15044-15049
51. Fujarewicz K, Jarzab M, Eszlinger M, Krohn K, Paschke R, Oczko-Wojciechowska M, Wiench M, Kukulska A, Jarzab B, and Swierniak A. A multi-gene approach to differentiate papillary thyroid carcinoma from benign lesions: gene selection using support vector machines with bootstrapping. Endocrine Related Cancer 2007 14 809-826
52. Fryknas M, Wickenberg-Bolin U, Goransson H, Gustafsson MG, Foukakis T, Lee JJ, Landegren U, Hoog A, Larsson C, Grimelius L, Wallin G, Pettersson U, and Isaksson A. Molecular markers for discrimination of benign and malignant follicular thyroid tumors. Tumour Biology 2006 27 211-220
53. Finley DJ, Lubitz CC, Wei C, Zhu B, and Fahey TJ, III. Advancing the molecular diagnosis of thyroid nodules: defining benign lesions by molecular profiling. Thyroid 2005 15 562-568
54. Eszlinger M, Wiench M, Jarzab B, Krohn K, Beck M, Lauter J, Gubala E, Fujarewicz K, Swierniak A, and Paschke R. Meta- and reanalysis of gene expression profiles of hot and cold thyroid nodules and papillary thyroid carcinoma for gene groups. Journal of Clinical Endocrinology and Metabolism 2006 91 1934-1942
55. Smith MA, Parkinson DR, Cheson BD, and Friedman MA. Retinoids in cancer therapy. Journal of Clinical Oncology 1992 10 839-864
56. Elisei R, Vivaldi A, Agate L, Ciampi R, Molinaro E, Piampiani P, Romei C, Faviana P, Basolo F, Miccoli P, Capodanno A, Collecchi P, Pacini F, and Pinchera A. All-trans-retinoic acid treatment inhibits the growth of retinoic acid receptor beta messenger ribonucleic acid expressing thyroid cancer cell lines but does not reinduce the expression of thyroid-specific genes. Journal of Clinical Endocrinology and Metabolism 2005 90 2403-241
57. Haugen BR, Larson LL, Pugazhenthi U, Hays WR, Klopper JP, Kramer CA, and Sharma V. Retinoic acid and retinoid X receptors are differentially expressed in thyroid cancer and thyroid carcinoma cell lines and predict response to treatment with retinoids. Journal of Clinical Endocrinology and Metabolism 2004 89 272-280
58. Koh CS, Ku JL, Park SY, Kim KH, Choi JS, Kim IJ, Park JH, Oh SK, Chung JK, Lee JH, Kim WH, Kim CW, Cho BY, and Park JG. Establishment and characterization of cell lines from three human thyroid carcinomas: responses to all-trans-retinoic acid and mutations in the BRAF gene. Molecular Cellular Endocrinology 2007 264 118-127
59. Rochaix P, Monteil-Onteniente S, Rochette-Egly C, Caratero C, Voigt JJ, and Jozan S. Reduced expression of retinoic acid receptor beta protein (RAR beta) in human papillary thyroid carcinoma: immunohistochemical and western blot study. Histopathology 1998 33 337-343

60. Schmutzler C, Brtko J, Winzer R, Jakobs TC, Meissner-Weigl J, Simon D, Goretzki PE, and Kohrle J. Functional retinoid and thyroid hormone receptors in human thyroid-carcinoma cell lines and tissues. International Journal of Cancer 1998 76 368-376
61. Schmutzler C, Hoang-Vu C, Ruger B, and Kohrle J. Human thyroid carcinoma cell lines show different retinoic acid receptor repertoires and retinoid responses. European Journal of Endocrinology 2004 150 547-556
62. Takiyama Y, Miyokawa N, Sugawara A, Kato S, Ito K, Sato K, Oikawa K, Kobayashi H, Kimura S, and Tateno M. Decreased expression of retinoid X receptor isoforms in human thyroid carcinomas. Journal of Clinical Endocrinology and Metabolism 2004 89 5851-5861
63. Havekes B, Schroder van der Elst JP, van der PG, Goslings BM, Romijn JA, and Smit JW. Beneficial effects of retinoic acid on extracellular matrix degradation and attachment behaviour in follicular thyroid carcinoma cell lines. Journal of Endocrinology 2000 167 229-238
64. Simon D, Koehrle J, Reiners C, Boerner AR, Schmutzler C, Mainz K, Goretzki PE, and Roeher HD. Redifferentiation therapy with retinoids: therapeutic option for advanced follicular and papillary thyroid carcinoma. World Journal of Surgery 1998 22 569-574
65. Simon D, Korber C, Krausch M, Segering J, Groth P, Gorges R, Grunwald F, Muller-Gartner HW, Schmutzler C, Kohrle J, Roher HD, and Reiners C. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. European Journal of Nuclear Medicine and Molecular Imaging 2002 29 775-782
66. Wittekind C, Compton CC, Greene FL, and Sobin LH. TNM residual tumor classification revisited. Cancer 2002 94 2511-2516
67. Kukkonen ST, Haapiainen RK, Franssila KO, and Sivula AH. Papillary thyroid carcinoma: the new, age-related TNM classification system in a retrospective analysis of 199 patients. World Journal Surgery 1990 14 837-841
68. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, and Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. European Journal of Endocrinology 2006 154 787-803
69. Demeure MJ and Clark OH. Surgery in the treatment of thyroid cancer. Endocrinology and Metabolism Clinics North America 1990 19 663-683
70. Baudin E, Travagli JP, Ropers J, Mancusi F, Bruno-Bossio G, Caillou B, Cailleux AF, Lumbroso JD, Parmentier C, and Schlumberger M. Microcarcinoma of the thyroid gland: the Gustave-Roussy Institute experience. Cancer 1998 83 553-559
71. DeGroot LJ, Kaplan EL, McCormick M, and Straus FH. Natural history, treatment, and course of papillary thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 1990 71 414-424
72. Mazzaferri EL and Kloos RT. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2001 86 1447-1463
73. Katoh R, Sasaki J, Kurihara H, Suzuki K, Iida Y, and Kawaoi A. Multiple thyroid involvement (intraglandular metastasis) in papillary thyroid carcinoma. A clinicopathologic study of 105 consecutive patients. Cancer 1992 70 1585-1590
74. Russell WO, IbaneZ ML, Clark RL, and White EC. Thyroid carcinoma classification, intraglandular dissemination and clinicopathological study bases upon whole organ sections of 80 glands. Cancer 1963 16 1425-1460
75. Friedman M and Pacella BL, Jr. Total versus subtotal thyroidectomy. Arguments, approaches, and recommendations. Otolaryngoli clinics of North America 1990 23 413-427

76. Friedman M. Surgical management of thyroid carcinoma with laryngotracheal invasion. Otolaryngologic Clinics of North America 1990 23 495-507
77. Sosa JA, Bowman HM, Tielsch JM, Powe NR, Gordon TA, and Udelsman R. The importance of surgeon experience for clinical and economic outcomes from thyroidectomy. Annals of Surgery 1998 228 320-330
78. Utiger RD. Follow-up of patients with thyroid carcinoma. New England Journal of Medicine 1997 337 928-930
79. Sherman SI, Tielens ET, Sostre S, Wharam MD, Jr., and Ladenson PW. Clinical utility of post-treatment radioiodine scans in the management of patients with thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 1994 78 629-634
80. Tenenbaum F, Corone C, Schlumberger M, and Parmentier C. Thyroglobulin measurement and postablative iodine-131 total body scan after total thyroidectomy for differentiated thyroid carcinoma in patients with no evidence of disease. European Journal of Cancer 1996 32A 1262
81. Simpson WJ, Panzarella T, Carruthers JS, Gospodarowicz MK, and Sutcliffe SB. Papillary and follicular thyroid cancer: impact of treatment in 1578 patients. International Journal of Radiation, Oncology, Biology & Physics 1988 14 1063-1075
82. Tubiana M, Schlumberger M, Rougier P, Laplanche A, Benhamou E, Gardet P, Caillou B, Travagli JP, and Parmentier C. Long-term results and prognostic factors in patients with differentiated thyroid carcinoma. Cancer 1985 55 794-804
83. Mazzaferri EL and Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. American Journal of Medicine 1994 97 418-428
84. Samaan NA, Schultz PN, Hickey RC, Goepfert H, Haynie TP, Johnston DA, and Ordonez NG. The results of various modalities of treatment of well differentiated thyroid carcinomas: a retrospective review of 1599 patients. Journal of Clinical Endocrinology and Metabolism 1992 75 714-720
85. Sawka AM, Thephamongkhol K, Brouwers M, Thabane L, Browman G, and Gerstein HC. Clinical review 170: A systematic review and metaanalysis of the effectiveness of radioactive iodine remnant ablation for well-differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2004 89 3668-3676
86. Taylor T, Specker B, Robbins J, Sperling M, Ho M, Ain K, Bigos ST, Brierley J, Cooper D, Haugen B, Hay I, Hertzberg V, Klein I, Klein H, Ladenson P, Nishiyama R, Ross D, Sherman S, and Maxon HR. Outcome after treatment of high-risk papillary and non-Hurthle-cell follicular thyroid carcinoma. Annals of Internal Medicine 1998 129 622-627
87. Hay ID, Grant CS, van Heerden JA, Goellner JR, Ebersold JR, and Bergstralh EJ. Papillary thyroid microcarcinoma: a study of 535 cases observed in a 50-year period. Surgery 1992 112 1139-1146
88. Hay ID, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, Maurer MS, McIver B, Mullan BP, Oberg AL, Powell CC, van Heerden JA, and Goellner JR. Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. World Journal of Surgery 2002 26 879-885
89. Jonklaas J, Sarlis NJ, Litofsky D, Ain KB, Bigos ST, Brierley JD, Cooper DS, Haugen BR, Ladenson PW, Magner J, Robbins J, Ross DS, Skarulis M, Maxon HR, and Sherman SI. Outcomes of patients with differentiated thyroid carcinoma following initial therapy. Thyroid 2006 16 1229-1242

90. Sanders LE and Cady B. Differentiated thyroid cancer: reexamination of risk groups and outcome of treatment. Archives of Surgery 1998 133 419-425
91. Jung TS, Kim TY, Kim KW, Oh YL, Park dJ, Cho BY, Shong YK, Kim WB, Park YJ, Jung JH, and Chung JH. Clinical features and prognostic factors for survival in patients with poorly differentiated thyroid carcinoma and comparison to the patients with the aggressive variants of papillary thyroid carcinoma. Endocrine Journal 2007 54 265-274
92. Brown AP, Chen J, Hitchcock YJ, Szabo A, Shrieve DC, and Tward JD. The risk of second primary malignancies up to three decades after the treatment of differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2008 93 504-515
93. Brown AP, Chen J, Hitchcock YJ, Szabo A, Shrieve DC, and Tward JD. The risk of second primary malignancies up to three decades after the treatment of differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2008 93 504-515
94. De Vathaire F, Schlumberger M, Delisle MJ, Francese C, Challeton C, de la GE, Meunier F, Parmentier C, Hill C, and Sancho-Garnier H. Leukaemias and cancers following iodine-131 administration for thyroid cancer. British Journal of Cancer 1997 75 734-739
95. Rubino C, De Vathaire F, Dottorini ME, Hall P, Schvartz C, Couette JE, Dondon MG, Abbas MT, Langlois C, and Schlumberger M. Second primary malignancies in thyroid cancer patients. British Journal of Cancer 2003 89 1638-1644
96. Sandeep TC, Strachan MW, Reynolds RM, Brewster DH, Scelo G, Pukkala E, Hemminki K, Anderson A, Tracey E, Friis S, McBride ML, Kee-Seng C, Pompe-Kirn V, Kliewer EV, Tonita JM, Jonasson JG, Martos C, Boffetta P, and Brennan P. Second primary cancers in thyroid cancer patients: a multinational record linkage study. Journal of Clinical Endocrinology and Metabolism 2006 91 1819-1825
97. Subramanian S, Goldstein DP, Parlea L, Thabane L, Ezzat S, Ibrahim-Zada I, Straus S, Brierley JD, Tsang RW, Gafni A, Rotstein L, and Sawka AM. Second primary malignancy risk in thyroid cancer survivors: a systematic review and meta-analysis. Thyroid 2007 17 1277-1288
98. Haugen BR, Pacini F, Reiners C, Schlumberger M, Ladenson PW, Sherman SI, Cooper DS, Graham KE, Braverman LE, Skarulis MC, Davies TF, DeGroot LJ, Mazzaferri EL, Daniels GH, Ross DS, Luster M, Samuels MH, Becker DV, Maxon HR, III, Cavalieri RR, Spencer CA, McEllin K, Weintraub BD, and Ridgway EC. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. Journal of Clinical Endocrinology and Metabolism 1999 84 3877-3885
99. Ladenson PW, Braverman LE, Mazzaferri EL, Brucker-Davis F, Cooper DS, Garber JR, Wondisford FE, Davies TF, DeGroot LJ, Daniels GH, Ross DS, and Weintraub BD. Comparison of administration of recombinant human thyrotropin with withdrawal of thyroid hormone for radioactive iodine scanning in patients with thyroid carcinoma. New England Journal of Medicine 1997 337 888-896
100. Eustatia-Rutten CF, Smit JW, Romijn JA, van der Kleij-Corssmit EP, Pereira AM, Stokkel MP, and Kievit J. Diagnostic value of serum thyroglobulin measurements in the follow-up of differentiated thyroid carcinoma, a structured meta-analysis. Clinical Endocrinology (Oxf) 2004 61 61-74
101. Goslings BM. Proceedings: Effect of a low iodine diet on 131-I therapy in follicular thyroid carcinomata. Journal of Endocrinology 1975 64 30P-
102. Maxon HR, Thomas SR, Boehringer A, Drilling J, Sperling MI, Sparks JC, and Chen IW. Low iodine diet in I-131 ablation of thyroid remnants. Clinical Nuclear Medicine 1983 8 123-126

103. Chianelli M, Todino V, Graziano FM, Panunzi C, Pace D, Guglielmi R, Signore A, and Papini E. Low-activity (2.0 GBq; 54 mCi) radioiodine post-surgical remnant ablation in thyroid cancer: comparison between hormone withdrawal and use of rhTSH in low-risk patients. European Journal of Endocrinology 2009 160 431-436
104. Taieb D, Sebag F, Cherenko M, Baumstarck-Barrau K, Fortanier C, Farman-Ara B, De Micco C, Vaillant J, Thomas S, Conte-Devolx B, Loundou A, Auquier P, Henry JF, and Mundler O. Quality of life changes and clinical outcomes in thyroid cancer patients undergoing radioiodine remnant ablation (RRA) with recombinant human TSH (rhTSH): a randomized controlled study. Clinical Endocrinology (Oxf) 2009 71 115-123
105. Goretzki PE, Frilling A, Simon D, and Roeher HD. Growth regulation of normal thyroids and thyroid tumors in man. Recent Results of Cancer Research 1990 118 48-63
106. McGriff NJ, Csako G, Gourgiotis L, Lori CG, Pucino F, and Sarlis NJ. Effects of thyroid hormone suppression therapy on adverse clinical outcomes in thyroid cancer. Ann Med 2002 34 554-564
107. Brabant G. Thyrotropin suppressive therapy in thyroid carcinoma: what are the targets? Journal of Clinical Endocrinology and Metabolism 2008 93 1167-1169
108. Hovens GC, Stokkel MP, Kievit J, Corssmit EP, Pereira AM, Romijn JA, and Smit JW. Associations of serum thyrotropin concentrations with recurrence and death in differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2007 92 2610-2615
109. Heemstra KA, Hamdy NA, Romijn JA, and Smit JW. The effects of thyrotropin-suppressive therapy on bone metabolism in patients with well-differentiated thyroid carcinoma. Thyroid 2006 16 583-591
110. Franklyn JA, Betteridge J, Daykin J, Holder R, Oates GD, Parle JV, Lilley J, Heath DA, and Sheppard MC. Long-term thyroxine treatment and bone mineral density. Lancet 1992 340 9-13
111. Diamond T, Nery L, and Hales I. A therapeutic dilemma: suppressive doses of thyroxine significantly reduce bone mineral measurements in both premenopausal and postmenopausal women with thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 1991 72 1184-1188
112. Dimitriadis G, Mitrou P, Lambadiari V, Boutati E, Maratou E, Koukkou E, Tzanela M, Thalassinos N, and Raptis SA. Glucose and lipid fluxes in the adipose tissue after meal ingestion in hyperthyroidism. Journal of Clinical Endocrinology and Metabolism 2006 91 1112-1118
113. Yavuz DG, Yuksel M, Deyneli O, Ozen Y, Aydin H, and Akalin S. Association of serum paraoxonase activity with insulin sensitivity and oxidative stress in hyperthyroid and TSH-suppressed nodular goitre patients. Clinical Endocrinology (Oxf) 2004 61 515-521
114. Biondi B, Palmieri EA, Fazio S, Cosco C, Nocera M, Sacca L, Filetti S, Lombardi G, and Perticone F. Endogenous subclinical hyperthyroidism affects quality of life and cardiac morphology and function in young and middle-aged patients. Journal of Clinical Endocrinology and Metabolism 2000 85 4701-4705
115. Smit JW, Eustatia-Rutten CF, Corssmit EP, Pereira AM, Frolich M, Bleeker GB, Holman ER, van der Wall EE, Romijn JA, and Bax JJ. Reversible diastolic dysfunction after long-term exogenous subclinical hyperthyroidism: a randomized, placebo-controlled study. Journal of Clinical Endocrinology and Metabolism 2005 90 6041-6047
116. Botella-Carretero JI, Galan JM, Caballero C, Sancho J, and Escobar-Morreale HF. Quality of life and psychometric functionality in patients with differentiated thyroid carcinoma. Endocrine Related Cancer 2003 10 601-610

117. Crevenna R, Zettinig G, Keilani M, Posch M, Schmidinger M, Pirich C, Nuhr M, Wolzt M, Quittan M, Fialka-Moser V, and Dudczak R. Quality of life in patients with non-metastatic differentiated thyroid cancer under thyroxine supplementation therapy. Supportive Care in Cancer 2003 11 597-603
118. Dagan T, Bedrin L, Horowitz Z, Chaushu G, Wolf M, Kronenberg J, and Talmi YP. Quality of life of well-differentiated thyroid carcinoma patients. Journal of Laryngology and Otology 2004 118 537-542
119. Schlumberger M, Pacini F, Wiersinga WM, Toft A, Smit JW, Sanchez FF, Lind P, Limbert E, Jarzab B, Jamar F, Duntas L, Cohen O, and Berg G. Follow-up and management of differentiated thyroid carcinoma: a European perspective in clinical practice. European Journal of Endocrinology 2004 151 539-548
120. Schlumberger M, Berg G, Cohen O, Duntas L, Jamar F, Jarzab B, Limbert E, Lind P, Pacini F, Reiners C, Sanchez FF, Toft A, and Wiersinga WM. Follow-up of low-risk patients with differentiated thyroid carcinoma: a European perspective. European Journal of Endocrinology 2004 150 105-112
121. Biondi B and Cooper DS. Benefits of thyrotropin suppression versus the risks of adverse effects in differentiated thyroid cancer. Thyroid 2010 20 135-146
122. Lameloise N, Siegrist-Kaiser C, O'Connell M, and Burger A. Differences between the effects of thyroxine and tetraiodothyroacetic acid on TSH suppression and cardiac hypertrophy. European Journal of Endocrinology 2001 144 145-154
123. Sherman SI, Gopal J, Haugen BR, Chiu AC, Whaley K, Nowlakha P, and Duvic M. Central hypothyroidism associated with retinoid X receptor-selective ligands. New England Journal of Medicine 1999 340 1075-1079
124. Spencer CA and Wang CC. Thyroglobulin measurement. Techniques, clinical benefits, and pitfalls. Endocrinology and Metabolism Clinics of North America 1995 24 841-863
125. Spencer CA. Dynamics of thyroid hormone suppression of serum thyrotropin: an invited commentary. European Journal of Endocrinology 1996 135 285-286
126. Ligabue A, Poggioli MC, and Zacchini A. Interference of specific autoantibodies in the assessment of serum thyroglobulin. Journal of Nuclear Biology Medicine 1993 37 273-279
127. Mariotti S, Barbesino G, Caturegli P, Marino M, Manetti L, Pacini F, Centoni R, and Pinchera A. Assay of thyroglobulin in serum with thyroglobulin autoantibodies: an unobtainable goal? Journal of Clinical Endocrinology and Metabolism 1995 80 468-472
128. Spencer CA. Recoveries cannot be used to authenticate thyroglobulin (Tg) measurements when sera contain Tg autoantibodies. Clinical Chemistry 1996 42 661-663
129. Van Herle AJ, Uller RP, Matthews NI, and Brown J. Radioimmunoassay for measurement of thyroglobulin in human serum. Journal of Clinical Investigation 1973 52 1320-1327
130. Castagna MG, Brilli L, Pilli T, Montanaro A, Cipri C, Fioravanti C, Sestini F, Capezzone M, and Pacini F. Limited value of repeat recombinant human thyrotropin (rhTSH)-stimulated thyroglobulin testing in differentiated thyroid carcinoma patients with previous negative rhTSH-stimulated thyroglobulin and undetectable basal serum thyroglobulin levels. Journal of Clinical Endocrinology and Metabolism 2008 93 76-81
131. Kloos RT and Mazzaferri EL. A single recombinant human thyrotropin-stimulated serum thyroglobulin measurement predicts differentiated thyroid carcinoma metastases three to five years later. Journal of Clinical Endocrinology and Metabolism 2005 90 5047-5057
132. Schlumberger M, Hitzel A, Toubert ME, Corone C, Troalen F, Schlageter MH, Claustrat F, Koscielny S, Taieb D, Toubeau M, Bonichon F, Borson-Chazot F, Leenhardt L, Schvartz C,

Dejax C, Brenot-Rossi I, Torlontano M, Tenenbaum F, Bardet S, Bussiere F, Girard JJ, Morel O, Schneegans O, Schlienger JL, Prost A, So D, Archambeaud F, Ricard M, and Benhamou E. Comparison of seven serum thyroglobulin assays in the follow-up of papillary and follicular thyroid cancer patients. Journal of Clinical Endocrinology and Metabolism 2007 92 2487-2495

133. Pacini F, Capezzone M, Elisei R, Ceccarelli C, Taddei D, and Pinchera A. Diagnostic 131-iodine whole-body scan may be avoided in thyroid cancer patients who have undetectable stimulated serum Tg levels after initial treatment. Journal of Clinical Endocrinology and Metabolism 2002 87 1499-1501
134. Torlontano M, Crocetti U, D'Aloiso L, Bonfitto N, Di Giorgio A, Modoni S, Valle G, Frusciante V, Bisceglia M, Filetti S, Schlumberger M, and Trischitta V. Serum thyroglobulin and 131I whole body scan after recombinant human TSH stimulation in the follow-up of low-risk patients with differentiated thyroid cancer. European Journal of Endocrinology 2003 148 19-24
135. Pacini F, Molinaro E, Castagna MG, Agate L, Elisei R, Ceccarelli C, Lippi F, Taddei D, Grasso L, and Pinchera A. Recombinant human thyrotropin-stimulated serum thyroglobulin combined with neck ultrasonography has the highest sensitivity in monitoring differentiated thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 2003 88 3668-3673
136. Frasoldati A, Pesenti M, Gallo M, Caroggio A, Salvo D, and Valcavi R. Diagnosis of neck recurrences in patients with differentiated thyroid carcinoma. Cancer 2003 97 90-96
137. Larson SM and Robbins R. Positron emission tomography in thyroid cancer management. Seminars in Roentgenology 2002 37 169-174
138. Leboulleux S, Schroeder PR, Busaidy NL, Auperin A, Corone C, Jacene HA, Ewertz ME, Bournaud C, Wahl RL, Sherman SI, Ladenson PW, and Schlumberger M. Assessment of the incremental value of recombinant thyrotropin stimulation before 2-[18F]-Fluoro-2-deoxy-D-glucose positron emission tomography/computed tomography imaging to localize residual differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2009 94 1310-1316
139. Schluter B, Bohuslavizki KH, Beyer W, Plotkin M, Buchert R, and Clausen M. Impact of FDG PET on patients with differentiated thyroid cancer who present with elevated thyroglobulin and negative 131I scan. Journal of Nuclear Medicine 2001 42 71-76
140. Robbins RJ, Wan Q, Grewal RK, Reibke R, Gonen M, Strauss HW, Tuttle RM, Drucker W, and Larson SM. Real-time prognosis for metastatic thyroid carcinoma based on 2-[18F]fluoro-2-deoxy-D-glucose-positron emission tomography scanning. Journal of Clinical Endocrinology and Metabolism 2006 91 498-505
141. Duntas LH and Biondi B. Short-term hypothyroidism after Levothyroxine-withdrawal in patients with differentiated thyroid cancer: clinical and quality of life consequences. European Journal of Endocrinology 2007 156 13-19
142. Schroeder PR, Haugen BR, Pacini F, Reiners C, Schlumberger M, Sherman SI, Cooper DS, Schuff KG, Braverman LE, Skarulis MC, Davies TF, Mazzaferri EL, Daniels GH, Ross DS, Luster M, Samuels MH, Weintraub BD, Ridgway EC and Ladenson PW. A comparison of short-term changes in health-related quality of life in thyroid carcinoma patients undergoing diagnostic evaluation with recombinant human thyrotropin compared with thyroid hormone withdrawal. Journal of Clinical Endocrinology and Metabolism 2006 91 878-884
143. Robbins RJ, Tuttle RM, Sharaf RN, Larson SM, Robbins HK, Ghossein RA, Smith A, and Drucker WD. Preparation by recombinant human thyrotropin or thyroid hormone withdrawal

are comparable for the detection of residual differentiated thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 2001 86 619-625

144. Pacini F, Ladenson PW, Schlumberger M, Driedger A, Luster M, Kloos RT, Sherman S, Haugen B, Corone C, Molinaro E, Elisei R, Ceccarelli C, Pinchera A, Wahl RL, Leboulleux S, Ricard M, Yoo J, Busaidy NL, Delpassand E, Hanscheid H, Felbinger R, Lassmann M, and Reiners C. Radioiodine ablation of thyroid remnants after preparation with recombinant human thyrotropin in differentiated thyroid carcinoma: results of an international, randomized, controlled study. Journal of Clinical Endocrinology and Metabolism 2006 91 926-932
145. Durante C, Haddy N, Baudin E, Leboulleux S, Hartl D, Travagli JP, Caillou B, Ricard M, Lumbroso JD, De Vathaire F, and Schlumberger M. Long-term outcome of 444 patients with distant metastases from papillary and follicular thyroid carcinoma: benefits and limits of radioiodine therapy. Journal of Clinical Endocrinology and Metabolism 2006 91 2892-2899
146. Pacini F, Agate L, Elisei R, Capezzone M, Ceccarelli C, Lippi F, Molinaro E, and Pinchera A. Outcome of differentiated thyroid cancer with detectable serum Tg and negative diagnostic (131)I whole body scan: comparison of patients treated with high (131)I activities versus untreated patients. Journal of Clinical Endocrinology and Metabolism 2001 86 4092-4097
147. Haugen BR. Management of the patient with progressive radioiodine non-responsive disease. Seminars in Surgical Oncology 1999 16 34-41
148. De Besi P, Busnardo B, Toso S, Girelli ME, Nacamulli D, Simioni N, Casara D, Zorat P, and Fiorentino MV. Combined chemotherapy with bleomycin, adriamycin, and platinum in advanced thyroid cancer. Journal of Endocrinology Investigations 1991 14 475-480
149. Shimaoka K, Schoenfeld DA, DeWys WD, Creech RH, and DeConti R. A randomized trial of doxorubicin versus doxorubicin plus cisplatin in patients with advanced thyroid carcinoma. Cancer 1985 56 2155-2160
150. Williams SD, Birch R, and Einhorn LH. Phase II evaluation of doxorubicin plus cisplatin in advanced thyroid cancer: a Southeastern Cancer Study Group Trial. Cancer Treatment Reports 1986 70 405-407
151. Koong SS, Reynolds JC, Movius EG, Keenan AM, Ain KB, Lakshmanan MC, and Robbins J. Lithium as a potential adjuvant to 131I therapy of metastatic, well differentiated thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 1999 84 912-916
152. Liu YY, van der PG, Karperien M, Stokkel MP, Pereira AM, Morreau J, Kievit J, Romijn JA, and Smit JW. Lithium as adjuvant to radioiodine therapy in differentiated thyroid carcinoma: clinical and in vitro studies. Clinical Endocrinology (Oxf) 2006 64 617-624
153. Kebebew E, Lindsay S, Clark OH, Woeber KA, Hawkins R, Greenspan FS. Results of rosiglitazone therapy in patients with thyroglobulin-positive and radioiodine-negative advanced differentiated thyroid carcinoma. Thyroid 2009 19 953-956
154. Schmutzler C, Brtko J, Bienert K, and Kohrle J. Effects of retinoids and role of retinoic acid receptors in human thyroid carcinomas and cell lines derived therefrom. Experimental Clinical Endocrinology and Diabetes 1996 104 Suppl 4 16-19
155. Schmutzler C and Kohrle J. Retinoic acid redifferentiation therapy for thyroid cancer. Thyroid 2000 10 393-406
156. Van Herle AJ, Agatep ML, Padua DN, III, Totanes TL, Canlapan DV, Van Herle HM, and Juillard GJ. Effects of 13 cis-retinoic acid on growth and differentiation of human follicular carcinoma cells (UCLA R0 82 W-1) in vitro. Journal of Clinical Endocrinology and Metabolism 1990 71 755-763

157. Schmutzler C, Schmitt TL, Glaser F, Loos U, and Kohrle J. The promoter of the human sodium/iodide-symporter gene responds to retinoic acid. Mol Cell Endocrinol 2002 189 145-155
158. Simon D, Kohrle J, Schmutzler C, Mainz K, Reiners C, and Roher HD. Redifferentiation therapy of differentiated thyroid carcinoma with retinoic acid: basics and first clinical results. Experiments in Clinical Endocrinology and Diabetes 1996 104 Suppl 4 13-15
159. Coelho SM, Corbo R, Buescu A, Carvalho DP, and Vaisman M. Retinoic acid in patients with radioiodine non-responsive thyroid carcinoma. Journal of Endocrinology Investigations 2004 27 334-339
160. Pennell NA, Daniels GH, Haddad RI, Ross DS, Evans T, Wirth LJ, Fidias PH, Temel JS, Gurubhagavatula S, Heist RS, Clark JR, and Lynch TJ. A phase II study of gefitinib in patients with advanced thyroid cancer. Thyroid 2008 18 317-323
161. Cohen EE, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, Kane MA, Sherman E, Kim S, Bycott P, Tortorici M, Shalinsky DR, Liau KF, and Cohen RB. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. Journal of Clinical Oncology 2008 26 4708-4713
162. Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, Licitra L, Eschenberg MJ, Sun YN, Juan T, Stepan DE, and Schlumberger MJ. Motesanib diphosphate in progressive differentiated thyroid cancer. New England Journal of Medicine 2008 359 31-42
163. Cohen EE, Needles BM, Cullen KJ, Wong SJ, Wade JL, Ivy SP, Villaflor VM, Seiwert TY, Nichols K, and Vokes EE. Phase 2 study of sunitinib in refractory thyroid cancer. Journal of Clinical Oncology 2008 26 (May 20 suppl; abstr 6025)
164. Carr L, Goulart B, Martins R, Keith E, Kell E, Wallace S, Capell P, and Mankoff D. Phase II trial of continuous dosing of sunitinib in advanced, FDG-PET avid, medullary thyroid carcinoma (MTC) and well-differentiated thyroid cancer (WDTC). Journal of Clinical Oncology 2009 27 15 (May 20 suppl; abstr 6056)
165. Riesco-Eizaguirre G, Rodriguez I, De l, V, Costamagna E, Carrasco N, Nistal M, and Santisteban P. The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. Cancer Research 2009 69 8317-8325
166. De Vita G, Zannini M, Cirafici AM, Melillo RM, Di Lauro R, Fusco A, and Santoro M. Expression of the RET/PTC1 oncogene impairs the activity of TTF-1 and Pax-8 thyroid transcription factors. Cellular Growth Differentiation 1998 9 97-103
167. Bible KC, Smallridge RC, Maples WJ, Molina JR, Menefee ME, Suman VJ, Burton JK, Bieber CC, Ivy SP, and Erlichman C. Phase II trial of pazopanib in progressive, metastatic, iodine-insensitive differentiated thyroid cancers. Journal of Clinical Oncology 2009 27:15s (suppl; abstr 3521)
168. Fenton MS, Marion K, Salem AK, Hogen R, Naeim F, and Hershman. Induction of cell cycle arrest by sunitinib through inhibition of the MEK/ERK pathway in RET/PTC1 papillary thyroid cancer cells. 90th Annual Meeting of the Endocrine Society, San Francisco (abstract) 1990
169. Desai J, Yassa L, Marqusee E, George S, Frates MC, Chen MH, Morgan JA, Dychter SS, Larsen PR, Demetri GD, and Alexander EK. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. Annals of Internal Medicine 2006 145 660-664
170. Mannavola D, Coco P, Vannucchi G, Bertuelli R, Carletto M, Casali PG, Beck-Peccoz P, and Fugazzola L. A novel tyrosine-kinase selective inhibitor, sunitinib, induces transient hypothyroidism by blocking iodine uptake. Journal of Clinical Endocrinology and Metabolism 2007 92 3531-3534

171. Rini BI, Tamaskar I, Shaheen P, Salas R, Garcia J, Wood L, Reddy S, Dreicer R, and Bukowski RM. Hypothyroidism in patients with metastatic renal cell carcinoma treated with sunitinib. Journal of the National Cancer Institute 2007 99 81-83
172. Wong E, Rosen LS, Mulay M, Vanvugt A, Dinolfo M, Tomoda C, Sugawara M, and Hershman JM. Sunitinib induces hypothyroidism in advanced cancer patients and may inhibit thyroid peroxidase activity. Thyroid 2007 17 351-355
173. Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L, Woulfe K, Pravda E, Cassiola F, Desai J, George S, Morgan JA, Harris DM, Ismail NS, Chen JH, Schoen FJ, Van den Abbeele AD, Demetri GD, Force T, and Chen MH. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet 2007 370 2011-2019
174. Wolter P, Stefan C, Decallonne B, Dumez H, Bex M, Carmeliet P, and Schoffski P. The clinical implications of sunitinib-induced hypothyroidism: a prospective evaluation. British Journal of Cancer 2008 99 448-454
175. Grossmann M, Premaratne E, Desai J, and Davis ID. Thyrotoxicosis during sunitinib treatment for renal cell carcinoma. Clinical Endocrinology (Oxf) 2008 69 669-672
176. Faris JE, Moore AF, and Daniels GH. Sunitinib (sutent)-induced thyrotoxicosis due to destructive thyroiditis: a case report. Thyroid 2007 17 1147-1149
177. Alexandrescu DT, Popoveniuc G, Farzanmehr H, Dasanu CA, Dawson N, and Wartofsky L. Sunitinib-associated lymphocytic thyroiditis without circulating antithyroid antibodies. Thyroid 2008 18 809-812
178. Kamba T, Tam BY, Hashizume H, Haskell A, Sennino B, Mancuso MR, Norberg SM, O'Brien SM, Davis RB, Gowen LC, Anderson KD, Thurston G, Joho S, Springer ML, Kuo CJ, and McDonald DM. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. American Journal of Physiology and Heart Circulatory Physiology 2006 290 H560-H576
179. Kamba T and McDonald DM. Mechanisms of adverse effects of anti-VEGF therapy for cancer. British Journal of Cancer 2007 96 1788-1795
180. Tamaskar I, Bukowski R, Elson P, Ioachimescu AG, Wood L, Dreicer R, Mekhail T, Garcia J, and Rini BI. Thyroid function test abnormalities in patients with metastatic renal cell carcinoma treated with sorafenib. Annals of Oncology 2008 19 265-268
181. de Groot JW, Zonnenberg BA, Plukker JT, Der Graaf WT, and Links TP. Imatinib induces hypothyroidism in patients receiving levothyroxine. Clinical Pharmacologic Therapy 2005 78 433-438
182. de Groot JW, Links TP, and van der Graaf WT. Tyrosine kinase inhibitors causing hypothyroidism in a patient on levothyroxine. Annals of Oncology 2006 17 1719-1720
183. Bianco AC, Salvatore D, Gereben B, Berry MJ, and Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocrine Reviews 2002 23 38-89
184. Eelkman Rooda SJ, Kaptein E, and Visser TJ. Serum triiodothyronine sulfate in man measured by radioimmunoassay. Journal of Clinical Endocrinology and Metabolism 1989 69 552-556
185. Cooper DS, Specker B, Ho M, Sperling M, Ladenson PW, Ross DS, Ain KB, Bigos ST, Brierley JD, Haugen BR, Klein I, Robbins J, Sherman SI, Taylor T, and Maxon HR, III. Thyrotropin suppression and disease progression in patients with differentiated thyroid cancer: results from the National Thyroid Cancer Treatment Cooperative Registry. Thyroid 1998 8 737-744

186. Sgarbi JA, Villaca FG, Garbeline B, Villar HE, and Romaldini JH. The effects of early antithyroid therapy for endogenous subclinical hyperthyroidism in clinical and heart abnormalities. Journal of Clinical Endocrinology and Metabolism 2003 88 1672-1677
187. Napoli R, Biondi B, Guardasole V, Matarazzo M, Pardo F, Angelini V, Fazio S, and Sacca L. Impact of hyperthyroidism and its correction on vascular reactivity in humans. Circulation 2001 104 3076-3080
188. Heemstra KA, Smit JW, Eustatia-Rutten CF, Heijboer AC, Frolich M, Romijn JA, and Corssmit EP. Glucose tolerance and lipid profile in longterm exogenous subclinical hyperthyroidism and the effects of restoration of euthyroidism, a randomised controlled trial. Clinical Endocrinology (Oxf) 2006 65 737-744
189. Giusti M, Sibilla F, Cappi C, Dellepiane M, Tombesi F, Ceresola E, Augeri C, Rasore E, and Minuto F. A case-controlled study on the quality of life in a cohort of patients with history of differentiated thyroid carcinoma. Journal of Endocrinologic Investigations 2005 28 599-608
190. Schultz PN, Stava C, and Vassilopoulou-Sellin R. Health profiles and quality of life of 518 survivors of thyroid cancer. Head&Neck 2003 25 349-356
191. Bianco AC and Kim BW. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 2006 116 2571-2579
192. de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ, and Breteler MM. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. Journal of Clinical Endocrinology and Metabolism 2007 92 636-640
193. Kohrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. Molecular and Cellular Endocrinology 1999 151 103-119
194. Maeda A, Toyoda N, Yasuzawa-Amano S, Iwasaka T, and Nishikawa M. Type 2 deiodinase expression is stimulated by growth factors in human vascular smooth muscle cells. Molecular and Cell Endocrinology 2003 200 111-117
195. Mizuma H, Murakami M, and Mori M. Thyroid hormone activation in human vascular smooth muscle cells: expression of type II iodothyronine deiodinase. Circulation Research 2001 88 313-318
196. Salvatore D, Bartha T, Harney JW, and Larsen PR. Molecular biological and biochemical characterization of the human type 2 selenodeiodinase. Endocrinology 1996 137 3308-3315
197. Courtin F, Zrouri H, Lamirand A, Li WW, Mercier G, Schumacher M, Goascogne CL, and Pierre M. Thyroid hormone deiodinases in the central and peripheral nervous system. Thyroid 2005 15 931-942
198. Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, and Maia AL. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 2005 90 3472-3478
199. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, and Celi FS. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. Diabetes 2002 51 880-883
200. Peeters RP, van der Deure WM, and Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. European Journal of Endocrinology 2006 155 655-662

201. Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, Visser TJ. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. Journal of Clinical Endocrinology and Metabolism 2003 88 2880-2888
202. Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S, and Trischitta V. Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. Journal of Clinical Endocrinology and Metabolism 2008 93 910-913
203. Peeters RP, van den Beld AW, Attalki H, Toor H, de Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG, Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. American Journal of Physiology, Endocrinology and Metabolism 2005 289 E75-E81
204. Hansen PS, Brix TH, Sorensen TI, Kyvik KO, Hegedus L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. Journal of Clinical Endocrinology and Metabolism 2004 89 1181-1187
205. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. Journal of Clinical Endocrinology and Metabolism 2002 87 1068-1072
206. Benhadi N, Fliers E, Visser TJ, Reitsma JB, Wiersinga WM. Pilot study on the assessment of the setpoint of the hypothalamus-pituitary-thyroid axis in healthy volunteers. European Journal of Endocrinology 2010 162 323-329
207. Rivkees SA, Bode HH, and Crawford JD. Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. New England Journal of Medicine 1988 318 599-602
208. Bassett JH and Williams GR. The molecular actions of thyroid hormone in bone. Trends in Endocrinology and Metabolism 2003 14 356-364
209. Bassett JH and Williams GR. Critical role of the hypothalamic-pituitary-thyroid axis in bone. Bone 2008 43 418-426
210. Segni M and Gorman CA. The aftermath of childhood hyperthyroidism. Journal of Pediatric Endocrinology and Metabolism 2001 14 Suppl 5 1277-1282
211. Kim DJ, Khang YH, Koh JM, Shong YK, and Kim GS. Low normal TSH levels are associated with low bone mineral density in healthy postmenopausal women. Clinical Endocrinology (Oxf) 2006 64 86-90
212. Bauer DC, Ettinger B, Nevitt MC, and Stone KL. Risk for fracture in women with low serum levels of thyroid-stimulating hormone. Annals of Internal Medicine 2001 134 561-568
213. Lee WY, Oh KW, Rhee EJ, Jung CH, Kim SW, Yun EJ, Tae HJ, Baek KH, Kang MI, Choi MG, Yoo HJ, and Park SW. Relationship between subclinical thyroid dysfunction and femoral neck bone mineral density in women. Archives of Medical Research 2006 37 511-516
214. Britto JM, Fenton AJ, Holloway WR, and Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994 134 169-176
215. Basset P, Okada A, Chenard MP, Kannan R, Stoll I, Anglard P, Bellocq JP, and Rio MC. Matrix metalloproteinases as stromal effectors of human carcinoma progression: therapeutic implications. Matrix Biology 1997 15 535-541

216. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, and Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. Journal of Cell Physiology 2004 201 17-25
217. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, and Nakao K. A novel interaction between thyroid hormones and 1,25(OH)(2)D(3) in osteoclast formation. Biochemical and Biophysical Research Communications 2002 291 987-994
218. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF, and Zaidi M. TSH is a negative regulator of skeletal remodeling. Cell 2003 115 151-162
219. Morimura T, Tsunekawa K, Kasahara T, Seki K, Ogiwara T, Mori M, and Murakami M. Expression of type 2 iodothyronine deiodinase in human osteoblast is stimulated by thyrotropin. Endocrinology 2005 146 2077-2084
220. Galliford TM, Murphy E, Williams AJ, Bassett JH, and Williams GR. Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinology 2005 30 237-246
221. Heemstra KA, van der Deure WM, Peeters RP, Hamdy NA, Stokkel MP, Corssmit EP, Romijn JA, Visser TJ, and Smit JW. Thyroid hormone independent associations between serum TSH levels and indicators of bone turnover in cured patients with differentiated thyroid carcinoma. European Journal of Endocrinology 2008 159 69-76
222. Bassett JH, Nordstrom K, Boyde A, Howell PG, Kelly S, Vennstrom B, and Williams GR. Thyroid status during skeletal development determines adult bone structure and mineralization. Molecular Endocrinology 2007 21 1893-1904
223. Bassett JH, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PG, Weiss RE, Roux JP, Malaval L, Clement-Lacroix P, Samarut J, Chassande O, and Williams GR. Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Molecular Endocrinology 2007 21 1095-1107
224. van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, and Visser TJ. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. Clinical Endocrinology (Oxf) 2008 68 175-181
225. Fazio S, Palmieri EA, Lombardi G, and Biondi B. Effects of thyroid hormone on the cardiovascular system. Recent Progress in Hormonal Research 2004 59 31-50
226. Forfar JC, Muir AL, Sawers SA, and Toft AD. Abnormal left ventricular function in hyperthyroidism: evidence for a possible reversible cardiomyopathy. New England Journal of Medicine 1982 307 1165-1170
227. Klein I and Ojamaa K. Thyroid hormone and the cardiovascular system. New England Journal of Medicine 2001 344 501-509
228. Biondi B, Fazio S, Carella C, Sabatini D, Amato G, Cittadini A, Bellastella A, Lombardi G, and Sacca L. Control of adrenergic overactivity by beta-blockade improves the quality of life in patients receiving long term suppressive therapy with levothyroxine. Journal of Clinical Endocrinology and Metabolism 1994 78 1028-1033
229. Heeringa J, Hoogendoorn EH, van der Deure WM, Hofman A, Peeters RP, Hop WC, den Heijer M, Visser TJ, and Witteman JC. High-normal thyroid function and risk of atrial fibrillation: the Rotterdam study. Archives of Internal Medicine 2008 168 2219-2224
230. Ladenson PW. Recognition and management of cardiovascular disease related to thyroid dysfunction. American Journal of Medicine 1990 88 638-641

231. Rodondi N, Bauer DC, Cappola AR, Cornuz J, Robbins J, Fried LP, Ladenson PW, Vittinghoff E, Gottdiener JS, and Newman AB. Subclinical thyroid dysfunction, cardiac function, and the risk of heart failure. The Cardiovascular Health study. Journal of the American College of Cardiology 2008 52 1152-1159
232. Osman F, Franklyn JA, Holder RL, Sheppard MC, and Gammage MD. Cardiovascular manifestations of hyperthyroidism before and after antithyroid therapy: a matched case-control study. Journal of the American College of Cardiology 2007 49 71-81
233. Osman F, Gammage MD, and Franklyn JA. Thyroid disease and its treatment: short-term and long-term cardiovascular consequences. Current Opinions in Pharmacology 2001 1 626-631
234. Tielens ET, Pillay M, Storm C, and Berghout A. Cardiac function at rest in hypothyroidism evaluated by equilibrium radionuclide angiography. Clinical Endocrinology (Oxf) 1999 50 497-502
235. Santos AD, Miller RP, Mathew PK, Wallace WA, Cave WT, Jr., and Hinojosa L. Echocardiographic characterization of the reversible cardiomyopathy of hypothyroidism. American Journal of Medicine 1980 68 675-682
236. Kahaly GJ and Dillmann WH. Thyroid hormone action in the heart. Endocrine Reviews 2005 26 704-728
237. Crowley WF, Jr., Ridgway EC, Bough EW, Francis GS, Daniels GH, Kourides IA, Myers GS, and Maloof F. Noninvasive evaluation of cardiac function in hypothyroidism. Response to gradual thyroxine replacement. New England Journal of Medicine 1977 296 1-6
238. Aydin M, Reyhan M, Sukan A, Yapar AF, and Aktas A. Gated SPECT findings revealing diastolic dysfunction in acute hypothyroidism. Clinical Nuclear Medicine 2007 32 94-100
239. Botella-Carretero JI, Gomez-Bueno M, Barrios V, Caballero C, Garcia-Robles R, Sancho J, and Escobar-Morreale HF. Chronic thyrotropin-suppressive therapy with levothyroxine and short-term overt hypothyroidism after thyroxine withdrawal are associated with undesirable cardiovascular effects in patients with differentiated thyroid carcinoma. Endocrine Related Cancer 2004 11 345-356
240. Chrisoulidou A, Pazaitou-Panayiotou K, Kaprara A, Platoyiannis D, Lafaras C, Boudina M, Georgiou E, Drimonitis A, Bischiniotis T, and Vainas I. Effects of thyroxine withdrawal in biochemical parameters and cardiac function and structure in patients with differentiated thyroid cancer. Minerva Endocrinology 2006 31 173-178
241. Di Paola R, Alagona C, Pezzino V, Mangiameli S, and Regalbuto C. Left ventricular function in acute hypothyroidism: a Doppler echocardiography study. Italian Heart Journal 2004 5 857-863
242. Donaghue K, Hales I, Allwright S, Cooper R, Edwards A, Grant S, Morrow A, and Wilmshurst E. Cardiac function in acute hypothyroidism. European Journal of Nuclear Medicine 1985 11 147-149
243. Grossmann G, Wieshammer S, Keck FS, Goller V, Giesler M, and Hombach V. Doppler echocardiographic evaluation of left ventricular diastolic function in acute hypothyroidism. Clinical Endocrinology(Oxf) 1994 40 227-233
244. Grossmann G, Keck FS, Wieshammer S, Goller V, Schmidt A, and Hombach V. Systolic ventricular function in acute hypothyroidism: a study using Doppler echocardiography. Experimental Clininical Endocrinology 1994 102 104-110
245. Kahaly G, Mohr-Kahaly S, Beyer J, and Meyer J. Left ventricular function analyzed by Doppler and echocardiographic methods in short-term hypothyroidism. American Journal of Cardiology 1995 75 645-648

246. Wieshammer S, Keck FS, Waitzinger J, Henze E, Loos U, Hombach V, and Pfeiffer EF. Acute hypothyroidism slows the rate of left ventricular diastolic relaxation. Canadian Journal of Physiolog and Pharmacology 1989 67 1007-1010
247. Gulseren S, Gulseren L, Hekimsoy Z, Cetinay P, Ozen C, and Tokatlioglu B. Depression, anxiety, health-related quality of life, and disability in patients with overt and subclinical thyroid dysfunction. Archives of Medical Research 2006 37 133-139
248. Tan LG, Nan L, Thumboo J, Sundram F, and Tan LK. Health-related quality of life in thyroid cancer survivors. Laryngoscope 2007 117 507-510

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Retinoic acid receptor and retinoid X receptor subtype expression for the differential diagnosis of thyroid neoplasms

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Abstract

Background

Although differential expression of retinoic acid receptor (RAR) subtypes between benign and malignant thyroid tissues has been described, their diagnostic value has not been reported.

Aim

To investigate the diagnostic accuracy of RAR and retinoid X receptor (RXR) subtype protein expression for the differential diagnosis of thyroid neoplasms.

Methods

We used a tissue array containing 93 benign thyroid tissues (normal thyroid, multinodular goiter, and follicular adenoma (FA)) and 77 thyroid carcinomas (papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, and follicular variant of PTC (FVPTC)). Immunostaining was done for RAR and RXR subtypes. Staining was analyzed semi quantitatively based on receiver operating curve analyses and using hierarchical cluster analysis.

Results

We found increased expression of cytoplasmic (c) RARalpha, cRARgamma, cRXRbeta and decreased expression of nuclear (n) RARbeta, nRARgamma, and nRXRalpha in thyroid carcinomas compared with benign tissues. We found three proteins differently expressed between FA and FTC and five proteins differentially expressed between FA and FVPTC, with high diagnostic accuracies. Using cluster analysis, the combination of negative staining of membranous RXRbeta and positive staining for cRXRbeta had a high positive predictive value (98%) for malignant thyroid disease, whereas the combination of positive nRXRalpha and negative cRXRbeta staining had a high predictive value (91%) for benign thyroid lesions.

Conclusion

We conclude that differences in RAR and RXR subtype protein expression may be valuable for the differential diagnosis of thyroid neoplasms. The results of this study and especially the value of cluster analysis have to be confirmed in subsequent studies.

Introduction

The microscopical distinction between benign and malignant neoplastic thyroid nodules by conventional histology is often difficult as these lesions may share overlapping histological characteristics. Therefore, it is important to identify new markers to distinguish benign from malignant thyroid tumors. In recent years, several immunohistochemical markers have been studied to improve the differential diagnosis of thyroid lesions, using both candidate markers and unbiased approaches (1–12).

The expression of retinoid receptors may be interesting for the differentiation between benign and malignant thyroid tissues. Retinoids are important for growth, differentiation, and morphogenesis in vertebrates (13). Retinoids are derivatives of vitamin A (i.e. retinol). Retinoid receptors belong to the family of nuclear receptors and can be distinguished in retinoic acid receptors (RAR) and retinoid X receptors (RXR). According to the literature, retinoid receptors appear to be differentially expressed in benign and malignant thyroid tissues, the general picture being decreased expression of retinoid receptor subtypes in thyroid cancer (**Table 1**) (14–20), which may also have therapeutic implications (17,18,21–23). However, in these publications on retinoid receptor expression in thyroid lesions, the question whether retinoid receptor expression could be used for the differential diagnosis of thyroid neoplasms was not addressed, probably because most studies included relatively small number of patient samples or the studies included only a subset of retinoid receptors (**Table 1**).

We therefore, decided to study the diagnostic value of RAR and RXR subtype expression in benign and malignant thyroid tissues, using receiver operating curve (ROC) analyses as well as hierarchical cluster analysis (12).

Materials and methods

Thyroid tissues

We obtained one hundred and seventy histological samples from surgically removed thyroid lesions representing five different histological thyroid disorders and adjacent thyroid normal tissue from the archive of the Department of Pathology of the Leiden University Medical Center. We selected 93 benign thyroid tissues (normal n=64, multinodular goiter n=16, follicular adenoma (FA) n=13), and 77 non-medullary thyroid carcinomas (papillary thyroid carcinoma (PTC) n=53, follicular thyroid carcinoma (FTC) n=13 and follicular variant of PTC (FVPTC) n=11). All original histological diagnoses were reviewed by two independent observers. Given the variability in phenotype of follicular lesions, only micro follicular FA and widely invasive FTC's were included on which both observers agreed. Likewise, we only included encapsulated FVPTC tumors with a typical PTC nuclear pattern on which both observers agreed.

Table 1: Overview of literature on retinoic acid receptor and retinoid X receptor expression in thyroid tissue samples and carcinoma cell lines

Study	# tissue samples / cell lines		Method	Retinoid receptor investigated	Results
Rochaix *et al.* (1998) (14)	58 samples	16 PTC 2 FTC 30 CTL 6 MNG 2 FA 2 toxic goiter	Immunohistochemistry Western blot	RARβ	Reduced RARβ expression in PTC compared to normal tissue Moderate RARβ expression in one FTC, none in the other
Schmutzler *et al.* (1998) (15)	4 cell lines	FTC-133 FTC-238 HTh74 (ATC) C643 (ATC)	RT-PCR Northern blot	RARα RARβ RARγ RXRα RXRβ RXRγ	Expression RARα, β, γ, RXR α and β on all carcinoma cell lines, however lower compared to goiterous cells. Lowest in FTC cells
	18 samples	2 CTL 2 adenomas 2 unknown Ca 3 FTC 3 OTC 6 PTC			9 of 12 tumor samples decreased or absent expression of RXRβ
Takiyama *et al.* (2003) (16)	176 samples	57 PTC 40 FTC 24 ATC 28 MTC 27 FA	Immunohistochemistry RT-PCR and Western blot	RXRα RXRβ RXRγ	Decreased nuclear expression of all RXR isoforms in carcinoma PTC and FTC low nuclear expression, moderate cytoplasmic expression ATC no expression RXRγ
	3 cell lines	ARO (ATC) WRO (FTC) NPA (PTC)			FA distinct nuclear staining RXRα and RXRβ RXRγ undetectable in WRO, RXRα and RXRβ detectable in all cell lines
Schmutzler *et al.* (2004) (17)	4 cell lines	FTC-133 FTC-238 HTh74 (ATC) C643 (ATC)	RT-PCR and Northern blot	RARα RARβ	Reduced level RARβ in FTC-238 Reduced level RARα in HTh74 and C643

Table 1: Continued

Study	# tissue samples / cell lines		Method	Retinoid receptor investigated	Results
Haugen *et al.* (2004) (18)	10 samples	5 PTC 1 FTC 1 insular 1 ATC 2 FA	RT-PCR and Western blot	RARα RARβ RARγ RXRα RXRβ	RARα and γ expressed in all cell lines RARβ not expressed in FTC cells RXRα and β decreased expressed in ATC cells RXRγ expressed only in ATC
	4 cell lines	MRO-87 (FTC) WRO-82 (FTC) TAD-2 (CTL) DRO-90 (ATC)		RXRγ	RARβ expression decreased in malignant tissues RXRγ expression decreased in benign tissue and increased in malignant tissue
Elisei *et at.* (2005) (19)	24 samples	10 PTC 10 CTL 4 ATC	Immunohistochemistry	RARβ	Decreased RARβ in PTC and ATC compared to controls
Koh *et al.* (2006) (20)	3 cell lines	SNU-80 (PTC) SNU-373 (PTC) SNU-790 (ATC)		RARα RARβ RARγ	RARβ not expressed RARα detected in all three cell lines RARγ detected in SNU-80 and SNU-373

PTC= papillary thyroid carcinoma, FTC= follicular thyroid carcinoma, CTL= normal thyroid tissue, MNG= multi nodular goiter, FA= follicular adenoma, OTC= oncocytic thyroid carcinoma, ATC= anaplastic thyroid carcinoma, RT-PCR= real time peroxidase chain reaction, RA= retinoic acid, RAR= retinoic acid receptor, RXR= retinoid X receptor

Tissue microarray

Formalin-fixed, paraffin-embedded blocks routinely prepared from surgical specimens of thyroid tumors were selected for this study. Representative areas containing tumor or adjacent normal tissue were identified by a pathologist. Triplicate tissue cores with a diameter of 0.6 mm were taken from each specimen (Beecher Instruments, Silver Springs, MD, USA) and arrayed on a recipient paraffin block, using standard procedures (24).

Immunohistochemistry methods

Four micrometer consecutive tissue sections were cut from each arrayed paraffin block and prepared on pathological slides. The sections were deparaffinized in xylene followed by 0.3% hydrogen peroxide in methanol at room temperature for 20 min to block endogenous peroxidase. After rehydration, antigen retrieval was performed by microwave treatment in 0.001 M citrate buffer (pH 6.0). The sections were incubated with the following primary antibodies against RAR and RXR subtypes: anti-RARalpha monoclonal antibody 9A9A6, dilution 1:3000; anti- RARbeta monoclonal antibody 8B10B2, dilution 1:200; anti-RARgamma monoclonal antibody. 4G-7A11, dilution 1:350; anti-RXRalpha monoclonal antibody 4RX3A2, dilution 1:1000 (all gifts of Dr C Rochette-Egly, IGBMC, Illkirch, France), anti-RXRbeta polyclonal antibody sc-831, dilution 1:650 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-RXRgamma polyclonal antibody sc-555, dilution 1:500 (Santa Cruz). Sections were incubated overnight at room temperature with the primary antibodies, dissolved in PBS with 1% bovine serum albumin. Subsequently, the sections were incubated for 30 min with either the biotinylated rabbit-anti-mouse conjugate, dilution 1:200 or goat-anti-rabbit, dilution 1:400 (DakoCytomation, Glostrup, Denmark), followed by incubation for 30 min with the streptavidin-biotin-peroxidase conjugate. This step was performed by 10-min incubation with 3,3′- diaminobenzidine-tetrachloride substrate in a buffered 0.05-M Tris/HCl (pH 7.6) solution containing 0.002% hydrogen peroxide. Negative controls were stained with the primary antibody omitted. The sections were counterstained with hematoxylin.

Immunohistochemical scoring

A semi quantitative assessment of immunohistochemical scoring was performed including both the intensity of staining and the percentage of positive cells. The percentage of cells with positive staining was scored as follows: >0–20%: ‘1’, >20–50%: ‘2’, >50–70%: ‘3’, and >70–100% ‘4’. The staining intensity was scored as faint: ‘1’, intermediate: ‘2’, and intense: ‘3’. Scores for proportion of positive cells and intensity were multiplied. Nuclear, cytoplasmic, and membranous staining was scored inde-

pendently. The total score per sample therefore ranged from 0 to 12. Score results for triplicate samples were averaged.

Statistical analyses

Statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Initially, staining scores for every individual antibody were expressed as mean ± SD per histological category (**Table 2**). The next step was the analysis of differences in staining scores for each antibody between malignant *versus* benign tissues, malignant *versus* normal tissues, FA *versus* FTC and FA *versus* FVPTC using the Mann-Whitney test. For each differentially expressed antibody between two histological categories, the optimal cut-off value for the distinction between the two categories was determined by receiver operating characteristic (ROC) analysis. In theory, this could give different cut-off values for one antibody for different comparisons. Only antibodies with sensitivities and specificities above 70% were included in further analyses. In addition to the individual protein markers, the analysis of the diagnostic accuracy of panels of antibodies was performed using hierarchical clustering analysis of tissue microarray data using Cluster and TreeView (Cluster and TreeView 2.11; Eisen Lab, University of California at Berkeley, CA, USA) (12,25). Input for these analyses was the individual staining score per sample for each antibody. A P-value of <0.05 was considered significant.

Table 2: Results of retinoid receptor staining

	Normal n=64	**Multi nodular goiter** n=16	**Follicular adenoma** n=13	**FTC** n=13	**FVPTC** n=11	**PTC** n=53
Nuclear RARα	4.47 ± 1.98	4.06 ± 1.46	4.88 ± 1.67	6.02 ± 3.71	1.12 ± 2.39	2.56 ± 2.43
Cytoplasmic RARα	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.43 ± 4.47	1.67 ± 2.42	1.21 ± 2.47
Nuclear RARβ	8.53 ± 2.20	8.62 ± 1.42	8.63 ± 2.21	5.62 ± 3.37	5.78 ± 3.12	6.21 ± 3.18
Nuclear RARγ	4.56 ± 2.59	3.17 ± 1.40	3.17 ± 2.26	1.61 ± 1.58	0.85 ± 1.92	2.00 ± 2.63
Cytoplasmic RARγ	0.00 ± 0.00	0.00 ± 0.00	0.55 ± 1.81	1.78 ± 3.54	0.54 ± 1.80	0.93 ± 2.58
Nuclear RXRα	2.29 ± 2.00	2.27 ± 2.03	2.64 ± 2.60	0.44 ± 0.12	0.00 ± 0.00	0.37 ± 1.10
Nuclear RXRβ	0.71 ± 1.62	0.36 ± 1.21	0.00 ± 0.00	0.00 ± 0.00	0.30 ± 0.95	0.32 ± 1.33
Cytoplasmic RXRβ	0.07 ± 0.53	0.00 ± 0.00	2.18 ± 4.85	5.99 ± 4.89	2.68 ± 4.54	6.80 ± 4.55
Membranous RXRβ	1.66 ± 2.06	3.34 ± 2.05	4.53 ± 2.69	1.88 ± 1.88	1.41 ± 2.84	0.92 ± 2.20

Scoring method: The percentage of cells with positive staining was scored: >0–20%: '1', >20–50%: '2', >50–70%: '3', and >70–100% '4'. The intensity was scored as faint: '1', intermediate: '2', and intense: '3'. These scores were multiplied by each other for a combination score. Score results for triplicate samples were averaged. Distinctive scores were categorized according to nuclear, cytoplasm and membranous staining patterns. Data are mean ± SD. RAR= Retinoic Acid Receptor RXR= Retinoid X Receptor

Results

RAR and RXR expression in thyroid lesions: benign versus malignant

The scores for expression of RAR and RXR receptor subclasses are shown in **Table 2**. Benign tissue samples had an overall lower expression of cytoplasmic RARalpha (cRARalpha), cytoplasmic RARgamma (cRARgamma), and cytoplasmic RXRbeta (cRXRbeta) and a higher expression of nuclear RARalpha (nRARalpha), nuclear RARbeta (nRARbeta), nuclear RARgamma (nRARgamma) and nuclear RXRalpha (nRXRalpha) compared with malignant tissues. FA scored particularly high for nuclear RXRalpha (nRXRalpha) and low for nuclear RXRbeta (nRXRbeta) expression. FVPTC also had a very low expression of nRXRbeta. RXRgamma staining did not reveal a positive result in all thyroid tissues, and was excluded from further analyses.

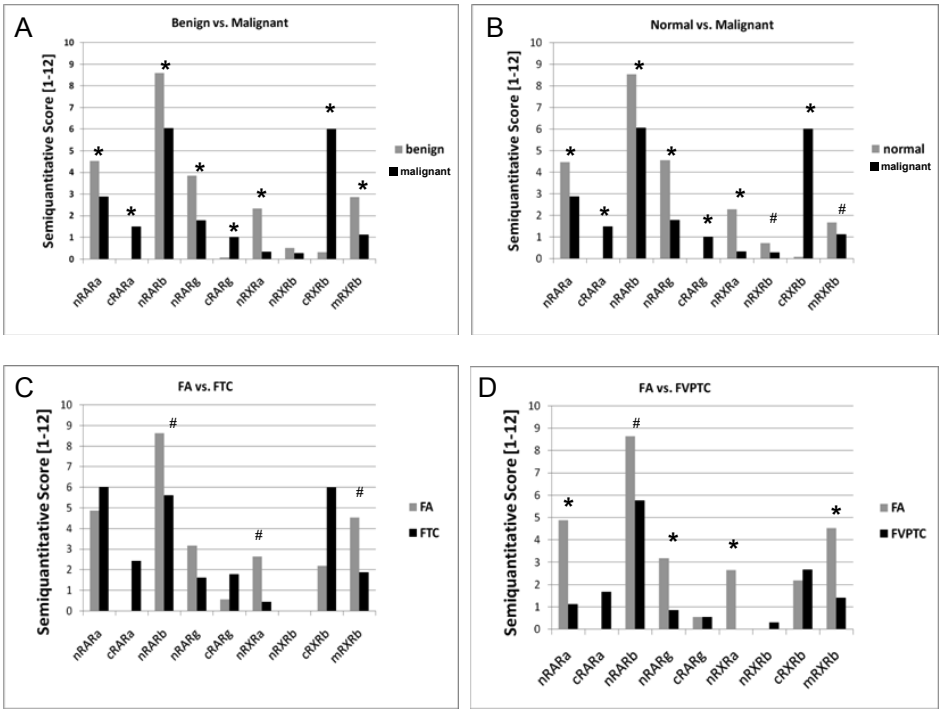


Figure 1

Immunostaining of RAR and RXR subtype antibodies in normal, benign, and malignant thyroid lesions. Immunohistochemistry scores were expressed semi-quantitatively (for explanation, see text). Data are expressed as mean ± S.D. Comparisons between (A) benign and malignant, (B) normal and malignant, follicular adenoma (FA) and follicular thyroid carcinoma (FTC), and (D) FA and follicular variant of papillary carcinoma (FVPTC) were performed with the Mann–Whitney test.
*P<0.005; # P<0.05; n= nuclear; c= cytoplasmic; m= membranous; RAR= Retinoic Acid Receptor; RXR= Retinoid X Receptor

Figure 1 shows the differences in expression patterns for different categories of thyroid tissues. All RAR and RXR subtypes appeared to be differentially expressed between malignant thyroid lesions and normal thyroid tissue.

RAR and RXR expression in thyroid lesions: follicular lesions

The differentiation between follicular lesions (FA, FTC, and FVPTC) is difficult. Therefore, we compared these subgroups separately. FTC had a significantly lower expression of nRARbeta, nRXRalpha, and mRXRbeta compared with FA. FVPTC had a significantly lower expression of nRARalpha, nRARbeta, nRARgamma, nRXRalpha, and mRXRbeta compared with FA (**Figure 1**).

ROC analyses

For each differentially expressed antibody between two categories, the optimal cut-off values for the distinction between the two histological classes were determined by ROC analysis. Only antibodies with sensitivities and specificities above 70% were used for further analyses (**Table 3**). Comparison of the expression between benign and malignant thyroid tissues revealed sensitivities and specificities >70% for nRXRalpha, cRXRbeta, and mRXRbeta, the highest sensitivity (89%) and specificity (96%) for nuclear RXRalpha (**Table 3**). NRXRalpha and cRXRbeta also discriminated reasonably between malignant and normal thyroid tissues.

In the comparison between FA and FTC, nRARbeta, nRARalpha, cRXRbeta, and mRXRbeta had sensitivities and specificities above 70%, the highest sensitivity for FTC found for nRARalpha (85%) and the highest specificity for nRARbeta (91%; **Table 3**).

In the comparison between FA and FVPTC, nRARbeta, nRARgamma, nRARalpha, nRXRalpha and mRXRbeta had sensitivities and specificities above 70%. The highest sensitivity for FVPTC was found for nRXRalpha (100%) and the highest specificities for both nuclear nRARalpha (91%) and nRARgamma (91%; **Table 3**).

Hierarchical cluster analysis

To identify the optimal combinations of RAR and RXR subtype expression for the differential diagnosis of thyroid neoplasms, we performed an unsupervised hierarchical cluster analysis, the results of which are shown in **Table 4** and **Figure 2**. We found that 98% of thyroid lesions in cluster 2 (negative staining of mRXRbeta and positive staining for cRXRbeta) were malignant; whereas 91% of the lesions in cluster 4 (positive staining for nRXRalpha and a negative staining for cRXRbeta) were benign. The diagnostic parameters are summarized in **Table 4**. In general, the follicular lesions

Table 3: Diagnostic value of RAR and RXR differentially expressed in thyroid tissues with sensitivity and specificity above 70%.

	Malignant versus benign			**Malignant versus normal**		
	Cut-off level [a]	Sensitivity for malignancy (%)	Specificity for malignancy (%)	Cut-off level [a]	Sensitivity for malignancy (%)	Specificity for malignancy (%)
Nuclear RAR β				<8.5	72	80
Nuclear RXR α	<1	89	96	<1	89	75
Cytoplasmic RXR β	>1	71	96	>1	71	89
Membranous RXR β	<1	74	72			
	FTC versus FA			**FVPTC versus FA**		
	Cut-off level [a]	Sensitivity for FTC (%)	Specificity for FTC (%)	Cut-off level [a]	Sensitivity for FVPTC (%)	Specificity for FVPTC (%)
Nuclear RAR α	<1	85	80	<3	92	91
Nuclear RAR β	<8	73	91	<8	91	82
Nuclear RAR γ				>1	82	91
Nuclear RXR α				<1	100	73
Cytoplasmic RXR β	>2	71	82			
Membranous RXR β	<4	82	75	<1	82	82

Benign thyroid tissues= multinodular goiter, follicular adenoma and normal
RAR= retinoic acid receptor, RXR= retinoid X receptor. [a] Obtained by ROC analyses of semi-quantitative immunohistochemistry scores.

did not cluster separately, but we found that only one FA was present in cluster 2 (high positive predictive value for malignancy), whereas in cluster 4 (high positive predictive value for benign lesions) only one FTC was present (**Figure 2**).

Discussion

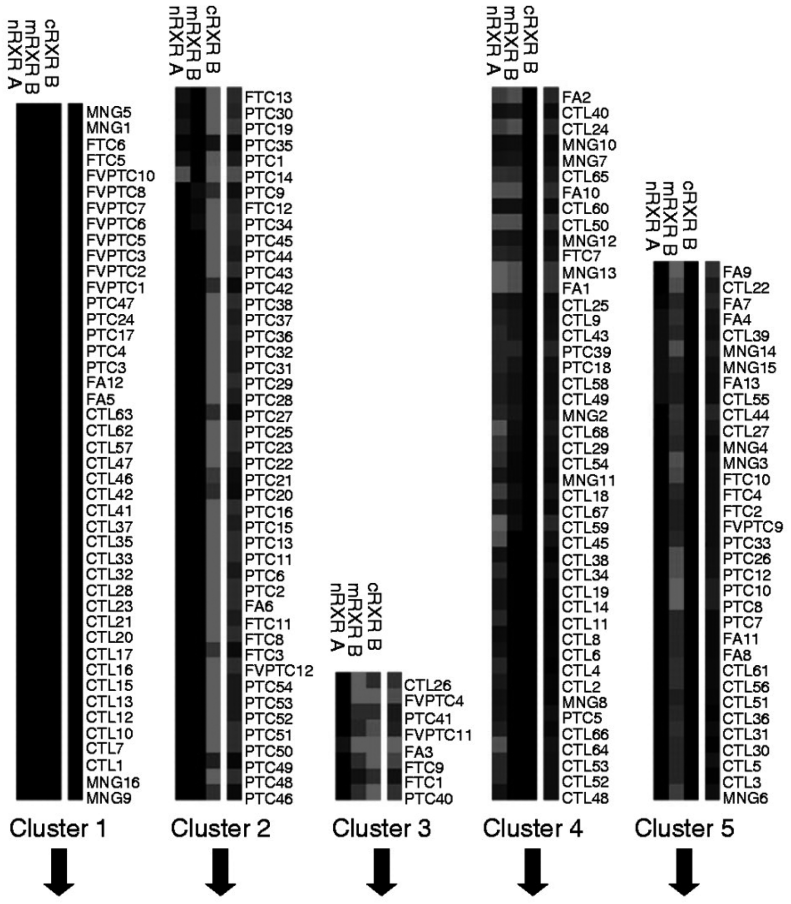
The present study was performed to evaluate the diagnostic value of the expression of RAR and RXR subtypes in a large panel of thyroid neoplasms. To our knowledge, the diagnostic value of RAR and RXR receptor expression for the differential diagnosis of thyroid neoplasms has not been published before (14–20). Our study also differed from earlier ones with regard to the identification of optimal semi quantitative cut-off levels using ROC analyses and hierarchical cluster analysis.

Table 4: Diagnostic value of combinations of retinoid receptor staining for benign *vs*. malignant thyroid lesions, based on hierarchical cluster analysis.

	Combination mRXRβ- and cRXRβ+	**Combination not present**	**Total**
(a) Diagnostic value of combinations of retinoid receptor staining for benign versus malignant thyroid lesions, based on cluster 2 in hierarchical cluster analysis			
Malignant	44	35	79
Benign	1 (FA)	96	98
Total	45	131	176
	PPV malignancy = 98%	NPV malignancy = 72%	LR malignant 56
	Combination nRXRα+ and cRXRβ -	**Combination not present**	**Total**
(b) Diagnostic value of nRXRα and cRXRβ staining for benign versus malignant thyroid lesions, based on cluster 4 in hierarchical cluster analysis			
Benign	41	56	97
Malignant	4 (3 PTC and 1 FTC)	75	79
Total	45	130	175
	PPV benign = 91%	NPV benign = 57%	LR benign 8.6

RAR= retinoic acid receptor, RXR= retinoid X receptor, PPV= positive predicting value, NPV= negative predicting value, LR= likelihood ratio

In general, we found an increased expression of cRARalpha, cRARgamma, cRXRbeta, and a decreased expression of nRARbeta, nRARgamma, and nRARalpha in thyroid carcinomas compared with benign thyroid tissue. The most challenging pathological differential diagnosis is between FA, FTC, and FVPTC. We found three proteins differentially expressed between FA and FTC and five proteins differentially expressed between FA and FVPTC. In the comparison between FA and FTC the highest sensitivity for FTC was found for nRARalpha and the highest specificity for nRARbeta. In the comparison between FA and FVPTC, the highest sensitivity for FVPTC was found for nRXRalpha and the highest specificities for nRARalpha and nRARgamma. Some of these observations are in line with other studies that investigated RAR and/or RXR expression in thyroid tissue samples (**Table 1**). Rochaix *et al*. (14) (Immunohistochemistry), Haugen *et al*. (18) (RT-PCR), and Elisei *et al*. (19) (RT-PCR) also found reduced RARbeta expression in PTC, compared with normal tissue. Rochaix *et al*. (14) only investigated two FTC samples of which one sample showed moderate RARbeta expression and the other did not. Our finding of higher nuclear and lower cytoplasm expression of RARgamma in malignant thyroid tissues was not reported before. Nuclear RXRalpha expression was low or absent in thyroid carcinomas in our study. This finding is confirmed by a paper by Takiyama *et al*. (16). We did not find positive RXRgamma staining in thyroid tissues, which is unexpected, given the results of Haugen *et al*. by western blot (18).



	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
nRXRA	–	+/–	–	+	–
mRXRB	–	–	+	+/–	+
cRXRB	–	+	+	–	–
Malignant	15	44	6	4	10
Benign	29	1	2	41	24

Figure 2

Hierarchical cluster analyses using RAR and RXR subtype antibodies in thyroid tissues. Nuclear RXRalpha, cytoplasmic RXRbeta, and membranous RXRbeta were identified as the best predictors of benign or malignant thyroid lesions. The absence of membranous (m) RXRbeta and the presence of cytoplasmic (c) RXRbeta had a high positive predictive value for malignancy (98%, cluster 2). The presence of nuclear (n) RXRalpha and absence of cytoplasmic RXRbeta had a high positive predictive value for benign lesions (91%, cluster 4). CTL= normal thyroid, FA= follicular adenoma, FTC= follicular thyroid carcinoma, PTC= papillary thyroid carcinoma, FVPTC= follicular variant PTC.

There are two studies on RXRbeta expression in thyroid neoplasms (15,16). They both found decreased or absent expression of RXRbeta in carcinomas. One of these studies, however, (15) used RT-PCR and contained only 12 human thyroid carcinoma samples. In our study, we differentiated between nuclear, cytoplasmic, and membranous staining. The only study that also differentiated between nuclear and cytoplasm staining pattern, only investigated RXR isoform expression (16).

We performed a cluster analysis including all studied tissues and antibodies. Our findings showed that the combination of negative staining of mRXRbeta and a positive staining for cRXRbeta had a high accuracy for the detection of malignant thyroid tissues, whereas the combination of a positive staining for nRXRalpha and a negative staining for cRXRbeta was present in most benign tissues.

There are some limitations to our study. Although we were able to distinguish between follicular lesions, the number of follicular lesions was relatively small. Therefore, additional studies should be performed with larger numbers of follicular lesions, also including histological subtypes of follicular lesions. Moreover, the findings of our study and the clinical usefulness of hierarchical cluster analysis have to be validated in subsequent studies and most importantly in cytological preparations. Also, other difficult-to-classify thyroid neoplasms such as minimally invasive follicular carcinomas as well as FA subclasses should be included in subsequent studies. The biological mechanisms responsible for the differential expression of RAR and RXR between thyroid tissues also remain to be elucidated. In conclusion, differences in RAR and RXR subtype protein expression as studied by immunohistochemistry may be of additional value in the differential diagnosis of thyroid neoplasms.

References

1. Huang Y, Prasad M, Lemon WJ, Hampel H, Wright FA, Kornacker K, LiVolsi V, Frankel W, Kloos RT, Eng C, Pellegata NS, de la Chapelle A. Gene expression in papillary thyroid carcinoma reveals highly consistent profiles. PNAS 2001 98 15044–15049.
2. Zhao J, Leonard C, Gemsenjager E, Heitz PU, Moch H, Odermatt B. Differentiation of human follicular thyroid adenomas from carcinomas by gene expression profiling. Oncology Reports 2008 19 329–337.
3. Zhao J, Leonard C, Brunner E, Gemsenjager E, Heitz PU, Odermatt B. Molecular characterization of well-differentiated human thyroid carcinomas by cDNA arrays. International Journal of Oncology 2006 29 1041–1051.
4. Lubitz CC, Fahey TJ III. Gene expression profiling of thyroid tumors – clinical applicability. Nature Clinical Practice. Endocrinology, Metabolism 2006 2 472–473.
5. Fryknas M, Wickenberg-Bolin U, Goransson H, Gustafsson MG, Foukakis T, Lee JJ, Landegren U, Hoog A, Larsson C, Grimelius L, Wallin G, Pettersson U, Isaksson A. Molecular markers for discrimination of benign and malignant follicular thyroid tumors. Tumour Biology 2006 27 211–220.
6. Eszlinger M, Wiench M, Jarzab B, Krohn K, Beck M, Lauter J, Gubala E, Fujarewicz K, Swierniak A, Paschke R. Meta- and reanalysis of gene expression profiles of hot and cold thyroid nodules and papillary thyroid carcinoma for gene groups. Journal of Clinical Endocrinology and Metabolism 2006 91 1934–1942.
7. Fujarewicz K, Jarzab M, Eszlinger M, Krohn K, Paschke R, Oczko- Wojciechowska M, Wiench M, Kukulska A, Jarzab B, Swierniak A. A multi-gene approach to differentiate papillary thyroid carcinoma from benign lesions: gene selection using support vector machines with bootstrapping. Endocrine-Related Cancer 2007 14 809–826.
8. Rosen J, He M, Umbricht C, Alexander HR, Dackiw AP, Zeiger MA, Libutti SK. A six-gene model for differentiating benign from malignant thyroid tumors on the basis of gene expression. Surgery 2005 138 1050–1056.
9. Stolf BS, Abreu CM, Mahler-Araujo MB, Dellamano M, Martins WK, de Carvalho MB, Curado MP, Diaz JP, Fabri A, Brentani H, Carvalho AF, Soares FA, Kowalski LP, Hirata R Jr, Reis LF. Expression profile of malignant and non-malignant diseases of the thyroid gland reveals altered expression of a common set of genes in goiter and papillary carcinomas. Cancer Letters 2005 227 59–73.
10. Finley DJ, Lubitz CC, Wei C, Zhu B, Fahey TJ III. Advancing the molecular diagnosis of thyroid nodules: defining benign lesions by molecular profiling. Thyroid 2005 15 562–568.
11. Wasenius VM, Hemmer S, Kettunen E, Knuutila S, Franssila K, Joensuu H. Hepatocyte growth factor receptor, matrix metalloproteinase-11, tissue inhibitor of metalloproteinase-1, and fibronectin are up-regulated in papillary thyroid carcinoma: a cDNAand tissue microarray study. Clinical Cancer Research 2003 9 68–75.
12. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED- 1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor-gamma, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. European Journal of Endocrinology 2008 158 375–384.
13. Smith MA, Parkinson DR, Cheson BD, Friedman MA. Retinoids in cancer therapy. Journal of Clinical Oncology 1992 10 839–864.

14. Rochaix P, Monteil-Onteniente S, Rochette-Egly C, Caratero C, Voigt JJ, Jozan S. Reduced expression of retinoic acid receptor beta protein (RAR beta) in human papillary thyroid carcinoma: immunohistochemical and western blot study. Histopathology 1998 33 337–343.
15. Schmutzler C, Brtko J, Winzer R, Jakobs TC, Meissner-Weigl J, Simon D, Goretzki PE, Kohrle J. Functional retinoid and thyroid hormone receptors in human thyroid-carcinoma cell lines and tissues. International Journal of Cancer 1998 76 368–376.
16. Takiyama Y, Miyokawa N, Sugawara A, Kato S, Ito K, Sato K, Oikawa K, Kobayashi H, Kimura S, Tateno M. Decreased expression of retinoid X receptor isoforms in human thyroid carcinomas. Journal of Clinical Endocrinology and Metabolism 2004 89 5851–5861.
17. Schmutzler C, Hoang-Vu C, Ruger B, Kohrle J. Human thyroid carcinoma cell lines show different retinoic acid receptor repertoires and retinoid responses. European Journal of Endocrinology 2004 150 547–556.
18. Haugen BR, Larson LL, Pugazhenthi U, Hays WR, Klopper JP, Kramer CA, Sharma V. Retinoic acid and retinoid X receptors are differentially expressed in thyroid cancer and thyroid carcinoma cell lines and predict response to treatment with retinoids. Journal of Clinical Endocrinology and Metabolism 2004 89 272–280.
19. Elisei R, Vivaldi A, Agate L, Ciampi R, Molinaro E, Piampiani P, Romei C, Faviana P, Basolo F, Miccoli P, Capodanno A, Collecchi P, Pacini F, Pinchera A. All-trans-retinoic acid treatment inhibits the growth of retinoic acid receptor beta messenger ribonucleic acid expressing thyroid cancer cell lines but does not reinduce the expression of thyroid-specific genes. Journal of Clinical Endocrinology and Metabolism 2005 90 2403–2411.
20. Koh CS, Ku JL, Park SY, Kim KH, Choi JS, Kim IJ, Park JH, Oh SK, Chung JK, Lee JH, Kim WH, Kim CW, Cho BY, Park JG. Establishment and characterization of cell lines from three human thyroid carcinomas: responses to all-trans-retinoic acid and mutations in the BRAF gene. Molecular and Cellular Endocrinology 2007 264 118–127.
21. Havekes B, Schroder Van Der Elst JP, van der PG, Goslings BM, Romijn JA, Smit JW. Beneficial effects of retinoic acid on extracellular matrix degradation and attachment behaviour in follicular thyroid carcinoma cell lines. Journal of Endocrinology 2000 167 229–238.
22. Simon D, Korber C, Krausch M, Segering J, Groth P, Gorges R, Grunwald F, Muller-Gartner HW, Schmutzler C, Kohrle J, Roher HD, Reiners C. Clinical impact of retinoids in redifferentiation therapy of advanced thyroid cancer: final results of a pilot study. European Journal of Nuclear Medicine and Molecular Imaging 2002 29 775–782.
23. Liu YY, Stokkel MP, Pereira AM, Corssmit EP, Morreau HA, Romijn JA, Smit JW. Bexarotene increases uptake of radioiodine in metastases of differentiated thyroid carcinoma. European Journal of Endocrinology 2006 154 525–531.
24. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nature Medicine 1998 4 844–847.
25. Toronen P. Selection of informative clusters from hierarchical cluster tree with gene classes. BMC Bioinformatics 2004 5 32.

3

Beneficial effects of sorafenib on tumor progression, but not on radioiodine uptake, in patients with differentiated thyroid carcinoma

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Abstract

Objective

Treatment options for patients with radioactive iodine (RaI) refractory metastases of differentiated thyroid carcinoma (DTC) are limited. We studied the effects of the multitarget tyrosine kinase inhibitor sorafenib on the reinduction of RaI uptake and tumor progression.

Design

Open, single center, single arm 26-week prospective phase II study with open-ended extension.

Methods

We treated 31 patients with progressive metastatic or locally advanced RaI refractory DTC with sorafenib 400 mg b.i.d.. The primary endpoint was reinduction of RaI uptake at 26 weeks. Additional endpoints were the radiological response and the influence on bone metastases.

Results

At 26 weeks of sorafenib therapy, no reinduction of RaI uptake at metastatic sites was observed, but 19 patients (59%) had a clinical beneficial response, eight of whom had a partial response (25%) and 11 had stable disease (34%). Seven patients had progressive disease (22%). Sorafenib was significantly less effective in patients with bone metastases. The estimated median progression free survival was 58 weeks (95% confidence interval, CI, 47–68). In general, thyroglobulin (Tg) response (both unstimulated and TSH stimulated) reflected radiological responses. The median time of the nadir of Tg levels was 3 months. Responses were not influenced by histological subtype, mutational status or other variables. No unusual side effects were observed.

Conclusions

Sorafenib has a beneficial effect on tumor progression in patients with metastatic DTC, but was less effective in patients with bone metastases. Diagnostic whole body scintigraphy did not reveal an effect of sorafenib on the reinduction of RaI uptake.

Introduction

The prognosis of differentiated thyroid carcinoma (DTC) in general is favorable due to the efficacy of the combined treatment of surgery and radioactive iodine (RaI) and the biological behavior of the tumor (1, 2). However, approximately 50% of patients with distant metastases of DTC die within 10 years of diagnosis (3). Although the role of RaI in recurrent or metastatic thyroid cancer is beyond dispute (4–6), the efficacy of this therapy is hampered by the decreased expression of the Sodium-Iodide-Symporter (NIS) in DTC during the process of dedifferentiation (7–9). Predictive factors for responsiveness to RaI treatment include a younger age, small metastases, well-differentiated thyroid tumor histology and the absence of uptake of 18-fluoro deoxyglucose (FDG) at FDG-positron emission tomography (PET) scanning. Especially in patients with bone metastases, the efficacy of RaI is limited to 7–20% of the patients (4,6,10,11). At present, there are no effective therapies available for RaI non-avid DTC. Conventional chemotherapy is hardly effective in DTC, and no longer recommended in international guidelines (12,13).

In DTC, many genetic alterations have been identified, involving tyrosine kinase signaling pathways (14–16). In nearly all cases of papillary thyroid carcinoma (PTC), genetic defects involving the RET, RAS, and RAF protein kinase signaling cascade are identified. The BRAFV600E mutation has been found in 29–69% of PTC and has been associated with aggressive features including extrathyroidal extension and advanced stage (17). Translocations of RET observed in PTC result in a chimeric protein consisting of an activated RET tyrosine kinase domain (15,18–32). Follicular thyroid carcinomas (FTC) frequently harbor mutations in one of the three RAS genes. The RET–RAS–RAF pathway is interconnected with the epithelial growth factor receptor (EFGR) activated cascade that among others leads to vascular endothelial growth factor (VEGF) and VEGF receptor (VEGFR) synthesis. Therefore, compounds targeting the activated RET–RAS–RAF pathway and beyond may be effective in non-RaI avid DTC. The anti-EGFR compound gefitinib was not successful in 27 patients with DTC, medullary or anaplastic thyroid carcinoma (33). In a phase II study in 60 thyroid carcinoma patients with various histologies, the VEGFR inhibitor axitinib showed a partial response of 30% (median progression free survival (PFS) 18 months) (34). Recently, 3 phase II studies have been published, using multi-kinase inhibitors (35–37). Motesanib diphosphate induced a partial response in 14% of 93 DTC patients (median PFS 40 weeks) (36). Two studies have been published using sorafenib. Sorafenib (BAY 43-9006) is an inhibitor of RET, CRAF, wild-type and mutant (V600E) BRAF, VEGFR1, -2, -3, Flt3, and c-KIT.

In the first study, including DTC, anaplastic and medullary thyroid carcinoma patients, sorafenib induced a partial response in 23% (median PFS 79 weeks). In

the second study, including patients with DTC and anaplastic thyroid carcinoma, a partial response was observed in 11% (median PFS 4.5–16 weeks) (37). As bone metastases of DTC are poorly responsive to RaI, the response of bone metastases to the above-mentioned therapies is of interest. However, there were no separate analyses for patients with bone metastases in the above-mentioned studies.

In DTC, a relationship has been identified between genetic alterations in the RET, RAS, RAF cascade and loss of NIS expression (17,38). Interestingly, in an *in-vitro* study, sunitinib was able to reinduce NIS expression in RET/PTC transformed thyroid cells (39). In addition, sunitinib also increased RaI uptake in FRTL-5 cells. We therefore hypothesized that treatment with a multiple kinase inhibitor not only reduces tumor progression, but may also restore RaI uptake in non-RaI avid DTC. Therefore, we performed a single arm phase II trial to determine the efficacy of sorafenib in patients with iodine refractory metastatic DTC, focusing on the reinduction of RaI uptake and the efficacy in bone metastases.

Patients and methods

Patients

Eligibility criteria for the present study were the presence of progressive metastases or unresectable local recurrence of DTC for which RaI therapy was no longer effective, as indicated by prior negative post-therapeutic whole body scintigraphy (WBS). Progressive disease was defined according to response evaluation criteria in solid tumors (RECIST) criteria in the year before initiation of treatment. Patients who were selected had to have undergone total thyroidectomy and RaI ablative therapy. Exclusion criteria were pregnancy, contraindications for the application of recombinant human thyrotropin (rhTSH) and contraindications for the use of sorafenib. In addition, no patients with poorly responsive DTC were included as it was hypothesized that the likelihood of reinduction of RaI uptake in this category of tumors would be very small. Other eligibility criteria included Eastern Cooperative Oncology Group performance status 0–2 and life expectancy more than 3 months. Patients were ineligible if they had previous exposure to biological therapies. All patients provided written informed consent before enrollment onto the trial. The study protocol was approved by the Institutional Review Board of the Leiden University Medical Center. This study has been registered at ClinicalTrials.gov (# NCT00887107).

Study design

The study was a 26-week open-label, single-arm, phase II study of oral sorafenib in patients with metastatic DTC. The primary objective was to study the effect of sorafenib treatment on reinduction of radioiodine uptake. Secondary objectives were radiological tumor response according to RECIST and the relation between the presence of bone metastases and therapy success. Sorafenib was initially administered at a dose of 400 mg orally twice a day. Eligibility assessments, including a review of medical history and prior treatments, physical examination and laboratory assessments were completed within 2 weeks before initiation of the sorafenib therapy. Baseline radiological evaluation by computed tomography (CT) of target lesions was performed 4 weeks prior to initiation of therapy. From all patients, tumor samples from the primary tumors were obtained for review by the institutional pathologist (H.M.). After inclusion, the patients underwent a diagnostic scintigraphy 3 days after i.v. administration of 185 MBq I-131 (Mallinckrodt BV, Petten, The Netherlands). Single photon emission computed tomography (SPECT) of the head and neck and chest was performed. Two experienced observers visually analyzed all images. An I-131 standard was used to quantify the uptake in the area of interest at WBS. Patients were prescribed a low iodide diet from 7 days prior to the administration of I-131 (40). The patients received i.m. injections with 0.9 mg rhTSH (Thyrogen, Genzyme, Naarden, The Netherlands) on two consecutive days before the RaI administration. The day after the first WBS, patients started treatment with sorafenib 400 mg bid. Twenty-six weeks after initiation of sorafenib therapy, CT scanning and RaI imaging studies were repeated. All target lesions were imaged using CT. According to RECIST criteria, bone lesions were not evaluated as target lesions. It was carefully planned that CT scans using i.v. contrast were performed after diagnostic RaI scintigraphies. Procedures to exclude the occurrence of new bone metastases included bone and FDG-PET scintigraphy. Patients visited the hospital every 4 weeks for physical examination and assessment of laboratory safety parameters. Patients were assessed for new symptoms, compliance with study medications, and concomitant medications. Dose adjustments were made as needed for toxicity. The target serum TSH levels were below 0.1 mU/l. After completion of 26-week treatment, patients were allowed to continue sorafenib treatment, when a favorable response (complete or partial remission, or stable disease) had been achieved, until progression according to RECIST criteria. Adverse events were graded with the use of Common Terminology Criteria for Adverse Events (version 3).

To study the relation between efficacy and tumor mutational state, tumor DNA from patients was isolated from the paraffin-embedded material by taking tissue punches (diameter, 0.6 mm) with a tissue microarrayer (Beecher Instruments, Silver Springs, MD, USA) from tumor and normal areas selected on the basis of a hematoxylin

eosin (HE)-stained slide. Mutational analysis was performed using PCR amplification followed by sequencing on an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA) ABI 3700. Codons investigated were: B-type Raf kinase (BRAF, V600E and exon 11), HRAS (codon 12/13 and 61), KRAS (codon 12/13 and 61), NRAS (codon 61), and phosphatidylinositol-3-kinase, catalytic, alpha polypeptide (PIK3CA, exons 9 and 20). Primer sequences are available on request.

Laboratory parameters

The following laboratory parameters were assessed: serum TSH, free-thyroxine (FT4), thyroglobulin (Tg), and Tg antibody levels were measured at all visits. TSH and Tg were also measured 1 and 3 days after the last rhTSH injection. Safety parameters were assessed at every visit and included a hematological profile as well as serum levels of sodium, potassium, creatinine, renal and liver function. Serum TSH was determined with a Modular Analytics E-170 system ((Roche Diagnostic Systems), intra-assay variability: 0.9–10.7%, interassay variability: 0.9–12.1%). Serum Tg was determined with IRMA ((Tg kit, Brahms, Berlin, Germany) on a Wallac (Wallac, Turku, Finland), intra-assay variability: 0.1–13.9%, interassay variability: 12.3–17.4%). Serum Tg antibodies were determined with IRMA (Sorin Biomedica, Amsterdam, The Netherlands) on a Wallac (Wallac, Turku, Finland, intra-assay variability: 3.6–4.1%, interassay variability: 11.6%).

Statistical methods

Using a Simon two-stage design, the study required 24 subjects to decide whether the proportion responding, P, is ≤10% or ≥40%. If the number of responses was six or more, the hypothesis that $P \leq 10\%$ is rejected with a target error rate of 5% and an actual error rate of 2.8%. If the number of responses was five or less, the hypothesis that $P \geq 40\%$ is rejected with a target error rate of 5% and an actual error rate of 4%. All enrolled patients were included in an intention-to-treat analysis. Data are reported as mean ± S.D., median (range) or proportions. Estimates of PFS (time from starting study drug to progression) with associated 95% CI's were obtained using the Kaplan–Meier method. Analyses of variables influencing response to sorafenib were analyzed with binomial logistic regression (26-week data). The calculations were performed using SPSS 16.0 for Windows (SPSS, Chicago, IL, USA).

Results

Patient characteristics

Between 13 October 2007 and 7 October 2008, a total of 31 patients started sorafenib treatment (**Figure 1**). One patient chose not to start sorafenib therapy after giving signed informed consent. Therefore, 32 patients were included in the intention-to-treat analysis. A total of 22 patients completed 26 weeks of treatment. The remaining patients discontinued treatment earlier because of disease progression (four patients, see efficacy section for details), drug-related adverse events (two patients, see adverse events section for details), non-drug related adverse events (two patients) or the patient's request (one patient). Eight of the 22 patients who completed the 26-week trial and continued sorafenib treatment, discontinued treatment later on, two because of adverse events and six because of disease progression. The median duration of sorafenib treatment for all patients was 32 weeks (range 3 days to 79 weeks). Median duration of follow-up was 63 weeks (range 28–80 weeks).

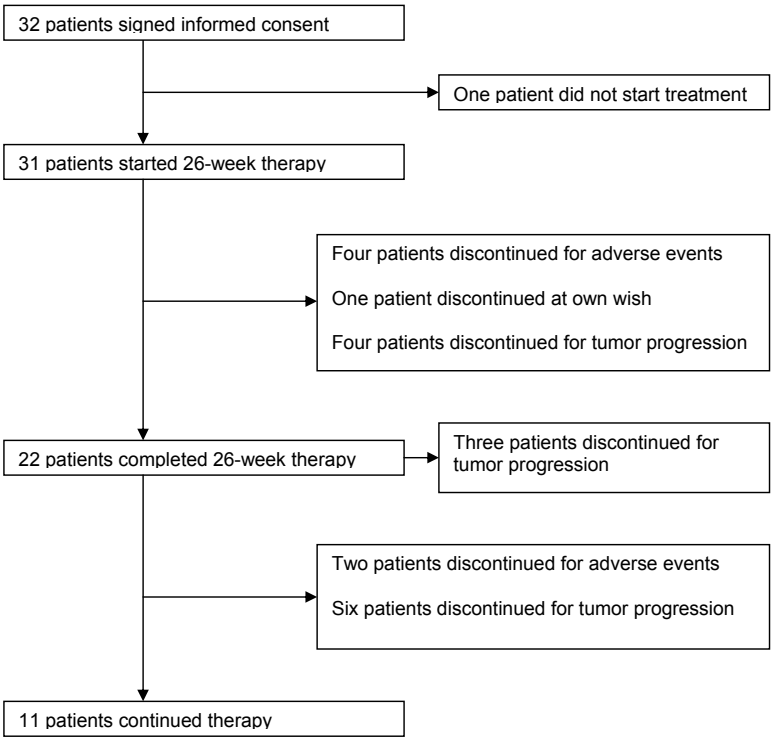


Figure 1
Study flowchart.

Baseline characteristics are given in **Table 1**. All patients had undergone prior RaI treatments with a median activity of 15.2 (range 3.7–30.0) GBq. The number of RaI treatments ranged from 1 to 4. None of the patients had RAI uptake before inclusion as indicated by post-therapeutic WBSs and at baseline. Fifteen patients had undergone external beam irradiation. In all patients, external beam irradiation was aimed at one or more bone metastases. As no bone metastases were target lesions this treatment did not interfere with the assessment of efficacy. Interestingly, the most common histologic carcinoma subtype was Hürthle cell carcinoma. In follicular thyroid with Hürthle cell metaplasia, the majority of cells were Hürthle cells. At study entry, almost all patients had lung metastases (n=30), whereas 14 patients had bone metastases. Most patients had metastases at multiple sites. In 10/13 patients with PTC, BRAFV600E mutations were identified, one of which (the tall cell variant) also

Table 1: Baseline Characteristics

	All patients (n=32)
Gender (No. ;%)	
Female	13 (39)
Male	19 (61)
Time from diagnosis (year) (median, range)	3 (0 – 18)
Age (year) (median, range)	65 (53 – 82)
Histology (no. ;%)	
Papillary	13 (41)
Tall cell	1 (3)
Hürthle cell metaplasia	2 (6)
Follicular variant papillary	3 (9)
Follicular	15 (47)
Hürthle cell metaplasia	11 (34)
Mixed papillary	1 (3)
Initial TNM stage (no. ;%) (5 unknown)	
T1-3 N 0-1 M0	12 (44)
T4 N 0-1 M0	6 (23)
M1	9 (33)
Tumor extent at study entry (no. ; %)	
Thyroid bed only	1 (3)
Lungs only	8 (25)
Lungs and bone only	7 (22)
Locally advanced * and distant metastasis	9 (33)
Other	7 (22)
Radioiodine dose (GBq) (median, range)	15.2 (3.7 – 30.0)
External beam radiation (no. ; %)	15 (47)

*Including both thyroid bed (n= 5) and neck lymph nodes (n= 4)

showed a PIK3CA mutation. Few additional mutations were identified: one KRAS in FVPTC; two NRAS mutations were found (one in a PTC without a BRAF mutation and one in a FTC); and one additional PIK3CA mutation was found in a FTC with Hürthle cell metaplasia.

Efficacy

Data on responses are given in **Table 2** and **Figure 2**. Disease progression led to treatment discontinuation of patients at 6 weeks (one patient with new bone metastases), 18 weeks (one patient with a new abdominal metastasis, one patient with new pulmonary metastases), 25 weeks (one patient with cerebral metastases), 26 weeks (end of 26-week trial: two patients with new bone metastases, one patient with a new pulmonary metastasis), 32 weeks (two patients with new bone metastases, one patient with progression of a hip lesion, one patient with new liver metastases and one patient with new lung lesions) and 40 weeks (one patient with a new neck lesion).

At 26 weeks, 19 patients (59%) had achieved a clinical beneficial response, eight of whom had a partial response (25%) and 11 had stable disease (34%; **Table 2**). There were no complete responses. At 26 weeks, the cumulative number of patients with progressive disease was seven. Interestingly, four patients with progressive disease based on new lesions had no progression or a considerable decrease in the sum of the longest diameter of target lesions. Radiological response at 26 weeks was not influenced by gender (P = 0.593), age (P = 0.172), initial tumor lymph nodes metastases (TNM) stage (P = 0.488), histology (P = 0.614), or the presence of the BRAFV600E mutation (P = 0.760). The prevalence of other mutations was too low to allow statistical analysis. However, when bone metastases were present, the response to sorafenib was significantly worse than in the absence of bone metastases (P = 0.004, **Table 2**).

Although this study was not formally designed for the establishment of PFS, estimation of median PFS was 58 weeks (95% CI, 47–68). Likewise, PFS was influenced by the presence of bone metastases. PFS was 69 weeks (CI: 58–80) in patients without bone metastases and 47 weeks (CI: 32–62) in patients with bone metastases (P = 0.046).

In general, Tg response reflected radiological response and there were no differences between the responses of unstimulated *versus* TSH stimulated Tg measurements (**Table 2, Figure 2**). The median time of the nadir of Tg levels was 3 months. The median change in Tg at 26 weeks *versus* baseline was -16 mg/l (range -2746 to 17.836). Remarkably, one patient with progression in a non-target lesion had a decrease in unstimulated Tg, but an increase in TSH stimulated Tg. In one 54-year-old woman with pulmonary metastases of a Hürthle cell FTC, a remarkable rise in serum

Table 2: Tumor response

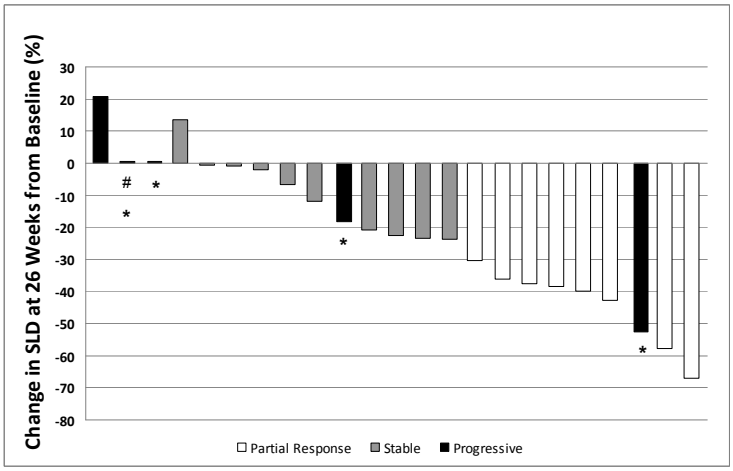
	All patients (n=32)	Bone metastases		Histology (a)				
		Absent	Present	PTC		FVPTC	FTC	
Reinduction of iodine uptake at 26 weeks	0			Total	Hürthle cell		Total	Hürthle cell
Radiological response (n; % of total patients)								
	(b)			(c)				
Complete response	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0
Partial response	8 (25)	5 (16)	3 (9)	2 (22)	0	2 (67)	4 (49)	4
Stable disease	11 (34)	7 (22)	4 (13)	6 (67)	1	0 (0)	5 (36)	3
Clinical benefit	19 (59)	12 (38)	7 (22)	8 (89)	1	2 (67)	9 (64)	7
Progressive disease	7 (22)	0 (0)	7 (22)*	1 (11)	0	1 (33)	5 (36)	3
Serum Thyroglobulin levels (µg/l)								
Baseline (median; range)	77 (4.4-8570)							
26-weeks (n=22)	46 (0.9-19500)							
Delta Tg (versus baseline, median; range)	-16 (-2746-17836)							
Nadir	29 (0.5-7634)							
Time to nadir (months, median; range)	3 (0-5)							
Progression free survival								
PFS (weeks, median; CI)	58 (47-68)							

* P= 0.005 versus no bone metastases
(a) One patient with mixed PTC and FTC left the study because of an adverse event and could not be evaluated
(b) Percentage of total (n= 32) patients
(c) Percentage of evaluable patients per histology class

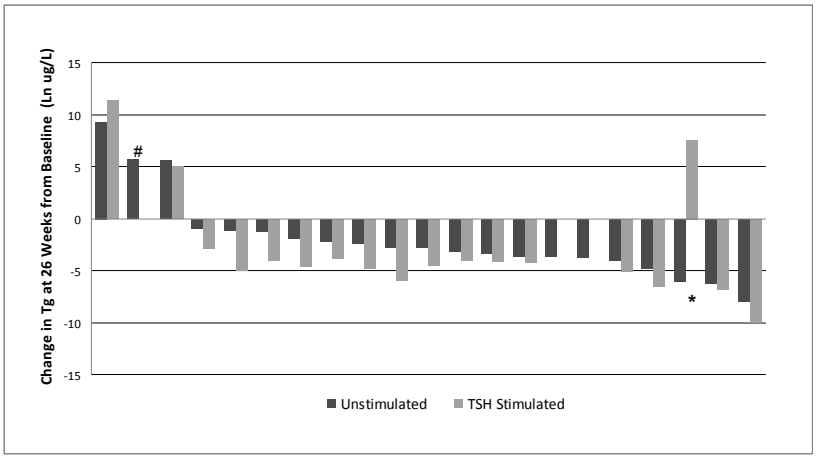
Tg from 1664 to 99 900 mg/l was observed 8 weeks after initiation of therapy. A chest CT scan revealed an impressive decrease in the number, size, and density of all pulmonary metastases.

Reinduction of radioiodine uptake could be evaluated in 21 patients who had completed 26-week treatment with sorafenib. In one patient, WBS could not be performed for technical reasons. In three patients with extensive metastases, both at baseline and at 26 weeks, RaI WBS revealed uptake at the thyroid bed, but not at the sites of metastases. In 20 patients, there was no RaI uptake at metastatic sites after 26 weeks of sorafenib therapy. In one patient with an occipital skeletal metastasis that did not accumulate RaI at baseline, very slight RaI uptake was observed at 26 weeks. However, a post-therapeutic WBS performed after subsequent 7600 MBq RaI therapy did not reveal any uptake, and consequently it was concluded that no clinically relevant reinduction of RaI therapy had occurred in this patient.

A


Change in SLD at 26 Weeks from Baseline (%)
30
20
10
0
-10
-20
-30
-40
-50
-60
-70
-80
#
*
*
*
*
Partial Response
Stable
Progressive

B


Change in Tg at 26 Weeks from Baseline (Ln ug/L)
15
10
5
0
-5
-10
-15
#
*
Unstimulated
TSH Stimulated

C

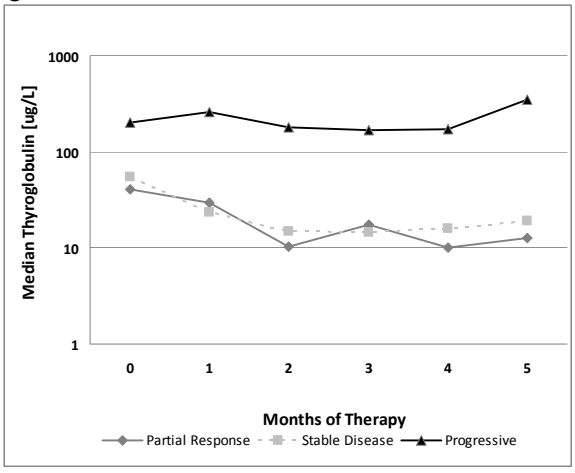

Median Thyroglobulin [ug/L]
1000
100
10
1
0
1
2
3
4
5
Months of Therapy
Partial Response
Stable Disease
Progressive

Figure 2 (page 73)
(A) The change from baseline in tumor dimensions (sum of longest diameters (SLD)) is shown for 22 patients who completed 26 weeks of treatment with sorafenib and one patient who discontinued treatment at 18 weeks for disease progression (new soft-tissue lesion (#)). Not shown are data for three patients with progressive disease due to new lesions. Four patients with progressive disease based on new lesions had no progression or a considerable decrease in SLD (*). Target and non-target lesions are defined according to the response evaluation criteria in solid tumors (RECIST). (B) The natural logarithm of the change at 26 weeks from baseline in unstimulated and TSH stimulated serum thyroglobulin (Tg) levels is shown for 22 patients who completed 26 weeks of treatment with sorafenib and one patient who discontinued treatment at 25 weeks for disease progression (new cerebral metastasis (#)). One patient with progression in a non-target lesion had a decrease in unstimulated Tg but an increase in TSH stimulated Tg (*). TSH stimulated Tg measurements were performed 3 days after the last injection of 0.9 mg rhTSH. (C) Medians of unstimulated serum thyroglobulin (Tg) levels of 25 evaluable patients during 26 weeks of treatment with sorafenib stratified according to radiological response. Three of these patients with progressive disease discontinued treatment before 26 weeks. From one other patient, who also discontinued treatment before 26 weeks for disease progression, no Tg levels were obtained.

Adverse events

Adverse events led to discontinuation of the study in six patients at respectively 3 weeks (angioedema), 5 weeks (myocardial infarction), 7 weeks (hematuria), 12 weeks (concomitant small cell lung carcinoma), 38 weeks (dyspnea, not related to tumor progression), and 50 weeks (congestive heart disease). Doses were reduced in 18 (56%) patients to control toxicities. The median dose at 26 weeks was 400 mg/day. The most prevalent adverse events are mentioned in **Table 3**. The hand–foot syndrome occurred in the first few weeks of treatment and subsided in most patients after dose reduction and topical treatment. Most patients received nutritional support for weight loss. In three patients with considerable weight loss, a percutaneous gastric feeding catheter had to be inserted. Mineral deficiencies were treated with supplementation. Hypertension was treated with antihypertensive drugs. Hematological abnormalities were mild and did not require treatment. In order to maintain serum-free T4 levels and TSH levels at the required values, five patients required a thyroxine dose reduction, whereas in six patients, the dose needed to be increased. No relationship was observed between toxicity and performance state and age.

Discussion

We studied the efficacy of the multiple target tyrosine kinase inhibitor sorafenib in patients with iodine-refractory metastatic DTC, focusing on the reinduction of RaI uptake and the efficacy in patients with bone metastases. After 26 weeks of treatment, we found a partial response rate of 25% and a clinical beneficial response in 59% of

Table 3: Adverse events

Event	All Number of patients (% of total n=32)	Grades (% of category) 1	2	3	4
Hand foot syndrome	21 (66)	33	33	33	0
Weight loss	18 (56)	35	50	15	0
Diarrhea	16 (50)	31	69	0	0
Alopecia	15 (47)	87	13	0	0
Rash	15 (47)	60	40	0	0
Mucositis	14 (44)	100	0	0	0
Hypertension	13 (41)	31	31	38	0
Hypocalcaemia	13 (41)	100	0	0	0
Thrombopenia	9 (28)	100	0	0	0
Hypophosphatemia	9 (28)	100	0	0	0
Anemia	8 (25)	100	0	0	0
Myocardial infarction	1 (3)	0	0	0	100
Congestive heart disease	1 (3)	0	0	100	0
Hematuria	1 (3)	0	100	0	0

According to Common Terminology Criteria for Adverse Events (version 3).

the patients. Patients with bone metastases responded significantly worse. Reinduction of RaI uptake as studied by diagnostic WBS was not observed in any patient.

RaI therapy is the only available conventional therapy for patients with metastases of DTC. Hürthle cell carcinomas respond less favorably to RaI, which is compatible with the fact that the most prevalent histology was Hürthle cell metaplasia. The fact that 10/13 PTC harbored BRAFV600E mutations also illustrates the unfavorable prognostic characteristic of our patient group (41). The extensive characterization in recent years of the molecular pathways involved in the pathogenesis of DTC has revealed potential targets for new therapies. The identification of tyrosine kinase activated pathways in DTC together with the advent of novel classes of tyrosine kinase inhibitors has provided new therapeutic perspectives for patients with non-RaI avid DTC. The results of our study with respect to radiological response are comparable with those observed in another study with sorafenib by Gupta *et al*. (35) and better than observed in the study of Kloos *et al*. (37). The latter study, however, included patients with anaplastic carcinoma. Comparison of the results of phase II studies with different tyrosine kinase inhibitors in DTC is hampered by differences in patient categories (including histologies, tumor stages, sites of metastases, and tumor extent), study design, and analytical methods. Nonetheless, the results obtained with sorafenib in different studies, including our own suggest that sorafenib is among the most successful and promising compounds for metastatic DTC. We found a signifi-

cant difference in the response to sorafenib between patients with or without bone metastases, the latter category responding worse. Nevertheless, 23% of patients with bone metastases had a favorable response. Those patients had metastases at multiple sites, mostly pulmonary that regressed under sorafenib, whereas the bone metastases were stable.

The explanation for a less favorable response of bone metastases is not clear. It can be hypothesized that both pharmacokinetic factors (lower tissue levels in bone) and tumor-related factors are involved. As it is hardly possible to obtain tissue from bone metastases, the relationship between the genetic profile of bone metastases and responsiveness to sorafenib is not easy to study. Another explanation may be that it has been observed that the role of VEGFR signal transduction in tumor propagation in conscript vascular systems such as soft-tissue metastases may differ from less well-defined vascular beds as the bone marrow. As a consequence, VEGFR targeted therapies may have less prominent beneficial effects in bone metastases. In addition it is also conceivable that the presence of bone metastases influences the response of soft-tissue metastases to sorafenib. Although the mechanism is not clear it could be hypothesized that the systemic release of proteins or cytokines, such as interleukin 6 or TGF beta from the bone microenvironment plays a role in this phenomenon.

The fact that there was a mixed response in some subjects (both regression and progression) is explained by the heterogeneous nature of metastases in thyroid carcinoma, which can be caused by tumor-related factors and the microenvironment of the metastatic site. This may lead to differences in tumor dependence on tyrosine kinase activated pathways, which may consequently lead to differences in response to sorafenib. RECIST does not take into account these phenomena, which are very important for the selection of lesions that may or may not respond to tyrosine kinase inhibitors.

Another purpose of our study was to assess the effects of sorafenib on RaI uptake. We did not find reinduction of RaI uptake in any patient. Although a clear relation has been found *in-vitro* between genetic alterations in DTC and decreased NIS gene expression (17,38), multiple mechanisms may be involved in decreased NIS functionality, including impaired NIS membrane trafficking (42,43), epigenetic changes in NIS and/or NIS promoter genes (44). Although *in-vitro* studies have shown that multiple target tyrosine kinase inhibitors may lead to reinduction of RaI uptake (39,45), it may well be that these additional mechanisms have prevented a beneficial effect of sorafenib on RaI uptake in our study. Another explanation for the lack of an effect of sorafenib on RaI uptake may be the lower sensitivity of diagnostic WBS as compared to post therapeutic scintigraphy. For this reason, we used SPECT acquisition in our diagnostic scintigraphies, which has a higher sensitivity than conventional WBS. Nevertheless, we cannot exclude that a limited sensitivity may indeed have

contributed to our observation of a failure of sorafenib to reinduce RaI uptake. As baseline CT scans were performed 4 weeks prior to radioiodine scintigraphies, i.v. contrast could have interfered with the diagnostic scintigraphy at baseline. As no urinary iodine measurements were performed, this cannot be entirely ruled out. However, the fact that all patients had negative post-therapeutic WBSs previously ascertains the true radioiodine non-avid nature of the lesions.

Although uptake of RaI is an important determinant of tumor response, another important factor is radio sensitivity. Indeed, a subgroup of thyroid carcinomas does not respond to RaI therapy, despite RaI uptake. Genetic alterations in radiation-induced apoptotic pathways, including p-53 or cdk-21 may be involved. Therefore, in the design of redifferentiation studies in thyroid carcinoma, this aspect should always be taken into account, ideally involving genetic studies in these pathways.

Serum Tg levels in general paralleled radiological changes. However, tumor lysis during sorafenib or comparable compounds can lead to elevated Tg levels as was observed in one patient. This could be due to tumor lysis, but it could in theory also be caused by increased Tg synthesis as a result of enhanced differentiated behavior of thyroid carcinoma cells.

Although sorafenib therapy had considerable adverse effects, no unexpected or new adverse effects were observed. Most of the adverse effects were temporary and could be managed with dose reduction or therapeutic measures. Weight loss was considerable, especially in patients with low weight at baseline, requiring dietary support or even catheter feeding.

In conclusion, the results of our study confirm the potential role of sorafenib in patients with non-RaI avid metastases of DTC. In addition, we found that patients with bone metastases respond less favorably, and that diagnostic WBS did not reveal an effect of sorafenib on the reinduction of RaI uptake in these patients. Future phase III studies should confirm the efficacy of sorafenib for DTC.

References

1. Schlumberger MJ. Papillary and follicular thyroid carcinoma. New England Journal of Medicine 1998 338 297–306.
2. Mazzaferri EL, Kloos RT. Clinical review 128: current approaches to primary therapy for papillary and follicular thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2001 86 1447–1463.
3. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A National Cancer Data Base Report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995. Cancer 1998 83 2638–2648.
4. Pacini F, Cetani F, Miccoli P, Mancusi F, Ceccarelli C, Lippi F, Martino E, Pinchera A. Outcome of 309 patients with metastatic differentiated thyroid carcinoma treated with radioiodine. World Journal of Surgery 1994 18 600–604.
5. Schlumberger M, Challeton C, De Vathaire F, Parmentier C. Treatment of distant metastases of differentiated thyroid carcinoma. Journal of Endocrinological Investigation 1995 18 170–172.
6. Schlumberger M, Challeton C, De Vathaire F, Travagli JP, Gardet P, Lumbroso JD, Francese C, Fontaine F, Ricard M, Parmentier C. Radioactive iodine treatment and external radiotherapy for lung and bone metastases from thyroid carcinoma. Journal of Nuclear Medicine 1996 37 598–605.
7. Arturi F, Russo D, Schlumberger M, du VJ, Caillou B, Vigneri P, Wicker R, Chiefari E, Suarez HG, Filetti S. Iodide symporter gene expression in human thyroid tumors. Journal of Clinical Endocrinology and Metabolism 1998 83 2493–2496.
8. Caillou B, Troalen F, Baudin E, Talbot M, Filetti S, Schlumberger M, Bidart JM. NaC/IK symporter distribution in human thyroid tissues: an immunohistochemical study. Journal of Clinical Endocrinology and Metabolism 1998 83 4102–4106.
9. Lazar V, Bidart JM, Caillou B, Mahe C, Lacroix L, Filetti S, Schlumberger M. Expression of the NaC/IK symporter gene in human thyroid tumors: a comparison study with other thyroid specific genes. Journal of Clinical Endocrinology and Metabolism 1999 84 3228–3234.
10. Marcocci C, Pacini F, Elisei R, Schipani E, Ceccarelli C, Miccoli P, Arganini M, Pinchera A. Clinical and biologic behavior of bone metastases from differentiated thyroid carcinoma. Surgery 1989 106 960–966.
11. Ruegemer JJ, Hay ID, Bergstralh EJ, Ryan JJ, Offord KP, Gorman CA. Distant metastases in differentiated thyroid carcinoma: a multivariate analysis of prognostic variables. Journal of Clinical Endocrinology and Metabolism 1988 67 501–508.
12. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. European Journal of Endocrinology 2006 154 787–803.
13. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, Tuttle RM. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006 16 109–142.
14. Sobrinho-Simoes M, Preto A, Rocha AS, Castro P, Maximo V, Fonseca E, Soares P. Molecular pathology of well-differentiated thyroid carcinomas. Virchows Archiv 2005 447 787–793.
15. Fagin JA. How thyroid tumors start and why it matters: kinase mutants as targets for solid cancer pharmacotherapy. Journal of Endocrinology 2004 183 249–256.

16. Soares P, Sobrinho-Simoes M. Recent advances in cytometry, cytogenetics and molecular genetics of thyroid tumours and tumour-like lesions. Pathology, Research and Practice 1995 191 304–317.
17. Riesco-Eizaguirre G, Gutierrez-Martinez P, Garcia-Cabezas MA, Nistal M, Santisteban P. The oncogene BRAFV600E is associated with a high risk of recurrence and less differentiated papillary thyroid carcinoma due to the impairment of NaC/IK targeting to the membrane. Endocrine-Related Cancer 2006 13 257–267.
18. Tallini G. Molecular pathobiology of thyroid neoplasms. Endocrine Pathology 2002 13 271–288.
19. Cinti R, Yin L, Ilc K, Berger N, Basolo F, Cuccato S, Giannini R, Torre G, Miccoli P, Amati P, Romeo G, Corvi R. RET rearrangements in papillary thyroid carcinomas and adenomas detected by interphase FISH. Cytogenetics and Cell Genetics 2000 88 56–61.
20. Corvi R, Berger N, Balczon R, Romeo G. RET/PCM-1: a novel fusion gene in papillary thyroid carcinoma. Oncogene 2000 19 4236–4242.
21. Klugbauer S, Jauch A, Lengfelder E, Demidchik E, Rabes HM. A novel type of RET rearrangement (PTC8) in childhood papillary thyroid carcinomas and characterization of the involved gene (RFG8). Cancer Research 2000 60 7028–7032.
22. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Research 1997 57 1690–1694.
23. Grieco M, Santoro M, Berlingieri MT, Melillo RM, Donghi R, Bongarzone I, Pierotti MA, Della PG, Fusco A, Vecchio G. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. Cell 1990 60 557–563.
24. Santoro M, Dathan NA, Berlingieri MT, Bongarzone I, Paulin C, Grieco M, Pierotti MA, Vecchio G, Fusco A. Molecular characterization of RET/PTC3; a novel rearranged version of the RETproto-oncogene in a human thyroid papillary carcinoma. Oncogene 1994 9 509–516.
25. Santoro M, Chiappetta G, Cerrato A, Salvatore D, Zhang L, Manzo G, Picone A, Portella G, Santelli G, Vecchio G, Fusco A. Development of thyroid papillary carcinomas secondary to tissuespecific expression of the RET/PTC1 oncogene in transgenic mice. Oncogene 1996 12 1821–1826.
26. Santoro M, Carlomagno F, Hay ID, Herrmann MA, Grieco M, Melillo R, Pierotti MA, Bongarzone I, Della PG, Berger N. Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. Journal of Clinical Investigation 1992 89 1517–1522.
27. Viglietto G, Chiappetta G, Martinez-Tello FJ, Fukunaga FH, Tallini G, Rigopoulou D, Visconti R, Mastro A, Santoro M, Fusco A. RET/PTC oncogene activation is an early event in thyroid carcinogenesis. Oncogene 1995 11 1207–1210
28. Greco A, Pierotti MA, Bongarzone I, Pagliardini S, Lanzi C, Della PG. TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. Oncogene 1992 7 237–242.
29. Xing M. BRAF mutation in thyroid cancer. Endocrine-Related Cancer 2005 12 245–262.
30. Soares P, Trovisco V, Rocha AS, Lima J, Castro P, Preto A, Maximo V, Botelho T, Seruca R, Sobrinho-Simoes M. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene 2003 22 4578–4580.
31. Puxeddu E, Moretti S, Elisei R, Romei C, Pascucci R, Martinelli M, Marino C, Avenia N, Rossi ED, Fadda G, Cavaliere A, Ribacchi R, Falorni A, Pontecorvi A, Pacini F, Pinchera A,

Santeusanio F. BRAF(V599E) mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. Journal of Clinical Endocrinology and Metabolism 2004 89 2414–2420.

32. Fukushima T, Suzuki S, Mashiko M, Ohtake T, Endo Y, Takebayashi Y, Sekikawa K, Hagiwara K, Takenoshita S. BRAF mutations in papillary carcinomas of the thyroid. Oncogene 2003 22 6455–6457.
33. Pennell NA, Daniels GH, Haddad RI, Ross DS, Evans T, Wirth LJ, Fidias PH, Temel JS, Gurubhagavatula S, Heist RS, Clark JR, Lynch TJ. A phase II study of Gefitinib in patients with advanced thyroid cancer. Thyroid 2008 18 317–323.
34. Cohen EEW, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, Kane MA, Sherman E, Kim S, Bycott P, Tortorici M, Shalinsky DR, Liau KF, Cohen RB. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. Journal of Clinical Oncology 2008 26 4708–4713.
35. Gupta-Abramson V, Troxel AB, Nellore A, Puttaswamy K, Redlinger M, Ransone K, Mandel SJ, Flaherty KT, Loevner LA, O'Dwyer PJ, Brose MS. Phase II trial of sorafenib in advanced thyroid cancer. Journal of Clinical Oncology 2008 26 4714–4719.
36. Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, Licitra L, Eschenberg MJ, Sun YN, Juan T, Stepan DE, Schlumberger MJ, The Motesanib Thyroid Cancer Study Group . Motesanib diphosphate in progressive differentiated thyroid cancer. New England Journal of Medicine 2008 359 31–42.
37. Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, Liang J, Wakely PEJ, Vasko VV, Saji M, Rittenberry J, Wei L, Arbogast D, Collamore M, Wright JJ, Grever M, Shah MH. Phase II trial of sorafenib in metastatic thyroid cancer. Journal of Clinical Oncology 2009 27 1675–1684.
38. De Vita G, Zannini M, Cirafici AM, Melillo RM, Di Lauro R, Fusco A, Santoro M. Expression of the RET/PTC1 oncogene impairs the activity of TTF-1 and Pax-8 thyroid transcription factors. Cell Growth, Differentiation 1998 9 97–103.
39. Fenton MS, Marion K, Salem AK, Hogen R, Naeim F, Hershman JM. Induction of cell cycle arrest by sunitinib through inhibition of the MEK/ERK pathway in RET/PTC1 papillary thyroid cancer cells. 90th Annual Meeting of the Endocrine Society, San Francisco (abstract), 1990.
40. Pluijmen MJ, Eustatia-Rutten C, Goslings BM, Stokkel MP, Arias AM, Diamant M, Romijn JA, Smit JW. Effects of low-iodide diet on postsurgical radioiodine ablation therapy in patients with differentiated thyroid carcinoma. Clinical Endocrinology 2003 58 428–435.
41. Elisei R, Ugolini C, Viola D, Lupi C, Biagini A, Giannini R, Romei C, Miccoli P, Pinchera A, Basolo F. BRAFV600E mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. Journal of Clinical Endocrinology and Metabolism 2008 93 3943–3949.
42. Wapnir IL, van de Rijn M, Nowels K, Amenta PS, Walton K, Montgomery K, Greco RS, Dohan O, Carrasco N. Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. Journal of Clinical Endocrinology and Metabolism 2003 88 1880–1888.
43. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED- 1, Hector Battifora mesothelial-1, cytokeratin-19, peroxisome proliferator-activated receptor-g, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. European Journal of Endocrinology 2008 158 375–384.

44. Furuya F, Shimura H, Suzuki H, Taki K, Ohta K, Haraguchi K, Onaya T, Endo T, Kobayashi T. Histone deacetylase inhibitors restore radioiodine uptake and retention in poorly differentiated and anaplastic thyroid cancer cells by expression of the sodium/iodide symporter thyroperoxidase and thyroglobulin. Endocrinology 2004 145 2865–2875.
45. Salem AK, Fenton MS, Marion KM, Hershman JM. Effect of sunitinib on growth and function of FRTL-5 thyroid cells. Thyroid 2008 18 631–635.

4

Sorafenib induced hypothyroidism is associated with increased type 3 deiodination

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Abstract

Background

Therapy with tyrosine kinase inhibitors is associated with thyroid dysfunction. Decreased serum thyroid hormone levels during tyrosine kinase inhibitors are also observed in athyreotic patients with thyroid carcinoma. We therefore hypothesized that tyrosine kinase inhibitors may influence thyroid hormone metabolism.

Aim

The aim was to study the effects of sorafenib therapy on serum thyroid hormone concentrations and iodothyronine deiodination in athyreotic patients.

Design

The design included a prospective open, single-center, single-arm 26-wk study.

Methods

We measured serum thyroxine (T4), free T4, 3,5,3-triiodothyronine (T3), free T3, reverse T3 (rT3), and TSH concentrations at baseline and after 26 wk in 21 patients with progressive non-medullary thyroid carcinoma treated with sorafenib. Ratios of T3/T4 and T3/rT3, which are independent of substrate availability and reflect iodothyronine deiodination, were calculated.

Results

Serum free T4 and T3 levels, adjusted for levothyroxine dose per kilogram body weight, decreased by 11 and 18%, respectively, whereas TSH levels increased. The serum T3/T4 and T3/rT3 ratios decreased by 18 and 22%, respectively, which is compatible with increased type 3 deiodination.

Conclusions

Sorafenib enhances T4 and T3 metabolism, which is probably caused by increased type 3 deiodination.

Introduction

Therapy with tyrosine kinase inhibitors in patients with malignancies is associated with alterations in thyroid hormone parameters. Sunitinib has been associated with hypothyroidism in 14–85% of the patients, ranging from transient increases in TSH to persistent hypothyroidism, requiring thyroxine substitution (1–6). In some patients, sunitinib induced hyperthyroidism as well (7–9). Both sunitinib induced hypothyroidism and hyperthyroidism may be caused by destructive thyroiditis, but other mechanisms, such as interference of sunitinib with thyroid peroxidase (4) or inhibition of thyroidal vascularization leading to thyroid atrophy (10, 11), have been proposed. Likewise, sorafenib was associated with TSH elevations in 18% of patients with metastatic renal cell carcinoma (12). All of the above mentioned studies were performed in patients with intact thyroid glands. However, thyroid function abnormalities have also been observed during therapy with imatinib (13, 14), motesanib (15), and sorafenib (16) in athyreotic patients with medullary thyroid carcinoma and non-medullary thyroid carcinoma. Imatinib increased TSH levels in all patients with medullary thyroid carcinoma (13, 14). Therapy with motesanib caused TSH elevations in 22% of 93 patients with non-medullary thyroid carcinoma. Sorafenib was associated with increased TSH levels in 33% of thyroid carcinoma patients (16). Because an increased need of thyroxine is observed in athyreotic patients who are treated with tyrosine kinase inhibitors, the mechanisms of hypothyroidism may include alterations in thyroid hormone metabolism. Stepwise deiodination is the major route of thyroid hormone degradation and is mediated by iodothyronine deiodinases (D1, D2, and D3) (17) and by hepatic conjugating enzymes (18). No human studies have been published to address the effect of sorafenib on peripheral thyroid hormone metabolism. We therefore studied the effects of sorafenib, administered for 26 weeks, on peripheral thyroid hormone metabolism in athyreotic humans.

Patients and Methods

Design

The effects of sorafenib on peripheral thyroid hormone metabolism were analysed in an open, single center, single arm 26-week prospective phase II study, intended to achieve reinduction of RaI uptake in athyreotic patients with progressive metastatic or locally advanced non-medullary thyroid carcinoma. Results of this study have been published recently (19). Sorafenib was initially administered at a dose of 400 mg orally twice daily. Exclusion criteria were pregnancy, contraindications for the application of recombinant human TSH (rhTSH), and contraindications for the use of

sorafenib. The institutional review board approved the study and all patients provided written informed consent.

Before initiation of sorafenib therapy and at 26 weeks, the patients underwent computed tomography scans for analysis of tumor dimensions and rhTSH (Thyrogen®, Genzyme, Naarden, The Netherlands) assisted 4 mCi diagnostic scintigraphies. During the study patients visited the hospital every month for assessment of thyroid function parameters, biochemical safety parameters and a physical examination. Fasting morning blood samples were stored at -80°C until analysis. All patients were treated with levothyroxine, which was adjusted if needed to maintain a target TSH concentration below 0.1 mU/L. Only patients who completed 26 weeks of treatment with sorafenib were included in the present analysis (per protocol analysis).

Study aims

The objective of this study was to evaluate the effects of sorafenib on thyroid hormone serum levels and to evaluate the effects of sorafenib on iodothyronine deiodinase activity.

Study parameters

The following thyroid function parameters were measured: serum TSH, free thyroxine (FT4), total thyroxine (T4), free 3,5,3- triiodothyronine (FT3), total 3,5,3-triiodothyronine (T3), and reverse 3,5,3-triiodothyronine (rT3). Because levothyroxine dosages were adjusted during the study according to serum TSH levels, serum thyroid hormone levels were corrected for the daily levothyroxine dose and body weight. Dose-adjusted FT4 was calculated as follows: (FT4 (pmol/liter) * weight (kg))/levothyroxine dose (µg).

To assess effects of sorafenib on iodothyronine deiodination, ratios of serum levels of T3/T4 and T3/rT3 were calculated. The T3/rT3 ratio is considered to be a sensitive indicator of the peripheral metabolism of thyroid hormone, being positively influenced by D1 and D2 deiodinase and negatively by D3 deiodinase. Serum thyroxine-binding globulin (TBG) levels and FT4/T4 and FT3/T3 ratios were calculated to exclude effects of sorafenib on thyroid hormone binding proteins.

Assays

Fasting blood samples were collected before daily levothyroxine ingestion at both baseline and 26 wk and stored at -80°C. TSH, FT4, T4, T3, and TBG were measured by chemiluminescence assays (Vitros ECI Immunodiagnostic System; Ortho-Clinical Diagnostics via GE Healthcare, Rochester, NY). FT3 was measured with a RIA (Trinity

Biotech, Bray, Ireland). rT3 was measured with an in-house RIA (20). The detection limit of the TSH assay was 0.005 mU/liter. Within-assay coefficients of variation amounted to 4% for TSH, 2% for T4, 2% for T3, and 3–4% for rT3.

Statistics

Data are reported as mean ± SD, median (range) or proportions. Differences in thyroid hormone parameters were analyzed using the two tailed Student's t-test for paired data, comparing measurements between baseline and after 26 weeks sorafenib therapy for normally distributed data or with a Wilcoxon test for non-normally distributed data. Differences were considered statistically significant at $P < 0.05$. All calculations were performed using SPSS 16.0 for windows (SPSS, Chicago, IL)

Results

Between October 2007 and October 2008, a total of 32 patients were included in the study. Twenty-two patients completed the 26 weeks because one patient chose not to start therapy and nine patients discontinued treatment during the 26 wk for disease progression (four patients), adverse events (four patients), and the patient's request (one patient) as reported previously (19). Because the thyroid hormone profile of one patient was not recorded, 21 patients were included in the present analysis: seven females, 14 males, with a median age of 65 yr (range, 53–82 yr), all with distant metastases of nonmedullary thyroid carcinoma (local, 4%; lungs only, 29%; lungs and bones, 24%; lungs and local, 14%; other, 29%). The efficacy of sorafenib with respect to tumor progression and the presence of adverse effects has been reported previously (19). Effects of sorafenib on study parameters are given in **Table 1**.

We observed a profound decrease in mean body weight of 6 kg. During the study, the levothyroxine dose per kilogram body weight was significantly increased (2.48 μg/kg before *vs.* 2.71 μg /kg after sorafenib; P=0.008) **(Table 1, Figure 1**). Nevertheless, TSH increased significantly from 0.051 to 0.545 mU/liter (P=0.023). Despite the increase in thyroxine dose, significant decreases in serum T3 (1.90 *vs.* 1.60 nmol/liter) and dose-adjusted free T3 levels (1.59 *vs.* 1.35 pmol*kg/μg) were observed. Absolute rT3 levels tended to increase after sorafenib therapy, and T3/T4, T3/rT3, and T4/rT3 ratios decreased significantly by 18, 22, and 7%, respectively, reflecting an increased conversion of T4 into rT3. Potential effects of sorafenib on thyroid hormone binding proteins were assessed by measuring TBG levels, which were not influenced by sorafenib, and by calculating ratios of free over total T4 and T3 concentrations, which did not differ before and after sorafenib.

Table 1: Thyroid hormone parameters

	N	Baseline	26-weeks sorafenib	P –value
Weight (kg)	21	77.3 ± 13.5	71.0 ± 14.2	<0.001
Sorafenib dose (mg/kg/day)	21	10.4 ± 2.4	8.4 ± 2.7	0.003
Levothyroxine dose (µg/kg/day)	21	2.48 ± 0.67	2.71 ± 0.61	0.008
T4 (nmol/L)	21	149.4 ± 31.9	152.9 ± 37.7	0.664
T3 (nmol/L)	20	1.90 ± 0.33	1.60 ± 0.34	0.007
FT4 (pmol/L)	21	27.3 ± 5.9	26.6 ± 6.9	0.587
FT4 (pmol/L)***weight** (kg) / levothyroxine dose (µg)	21	11.7 ± 3.6	10.4 ± 3.6	0.034
FT3 (pmol/L)	13	5.43 ± 0.71	4.80 ± 1.09	0.075
FT3 (pmol/L)***weight** (kg) / levothyroxine dose (µg)	13	1.59 ± 1.38	1.35 ± 1.15	0.005
rT3 (nmol/L)	20	0.68 (0.50-1.02)	0.79 (0.63-1.12)	0.091 *
TSH (mU/L)	21	0.01 (0.005-0.33)	0.100 (0.005-4.670)	0.023 *
TBG (mg/L)	21	19.8 ± 4.3	19.5 ± 4.0	0.620
FT3/T3 * 10^3	13	2.73 ± 0.33	2.93 ± 0.31	0.279
FT4/T4 * 10^3	21	5.55 ± 0.94	5.84 ± 0.95	0.124
T3/T4 * 100	20	1.28 ± 0.00	1.05 ± 0.00	<0.001
T3/rT3	20	2.74 ± 0.50	2.16 ± 0.53	<0.001
T4/rT3	20	220 ± 27	205 ± 32	0.036

Data expressed as mean ± SD or median (range)
* Wilcoxon -test

Discussion

In this study, we assessed the relationship between treatment with the multiple target kinase inhibitor sorafenib and alterations in thyroid hormone parameters. Therapy with tyrosine kinase inhibitors is associated with hypothyroidism. Proposed mechanisms include direct effects of these drugs on the thyroid, including destructive thyroiditis (4,10,11). Because thyroid function abnormalities have also been observed in athyreotic patients on thyroxine substitution, the mechanisms of hypothyroidism may include alterations in thyroid hormone metabolism as well. We hypothesized that sorafenib may influence the activities of iodothyronine deiodinases (D1, D2, and D3) (17), which has not been studied in humans so far. We found that a higher substitution dose of thyroxine was needed to maintain serum FT4 levels. These findings are consistent with a previous report in which eight imatinib treated patients that had undergone thyroidectomy required substantial increases in levothyroxine replacement dose (13). Indeed, the increased need of levothyroxine may be underestimated because some patients may have been overtreated before initiation of the trial. In addition, we found a clear decrease in serum T3/T4, T3/rT3, and T4/rT3 ratios. These

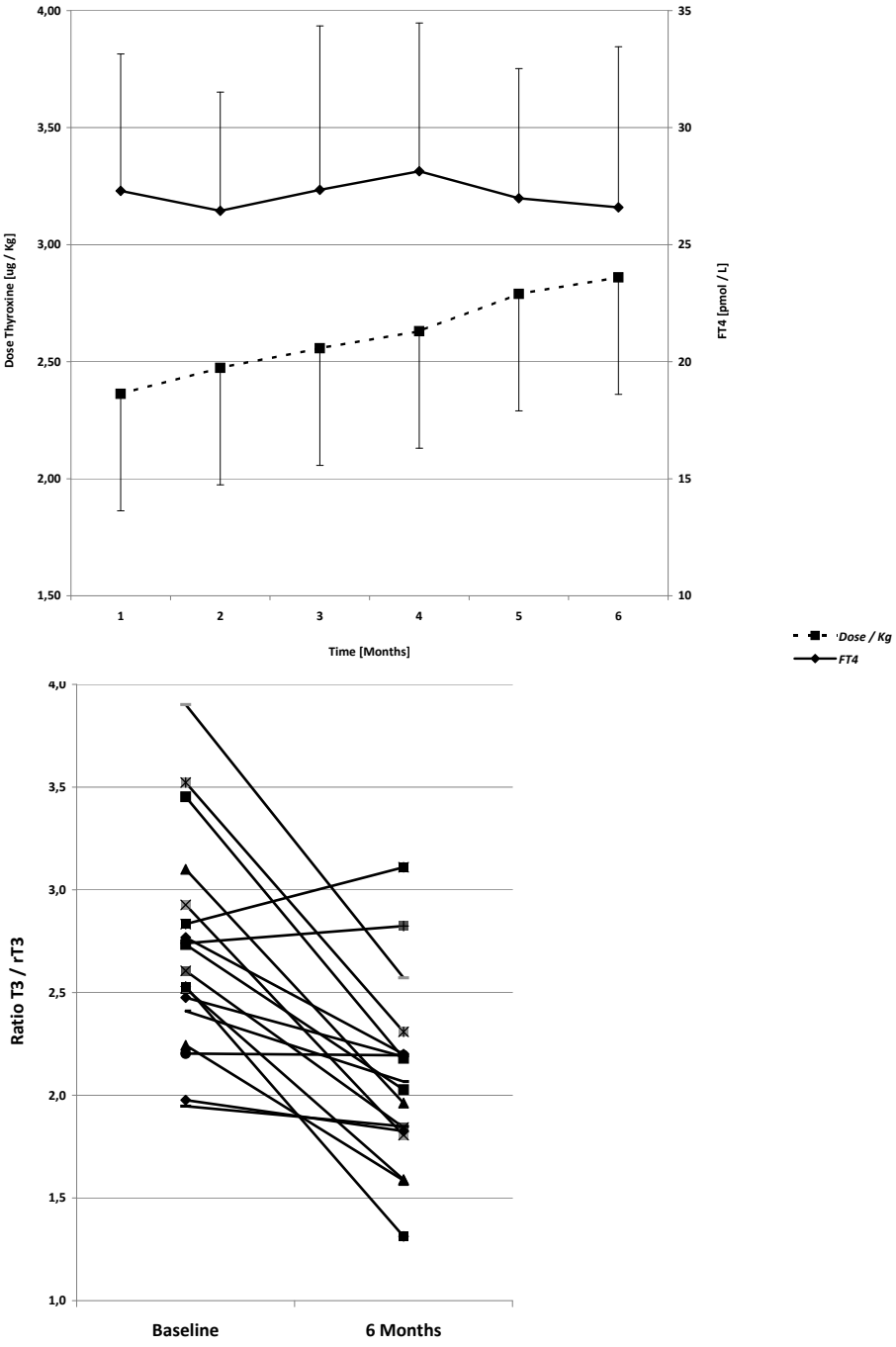

Figure 1

(A) Relation between levothyroxine dose adjusted for body weight (Dose Thyroxine) and serum FT4 concentrations during 6 months of treatment with sorafenib. (B) Individual T3/rT3 ratios before and 6 months after treatment with sorafenib.

ratios reflect alterations in the peripheral metabolism of thyroid hormone, being positively influenced by deiodinases D1 and D2 and negatively by D3 (17). Moreover, they are independent of levothyroxine administration. The decreased T3/T4 and T3/rT3 ratios may be caused by a decrease in D1 and/or D2 activity. However, this would be associated with a decreased rather than an increased T4 metabolism. Therefore, the decreased T3/T4 and T3/rT3 ratios are best explained by an increased D3 activity. The fact that, 2 months after the start of sorafenib, a significantly higher T4 dose per kilogram body weight was necessary to maintain FT4 concentrations strongly suggests that the phenomenon is already present early after initiation of sorafenib therapy. It is worthwhile to further elucidate the effects of sorafenib on D3 in *in-vitro* studies. It is unlikely that the increased D3 activity reflects a state of non thyroidal illness because serum TSH increased during sorafenib, whereas in non thyroidal illness, decreased rather than increased TSH levels would be expected. Although it can be hypothesized that decreased absorption of T4 could also have played a role, the interval between sorafenib and thyroxine intake was approximately 12 h. In addition, decreased T4 absorption would not affect T3/T4 and T3/rT3 ratios. No changes in TBG levels and the ratios between free and bound thyroid hormones were observed, ruling out effects of sorafenib on thyroid hormone binding proteins, which again, even if present, would not have affected the T3/rT3 ratio. It may hypothesized that sorafenib may also influence conjugation of thyroid hormone with glucuronates and sulfates by hepatic microsomal enzymes. However, altered conjugation would not influence T3/rT3 ratios. The fact that the increase in overall rT3 levels was non significant may suggest that rT3 degradation may be enhanced as well. It is likely that, because rT3 is the preferred substrate for type 1 deiodinase (in the absence of hyperthyroidism), increased rT3 may lead to enhanced type 1 deiodinase-mediated rT3 degradation.

In conclusion, this study shows that, in addition to direct effects of tyrosine kinase inhibitors on the thyroid gland, enhanced peripheral metabolism of thyroid hormone, likely by activity of type 3 deiodinase, may contribute to hypothyroidism during therapy with these drugs.

References

1. Desai J, Yassa L, Marqusee E, George S, Frates MC, Chen MH, Morgan JA, Dychter SS, Larsen PR, Demetri GD, Alexander EK. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. Annals of Internal Medicine 2006 145 660-664
2. Mannavola D, Coco P, Vannucchi G, Bertuelli R, Carletto M, Casali PG, Beck-Peccoz P, Fugazzola L. A novel tyrosine-kinase selective inhibitor, sunitinib, induces transient hypothyroidism by blocking iodine uptake. Journal of Clinical Endocrinology and Metabolism 2007 92 3531-3534
3. Rini BI, Tamaskar I, Shaheen P, Salas R, Garcia J, Wood L, Reddy S, Dreicer R, Bukowski RM. Hypothyroidism in patients with metastatic renal cell carcinoma treated with sunitinib. Journal of the National Cancer Institute 2007 99 81-83
4. Wong E, Rosen LS, Mulay M, Vanvugt A, Dinolfo M, Tomoda C, Sugawara M, Hershman JM. Sunitinib induces hypothyroidism in advanced cancer patients and may inhibit thyroid peroxidase activity. Thyroid 2007 17 351-355
5. Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L, Woulfe K, Pravda E, Cassiola F, Desai J, George S, Morgan JA, Harris DM, Ismail NS, Chen JH, Schoen FJ, Van den Abbeele AD, Demetri GD, Force T, Chen MH. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet 2007 370 2011-2019
6. Wolter P, Stefan C, Decallonne B, Dumez H, Bex M, Carmeliet P, Schoffski P. The clinical implications of sunitinib-induced hypothyroidism: a prospective evaluation. British journal of Cancer 2007 99 448-454
7. Grossmann M, Premaratne E, Desai J, Davis ID. Thyrotoxicosis during sunitinib treatment for renal cell carcinoma. Clinical Endocrinology (Oxf) 2008 69 669-672
8. Faris JE, Moore AF, Daniels GH. Sunitinib (sutent)-induced thyrotoxicosis due to destructive thyroiditis: a case report. Thyroid 2007 17 1147-1149
9. Alexandrescu DT, Popoveniuc G, Farzanmehr H, Dasanu CA, Dawson N, Wartofsky L. Sunitinib-associated lymphocytic thyroiditis without circulating anti thyroid antibodies. Thyroid 2008 18 809-812
10. Kamba T, McDonald DM. Mechanisms of adverse effects of anti-VEGF therapy for cancer. British Jounal of Cancer 2007 96 1788-1795
11. Kamba T, Tam BY, Hashizume H, Haskell A, Sennino B, Mancuso MR, Norberg SM, O'Brien SM, Davis RB, Gowen LC, Anderson KD, Thurston G, Joho S, Springer ML, Kuo CJ, McDonald DM. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. American Journal Physiology, Heart and Circulatory Physiology 2006 290 H560-H576
12. Tamaskar I, Bukowski R, Elson P, Ioachimescu AG, Wood L, Dreicer R, Mekhail T, Garcia J, Rini BI. Thyroid function test abnormalities in patients with metastatic renal cell carcinoma treated with sorafenib. Annals of Oncology 2008 19 265-268
13. de Groot JW, Zonnenberg BA, Plukker JT, van der Graaf WT, Links TP. Imatinib induces hypothyroidism in patients receiving levothyroxine. Clinical Pharmacology and Therapeutics 2005 78 433-438
14. de Groot JW, Links TP, van der Graaf WT. Tyrosine kinase inhibitors causing hypothyroidism in a patient on levothyroxine. Annals of Oncology 2006 17 1719-1720
15. Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, Licitra L, Eschenberg MJ, Sun YN, Juan T, Stepan DE, Schlumberger MJ, the Motesanib Thyroid Cancer Study Group.

Motesanib Diphosphate in Progressive Differentiated Thyroid Cancer. New England Journal of Medicine 2008 359 31-42

16. Gupta-Abramson V, Troxel AB, Nellore A, Puttaswamy K, Redlinger M, Ransone K, Mandel SJ, Flaherty KT, Loevner LA, O'Dwyer PJ, Brose MS. Phase II Trial of Sorafenib in Advanced Thyroid Cancer. Journal of Clinical Oncology 2008 26 4714-4719.
17. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, Cellular and Molecular Biology, and Physiological Roles of the Iodothyronine Selenodeiodinases. Endocrine Reviews 2002 23 38-89
18. Eelkman Rooda SJ, Kaptein E, Visser TJ. Serum triiodothyronine sulfate in man measured by radioimmunoassay. Journal of Clinical Endocrinology and Metabolism 1989 69 552-556
19. Hoftijzer H, Heemstra KA, Morreau H, Stokkel M, Corssmit EP, Gelderblom AJ, Weijers K, Pereira AM, Huijberts M, Kapiteijn E, Romijn JA, Smit JW. Beneficial effects of sorafenib on tumor progression, but not on radioiodine uptake, in patients with differentiated thyroid carcinoma. European Journal of Endocrinology 2009 16 923-31
20. Visser TJ, Docter R, Hennemann G Radioimmunoassay of reverse tri-iodothyronine. Journal of Endocrinology 1977 73 395-396

5

The type 2 deiodinase Thr92Ala polymorphism is not associated with T4 dose in athyroid patients treated for differentiated thyroid carcinoma or patients with Hashimoto thyroiditis

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Abstract

Objective

The type 2 deiodinase (D2)-Thr92Ala polymorphism has been associated with decreased D2 activity in some *in-vitro* experiments but not in others. So far no association between the D2-Thr92Ala polymorphism and serum thyroid hormone levels has been observed in humans, but in a recent study in athyroid patients, it was suggested that patients homozygous for the 92Ala allele needed higher T4 doses to achieve TSH suppression. We studied the association between the D2-Thr92Ala polymorphism with thyroid hormone levels and T4 dosage, in patients treated for differentiated thyroid carcinoma (DTC) and in a group of patients treated for Hashimoto thyroiditis.

Patients

We studied 154 patients with DTC treated with TSH suppressive thyroid hormone replacement therapy for longer than 3 years and 141 patients with Hashimoto thyroiditis treated for at least 6 months with T4.

Measurements

In all patients, serum levels of TSH, free T4, T3 and reverse T3 were measured and genotypes of the D2-Thr92Ala polymorphism were determined by Taqman assay. Univariate regression analysis was performed to determine the relation between T4 dosages and the D2-Thr92Ala polymorphism corrected for age, gender, BMI and serum TSH levels.

Results

Both in DTC patients and Hashimoto patients, no association was observed between serum thyroid hormone levels or T4 dosages in presence of the D2-Thr92Ala polymorphism. Categorization of DTC patients according to degree of TSH suppression did not change these results.

Conclusion

The D2-Thr92Ala polymorphism is not associated with thyroid hormone levels or T4 dose in patients treated for DTC or Hashimoto thyroiditis.

Introduction

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T3. Serum and local T3 concentrations are mainly regulated by the iodothyronine deiodinases D1, D2 and D3 (1). D2 is essential for the local production of T3 through deiodination of T4. D2 is thus essential for the negative feedback regulation of thyroid hormone on TSH production in the pituitary. Several polymorphisms in D2 have been described (2–5). Controversy exists about the functional implications of the D2-Thr92Ala polymorphism, which has been associated with a decreased D2 activity in some *in-vitro* experiments (2) but not in others (5). So far no associations were found between the D2-Thr92Ala polymorphism and serum thyroid hormone levels in studies in healthy subjects (4,6,7). Torlontano *et al.* reported in thyroidectomized differentiated thyroid carcinoma (DTC) patients that homozygous carriers of the D2-Ala92 allele needed higher dosages of T4 (8). This difference was most prominently observed in the group with near-suppressed TSH (TSH values between 0.1 and 0.5 mU/l). Limitations of this study were that actual values of serum TSH levels for wild-type and homozygous groups within the near-suppressed TSH group were not given. It is therefore unclear whether TSH levels in both groups were indeed identical, which would be a key finding to ascribe the slight differences in T4 dose indeed to the polymorphism. The fact that serum T4 and T3 levels did not differ between the wild-type group and D2-Thr92Ala homozygotes is also remarkable. Moreover, as TSH is a continuous variable, we believe that the optimal analytic strategy would be by regression analysis, rather than a categorized approach. We therefore performed this study to reconfirm the findings of Torlontano *et al.* For this reason, we studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and T4 dosage in 154 patients treated for DTC and 141 patients substituted with T4 for Hashimoto thyroiditis, using a linear regression model. In addition, we performed a categorized analysis to allow maximal comparability with the Torlontano study.

Patients and methods

Patients

Patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. All patients had been treated by near-total thyroidectomy followed by radioiodine ablation. After initial treatment, T4 therapy was started in a dose intended to suppress TSH levels below 0.4 mU/l for 15 years. All patients were cured as defined by the absence of 131-Iodine accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations

below 2 µg/l after TSH stimulation in the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease. Patients with tumor relapse were only included if they had been subsequently cured. The Local Ethics Committee of the Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

We also included 141 patients treated for at least 6 months with T4 therapy for Hashimoto thyroiditis. Serum TSH levels were between 0.11 and 4.0 mU/l. These patients were described earlier by Appelhof *et al.* (9).

Study design

After an overnight fast, patients had a physical examination, including, height (meters [m]) and weight (kilograms [kg]). Blood was collected for determination of TSH, free T4 (FT4), T3 and reverse T3 (rT3). Serum samples were handled immediately and stored at –80 ° C in Sarstedt tubes. DNA was collected for genotyping of the D2-Thr92Ala polymorphism. To be able to compare our study with the study of Torlontano *et al.* (8) patients were categorized in groups with a suppressed TSH (< 0.1 mU/l), near-suppressed TSH (0.1–0.5 mU/l) or non-suppressed TSH (> 0.5 mU/l).

Serum biochemistry

In the patients treated for DTC, serum FT4 and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intra-assay CV of 1.6–2.2% and 1.3–5.0%, respectively (Roche, Almere, the Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5–9.0%, on an ImX system (Abbott, Abbott Park, IL). Reverse T3 was measured using a RIA as described previously (10). In the patients treated for Hashimoto thyroiditis, serum TSH and FT4 were measured by time-resolved fluoroimmunoassay and serum T4 and T3 by in-house RIA methods (6).

Genotyping

DNA was isolated from peripheral leucocytes by the salting out procedure (11). Genotypes of the D2-Thr92Ala polymorphism (rs225014) were determined using 5 ng genomic DNA in a 5′ fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2 × 384-well PCR machines with end-point reading on the ABI 7900HT TaqMan® machine (Applied Biosystems, Nieuwerkerk aan den IJssel, the Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by design service of Applied Biosystems.

Statistical analysis

Values are presented as mean ± standard deviation (SD), median (range) or as numbers or proportions of patients. Deviation from Hardy–Weinberg Equilibrium was analyzed using a χ 2 -test. Dominant (Thr/Thr *vs.* Ala/X) and recessive (Thr/X *vs.* Ala/Ala) effects of the polymorphism were analyzed. The association between D2-Thr92Ala genotypes and T4 dosages or thyroid hormone levels was analyzed using multivariate regression analyses. This was corrected for age, gender, BMI and the natural logarithm of TSH levels. In addition, differences between the different D2 genotype groups were analyzed using unpaired *t* -test or ANCOVA. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P < 0.05$.

Results

Patient characteristics

We studied 154 DTC patients. Mean duration of TSH suppressive therapy was 9.2 years (range 0.5–42.6 years). Median duration of cure was 8.9 years (range 1.0–41.8 years). The mean dose of T4 was 183 ± 51 µg/day. Mean T4 dose was 2.2 ± 1.0 µg/kg body weight. We also studied 141 patients with Hashimoto thyroiditis on T4 replacement therapy. Genotyping of the D2-Thr92Ala polymorphism failed in two subjects. The remaining 139 patients were treated with T4 for a mean duration of 7.3 ± 5.8 years. Mean T4 dose was 125 ± 46 µg/day.

Thyroid hormone parameters and D2-Thr92Ala

Allele frequencies of the D2-Thr92Ala polymorphism in the DTC patients and Hashimoto thyroiditis patients were 39.6% and 40.3%, respectively. The genotype distributions did not deviate from Hardy–Weinberg equilibrium. Thyroid hormone levels and T4 dose for patients with DTC and Hashimoto thyroiditis are presented in **Table 1**. No differences were observed in thyroid hormone levels and T4 dose, corrected for BMI and TSH levels between wild-type, heterozygous and homozygous carriers of the D2-Thr92Ala polymorphism. Analyses were comparable when T4 dose was corrected for BMI. No differences were observed in the correlation between lnTSH and T4 dose/kg or FT4 level for the different carriers of the D2-thr92ala polymorphism (**Figure 1a–d**).

Table 1: Deiodinase type 2 genotypes and thyroid hormone parameters

	Genotype	Patients (n)	Age (yr)	Gender (m/f)	Weight (kg)	BMI (kg/m²)	TSH (mU/l)	FT4 (pmol/l)	T3 (nmol/l)	rT3 (nmol/l)	T4 dose (µg/day)	T4 dose (µg/kg)	Dose / kg x lnTSH
DTC	WT Thr/Thr	60	47.2±12.4	13/47	76.1±15.3	25.6±4.7	0.05 (0.003-4.6)	22.72±3.89	1.49±0.28	0.60±0.23	186.3±58.2	2.09±1.04	-6.74±5.28
	HeZ Ala/Thr	66	51.5±13.5	11/55	75.7±12.2	26.2±3.5	0.03 (0.003-4.9)	22.42±4.48	1.46±0.38	0.51±0.21	178.2±41.5	2.22±0.87	-6.81±4.96
	HoZ Ala/Ala	28	48.3±10.2	5/23	74.7±14.8	25.8±5.9	0.05 (0.003-6.8)	21.66±4.27	1.40±0.33	0.56±0.19	185.9±58.4	2.19±1.07	-7.82±5.67
	P-value		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
HT	WT Thr/Thr	47	46.6±8.6	5/42	78.5±17.8	27.8±5.4	2.02±1.76	14.46±2.87	1.73±0.36	ND	124.2±41.4	1.64±0.62	0.24±1.81
	HeZ Ala/Thr	72	47.3±10.9	11/61	80.4±18.3	28.2±5.6	1.59±1.41	15.16±3.05	1.73±0.30	ND	127.6±50.6	1.64±0.71	-0.22±2.01
	HoZ Ala/Ala	20	52.1±8.2	4/16	91.0±31.3	32.2±9.1	1.96±1.68	14.42±2.48	1.71±0.36	ND	116.3±38.1	1.36±0.51	0.17±1.45
	P-value		NS	NS	0.046	0.026	NS	NS	NS		NS	NS	NS

DTC= Differentiated thyroid carcinoma, HT= Hashimoto thyroiditis, WT= wild-type, HeZ= Heterozygous, HoZ= Homozygous, NS= Not significant, ND = no data available

Data are expressed as mean ± SD or number of patients, except for TSH which is median (range)

Analyses for TSH, FT4, T3 and T4 dose in HT patients are corrected for age, gender and BMI.

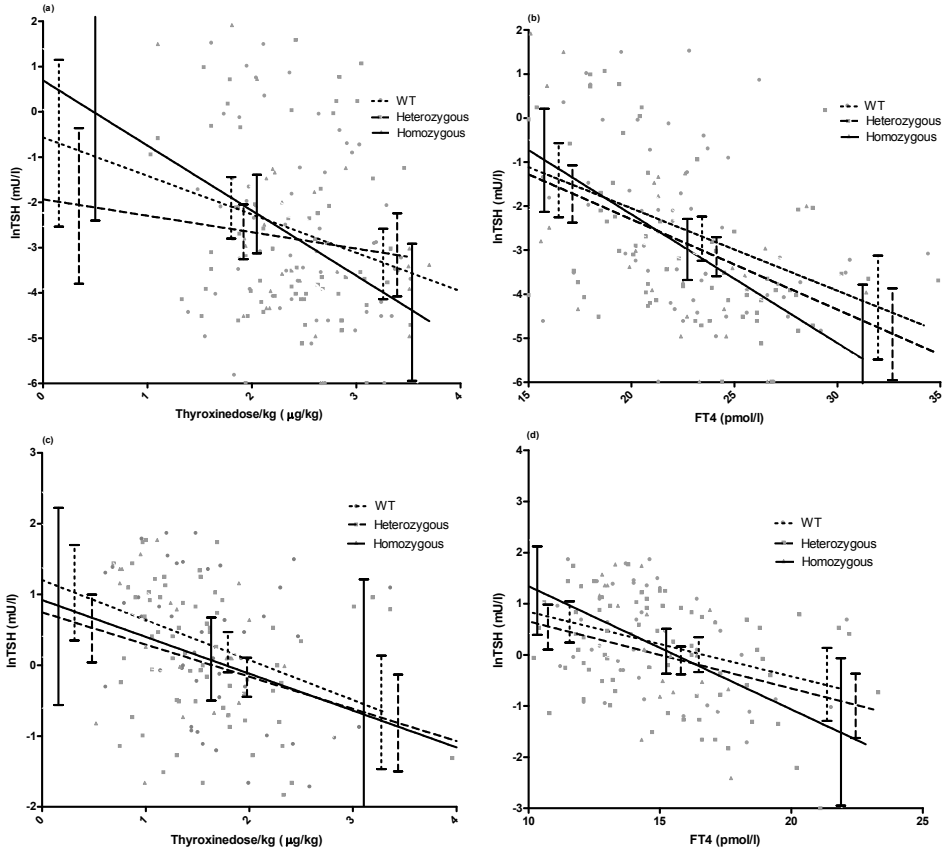

Figure 1

(A) Correlation between the natural logarithm of TSH and T4 dosage/kg for the different alleles of D2-Thr92Ala polymorphism in 154 patients with differentiated thyroid carcinoma. Lines: regression lines; bars: 95% confidence intervals of regression lines. (B) Correlation between the natural logarithm of TSH and FT4 for the different alleles of D2-Thr92Ala polymorphism in 154 patients with differentiated thyroid carcinoma. Lines: regression lines; bars: 95% confidence intervals of regression lines. (C) Correlation between the natural logarithm of TSH and T4 dosage/kg for the different alleles of D2-Thr92Ala polymorphism in 139 patients treated for Hashimoto thyroiditis. (D) Correlation between the natural logarithm of TSH and FT4 for the different alleles of D2-Thr92Ala polymorphism in 139 patients treated for Hashimoto thyroiditis.

Discussion

We studied the association between the D2-Thr92Ala polymorphism and thyroid hormone levels and T4 dose in two separate groups of patients, treated for DTC or Hashimoto thyroiditis. Frequencies of the alleles of D2-Thr92Ala are in agreement with previous studies varying between 30.0% and 38.8% in patient with normal thyroid

function or not taking thyroid replacement or thyreostatic medication (3,4,6,7). The D2-Thr92Ala polymorphism was not associated with thyroid hormone parameters or T4 dosages in the two separate groups of patients included in our analyses. This is in accordance with previous studies (4–7). Torlontano *et al*. found that homozygous DTC carriers of the D2-Ala92 allele need higher T4 dosages (8). This association was observed in the near-suppressed TSH group, but not in the suppressed group. The study of Torlontano *et al*. has however, several limitations. TSH levels in the near-suppressed group of the different alleles were not given, which would have been useful to investigate whether the differences in T4 dose are not caused by alterations in TSH levels. In our study, no differences were observed in TSH levels or T4 dose for the different alleles with and without categorization according to the degree of TSH suppression in the DTC patients or Hashimoto patients. In addition, we believe that the analysis strategy should be primarily based on regression analysis rather than TSH categories, because for alterations in TSH levels should be corrected.

Remarkably, they did not find any differences in thyroid hormone levels suggesting that patients with D2-Ala92 alleles need a higher T4 dose to reach the same serum FT4 level. By inference, the Ala allele would not affect T4 feedback but rather T4 resorption. Torlontano *et al*. explain the discrepancies of their findings with previous studies by two arguments. First, they state that in previous studies thyroid hormone levels were within the wide reference range, which makes is difficult to detect subtle differences in thyroid hormone levels for different carriers of the D2-Thr92Ala polymorphism. However, they found this difference only in the near-suppressed group, which is an ill-defined group with a wide plasma TSH range including patients with normal TSH levels. Second, Torlontano *et al*. argue that the difference between their finding and earlier studies may be explained by the absence of a thyroid gland in their patients. However, in our analysis with athyroid DTC and Hashimoto patients, we could not confirm this. A *post hoc* power analysis for T4 dose and T4/kg showed a sufficient power of 100%. Therefore, it seems unlikely that underpowering of our study plays a major role in the negative findings.

In summary, we found no association between the D2- Thr92Ala polymorphism and thyroid hormone levels and T4 dose in two separate groups of patients treated for DTC or Hashimoto thyroiditis.

References

1. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation. 2006 116 2571–2579.
2. Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, Maia AL.. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 2005 90 3472–3478.
3. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi FS. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the β -3-adrenergic receptor. Diabetes 2002 51 880–883.
4. Mentuccia D, Thomas MJ, Coppotelli G, Reinhart LJ, Mitchell BD, Shuldiner AR, Celi FS. The-Thr92Ala deiodinase type 2 (DIO2) variant is not associated with type 2 diabetes or indices of insulin resistance in the old order of Amish. Thyroid 2005 15 1223–1227.
5. Peeters RP, van den Beld AW, Attalki H, Toor H, de Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG, Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. American Journal of Physiology-Endocrinology and Metabolism 2005 289 E75–E81.
6. de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ, Breteler MM. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. Journal of Clinical Endocrinology and Metabolism 2007 92 636–640.
7. Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, Visser TJ. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. Journal of Clinical Endocrinology and Metabolism 2003 88 2880–2888.
8. Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S, Trischitta V. Type 2 deiodinase polymorphism (threonine 92 alanine) predicts 1-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. Journal of Clinical Endocrinology and Metabolism 2008 93 910–913.
9. Appelhof BC, Peeters RP, Wiersinga WM, Visser TJ, Wekking EM, Huyser J, Schene AH, Tijssen JG, Hoogendijk WJ, Fliers E. Polymorphisms in type 2 deiodinase are not associated with well-being, neurocognitive functioning, and preference for combined thyroxine/ 3,5,3 ′ -triiodothyronine therapy. Journal of Clinical Endocrinology and Metabolism 2005 90 6296– 299.
10. Visser TJ, Docter R, Hennemann G. Radioimmunoassay of reverse tri-iodothyronine. Journal of Endocrinology 1977 73 395–396.
11. Miller SA, Dykes DD, Polesky HF A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 1988 16 1215–1215.

6

The type 2 deiodinase ORFa-Gly3Asp polymorphism influences the setpoint of the hypothalamus-pituitary-thyroid axis in patients treated for differentiated thyroid carcinoma

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Submitted

Abstract

Context

Iodothyronine deiodinases D1, D2 and D3 play an important role in synthesis and degradation of T3. The relationship between serum TSH and T3 levels is determined by an individual setpoint of the hypothalamus-pituitary-thyroid axis.

Objective

Several polymorphisms have been described in D1 and D2 of which some are associated with serum TSH and iodothyronine levels. In this study we investigate whether polymorphisms of D1 and D2 influence the setpoint of the hypothalamus-pituitary-thyroid-axis.

Design

We collected 1905 serum FT4 and TSH measurements during 11.5±8.8 years of follow-up in patients treated for differentiated thyroid carcinoma (DTC). We determined these polymorphisms: D1-rs11206244, D1-rs12095080, D2-rs225014 and D2-rs12885300. Effects of these polymorphisms on the setpoints of the hypothalamus-pituitary-thyroid-axis were analysed with a linear mixed model.

Patients

151 consecutive patients treated and cured for DTC were included

Results

DTC patients homozygous for the D2-rs12885300 T allele have an altered setpoint of the hypothalamus-pituitary-thyroid axis. The slope of the regression line (corrected for age, BMI and gender) for wild-type patients was -0.32±0.028 (ln[TSH mU/l]/[FT4 pmol/l]), the intercept 4.95. For heterozygous patients the slope was -0.30±0.028 (ln[TSH mU/l]/[FT4 pmol/l]), the intercept 4.23. The slope of homozygous patients was -0.35±0.026 (ln[TSH mU/l]/[FT4 pmol/l]) and the intercept 6.07 (P =0.036 compared to wild-type and heterozygous patients).

Conclusion

Our data suggest that the negative feedback of FT4 on TSH is weaker in patients homozygous for the D2-ORFa-Gly3Asp than in wild-type and heterozygous subjects.

Background

Thyroid hormone has an important role in a wide range of physiological processes: from growth and development in children to energy homeostasis in the adult (1). Most actions of thyroid hormone are mediated by the active form of thyroid hormone, triiodothyronine (T3). Serum T3 levels are relatively constant in healthy subjects. Serum and local T3 concentrations are mainly regulated by the iodothyronine deiodinases D1, D2 and D3 (2). D1 is mainly involved in serum T3 production. In addition, it plays a role in the breakdown of rT3 (3,4). D2 catalyzes local T3 production through deiodination of T4 production in various tissues and is necessary for the negative feedback regulation of thyroid hormone on thyrotropin (TSH) production in the pituitary (5). Approximately 80% of T3 is produced by extrathyroidal pathways (2). D3 inactivates T3 and T4 and thus regulates the clearance of T3 and T4. It is thought to contribute to thyroid hormone metabolism by protecting tissues from excess thyroid hormone. The deiodinases adjust the thyroid hormone levels of individual tissues in response to various conditions (6,7).

Several polymorphisms have been described in D1 and D2 of which some are associated with circulating levels of T4, T3 and TSH (8,9,10,11). Two polymorphisms in the D1 gene, D1-rs11206244 (previously D1-C785T) and D1-rs12095080 (previously D1-A1814G), have been associated with changes in the balance of thyroid hormones in the serum, with raised T3, low T4 and low rT3 levels, but these changes have not been associated with differences in TSH. This implies that the net effect is perceived by the hypothalamus and pituitary as "neutral" (1,12,13,14). However, the D1-rs11206244 is in linkage disequilibrium with another single-nucleotide polymorphism (SNP) rs2235544 which did show a distinct association with circulating FT3/FT4 ratio (11).

Controversy exists about the functional implications of the D2-rs225014 (previously D2-Thr92Ala) polymorphism, which has been associated with a decreased D2 activity in some in-vitro experiments (9), but not in others (13). So far only one study found an association between the D2-rs225014 polymorphism and serum thyroid hormone levels (15), however a previous study performed by our department could not confirm these data (16). A recent study by Butler *et al*. performed a prospective intervention study which aimed at demonstrating in-vivo effects of the Thr92Ala D2 variant. They found subtle differences in the serum changes of total T3 in the Ala/Ala subjects 60 minutes after TRH injection. However, there were no significant differences in the response of FT4 and TSH after TRH injection (17).

In healthy blood donors the D2-ORFa-Asp variant (rs12885300) was associated with lower levels of serum T4, fT4 and rT3, but not with plasma T3 and TSH levels (8). This suggests that the D2-rs1288530 polymorphism leads to higher activity of D2 at the pituitary level. However, this association was not seen in elderly men (8).

Intraindividual variation in serum T4, T3 and TSH is narrow; however there is a considerable interindividual variability (18). A large body of evidence suggests that every individual has a unique thyroid function setpoint, compatible with a genetic influence on the regulation of the pituitary-thyroid axis (18,19,20). We hypothesized that polymorphisms in D1 and D2 could influence the setpoint of the hypothalamus-pituitary-thyroid axis. We tested this hypothesis in 151 patients treated for differentiated thyroid carcinoma (DTC). These individuals have no endogenous thyroid hormone production and thus no interference of the intrinsic T3 production of the thyroid. During follow-up of DTC, patients are treated with thyroxine substitution therapy in a dose intended to suppress TSH level, which results in a constant and precisely measurable supply of T4. During this period they regularly come for routine measurements of TSH and FT4, moreover they are regularly withdrawn from thyroxine to perform TSH stimulated radioactive iodine-131 whole body imaging, which leads to a wide individual range of combined measurements of TSH and FT4. These patients are therefore an ideal group to assess the relationship between polymorphisms in deiodinases and the setpoint of the hypothalamus-pituitary-thyroid axis.

Patients and Methods

Patients

One hundred and fifty-one consecutive patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center, a tertiary referral center for thyroid carcinoma. All patients had been treated by near-total thyroidectomy followed by radioiodine ablation between 1992 and 2007. After initial treatment, thyroxine therapy was started in a dose intended to suppress TSH levels below 0.4 mU/l for 15 years. All patients were cured as defined by the absence of I-131 accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 µg/l after TSH stimulation in the absence of Tg antibodies, a normal neck ultrasound and no other indication for disease. Patients with tumor relapse were only included if they had been subsequently cured. Patients that used corticosteroids or psychopharmaca that could alter serum TSH or FT4 levels (amiodarone, dopamine, phenytoin, lithium) were excluded from the study. The Local Ethics Committee of the Leiden University Medical Centre approved the study, and written informed consent was obtained from all subjects.

Study design

We collected all FT4 and TSH measurements since the initial diagnosis of the patients with the exception of recombinant human (Thyrogen) stimulated TSH levels. Blood

was collected for regular outpatient clinic appointments from 1992 till 2007. In 2007, blood was collected for DNA isolation for genotyping of the deiodinase type 1 and 2 polymorphisms, and for measurements of T3 and rT3 in addition to TSH and FT4.

Serum biochemistry

Serum free T4 concentrations were measured throughout the study period on an IMx system (Abbott, Abbott Park, IL) (intra-assay variability of 2.5–7.6% and interassay variability of 5.6–12.4% at different levels). Serum TSH concentrations were determined throughout the study period with Elecsys E-170 on a Modular Analytics E-170 system (Roche Diagnostic Systems, Basel, Switzerland; reference range 0.4–4.5 mU/liter, detection limit 0.005 mU/liter, intraassay variability 0.9–10.7%, and interassay variability 0.9–12.1%). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2·5–9·0%, on an ImX system (Abbott, Abbott Park, IL). Reverse T3 was measured using a RIA as described previously (20).

Genotyping

DNA was isolated from peripheral leucocytes by the salting out procedure (21). Genotypes of the D1 and D2 polymorphisms were determined using 5 ng genomic DNA in a 5′ fluoregenic Taqman assay and reactions were performed in 384-wells format on ABI9700 2x384well PCR machines with endpoint reading on the ABI 7900HT TaqManÒ machine (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Primer and probe sequences were optimized using the single nucleotide polymorphism assay-by-design service of Applied Biosystems.

Statistical Analysis

Values are presented as mean ± standard deviation (SD), median (range) or as numbers or proportions of patients. Deviation from the Hardy-Weinberg Equilibrium was analysed using a X^2-test. For the comparison of setpoints of the hypothalamus-pituitary-thyroid axis, we used a regression analysis with a general mixed model with a random intercept and random slope to assess the correlation between the natural logarithm of TSH (lnTSH) and FT4 for the different alleles of the polymorphisms. Herewith, we tested if the regression lines (with the equation $\ln TSH = \beta \cdot FT4 + \alpha$, where β is the slope and α is the intercept) were similar in slopes given a random intercept. We had different numbers of combined TSH/FT4 measurements for each individual. With our model the regression lines of patients were compared independently of the number of blood samples (combined TSH/FT4 measurements) available. However,

regression lines are expected to be more accurate if an individual patient had more TSH/FT4 measurements. In our model, we corrected for sex, BMI and age at time of the blood sampling, since these parameters can influence the lnTSH/FT4 relationship.

All calculations were performed using SPSS 17.0 for windows (SPSS, Inc., Chicago, IL). Differences were considered statistically significant at $P<0.05$.

Results

Patient characteristics

Patient characteristics are summarized in **Table 1**. We studied 151 DTC patients, 28 males and 123 females. At the timepoint of the blood sampling for the determination of the different alleles of the deiodinase type 1 and 2 polymorphisms, mean age was 49.1 ± 12.9, mean dose of thyroxine 183 ± 51 µg/day and mean duration of follow-up 11.5 ± 8.8 years.

We identified a total of 3121 blood samples routinely obtained during follow up between 1992 and 2007. In 1905 of these samples both TSH and FT4 were measured and these values were used for this study. Mean number of measurements per patient was 12.53 ± 5.49 (range 4-32).

During this period, patients were regularly withdrawn from thyroxine for routine TSH stimulated radioiodine-131 whole body scanning. Therefore, the TSH (and FT4) values have a very wide range (see **Table 2**).

Genotyping failed in 3 patients for the D1-rs11206244 polymorphism, in 4 patients for the D1-rs12095080 polymorphism, in 3 patients for the D2-rs225014 polymorphism and in 4 patients for the D2-rs12885300 polymorphism.

Polymorphisms in deiodinases and values of TSH and FT4

Detailed information on genotype frequencies of the two deiodinases is given in Table 2.

For the D1-rs11206244 (D2-C785T) polymorphism 66 patients were wild type (C/C), 58 patients were heterozygous (C/T) and 24 patients were homozygous variant carriers (T/T). For the D1-rs12095080 (D1-A1814G) polymorphism, 125 patients were wild type (A/A), 19 patients were heterozygous (A/G) and only 3 were homozygous (G/G). The D2-rs225014 polymorphism (D2-Thr92Ala) was more equally distributed: 56 patients were wild type (Thr/Thr), 64 heterozygous (Thr/Ala) and 28 homozygous (Ala/Ala). For the D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphism, 70 patients were wild type (Gly/Gly), 64 patients were heterozygous (Gly/Asp) and 13 patients were homozygous (Asp/Asp). The genotype distributions did not deviate from the Hardy-Weinberg equilibrium.

Table 1: Patient characteristics

	Patients (n=151)
Age (yr, mean ± SD)	49.10 ± 12.93
Sex (M/F)	28/123
Tumor stage	
Unknown	6
T0 N1 M0	1
T0 N1 M1	1
IA T1 N0 M0	8
IB T2 N0 M0	72
IIA T1 N1 M0	7
IIB T2-3 N0-1 M0	28
IIIA T1-3 N1-2 M0	6
IIIB T1-3 N3 M0, T4, any N M0	18
IV Any T, any N, M1	4
Histology Tumor	
Papillary (n)	104
Papillary-follicular variant (n)	21
Follicular (n)	25
Follicular-Hurtle cell (n)	1
Duration of follow-up (yr) (mean ± SD)	11.45 ± 8.86
Total number of blood samples	3121
Number of blood samples with both TSH and FT4	1905
Number of measurements per patient	12.53 ± 5.49 (range 4-32)
L-thyroxin dose (µg/day) (mean±SD)	182.87 ± 50.84

Correlation between TSH and FT4 for the different alleles of the D1 and the D2 polymorphisms

We used a linear mixed model analysis to compare the slopes of the regression lines between lnTSH and FT4 for the different alleles of the D1 and D2 polymorphisms. Interestingly, the regression lines of the D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphism for wild type and heterozygous patients were significantly different from the regression line of homozygous subjects (Table 2, Figure 1). The slopes were comparable; however the intercept for homozygous patient was significantly higher. The slope of the regression line for wild type patients was -0.322 ± 0.028 (ln[TSH mU/l]/[FT4 pmol/l]) with an intercept of 4.95. For heterozygous patients the slope was also -0.299 ± 0.028 (ln[TSH mU/l]/[FT4 pmol/l]) with an intercept of 4.23. The slope of the homozygous patients was -0.347 ± 0.026 (ln[TSH mU/l]/[FT4 pmol/l]), whereas the intercept was 6.07 ($p = 0.036$ vs. wild-type and heterozygous subjects).

Table 2: Distribution of sample size, thyroid hormone parameters

Deiodinase type 1 C785T polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (3 missing polymorphisms)	66	58	24
Number of blood samples	885	739	245
FT4 (pmol/l) (mean ± SD)	21.17 ± 5.63	21.27 ± 6.26	22.08 ± 5.219
TSH (mU/l) (median(range))	0.13 (0.004-289)	0.14 (0.005-365)	0.07 (0.005-256)
LnTSH (median(range))	-2.04 (-5.52-5.67)	-1.96 (-5.3-5.9)	-2.66 (-5.3-5.55)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.269 ± 0.023	-0.275 ± 0.024	-0.296 ± 0.021
Intercept (mean ± SD)	5.82 ± 0.58	5.53 ± 0.58	5.08 ± 0.82
Cross-sectional data 2007			
TSH (mU/l)	0.63 ± 1.36	0.28 ± 0.64	0.41 ± 1.01
T4 (nmol/l)	138.33 ± 33.67	141.78 ± 45.07	133.04 ± 41.51
T3 (nmol/l)	1.47 ± 0.31	1.50 ± 0.36	1.37 ± 0.33
T3/T4 ratio	0.011 ± 0.002	0.011 ± 0.002	0.011 ± 0.004
rT3 (nmol/l)	0.57 ± 0.24	0.51 ± 0.19	0.54 ± 0.20
Deiodinase type 1 A1814G polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (4 missing polymorphisms)	125	19	3
Number of blood samples	1570	260	33
FT4 (pmol/l) (mean ± SD)	21.61 ± 5.83	21.27 ± 5.73	22.96 ± 6.57
TSH (mU/l) (median(range))	0.12(0.04-364)	0.17 (0.05-324)	0.10 (0.05-93)
LnTSH (median(range))	-2.12 (-5.52-5.90)	-1.77 (-5.30-5.78)	-2.32 (-5.30-4.53)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.338 ± 0.061	-0.323 ± 0.023	-0.317 ± 0.008
Intercept (mean ± SD)	4.96 ± 1.48	5.70 ± 0.55	5.52 ± 0.67
Cross-sectional data 2007			
TSH (mU/l)	0.44 ± 1.01	0.63 ± 1.23	0.09 ± 0.13
T4 (nmol/l)	137 ± 37.64	151.70 ± 50.05	115.37 ± 19.09
T3 (nmol/l)	1.46 ± 0.33	1.46 ± 0.40	1.55 ± 0.43
T3/T4 ratio	0.011 ± 0.003	0.010 ± 0.002	0.013 ± 0.003
rT3 (nmol/l)	0.56 ± 0.23	0.52 ± 0.14	0.41 ± 0.05
Deiodinase type 2 Thr92Ala polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (3 missing polymorphisms)	56	64	28
Number of blood samples	781	777	311
FT4 (pmol/l) (mean ± SD)	21.73 ± 5.93	21.63 ± 5.79	21.10 ± 5.73
TSH (mU/l) (median(range))	0.12 (0.05-364)	0.10 (0.04-365)	0.14 (0.05-324)
LnTSH (median(range))	-1.85 (-5.30-5.67)	-2.30 (-5.50-5.90)	-1.96 (-5.30-5.78)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.313 ± 0.022	-0.319 ± 0.022	-0.315 ± 0.018
Intercept (mean ± SD)	5.64 ± 0.54	5.47 ±0.53	5.29 ± 0.77

Table 2: Continued

Cross-sectional data 2007			
TSH (mU/l)	0.44 ± 0.99	0.37 ± 0.87	0.66 ± 1.60
T4 (nmol/l)	144.80 ± 34.96	135.30 ± 44.38	133.26 ± 34.29
T3 (nmol/l)	1.49 ± 0.28	1.46 ± 0.38	1.40 ± 0.33
T3/T4 ratio	0.011 ± 0.001	0.011 ± 0.003	0.011 ± 0.002
rT3 (nmol/l)	0.58 ± 0.23	0.52 ± 0.21	0.55 ± 0.19
Deiodinase type 2 ORFa-Gly3Asp polymorphism			
Setpoint analysis 1992-2007	**Wild type**	**Heterozygous**	**Homozygous**
N (3 missing polymorphisms)	70	64	13
Number of blood samples	865	818	199
FT4 (pmol/l) (mean ± SD)	21.90 ± 5.95	21.44 ± 5.68	21.02 ± 5.71
TSH (mU/l) (median(range))	0.12 (0.05-364)	0.11(0.05-218)	0.24 (0.05-131)
LnTSH (median(range))	-2.12 (-5.30-5.90)	-2.21 (-5.52-5.39)	-1.42 (-5.30-4.88)
β (slope) (ln[TSH mU/l]/[FT4 pmol/l]) (mean ± SD)	-0.322 ± 0.028	-0.299 ± 0.028	-0.347 ± 0.026
Intercept (mean ± SD)	4.95 ± 0.66	4.23 ± 0.66	6.07 ± 0.86
Cross-sectional data 2007			
TSH (mU/l)	0.51 ± 1.15	0.39 ± 1.05	0.52 ± 0.90
T4 (nmol/l)	138.03 ± 42.98	136.68 ± 37.29	152.67 ± 20.13
T3 (nmol/l)	1.45 ± 0.33	1.45 ± 0.36	1.58 ± 0.23
T3/T4 ratio	0.011 ± 0.003	0.011 ± 0.002	0.010 ± 0.001
rT3 (nmol/l)	0.56 ± 0.21	0.50 ± 0.19	0.71 ± 0.33

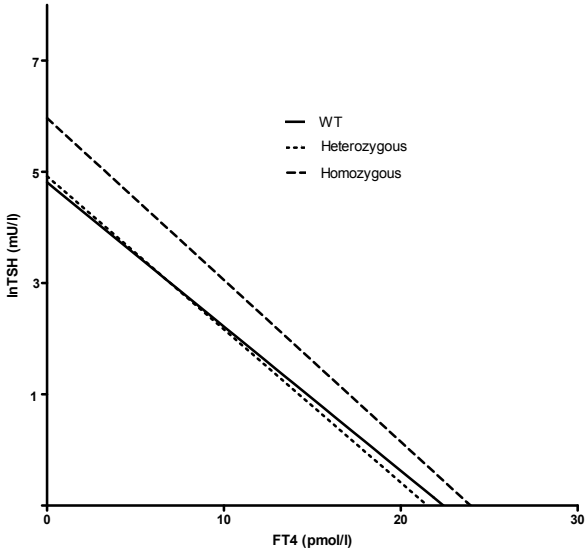

Figure 1
Correlation between the natural logarithm of serum levels of TSH and serum levels of FT4 for the D2-ORFa-Gly3Asp polymorphism in 151 patients with differentiated thyroid carcinoma.

Discussion

This study demonstrates that thyroidectomised DTC patients on thyroxine substitution who are homozygous for the D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphism have an altered setpoint of the hypothalamus-pituitary-thyroid axis. This study comprises a unique series of 1905 combined TSH and FT4 measurements. The mixed model analysis of the TSH/FT4 ratios is a precise approach to determine differences in individual setpoints. Our data suggest that the negative feedback of T4 on TSH is weaker in patients homozygous for the D2-rs12885300 (D2-ORFa-Gly3Asp) than in wild-type and heterozygous subjects. This is demonstrated by a higher lnTSH in combination with equal FT4 levels for homozygous patients.

Patients treated for DTC are ideal to investigate thyroid hormone metabolism, because they have been treated with total thyroidectomy and radioiodine ablation therapy. Because of this treatment they have no intrinsic T3 production. Therefore T3 levels are dependent on production at the tissue level through deiodination of exogenous T4 by D1 and D2. The negative feedback regulation of pituitary TSH secretion by T3, which in our patients is completely produced outside the thyroid, is mainly dependent on pituitary D2.

Although we have found a clear difference in the setpoint of the hypothalamus-pituitary-thyroid axis for the different D2-rs12885300 (D2-ORFa-Gly3Asp) polymorphisms, there are some unknown factors that could have also influenced TSH/FT4 ratios. We will discuss these factors briefly. First, unfortunately, because samples were collected as routine clinical follow-up, only TSH and FT4 levels were available, hence T3 and rT3 are only measured at one time point in 2007. Therefore we are not able to speculate about the serum values of T3 and rT3, and with that not the complete metabolic cycle of thyroid hormones during the entire period of the sample collection.

Second, we did not correct for a possible seasonal influence on setpoints. Two studies showed higher serum T3 and T4 values during the winter and lower values of TSH and total T3 during spring in healthy volunteers (23,24). However, given the huge number of random samples we assume that the seasons in which blood samples were collected will probably be equally distributed. Moreover, a previous study suggests a very limited effect of seasonal variation in thyroid function tests (20). In addition to this, we think that healthy volunteers in these studies and our thyroidectomized patients are not easily compared, since healthy persons have intrinsic thyroid hormone production possibly dependent on the seasonal feedback variation, whereas our patient population is treated with a fixed dose of thyroxine substitution. We therefore think that the potential contribution of seasonal influence is limited.

Our observations are however in contrast to the findings of Coppotelli *et al.* (25) who found an increased D2 activity of the D2-rs12885300 polymorphism in an in-vitro study and with the results of the study by Peeters *et al.* (8), who found that healthy blood donors with a D2-rs12885300 mutation needed less T4 to produce local T3 for the negative feedback action on the pituitary. These results were not confirmed in a group of healthy elderly men (8). However their observations in healthy blood donors with intrinsic thyroid function cannot be easily compared to DTC patients on TSH-suppressive thyroxine therapy.

Our patients are treated with a TSH suppressive dose of thyroxine (26,27). Another factor could be that long term subclinical hyperthyroidism may result in down-regulation of D1 and D2 and/or upregulation of D3 (2). However, we did not find a significant contribution of follow-up time and age at presentation to the observed effects of the D2-rs12885300 polymorphism on the setpoint of the hypothalamus-pituitary-thyroid axis.

In conclusion, we have found an altered setpoint of the hypothalamus-pituitary-thyroid axis for patients homozygous for the D2-rs12885300 polymorphism. However, it is unknown what the clinical significance of this altered setpoint will be. In the future, it would be interesting to investigate the proof of functionality of this D2 polymorphism and differences in biological variability in cell lines containing the different alleles of the D2-rs12885300 polymorphism.

References

1. Dayan CM, Panicker V. Novel insights into thyroid hormones from the study of common genetic variation. Nature Reviews Endocrinology 2009 5 211-218
2. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 2006 116 2571-2579
3. Kohrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. Molecular and Cellular Endocrinology 1999 151 103-119
4. Maeda A, Toyoda N, Yasuzawa-Amano S, Iwasaka T, Nishikawa M. Type 2 deiodinase expression is stimulated by growth factors in human vascular smooth muscle cells. Molecular and Cellular Endocrinology 2003 200 111-117
5. Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocrine Reviews 2002 23 38-89
6. Boelen A, Wiersinga WM, Fliers E. Fasting-induced changes in the hypothalamus-pituitary-thyroid axis. Thyroid 2008 18 123-129
7. Boelen A, Kwakkel J, Chassande O, Fliers E. Thyroid Hormone Receptor beta mediates acute illness-induced alterations in central thyroid hormone metabolism. Journal of Neuroendocrinology 2009 [Epub ahead of print]
8. Peeters RP, van den Beld AW, Attalki H, Toor H, de Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG, Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. American Journal of Physiology, Endocrinology and Metabolism 2005 289 E75-E81
9. Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, Maia AL. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 2005 90 3472-3478
10. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi FS. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. Diabetes 2002 51 880-883
11. Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JR, Weedon MN, Singleton A, Hernandez D, Evans J, Durant C, Ferrucci L, Melzer D, Saravanan P, Visser TJ, Ceresini G, Hattersley AT, Vaidya B, Dayan CM, Frayling TM. A common variation in deiodinase 1 gene DIO1 is associated with the relative levels of free thyroxine and triiodothyronine. Journal of Clinical Endocrinology and Metabolism 2008 93 3075-3081
12. de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ, Breteler MM. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. Journal of Clinical Endocrinology and Metabolism 2007 92 636-640
13. Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, Visser TJ. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. Journal of Clinical Endocrinology and Metabolism 2003 88 2880-2888

14. van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, Visser TJ. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. Clinical Endocrinology (Oxf) 2008 68 175-181
15. Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S, Trischitta V. Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. Journal of Clinical Endocrinology and Metabolism 2008 93 910-913
16. Heemstra KA, Hoftijzer HC, van der Deure WM, Peeters RP, Fliers E, Appelhof BC, Wiersinga WM, Corssmit EP, Visser TJ, Smit JW. Thr92Ala polymorphism in the type 2 deiodinase is not associated with T4 dose in athyroid patients or patients with Hashimoto thyroiditis. Clinical Endocrinology 2009 (Oxf) 71 279-283
17. Butler PW, Smith SM, Linderman JD, Brychta RJ, Alberobello AT, Dubaz OM, Luzon JA, Skarulis MC, Cochran CS, Wesley RA, Pucino F, Celi FS. The Thr92Ala 5′ type 2 deiodinase gene polymorphism is associated with a delayed triiodothyronine secretion in response to the thyrotropin-releasing hormone-stimulation test: a pharmacogenomic study. Thyroid 2010 20 1407-1412
18. Hansen PS, Brix TH, Sorensen TI, Kyvik KO, Hegedus L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. Journal of Clinical Endocrinology and Metabolism 2004 89 1181-1187
19. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. Journal of Clinical Endocrinology and Metabolism 2002 87 1068-1072
20. Benhadi N, Fliers E, Visser TJ, Reitsma JB, Wiersinga WM. Pilot study on the assessment of the setpoint of the hypothalamus-pituitary-thyroid axis in healthy volunteers. Eurean Journal of Endocrinology 2010 162 323-329
21. Visser TJ, Docter R, Hennemann G. Radioimmunoassay of reverse tri-iodothyronine. Journal of Endocrinology 1977 73 395-396
22. Miller SA, Dykes DD, Polesky HF.A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 1988 16 1215-
23. Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for interpretation of thyroid function tests. Thyroid 2003 13 1069-1078
24. Maes M, Mommen K, Hendrickx D, Peeters D, D'Hondt P, Ranjan R, De MF, Scharpe S. Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clinical Endocrinology (Oxf) 1997 46 587-598
25. Coppotelli G, Summers A, Chidakel A, Ross JM, Celi FS. Functional characterization of the 258 A/G (D2-ORFa-Gly3Asp) human type-2 deiodinase polymorphism: a naturally occurring variant increases the enzymatic activity by removing a putative repressor sit in the 5′ UTR of the gene. Thyroid 2006 16 625-632
26. Hovens GC, Stokkel MP, Kievit J, Corssmit EP, Pereira AM, Romijn JA, Smit JW. Associations of serum thyrotropin concentrations with recurrence and death in differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2007 92 2610-2615
27. McGriff NJ, Csako G, Gourgiotis L, Lori CG, Pucino F, Sarlis NJ. Effects of thyroid hormone suppression therapy on adverse clinical outcomes in thyroid cancer. Annals of Medicine 2002 34 554-564

7

The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density

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Abstract

The role of type 2 deiodinase (D2) in the human skeleton remains unclear. The D2 polymorphism Thr92Ala has been associated with lower enzymatic activity, which could result in lower local triiodothyronine (T3) availability in bone. We therefore hypothesized that the D2-Thr92Ala polymorphism may influence bone mineral density (BMD) and bone turnover. We studied 154 patients (29 men, 125 women: 79 estrogen-replete, 46 estrogen-deficient) with cured differentiated thyroid carcinoma. BMD and bone turnover markers [bone-specific alkaline phosphatase (BAP), cross-linking terminal C-telopeptide of type I collagen (CTX), procollagen type 1 aminoterminal propeptide (P1NP), and cross-linked N-telopeptide of type I collagen (NTX)] were measured. Effects of the D2-Thr92Ala polymorphism on BMD and bone turnover markers were assessed by a linear regression model, with age, gender, estrogen state, body mass index (BMI), serum calcium, 25-hydroxyvitamin D, parathyroid hormone (PTH), thyroid-stimulating hormone (TSH), and free thyroxine (T4) as covariables. Sixty patients were wild type (Thr/Thr), 66 were heterozygous (Thr/Ala), and 28 were homozygous (Ala/Ala) for the D2 polymorphism. There were no significant differences in any covariables between the three genotypes. Subjects carrying the D2-Thr92Ala polymorphism had consistently lower femoral neck and total hip densities than wild-type subjects ($p = 0.028$), and this was accompanied by significantly higher serum P1NP and CTX and urinary NTX/creatinine levels. We conclude that in patients with cured differentiated thyroid carcinoma, the D2-Thr92Ala polymorphism is associated with a decreased femoral neck BMD and higher bone turnover independent of serum thyroid hormone levels, which points to a potential functional role for D2 in bone.

Introduction

The involvement of thyroid hormone in bone metabolism has been well documented clinically, ranging from decreased skeletal development in childhood hypothyroidism (1–3), to accelerated growth in childhood hyperthyroidism (4), to an increased risk for osteoporosis in overt and subclinical hyperthyroidism (5–8). Although clinical observations suggest a clear involvement of thyroid hormone in bone metabolism, the molecular mechanisms by which thyroid hormone acts on bone so far have been only partially uncovered. Triiodothyronine (T3) promotes osteoblastic proliferation, differentiation, and apoptosis and, by induction of interleukin 6 (IL-6), prostaglandins, and RANKL, probably also promotes osteoclast formation and activation. This suggests that osteoblasts are the primary target cells for T3 in the regulation of bone remodeling (1,2,9–12). A functional role of thyroid stimulating hormone (TSH) on skeletal development and metabolism has been proposed on the basis of data obtained in animal studies (13–15) and in humans (16,17). This was disputed, however, by data obtained in thyroid hormone receptor (TR)–deficient mice, which indicated that bone remodeling was predominantly mediated by T3 via TRalpha (18,19). It also has been reported recently that in humans there is a significant association between bone mineral density (BMD) and serum thyroid hormone concentrations rather than TSH (20).

Most actions of thyroid hormone are mediated by the active form of thyroid hormone, T3. Circulating and local T3 concentrations are regulated mainly by the iodothyronine deiodinases D1, D2, and D3 (21). D2 is essential for the local production of T3 through deiodination of triiodothyroxine (T4). Although earlier studies on the role and functional expression of iodothyronine deiodinase enzymes in the skeleton have been equivocal (12,14,22–25), a recent study reported normal growth in mice with deficiencies in D1 and D2, indicating that D2 may not be critical in skeletal development (26). This notion was supported in a recent study that demonstrated that D2 activity is restricted to mature osteoblasts, suggesting a possible role for D2 in mature osteoblast function (27). Devising a study to address the potential role of deiodinases, including D2, on skeletal metabolism is difficult in humans, but study of the effects of functional D2 polymorphisms on BMD and bone turnover in humans may shed light on this role.

Several polymorphisms in D2 have been described (28–30). The single-nucleotide polymorphism (SNP) in D2-Thr92Ala has been associated with body mass index (BMI) and insulin resistance in subjects with obesity and type 2 diabetes mellitus (28,29), although this was not confirmed in the Framingham Offspring Study (31). In a study by Canani and colleagues (28), the maximal velocity of D2 was decreased by 3- to 10-fold in thyroid and skeletal muscle of carriers of the D2-Thr92Ala polymorphism.

This effect was observed in the absence of differences in D2 mRNA level or in the biochemical protein properties of the 92Ala allele. It was therefore suggested that either a functionally relevant SNP occurs in linkage disequilibrium in the Thr92Ala polymorphism or the 92Ala allele affects protein translation or stability.

The objective of this study was to try to elucidate a potential role for D2 in skeletal metabolism and BMD by evaluating the relationship between the D2-Thr92Ala polymorphism, BMD, and bone turnover markers in cured thyroidectomized differentiated thyroid carcinoma patients receiving thyroid hormone substitution. This human model has the advantage of strictly regulated serum thyroid hormone levels that are kept in a relatively narrow range.

Patients and Methods

Patients

Patients included in the study were all under control of the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. All patients had a diagnosis of differentiated thyroid carcinoma, for which they had been treated by near-total thyroidectomy followed by standard postoperative [131I] radioiodine ablation therapy. All patients were cured as defined by the absence of 131-Iodine accumulation at diagnostic scintigraphy, serum thyroglobulin (Tg) concentrations below 2 mg/L after TSH stimulation, in the absence of Tg antibodies, a normal neck ultrasound, and no other indication for disease (32). Patients with tumor relapse were included only if they were subsequently cured. None of the patients used any drug or had a disease known to influence bone metabolism. The Leiden University Medical Center Local Ethics Committees approved the study, and written informed consent was obtained from all subjects.

Study design

On the day of the study, patients underwent a full clinical examination, including height (meters) and weight (kilograms). Blood was collected after an overnight fast and measured for TSH, serum free T4 (FT4), T3, calcium, parathyroid hormone (PTH), 25-hydroxyvitamin D [25(OH)D], bone-specific alkaline phosphatase (BAP), cross-linking terminal C-telopeptide of type I collagen (CTX), and procollagen type 1 amino-terminal propeptide (P1NP). A second-morning-void urine was measured for excretion of cross-linked N-telopeptide of type I collagen (NTX). Plasma, serum, and urine samples were handled immediately and stored at -80°C in Sarstedt tubes. BMD (expressed in grams per square centimeter) was measured at the femoral neck and

lumbar spine (vertebrae L2–L4) by dual-energy X-ray absorptiometry (DXA, NHANES III–adjusted; Hologic 4500, Hologic, Inc., Bedford, MA, USA). Following World Health Organization (WHO) criteria, osteopenia was defined as a T-score between -1 and -2.5 and osteoporosis as a T-score below -2.5. The following data also were recorded: smoking habits, alcohol use, physical activity, calcium intake, medications (including self-prescription drugs) or vitamin or mineral supplements, and daily calcium intake and for females: date of first menstruation (menarche), date of last menstruation, cycle regularity, and estrogen substitution if applicable.

Biochemical parameters

Serum free T4 (FT4) and TSH were measured using a chemoluminescence immunoassay with a Modular Analytics E-170 system (intra-assay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche, Almere, The Netherlands). Serum T3 was measured with a fluorescence polarization immunoassay, CV 2.5-9.0 %, on an ImX system (Abbott, Abbott Park, IL, USA). Thyroglobulin was measured by Dynotest TG-s (Brahms Diagnostica GmbH, Germany). Plasma PTH was measured using an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands). Calcium was measured by colorimetry and 25(OH)-vitamin D by RIA (Incstar/DiaSorin, Stillwater, MN, USA). Serum BAP was measured by RIA (Hybritech Europe, Liege, Belgium). Serum CTX and P1NP were measured by chemoluminescence immunoassay using the Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). NTX was measured by ELISA (Ostex International Inc., Seattle, WA, USA). NTX was expressed as the ratio between NTX and urine creatinine excretion (NTX/creatinine) to correct for differences in creatinine excretion. Insulin sensitivity was estimated by homeostasis model assessment [HOMA: fasting insulin (milliunits per milliliter) - fasting glucose (millimoles per liter)/22.5].

Genetic analyses

DNA was isolated from peripheral leukocytes by the salting-out procedure. Genotypes were determined using 5 ng of genomic DNA by a 5′ fluoregenic TaqMan assay, and reactions were performed in 384-well format on an ABI9700 2x384-well PCR machine with endpoint reading on the ABI 7900HT TaqMan machine (Applied Biosystems, Nieuwerkerk aan den IJssel, The Netherlands). Primer and probe sequences were optimized using the SNP assay-by-design service of Applied Biosystems.

Statistical Analyses

Values are presented as mean ± SE, median (range), or as numbers or proportions of patients. Nonnormally distributed data (TSH and PTH) were log-transformed before analyses. Comparisons between groups were analyzed by ANOVA or chi-square tests. The relation between the three D2-Thr92Ala genotypes [Thr/Thr (wild type), Thr/Ala (heterozygote), and Ala/Ala (homozygote)], BMD, and markers of bone turnover were studied by a stepwise univariate regression analysis. After correction for age, gender, and estrogen status (ie, estrogen deplete or replete), the following covariables were entered: BMI, serum levels of calcium (corrected for an albumin concentration of 42 g/L), 25(OH)-vitamin D, lnPTH, FT4, T3, and lnTSH. We calculated that to detect an effect size of 0.15 (corresponding to an r2 of 0.13), adopting an alpha value of 0.05 and a beta value of 0.80, the number of subjects needed is 108. Because it has been documented that the D2-Thr92Ala polymorphism is associated with insulin resistance (28), we also compared insulin sensitivity (HOMA) in the three genotypes. Deviation from Hardy-Weinberg equilibrium was analyzed using a chi-square test. All calculations were performed using SPSS 12.0 for windows (SPSS, Inc., Chicago, IL, USA). Differences were considered statistically significant at $p<0.05$.

Results

Patient characteristics

Of a potential of 330 patients with cured differentiated thyroid carcinoma, 105 were excluded for various reasons (**Figure 1**). Sixty-nine patients were not willing or able to participate in the study for different reasons. A total of 156 patients thus were included in the study. Two patients were left out from the analyses because of incomplete data. Thirteen patients had postoperative hypoparathyroidism for which they were adequately supplemented with active vitamin D metabolites and calcium as required. Additional analyses were performed leaving out these patients (see below and **Table 2**). In addition, serum PTH levels were included as a covariable in the analyses (see below) to correct for the potentially confounding effects of hypoparathyroidism. The basal characteristics of the 154 patients included in the study are shown in **Table 1**. All patients were receiving L-thyroxine treatment at a mean dose of 183 ± 4 µg/day.

The D2 Thr92Ala polymorphism, BMD and biochemical parameters of skeletal metabolism

Genotype frequencies of the D2-Thr92Ala polymorphism [Thr/Thr = 60 (39%), Thr/Ala = 66 (43%), and Ala/Ala = 28 (18%)] did not deviate from Hardy-Weinberg

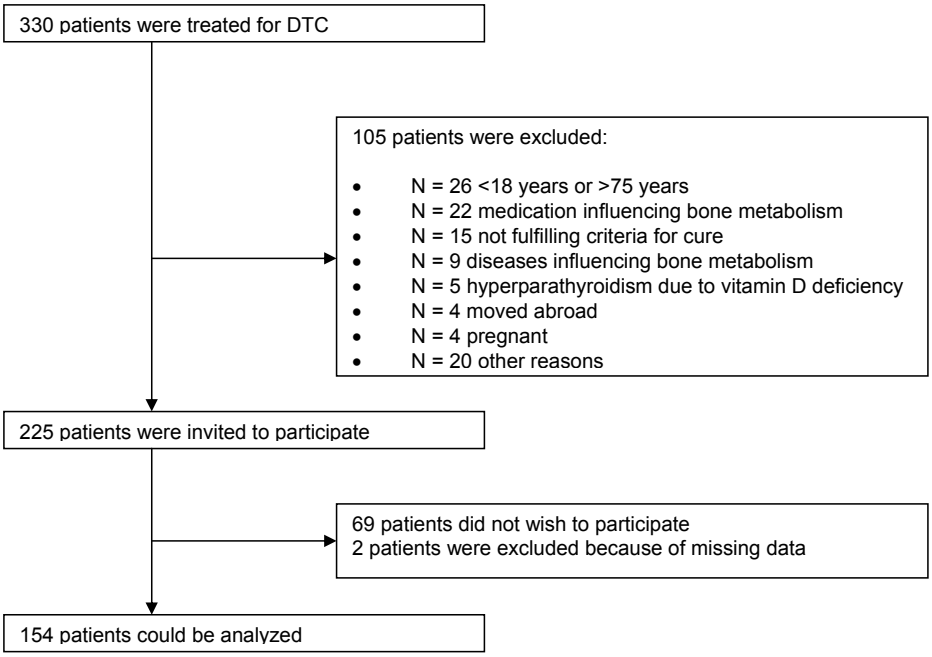

Figure 1
Flowchart of the study

Table 1: Patient characteristics

	Total (n=154)
Age (years)	49.2 ± 1.0
Males	29 (18.8 %)
Females: Estrogen Replete / Deplete	79 (51.3 %) / 46 (29.9 %)
Age at diagnosis	36.6 ± 1.1
Histology	
Papillary Thyroid Carcinoma (PTC)	107 (69 %)
Follicular Thyroid Carcinoma	25 (16 %)
Follicular variant PTC	21 (14 %)
Hürthle cell Thyroid Carcinoma	1 (1 %)
Total Activity Radioiodine	8067 ± 699 MBq
Lymph Node Surgery	14 (9 %)
TNM Stage	
T1-3 N0 M0	90 (63 %)
T1-3 N1 M0	30 (21%)
T4 or M1 n=143	23 (16 %)
Relapse DTC *(all were cured after relapse)*	20 (13 %)

Table 2: Characteristicsw of patients by D2-Thr92Ala Genotype

	Thr / Thr (60)	Thr / Ala (66)	Ala / Ala (28)	P
Men (n)	13	11	5	0.861[1]
Women (n) Estrogen Replete / Deplete	32 / 15	33 / 22	14 / 9	0.894[1]
Age (years)	47.2 ± 1.6	51.2 ± 1.7	48.3 ± 1.9	0.148[1]
Height (m)	1.72 ±0.01	1.70 ± 0.01	1.71 ± 0.02	0.307[1]
BMI (kg/m2)	25.6 ± 0.6	26.2 ± 0.4	25.8 ± 1.1	0.773[1]
Sports (hrs/week)	3.1 ± 1.1	5.0 ± 1.6	4.5 ± 2.3	0.654[1]
Smoking (n)	12 (9%)	7 (5%)	5 (1%)	0.092[1]
Menarche (age)	13.4 ± 0.2	13.1 ± 0.2	13.6 ± 0.3	0.399[1]
Menopause (age)	48.2 ± 1.5	47.7 ± 1.1	50.1 ± 1.5	0.484[1]
Follow-up duration (years)	13.1 ± 1.2	10.5 ± 1.0	11.3 ± 1.5	0.241[1]
Hypoparathyroidism (n)	5 (3%)	6 (4%)	2 (1%)	0.952[1]
Vertebral fractures (n)	1 (1%)	2 (1%)	1 (1%)	0.832[1]
HOMA (mmol*22.5/L)	1.75 ± 0.20	2.16 ± 0.21	1.86 ± 0.32	0.361[1]
Calcium (mmol/L)	2.39 ± 0.02 (59)	2.38 ± 0.01	2.39 ± 0.02	0.943[1]
25 OH vitD (nmol/L)	64.5 ± 3.9 (59)	60.4 ± 2.9	69.9 ± 4.8	0.277[1]
PTH (pmol/L)	4.88 ± 0.36 (58)	5.27 ± 0.43 (65)	6.19 ± 0.83	0.250[1]
TSH (mU/L)	0.051 (0.003-4.620)	0.031 (0.003-4.910)	0.051 (0.003-6.830)	0.753[1]
Dose thyroxine (µg/kg)	2.09 ± 1.04	2.23 ± 0.87	2.19 ± 1.03	0.398[1]
Free T4 (pmol/L)	22.7 ± 0.1	22.4 ± 0.1	21.6 ± 0.2	0.562[1]
T3 (nmol/L)	1.49 ± 0.04 (54)	1.47 ± 0.05 (59)	1.40 ± 0.07 (23)	0.624[1]
T3/T4 ratio * 10	6.6 ± 0.2 (54)	6.7 ± 0.2 (59)	6.6 ± 0.4 (23)	0.903[1]
BMD femoral neck (g/cm^2)	0.90 ± 0.02	0.84 ± 0.01	0.85 ± 0.03	0.028 /0.015[2]
BMD total hip (g/cm^2)	0.97 ± 0.02	0.92 ± 0.02	0.92 ± 0.03	0.064 /0.049[2]
BMD lumbar spine (g/cm^2)	1.08 ± 0.03	1.04 ± 0.02	1.07 ± 0.04	0.741 /0.094[2]
NTX / Creatinine * 1/1000	44.0 ± 4.1	56.5 ± 5.8	67.7 ± 10.6	0.008 /0.002[2]
BAP (ng/mL)	12.5 ± 0.5	13.5 ± 0.6	13.9 ± 0.7	0.063 /0.085[2]
P1NP (ng/mL)	40.0 ± 2.6	42.9 ± 3.4	50.9 ± 5.5	0.028 /0.032[2]
CTX (mg/mL)	0.28 ± 0.02	0.28 ± 0.02 [#]	0.37 ± 0.05	0.043 /0.036[2]

Values are presented as mean ± standard error (SE), median (range) or as numbers or proportions of patients. PTH= Parathyroid hormone, BAP= Bone Specific Alkaline Phosphatase, P1NP= Procollagen type 1 Aminoterminal Propeptide, CTX= C-crosslinking Terminal Telopeptide of Type I collagen, NTX/Creatinine= Ratio of Urinary N-Telopeptide of Collagen Cross-links and Creatinine Concentration; [1] = One-way ANOVA, [2] = general linear model, univariate with age, gender, estrogen state, BMI, Ca, lnPTH, 25-OHvitD, lnTSH and Free T4 as covariables, second value= patients with postoperative hypoparathyroidism left out.

equilibrium proportions. The 92Ala allele had a frequency of 45%, which is similar to previous studies in Caucasians (33,31). The characteristics of the three genotype subgroups are given in **Table 2**. The three groups were comparable with respect to age, gender, estrogen state (including ages at menarche and menopause), and BMI.

Physical activity and smoking habits did not differ either. Biochemical covariables for bone metabolism [ie, serum calcium, 25(OH)-vitamin D and PTH] were not different, as were serum FT4 and T3 levels, serum T3/T4 ratio, and TSH levels.

Because it has been documented that the D2-Thr92Ala polymorphism is associated with insulin resistance (28), we also compared insulin sensitivity by HOMA in the three genotypes, which again did not differ ($p = 0.361$). We also calculated whether HOMA was a significant determinant of BMD and of biochemical parameters of skeletal metabolism (corrected for age, gender, estrogen state, and BMI). Univariate analyses revealed that p values for HOMA as an independent variable were, respectively, 0.912 for femoral neck BMD, 0.583 for lumbar vertebral BMD, 0.826 for NTX/creatinine, 0.575 for BAP, 0.798 for P1NP, and 0.906 for CTX. HOMA therefore was not a determinant of BMD or bone turnover markers.

The relation between the three D2-Thr92Ala genotypes, BMD, and biochemical parameters of skeletal metabolism were studied by a stepwise univariate regression analysis. After correction for age, gender, estrogen status, and BMI, the following covariables were entered subsequently: serum levels of calcium, 25(OH)-vitamin D, lnPTH, FT4, and lnTSH. We found a significant independent relationship between the Thr92Ala genotypes and femoral neck BMD ($p = 0.022$) (**Table 2, Figure 2**) with a 6% lower BMD in homozygotes than in wild-type patients. This relationship was also present when total-hip BMD was measured. We also found independent relationships between the D2-Thr92Ala genotypes and biochemical parameters of skeletal metabolism: P1NP ($p = 0.028$), CTX ($p = 0.043$), and NTX/creatinine ($p = 0.008$), which were higher in homozygotes than in wild-type patients. Data for analyses leaving out patients with postoperative hypoparathyroidism did not influence these results (**Table 2**). The largest difference was observed for NTX/creatinine, which was 54% higher in homozygotes than in wild types.

Discussion

The main objective of this study was to investigate a potential role for the deiodinase D2 in bone metabolism in humans by studying the relationship between the D2-Thr92Ala polymorphism, BMD, and bone turnover. The D2-Thr92Ala polymorphism is associated with a lower D2 Vmax and therefore may lead to decreased local availability of T3 (28), which, in turn, may affect skeletal metabolism. We studied this relationship in a human model of thyroidectomized patients cured from differentiated thyroid carcinoma receiving thyroid hormone substitution. The advantage of this model is that study subjects have more uniform FT4 levels, which fell between the 25^{th} and 75^{th} percentiles for FT4 (19.5 and 24.9 pmol/L) in our group of patients.

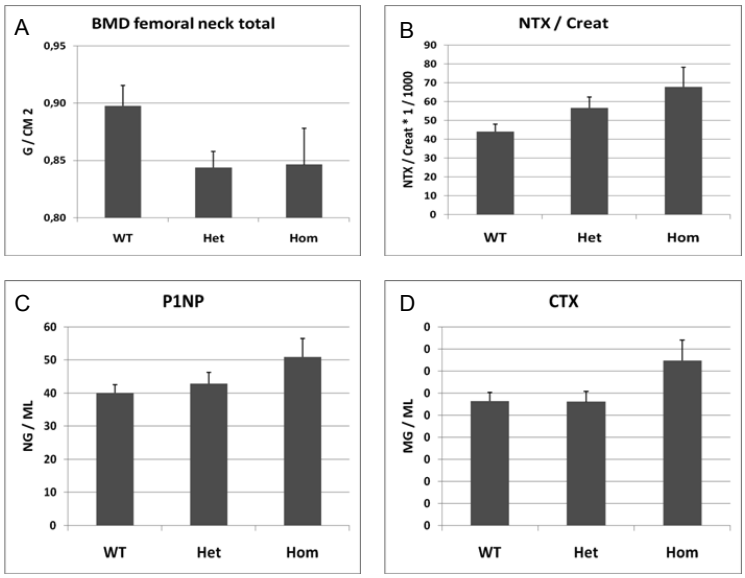

Figure 2
Relationships between D2 Thr92ALA genotypes and indicators of bone turnover.
(A) Femoral neck BMD.
(B) Ratio of urinary N-telopeptide of collagen cross-links and creatinine concentration.
(C) Procollagen type 1 amino-terminal propeptide (P1NP) levels.
(D) Cross-linking terminal C-telopeptide of type I collagen.
For levels of significance, see text and Table 2.

In support of the involvement of D2 in bone metabolism was the observation of a 6% decrease in femoral neck BMD and increased levels of P1NP (32%), CTX (27%), and NTX/creatinine (54%) in the Ala/Ala subgroup compared with wild-type subgroup. These effects were independent of factors known to influence BMD and bone metabolism, such as age, gender, BMI, estrogen state, PTH, and vitamin D. These effects were also independent of circulating levels of T3 and TSH and thus were indicative of an independent role of D2 in bone metabolism. We did not find an association of the D2 polymorphism with lumbar spine BMD, possibly owing to a differential effect of the polymorphism on predominantly trabecular bone at the lumbar spine *versus* predominantly cortical bone at the femoral neck. Our data did not confirm earlier observations of an association of the D2-Thr92Ala polymorphism with insulin sensitivity (28,29). This discrepancy may be explained by differences in the populations studied, with a low prevalence of obesity or insulin resistance in our subjects. Our data, however, are in keeping with the Framingham Offspring Study, which found no relation between the D2-Thr92Ala polymorphism and insulin resistance (31). We did not observe differences in height, indicating no difference in skeletal development among the three genotype subgroups. This is in line with

recent observations in C3H/HeJ D2-/- compound mutant mice with D1 deficiency and deletion of D2, which were shown to maintain normal growth (26). This notion is supported by a recent study suggesting that D2 may not play a physiologic role in growth plate chondrocytes (27).

The observed effects of the D2-Thr92Ala polymorphism on femoral neck BMD are in line with the importance of local availability of T3 for bone formation. D2 activity has been found on mature osteoblasts (34), which are the primary target cells for T3 regulatory effects on bone formation (1,2,10–12).

The effects of the D2-Thr92Ala polymorphism on bone turnover markers are not easy to explain. It is conventionally accepted that higher rather than lower circulating thyroid hormone levels result in higher bone turnover and decreased bone mass. However, the model we used is unique in the sense that circulating T3 levels were similar among the three D2 genotypes, allowing us to specifically study the consequences of the polymorphism for local T3 availability in the bone microenvironment. Williams and colleagues (27) showed no D2 activity in osteoclasts. The effects of the polymorphism on the markers of bone degradation (NTX/creatinine and CTX) therefore may not be explained by direct effects on osteoclasts but are more likely to result from changes in the interaction between osteoblasts and osteoclasts, possibly by alterations in the RANK/RANKL/OPG signaling pathway, which potentially can be modulated by local T3 availability in the bone microenvironment. In the context of conflicting data on a functional role for TSH in skeletal development, our data, which were corrected for serum TSH levels, outline the importance of local T3 for bone metabolism (13–17,35–38). Two recent papers by Bassett and colleagues (18,19), who studied mice with complete or haploinsufficiency of TRalpha and -beta, concluded that TRalpha regulates both skeletal development and adult bone maintenance.

Whereas a limitation of our study may be its relatively small size and its cross-sectional design, one of its clear strengths is that all subjects were phenotyped for factors other than thyroid status known to modulate bone metabolism. This design enabled us to use regression models, including relevant covariables, the feasibility of which is difficult in large cohort studies. In addition, according to the power calculation, the study had sufficient power, which was confirmed by a post hoc power analysis revealing that the power was 97% or higher for the dependent variables studied. A potential further limitation of our study is that thyroid hormone parameters measured at one point in time may not reflect the overall thyroid status over time. To address this issue, we calculated the slope of all TSH measurements routinely obtained after initial therapy in every patient participating in the study to verify the stability over time. An average of 15 TSH measurements were obtained per patient, and the slope of TSH values was -0.0001 (range -0.004 to 0) mU/L per year, thus indicating stable TSH levels over time.

In summary our data suggest that a decrease in local availability of T3 potentially owing to a D2 polymorphism may result in increased bone turnover and decreased bone mass at the predominantly cortical femoral neck. We believe that our study provides additional information on the role of D2 in bone metabolism and the functional consequences of the D2-Thr92Ala polymorphism, supporting a role for D2 in mature bone cells (27).

References

1. Bassett JH, Williams GR. The molecular actions of thyroid hormone in bone. Trends in Endocrinology and Metabolism 2003 14 356-364
2. Bassett JH, Williams GR Critical role of the hypothalamic-pituitary-thyroid axis in bone. Bone Bone. 2008 43 418-426.
3. Rivkees SA, Bode HH, Crawford JD.Long-term growth in juvenile acquired hypothyroidism: the failure to achieve normal adult stature. New England Journal of Medicine 1988 318: 599-602
4. Segni M, Gorman CA. The aftermath of childhood hyperthyroidism. Journal of Pediatric Endocrinology and Metabolism 200114 Suppl 5 1277-1282; discussion 1297-1298
5. Heemstra KA, Hamdy NA, Romijn JA, Smit JW. The effects of thyrotropin-suppressive therapy on bone metabolism in patients with well-differentiated thyroid carcinoma. Thyroid 2006 16 583-591
6. Kim DJ, Khang YH, Koh JM, Shong YK, Kim GS. Low normal TSH levels are associated with low bone mineral density in healthy postmenopausal women. Clinical Endocrinology (Oxford) 2006 64 86-90
7. Bauer DC, Ettinger B, Nevitt MC, Stone KL. Risk for fracture in women with low serum levels of thyroid-stimulating hormone. Annals of Internal Medicine 2001 134 561-568
8. Lee WY, Oh KW, Rhee EJ, Jung CH, Kim SW, Yun EJ, Tae HJ, Baek KH, Kang MI, Choi MG, Yoo HJ, Park SW. Relationship between subclinical thyroid dysfunction and femoral neck bone mineral density in women. Archives of Medical Research 2006 37 511-516
9. Britto JM, Fenton AJ, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994 134 169-176
10. Basset P, Okada A, Chenard MP, Kannan R, Stoll I, Anglard P, Bellocq JP, Rio MC. Matrix metalloproteinases as stromal effectors of human carcinoma progression: therapeutic implications. Matrix Biololgy 1997 15 535-541
11. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. Journal of Cell Physiology 2004 201 17-25
12. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, Nakao K. A novel interaction between thyroid hormones and 1,25(OH)(2)D(3) in osteoclast formation. Biochemical and Biophysical Research Communication 2002 291 987-994
13. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, DaviesTF, Zaidi M. TSH is a negative regulator of skeletal remodeling. Cell 2003 115 151-162
14. Morimura T, Tsunekawa K, Kasahara T, Seki K, Ogiwara T, Mori M, Murakami M. Expression of type 2 iodothyronine deiodinase in human osteoblast is stimulated by thyrotropin. Endocrinology 2005 146 2077-2084
15. Galliford TM, Murphy E, Williams AJ, Bassett JH, Williams GR. Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinology 2005 30 237-246
16. Heemstra KA, van der Deure WM, Peeters RP, Hamdy NA, Stokkel MP, Corssmit EP, Romijn JA, Visser TJ, Smit JW. Thyroid hormone independent associations between serum TSH levels and indicators of bone turnover in cured patients with differentiated thyroid carcinoma. European Journal of Endocrinology 2008 159 69-76

17. Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Biondi B, Iorio S, Giustina A, Amato G, Carella C. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. Journal of Bone Mineral Research 2005 20 480-486
18. Bassett JH, Nordstrom K, Boyde A, Howell PG, Kelly S, Vennstrom B, Williams GR. Thyroid status during skeletal development determines adult bone structure and mineralization. Molecular Endocrinology 2007 21 1893-1904
19. Bassett JH, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PG, Weiss RE, Roux JP, Malaval L, Clement-Lacroix P, Samarut J, Chassande O, Williams GR. Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Molecular Endocrinology 2007 21 1095-1107
20. van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, Visser TJ. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. Clinical Endocrinology (Oxford). 2008 68 175-181.
21. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 2006 116 2571-2579
22. Gouveia CH, Christoffolete MA, Zaitune CR, Dora JM, Harney JW, Maia AL, Bianco AC. Type 2 iodothyronine selenodeiodinase is expressed throughout the mouse skeleton and in the MC3T3-E1 mouse osteoblastic cell line during differentiation. Endocrinology 2005 146 195-200
23. LeBron BA, Pekary AE, Mirell C, Hahn TJ, Hershman JM. Thyroid hormone 5'-deiodinase activity, nuclear binding, and effects on mitogenesis in UMR-106 osteoblastic osteosarcoma cells. Journal of Bone and Mineral Research 1989 4 173-178
24. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, Nakao K. Thyroid hormones promote chondrocyte differentiation in mouse ATDC5 cells and stimulate endochondral ossification in fetal mouse tibias through iodothyronine deiodinases in the growth plate. Journal of Bone and Mineral Research 2002 17 443-454
25. Shen S, Berry W, Jaques S, Pillai S, Zhu J. Differential expression of iodothyronine deiodinase type 2 in growth plates of chickens divergently selected for incidence of tibial dyschondroplasia. Animal Genetics 2004 5 114-118
26. Christoffolete MA, Arrojo e Drigo, Gazoni F, Tente SM, Goncalves V, Amorim BS, Larsen PR, Bianco AC, Zavacki AM. Mice with impaired extrathyroidal thyroxine to 3,5,3'- triiodothyronine conversion maintain normal serum 3,5,3'-triiodothyronine concentrations. Endocrinology 2007 148 954- 960
27. Williams AJ, Robson H, Kester MH, van Leeuwen JP, Shalet SM, Visser TJ, Williams GR. Iodothyronine deiodinase enzyme activities in bone. Bone 2008 43 126-134
28. Canani LH, Capp C, Dora JM, Meyer ELS, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, Maia AL. The Type 2 Deiodinase A/G (Thr92Ala) Polymorphism Is Associated with Decreased Enzyme Velocity and Increased Insulin Resistance in Patients with Type 2 Diabetes Mellitus. Journal of Clinical Endocrinology and Metabolism 2005 90 3472-3478
29. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, Celi S. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3- adrenergic receptor. Diabetes 2002 51 880-883

30. Peeters RP, van der Deure WM, Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. European Journal of Endocrinology 2006 55 655-662
31. Maia AL, Dupuis J, Manning A, Liu C, Meigs JB, Cupples LA, Larsen PR, Fox CS. The type 2 deiodinase (DIO2) A/G polymorphism is not associated with glycemic traits: the Framingham Heart study. Thyroid 2007 17 199-202
32. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, Tuttle RM. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006 16 109-142
33. Peeters RP, van den Beld AW, Attalki H, Toor Hv, de Rijke YB, Kuiper GGJM, Lamberts SWJ, Janssen JAMJ, Uitterlinden AG, Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. American Journal of Physiology Endocrinoly and Metabolism 2005 289 E75-E81
34. Burger H, van Daele PL, Algra D, van den Ouweland FA, Grobbee DE, Hofman A, van Kuijk C, Schutte HE, Birkenhager JC, Pols HA. The association between age and bone mineral density in men and women aged 55 years and over: the Rotterdam Study. Bone and Mineral 1994 25 1-13
35. Inoue M, Tawata M, Yokomori N, Endo T, Onaya T. Expression of thyrotropin receptor on clonal osteoblast-like rat osteosarcoma cells. Thyroid 1998 8 1059-1064
36. Sun L, Davies TF, Blair HC, Abe E, Zaidi M. TSH and bone loss. Annals of the New York Academy of Science 2006 1068 309- 318
37. Davies T, Marians R, Latif R. The TSH receptor reveals itself. Journal of Clinical Investigation 2002 110 161-164
38. Sampath TK, Simic P, Sendak R, Draca N, Bowe AE, O'Brien S, Schiavi SC, McPherson JM, Vukicevic S. Thyroid-stimulating hormone restores bone volume, microarchitecture, and strength in aged ovariectomized rats. Journal of Bone Mineral Research 2007 22 849-859

8

Thyroid hormone rather than TSH decreases bone turnover during hypothyroidism in athyroid patients with differentiated thyroid carcinoma

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Submitted

Abstract

Background

Primary hypothyroidism affects bone metabolism. It is not clear whether this has to be attributed to decreased serum thyroid hormone levels per se or to increased TSH levels.

Objective

To document the effects of primary hypothyroidism on bone metabolism and to discriminate between effects mediated by decreased thyroid hormone levels *versus* those mediated by increased TSH levels.

Patients and methods

We studied the effects of recombinant human TSH (rhTSH) in 11 athyroid DTC patients on thyroxine substitution. In addition, we included 11 age-, gender- and BMI-matched athyroid patients previously treated for differentiated thyroid carcinoma (DTC), who were studied after 4 weeks of thyroxine withdrawal and during thyroxine replacement therapy. We measured plasma levels of PTH, 25-OH-vitamin D, procollagen type 1 aminoterminal propeptide levels (P1NP), C-cross-linking terminal telopeptide of type I collagen (CTX), receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG).

Results

No differences were observed on parameters of bone turnover after rhTSH administration. During thyroxine withdrawal, levels of C-cross-linking terminal telopeptide of type I collagen were significantly lower, whereas levels of osteoprotegerin were significantly higher compared to thyroxine replacement therapy.

Conclusion

Hypothyroidism results in decreased bone turnover. As rhTSH had no impact on bone turnover, it seems that low thyroid hormone levels instead of the increased TSH levels are responsible for the changes in bone turnover during hypothyroidism in DTC patients.

Introduction

The effects of thyroid hormone on bone are established and the conventional view is that hyperthyroidism result in bone loss (1).

But the consequences of hypothyroidism on bone metabolism remain unclear (**Table 1**). Some studies document low bone turnover as evidenced by decreased markers of bone resorption and formation (2-5), whereas others report normal bone turnover (6-8). Most studies, however, included patients with Hashimoto thyroiditis, in whom the duration and the extend of hypothyroidism is not known (3-5,7). Moreover, it is not clear, given the recent suggestion that TSH may be a negative regulator of bone remodelling through direct effect on bone independent of thyroid hormone levels (9-11), if the effects of hypothyroidism must be attributed to increased TSH levels or decreased thyroid hormone levels. It has been reported that TSHR knockout and haploinsufficient mice with normal thyroid hormone levels have decreased bone mass, suggesting that TSH might directly influence bone remodelling (10,12,13). However, other studies question the role of TSH in bone metabolism (14,15).

Three studies in humans have investigated the effect of recombinant human TSH (rhTSH) on bone metabolism, but their results are inconclusive. They showed either no impact on bone turnover (16), increased markers of bone formation (17,18) or decreased markers of bone resorption (18). In hypothyroidism the relative importance of decreased thyroid hormone levels or increased TSH levels on bone remains thus to be established.

The present study was designed in an attempt to discriminate between potential effects mediated by decreased thyroid hormone levels from those mediated by increased TSH levels in a human model in which the reciprocal relationship between thyroid hormone and TSH was interrupted. To this effect, we studied parameters of bone metabolism after parenteral administration of recombinant human TSH (rhTSH) resulting in exogenously increased TSH levels while preserving normal thyroid hormone levels by uninterrupted thyroid hormone substitution in athyroid differentiated thyroid carcinoma (DTC) patients. We studied the same parameters in age-, gender- and BMI matched athyroid DTC patients during short-term thyroxine withdrawal, resulting in decreased thyroid hormone levels and endogenously increased TSH levels and after reestablishment of thyroid hormone substitution.

Table 1: Overview of the literature on the effects of hypothyroidism and rhTSH on parameters of bone turnover

Article	Number of patients	Diagnosis	Control group	Design	Outcome								
					AF	*OC*	*P1NP*	*P1CP*	*OPG*	*CTX*	*U-DPD*	*U-PD*	*RANKL*
Effects of Hypothyroidism													
Botello-Carretero *et al.* (2)	19	DTC Thyroxine withdrawal	18 controls	Prospective		↓			↑		↓	↓	
Toivonen *et al.* (32)	14	DTC Thyroxine withdrawal	38 controls	Prospective		↓	↓	↑		↓			
Sabancu *et al.* (7)	27 20	Hypothyroidism HT + 3 months T4	5 controls	Cross-sectional	=						=		
Sekeroglu *et al.* (8)	16	Hypothyroidism heterogeneous)	15 controls	Cross-sectional	=	=					=		
Nakamura *et al.*(5)	8	Hypothyroidism (heterogeneous)	-	Prospective							↓	↓	
Guang-Da *et al.* (3)	20	Hashimoto thyroiditis	20 controls	Prospective					↑				
Nagasaki *et al.*(4)	53	Hashimoto thyroiditis	53 controls	Prospective					↑				
Effects of Recombinant human TSH													
Mazziotti *et al.* (18)	66	DTC + rhTSH	71 controls	Prospective	↑[B*]				=	↓[*]			
Giusti *et al.* (16)	24	DTC + rhTSH	Reference population	Prospective					=		=		=
Martini *et al.* (17)	30	DTC + rhTSH	80 controls	Prospective			↑[*]		=	=			↓[**]

AF= Alkaline Phosphatase, OC= osteocalcin, P1NP= procollagen type 1 aminoterminal propeptide, P1CP= procollagen type 1 carboxyterminal, OPG= osteoprotegerin (inhibits bone resorption), CTX= C-terminal telopeptide of collagen I, U-PD= Urinary Pyridinium crosslinks, U-DPD= Urinary excretion of Deoxypyridinoline, RANKL= receptor activator nuclear factor κB ligand [B] = Bone specific Alkaline Phosphatase, [*] = in postmenopausal women, [**]= in men and postmenopausal women

Patients and Methods

Subjects

Patients were recruited from the outpatient clinic of the Department of Endocrinology & Metabolic Diseases of Leiden University Medical Center, which is a tertiary referral center for differentiated thyroid carcinoma (DTC). Patients included in the study had a diagnosis of DTC for which they had been treated with near-total thyroidectomy, followed by routine postoperative I-131 radioiodine ablation therapy. Only patients cured for DTC were included, documented by the absence of measurable serum thyroglobulin (Tg) levels during TSH stimulation as well as by negative total-body RaI scintigraphy. Patients with DTC planned for a TSH-stimulated diagnostic protocol were asked to participate in the study. High TSH levels were achieved either by recombinant human TSH stimulation or by 4 week withdrawal of thyroxine. Patients with diabetes mellitus, body mass index (BMI) >35 kg/m^2 or other endocrine diseases were excluded. Patients who used any drugs known to influence bone turnover, such as bisphosphonates, corticosteroids or thiazide diuretics, were also excluded.

The local Ethics Committees of the Leiden University Medical Center approved the study, and written informed consent was obtained from all subjects.

Two groups matched for age, gender and BMI were studied. The first group consisted of 11 athyroid DTC patients who received uninterrupted thyroxine replacement therapy and underwent a TSH stimulation test in the course of monitoring disease state by injections of rhTSH. This resulted in exogenously increased TSH levels with unchanged normal FT4 levels (rhTSH group). The second group also consisted of 11 athyroid DTC patients with short-term thyroxine withdrawal resulting in decreased FT4 levels and endogenously increased TSH levels (thyroxine withdrawal group).

Study Design

Patients in the rhTSH group continued to receive thyroxine substitution and were evaluated prior to recombinant human TSH (Thyrogen, 0.9 mg) which was injected intramuscularly once daily for two consecutive days and patients were also evaluated 1 and 3 days after the last injection of rhTSH.

Patients in the thyroxine withdrawal group were evaluated four weeks after withdrawal of thyroxine substitution and again 8 weeks after restoration of thyroxine replacement therapy.

All patients were assessed at 8.00 a.m. after a 12 hour fast. Height (meters [m]) and weight (kilograms [kg]) were measured and BMI (weight [kg]/lenght^2 [m]) was calculated. Plasma samples were obtained for measurement of FT4, TSH, T3, PTH,

25-OH-vitamin D, procollagen type 1 aminoterminal propeptide levels (P1NP), C-cross-linking terminal telopeptide of type I collagen (CTX), receptor activator for nuclear factor κ B ligand (RANKL) and osteoprotegerin (OPG). Plasma samples were handled immediately and stored at –20° C in Sarstedt tubes.

Biochemical parameters

All plasma and serum samples were measured in one batch. Serum free thyroxine (FT4) and TSH were measured using an electrochemiluminescent immunoassay with a Modular Analytics E-170 system with an intra-assay CV of 1.6-2.2 % and 1.3-5.0 % respectively (Roche Diagnostics, Almere, The Netherlands). Serum T3 was measured using a fluorescent polarisation immunoassay on an AxSYM system (Abbott, Abbott Park, IL, USA CV 2.5-9.0 %). Plasma Parathyroid Hormone (PTH) was measured by an immunoradiometric assay (Nichols Diagnostic Institutes, Wijchen, The Netherlands), calcium and alkaline phosphatase activity by colorimetry on a fully automated Modular P800 system (Roche, Almere, The Netherlands) and 25(OH) vitamin D by RIA (Incstar/DiaSorin, Stillwater, MN, USA). CTX and P1NP were measured by electrochemiluminescent immunoassays using a Modular Analytics E-170 system (Roche Diagnostics, Almere, The Netherlands). RANKL was measured using the ampli sRANKL human kit (Biomedica, Vienna, Austria), an enzyme linked immunoassay with a detection limit of 0.02 pmol/l (intra-assay CV 8-9%, interassay CV 3-6%). Osteoprotegerin was measured by ELISA (Meso Scale Discovery, Gaithersburg, Maryland, USA) with a detection limit of 5.9 pg/ml. In our hands, the range was 206 to 404 pg/ml; CVs were 0.6-16.2%, with an average of 4.6%. All samples were measured in triplo in single batches for the levels of RANKL and osteoprotegerin.

Statistical Analyses

SPSS 15.0 for windows was used for statistical analyses (SPSS. Inc., Chicago, IL, USA). Values are expressed as mean ± SE. Data within subjects were analysed with the paired samples t-test or the ANOVA for repeated measures. Data between subjects were measured with the Mann-Whitney test. Differences were considered statistically significant at $P<0.05$.

Results

Patient demographic characteristics are shown in **Table 2**. Patients in the thyroxine withdrawal group and rhTSH group were well matched and there were no differences in age, gender, BMI, thyroxine dose or duration of follow-up between groups.

Table 2: Patient characteristics

	Thyroxine withdrawal-study (n=11)	rhTSH stimulation study (n = 11)	P-value
Age (years)	45.5 ± 3.0	47.0 ± 2.8	0.65
Sex (m/f)	4 : 7	4 : 7	0.67
BMI (kg/m^2)	28.1 ± 1.3	29.7 ± 2.6	0.75
Thyroxine dose (µg/day)	197 ± 13	200 ± 12	0.70
Duration of TSH suppression (years, (range))	5.0 ± 2.1 (0.6-24.3)	6.7 ± 2.4 (1.2 -25.3)	0.33

Data are expressed as mean ± SE (range) or number of patients

Eleven patients (4 male and 7 female patients) were included in the rhTSH group. Mean thyroxine dose at time of the evaluation was 200 ± 12 µg/day. TSH levels were significantly increased without any changes in FT4 levels 1 and 3 days after rhTSH was administered (**Table 3**).

There were no differences in the levels of calcium, PTH, 25-OH-vitamin D, alkaline phosphatase activity, P1NP, CTX, OPG, RANKL and in the RANKL/OPG ratio between baseline and time points after rhTSH administration.

Eleven patients (4 male and 7 female patients) were included in the thyroxine withdrawal group. Mean thyroxine dose prior to withdrawal was 197 ± 13 µg/day. Four weeks after thyroxine withdrawal, TSH levels were significantly increased at 142.4 ± 10.4 mU/L (normal laboratory reference range 0.3-4.8 mU/L) and FT4 levels were significantly decreased at 1.4 ± 0.2 pmol/L (normal laboratory reference range 10-24 pmol/L). Eight weeks after restoration of thyroxine replacement therapy, six patients had TSH levels within the normal laboratory reference range and five patients had suppressed TSH levels.

There were no significant differences in levels of calcium, PTH, 25-OH-vitamin D, alkaline phosphatase activity, P1NP, RANKL and the RANKL/OPG ratio between thyroxine withdrawal status and 8 weeks after reintroduction of thyroxine replacement therapy (**Table 3**). Serum concentrations of CTX were significantly lower and OPG levels significantly higher during hypothyroidism compared to 8 weeks after reintroduction of thyroxine replacement therapy. There was no significant difference between endogenously and exogenously increased TSH levels respectively obtained 4 weeks after thyroxine withdrawal and 1 day after rhTSH-administration. As expected,

Table 3: Effects of hypothyroidism and rhTSH injections in 11 matched athyroid patients on parameters of bone turnover

	Thyroxine withdrawal group			rhTSH group				
	Thyroxine replacement therapy	**Hypothyroidism**	**P-value[$]**	**Thyroxine replacement therapy**	**rhTSH day 1**	**rhTSH day 2**	**P-value[@]**	**P-value difference hypothyroidism *vs.* difference rhTSH**
TSH (Mu/L)	0.8 ± 0.3[&]	142.4 ± 10.4[#]	0.000	0.06 ± 0.2	143.4 ± 13.6	19.3 ± 2.5	0.00	0.90
FT4 (pmol/L)	24.8 ± 1.2	1.4 ± 0.2[# *]	0.000	23.4 ± 0.8	24.0 ± 0.9	24.3 ± 1.0	0.13	0.00
T3 (pmol/L)	1.3 ± 0.1	0.3 ± 0.1	0.000	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	0.25	0.00
Parameters of Bone turnover								
Calcium (mmol/L)	2.20 ± 0.02	2.18 ±0.03[*]	0.68	2.25 ± 0.04	2.28 ± 0.3	2.21 ± 0.06	0.17	0.43
PTH (pmol/L)	3.4 ± 0.5	4.0 ± 0.6	0.13	3.7 ± 0.6	3.5 ± 0.6	3.4 ± 0.6	0.55	0.12
25(OH)Vit D (nmol/L)	59 ± 6	57 ± 6	0.52	68 ± 8	67 ± 8	65 ± 9	0.27	1.00
P1NP (ng/ml)	28 ± 5	29 ± 6	0.27	38 ± 4	36 ± 4	37 ± 4	0.18	0.28
CTX (mg/ml)	0.28 ± 0.5	0.24 ± 0.4	0.00	0.33 ± 0.06	0.35 ± 0.06	0.33 ± 0.06	0.23	0.00
OPG (pg/ml)	193 ± 17	246 ± 22	0.00	174.4 ± 11.8	210.4 ± 21.9	198.7 ± 16.5	0.47	0.01
RANKL (pg/ml)	1.1 ± 0.3	1.1 ± 0.3	0.59	1.1 ± 0.3	1.1 ±0.3	1.0 ± 0.3	0.27	1.00
RANKL/OPG ratio	0.006 ± 0.002	0.005 ± 0.002	0.61	0.006 ± 0.002	0.006 ± 0.002	0.006 ± 0.002	0.74	0.44
Alk. Phosphates (U/L)	66 ± 5	66 ± 5	0.81	76 ± 8	77 ± 8	75 ± 7	0.60	0.70

Data is expresses as mean ± SD. [$] Paired samples t-test. [@] ANOVA for repeated measurements, [*] Significantly different *vs.* rhTSH day 1, [#] Significantly different *vs.* rhTSH day 3, [&] Significantly different *vs.* rhTSH thyroxine replacement therapy

FT4 levels were significantly decreased during thyroxine withdrawal compared to the normal levels attained by thyroxine substitution therapy 1 and 3 days after rhTSH administration. The differences in CTX levels and OPG levels were significantly different between the thyroxine withdrawal group and rhTSH group. Calcium levels were significant lower during hypothyroidism compared to rhTSH administration. There were no significant differences observed in any other parameters measured between groups.

Discussion

In this study, we have attempted to dissect the effects of increased TSH levels from those of decreased thyroid hormone levels on bone by studying athyroid DTC patients in which the relationship between thyroid hormone levels and TSH is disrupted. Our findings suggest that acute changes in TSH in the presence of stable thyroid hormone levels obtained by rhTSH administration do not significantly affect skeletal metabolism. The data from our second model suggest that hypothyroidism results in decreased bone turnover rather by decreased plasma thyroid hormone concentrations than by increased TSH concentrations, because rhTSH did not impact on bone turnover in DTC patients. To our knowledge, this is the first study comparing thyroxine withdrawal *versus* rhTSH-injection in age-, gender- and BMI matched DTC patients.

It has been proposed that TSH may modulate bone remodelling independently of thyroid hormones through binding to the TSH receptor on osteoblasts and osteoclasts (10). However, other studies question these findings. Bassett *et al.* reported that Pax $^{-/-}$ mice and hyt/hyt mice, two mouse models of congenital hypothyroidism in which the feedback between TSH and thyroid hormones was intact or disrupted, both displayed delayed ossification, reduced cortical bone, trabecular bone remodelling defects and reduced bone mineralization, indicating that the effects of congenital hypothyroidism on bone are independent of TSH (14). Moreover, Bassett *et al.* showed that osteoblasts and osteoclasts express TSH-receptors, but TSH did not affect a cAMP response or the differentiation or function (14). We used the model of athyroid DTC patients in whom a rhTSH simulation test was performed in an attempt to discriminate between the effects of TSH and those of FT4 on bone metabolism. These patients have no endogenous thyroid hormone production and are therefore an excellent model to study the effects of TSH without interfering effects of changes in thyroid hormone concentrations. However, acute treatment with rhTSH did not affect bone turnover. This is in keeping with a study using the same model (16), but ad odds with two others studies (17,18).

Mazzioti *et al.* found significantly increased levels of bone specific alkaline phosphatase with decreased levels of cross-linking terminal telopeptide of type I collagen in postmenopausal women after rhTSH administration (18). They found no changes in premenopausal women. Martini *et al.* found significantly increased levels of P1NP and RANKL after rhTSH administration (17). These differences were only significant in postmenopausal women for P1NP levels and in postmenopausal women and men for RANKL levels after stratification for gender and menopausal state. We studied only 2 postmenopausal women. This might explain the differences in outcome. We found no differences in osteoprotegerin levels, which is consistent with previous studies (16-18) and in agreement with the finding that TSH regulates bone turnover by different mechanisms than OPG (10,14).

Osteoprotegerin is a member of the TNF receptor superfamily. It inhibits osteoclastogenesis by interrupting the cell-to-cell interaction (19-21). Osteoprotegerin binds to RANKL (22), which is important for osteoclast differentiation. RANKL binds to its receptor, RANK, which is expressed on dendritic cells, T cells, osteoclast precursors and mature osteoclasts (23,24). RANKL increases the survival of RANK positive T cells (23), promotes osteoclast differentiation (22,25-28), stimulates the activity of mature osteoclasts (26,29,30) and promotes survival of osteoclasts by preventing apoptosis.

We also studied the effects of thyroxine replacement therapy after short-term hypothyroidism due to thyroxine withdrawal in age-, gender and BMI matched athyroid DTC patients. Levels of C-cross linking terminal telopeptide of type 1 collagen were lower during hypothyroidism after thyroxine withdrawal compared to 8 weeks after restoration of thyroxine replacement therapy. This is consistent with most reports on hypothyroidism (2,3,31), although Sabancu *et al.* reported no differences in markers of bone turnover during hypothyroidism in a heterogeneous patient population including patients with Hashimoto thyroiditis (7). A disadvantage of the inclusion of patients with Hashimoto thyroiditis may be that the duration and extent of hypothyroidism are not known. OPG levels were also significant higher during hypothyroidism compared to thyroxine replacement therapy. This is consistent with previous studies (2-4) and strengthens our finding that thyroxine withdrawal decreases bone turnover.

In summary, bone turnover is decreased during hypothyroidism after thyroxine withdrawal in DTC patients. We conclude that the low thyroid hormone levels instead of the increased TSH levels are responsible for the decreased bone resorption during hypothyroidism in DTC patients. However, these results must be confirmed in a wider population of men and pre-and postmenopausal women.

References

1. Greenspan SL, Greenspan FS. The effect of thyroid hormone on skeletal integrity. Annals of Internal Medicine 1999 130 750-758.
2. Botella-Carretero JI, varez-Blasco F, San Millan JL, Escobar-Morreale HF. Thyroid hormone deficiency and postmenopausal status independently increase serum osteoprotegerin concentrations in women. European Journal of Endocrinology 2007 156 539-545.
3. Guang-Da X, Hui-Ling S, Zhi-Song C, Lin-Shuang Z. Changes in plasma concentrations of osteoprotegerin before and after levothyroxine replacement therapy in hypothyroid patients. Journal of Clinical Endocrinology and Metabolism 2005 90 5765-5768.
4. Nagasaki T, Inaba M, Jono S, Hiura Y, Tahara H, Shirakawa K, Onoda N, Ishikawa T, Ishimura E, Nishizawa Y. Increased levels of serum osteoprotegerin in hypothyroid patients and its normalization with restoration of normal thyroid function. European Journal of Endocrinology 2005 152 347-353.
5. Nakamura H, Mori T, Genma R, Suzuki Y, Natsume H, Andoh S, Kitahara R, Nagasawa S, Nishiyama K, Yoshimi T. Urinary excretion of pyridinoline and deoxypyridinoline measured by immunoassay in hypothyroidism. Clinical Endocrinology (Oxford) 1996 44 447-451.
6. Engler H, Oettli RE, Riesen WF. Biochemical markers of bone turnover in patients with thyroid dysfunctions and in euthyroid controls: a cross-sectional study. Clinica Chimica Acta 1999 289 159-172.
7. Sabuncu T, Aksoy N, Arikan E, Ugur B, Tasan E, Hatemi H. Early changes in parameters of bone and mineral metabolism during therapy for hyper- and hypothyroidism. Endocrine Research 2001 27 203-213.
8. Sekeroglu MR, Altun ZB, Algün E, Dülger H, Noyan T, Balaharoglu R, Oztürk M. Serum cytokines and bone metabolism in patients with thyroid dysfunction. Advances in Therapy 2006 23 475-480.
9. Inoue M, Tawata M, Yokomori N, Endo T, Onaya T. Expression of thyrotropin receptor on clonal osteoblast-like rat osteosarcoma cells. Thyroid 1998 8 1059-1064.
10. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF, Zaidi M. TSH is a negative regulator of skeletal remodeling. Cell 2003 115 151-162.
11. Morimura T, Tsunekawa K, Kasahara T, Seki K, Ogiwara T, Mori M, Murakami M. Expression of type 2 iodothyronine deiodinase in human osteoblast is stimulated by thyrotropin. Endocrinology 2005 146 2077-2084.
12. Galliford TM, Murphy E, Williams AJ, Bassett JH, Williams GR. Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinology 2005 30 237-246.
13. Sun L, Davies TF, Blair HC, Abe E, Zaidi M. TSH and bone loss. Annals of the New York Acadademy of Science 2006 1068 309-318.
14. Bassett JH, Williams AJ, Murphy E, Boyde A, Howell PG, Swinhoe R, Archanco M, Flamant F, Samarut J, Costagliola S, Vassart G, Weiss RE, Refetoff S, Williams GR. A lack of thyroid hormones rather than excess TSH causes abnormal skeletal development in hypothyroidism. Molecular Endocrinology 2008 22 501-12
15. Bassett JH, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PG, Weiss RE, Roux JP, Malaval L, Clement-Lacroix P, Samarut J, Chassande O, Williams GR. Thyroid hormone

excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Molecular Endocrinology 2007 21 1095-1107.

16. Giusti M, Cecoli F, Ghiara C, Rubinacci A, Villa I, Cavallero D, Mazzuoli L, Mussap M, Lanzi R, Minuto F. Recombinant human thyroid stimulating hormone does not acutely change serum osteoprotegerin and soluble receptor activator of nuclear factor-kappaBeta ligand in patients under evaluation for differentiated thyroid carcinoma. Hormones (Athens) 2007 6 304-313.
17. Martini G, Gennari L, De Paola V, Pilli T, Salvadori S, Merlotti D, Valleggi F, Campagna S, Franci B, Avanzati A, Nuti R, Pacini F. The effects of recombinant TSH on bone turnover markers and serum osteoprotegerin and RANKL levels. Thyroid 2008 18 455-460.
18. Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, Biondi B, Iorio S, Giustina A, Amato G, Carella C. Recombinant human TSH modulates in vivo C-telopeptides of type-1 collagen and bone alkaline phosphatase, but not osteoprotegerin production in postmenopausal women monitored for differentiated thyroid carcinoma. Journal of Bone and Mineral Research 2005 20 480-486.
19. Hase H, Ando T, Eldeiry L, Brebene A, Peng Y, Liu L, Amano H, Davies TF, Sun L, Zaidi M, Abe E. TNFalpha mediates the skeletal effects of thyroid-stimulating hormone. Proceedings of the National Academy of Sciences 2006 103 12849-12854.
20. Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Lüthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 1997 89 309-319.
21. Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, Higashio K. Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochemical and Biophysical Research Communications 1997 234 137-142.
22. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Goto M, Mochizuki SI, Tsuda E, Morinaga T, Udagawa N, Takahashi N, Suda T, Higashio K. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proceedings of the National Academy of Sciences 1998 95 3597-3602.
23. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 1997 390 175-179.
24. Hsu H, Lacey DL, Dunstan CR, Solovyev I, Colombero A, Timms E, Tan HL, Elliott G, Kelley MJ, Sarosi I, Wang L, Xia XZ, Elliott R, Chiu L, Black T, Scully S, Capparelli C, Morony S, Shimamoto G, Bass MB, Boyle WJ. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. Proceedings of the National Academy of Sciences 1999 96 3540-3545.
25. Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM. Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 1999 402 304-309.
26. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 1998 93 165-176.

27. Matsuzaki K, Udagawa N, Takahashi N, Yamaguchi K, Yasuda H, Shima N, Morinaga T, Toyama Y, Yabe Y, Higashio K, Suda T. Osteoclast differentiation factor (ODF) induces osteoclast-like cell formation in human peripheral blood mononuclear cell cultures. Biochemical and Biophysical Research Communications 1998 246 199-204.
28. Quinn JM, Elliott J, Gillespie MT, Martin TJ. A combination of osteoclast differentiation factor and macrophage-colony stimulating factor is sufficient for both human and mouse osteoclast formation in vitro. Endocrinology 1998 139 4424-4427.
29. Burgess TL, Qian Y, Kaufman S, Ring BD, Van G, Capparelli C, Kelley M, Hsu H, Boyle WJ, Dunstan CR, Hu S, Lacey DL. The ligand for osteoprotegerin (OPGL) directly activates mature osteoclasts. Journal of Cell Biology 1999 145 527-538.
30. Fuller K, Wong B, Fox S, Choi Y, Chambers TJ. TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. Journal of Experimental Medicine 1998 188 997-1001.
31. Toivonen J, Tahtela R, Laitinen K, Risteli J, Valimaki MJ. Markers of bone turnover in patients with differentiated thyroid cancer with and following withdrawal of thyroxine suppressive therapy. European Journal of Endocrinology 1998 138 667-673.

9

Short term overt hypothyroidism induces discrete diastolic dysfunction in patients treated for differentiated thyroid carcinoma

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Abstract

Background

Thyroid hormone has important effects on the cardiovascular system. The consequences of episodes of acute hypothyroidism on cardiac function have been investigated in only a few studies, and their results are inconclusive. Our objective was to investigate the effects of acute hypothyroidism on cardiac function in patients with iatrogenically induced subclinical hyperthyroidism after treatment for differentiated thyroid carcinoma.

Material and methods

Fourteen patients with a history of differentiated thyroid carcinoma on thyroid stimulating hormone (TSH)-suppressive thyroxine replacement therapy were studied. We assessed cardiac function before, and 1 and 4 weeks after withdrawal of thyroxine substitution. We measured serum levels of free thyroxin, triiodothyronine and TSH and used a new sophisticated Doppler echocardiography technique, tissue Doppler imaging (TDI), to assess detailed and quantitative assessment of systolic and diastolic cardiac function. Echocardiographic parameters in patients were compared to controls.

Results

Compared to controls, patients had higher left ventricular mass and wall thickness and decreased diastolic function during TSH-suppressive L-thyroxine substitution therapy. Thyroxine withdrawal resulted in a decrease in both early (E) and late (A) diastolic mitral inflow velocities, without impact on E/A ratio. Using TDI, late diastolic velocity (A′) decreased without impact on E′/A′ ratio. Left ventricular dimensions, wall thickness and mass did not change during thyroxine withdrawal.

Conclusions

Subclinical hyperthyroidism is accompanied by diastolic dysfunction. Subsequent acute hypothyroidism induces only subtle changes in diastolic function.

Introduction

Thyroid hormone has profound effects on the cardiovascular system. Hyperthyroidism induces cardiac arrhythmias, left ventricular (LV) hypertrophy and diastolic dysfunction, and enhances systolic function (1–3). Subclinical hyperthyroidism – i.e. suppressed thyroid-stimulating hormone (TSH) levels with normal free thyroxine (FT4) levels – is associated with increased heart rate and supraventricular arrhythmias, including atrial fibrillation, increased LV mass (LVM) with a slightly enhanced systolic function, and diastolic dysfunction. Diastolic dysfunction is at least partly reversible after restoration of euthyroidism and is associated with an increase in mortality (4–7). Conversely, hypothyroidism is associated with bradycardia, mild hypertension, increased peripheral cardiovascular resistance, heart failure (1,3,8,9), decreased cardiac output and diastolic dysfunction (1,3,10,11). Long-standing hypothyroidism can even result in asymmetrical septal hypertrophy (12) and pericardial effusion (6). Hypothyroidism is also associated with coronary artery disease, presumably because of associated hypercholesterolemia, hypertriglyceridemia and hypertension (1,3,13). Thyroxine substitution reverses most cardiovascular alterations associated with hypothyroidism (3,6,9,14).

Patients with differentiated thyroid carcinoma (DTC) are treated with total thyroidectomy and radioiodine ablative therapy, followed by long-term TSH-suppressive thyroxine replacement therapy (15–17). During the first period after diagnosis, patients are regularly withdrawn from thyroxine for TSH-stimulated thyroglobulin measurements and diagnostic 185-megabecquerel iodine-131 scintigraphy. The consequences of these episodes of acute hypothyroidism on cardiac function have been investigated in only a few studies up to now. However, the results of those studies have been inconclusive (18–27), varying from mainly decreased diastolic function (18,19,23,25,26), to mainly altered systolic function (21,24). In these studies, without control groups, these parameters were measured by different techniques (echocardiography, radionuclide imaging), without blinding the observers with regards to treatment modalities.

Therefore, we performed a prospective study in a homogeneous group of athyreotic DTC patients to assess the impact of overt hypothyroidism induced by short-term thyroxine withdrawal on cardiac function measured by a new sophisticated echocardiography technique: tissue Doppler imaging (TDI). This technique allows for detailed and quantitative assessment of cardiac parameters, including diastolic and systolic function (28,29). In addition, the researchers who collected and analyzed the echocardiographic data were blinded with regard to treatment modalities, and cardiac parameters of the patients were also compared to a matched group of controls who have no cardiovascular co-morbidities.

Subjects and Methods

Subjects

Patients were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. The Department of Endocrinology is a tertiary referral center for DTC. Patients included were those who had been diagnosed with DTC, had received initial therapy consisting of total thyroidectomy and radioiodine ablative treatment, and were planned for TSH stimulated iodine-131 whole body scanning for evaluation of the effect of prior radioiodine therapy or screening in case of positive thyroglobulin antibodies. The patients were on TSH-suppressive therapy, aiming at TSH levels below 0.1 mU/L (normal reference values for TSH 0.4–4.4 mU/L). No drugs known to influence cardiovascular parameters were allowed. None of the patients had hemodynamic instability, previous myocardial infarction, rheumatic fever, endocarditis, diabetes mellitus, or connective tissue disease. The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

Study design

Fifteen DTC patients undergoing TSH-stimulated iodine-131 whole body scanning for follow-up were prospectively asked to participate in this study. On the last day of thyroxine therapy, on day 7 and on day 28 after withdrawal, hormonal and biochemical parameters were measured and echocardiography was performed. At each visit patients came to the outpatient clinic after an overnight fast and blood was collected for the measurement of TSH, FT4, triiodothyronine (T3) and creatinine concentrations. Height (m), weight (kg), resting blood pressure (mmHg) and heart rate (beats per minute) were documented. An independent cardiologist performed echocardiography at each visit.

Echocardiography: data acquisition

Echocardiography was performed with the patients in the left lateral decubitus position using a commercially available system (Vingmed System Vivid 7, General Electric/Vingmed, Milwaukee, WI, USA). Images were obtained using a 3.5-MHz transducer, at a depth of 16 cm in the parasternal (long- and short-axis) and apical (2- and 4-chamber, long axis) views. Standard two-dimensional and colour Doppler data, triggered to the QRS complex, were saved in Cineloop format. A minimum of three consecutive beats were acquired from each view and the images were stored for offline analysis (EchoPac 6.0.1, General Electric/Vingmed Ultrasound).

Left ventricular dimensions, fractional shortening and LV ejection fraction (LVEF) were measured from the M-mode recordings at the parasternal long-axis views (27).

LVM was calculated by the cube formula and using the correction formula proposed by Devereux *et al*. (31): 0.8 x {1.04[(LVEDD + PWT + IVST)^3 – (LVEDD)^3]} + 0.6, where, LVEDD is LV end-diastolic diameter, PWT is the posterior wall thickness, and IVST is interventricular septum thickness. LVM was corrected for body surface area to obtain LVM index (LVMI). LV hypertrophy was defined as LVMI > 120 g m^2 for men and > 116 g m^2 for women (31,32). Systolic function was evaluated by measurements of fractional shortening and LVEF (33).

The following parameters of diastolic function were measured: diastolic transmitral peak velocities (E and A wave) and the E/A ratio, the isovolumetric relaxation time and the deceleration time of the E-wave. In addition, left atrium anteroposterior diameter was measured from the M-mode parasternal long-axis recordings. Quantitative diastolic data were derived from TDI data. For TDI data analysis, the digital Cineloops were analysed using commercial software (EchoPac 6.0.1, General Electric/Vingmed Ultrasound). The sample volume (4 mm^3) was placed in the LV basal portions of the anterior, inferior, septal and lateral walls (using the 2- and 4-chamber images). The following parameters (mean values calculated from three consecutive beats) were derived: early diastolic velocity (E′) and late diastolic velocity (A′) and the E′/A′ ratio.

Baseline echocardiographic parameters from patients were compared to a control group consisting of 24 individuals matched for age, gender, body surface area and LVEF. The controls were selected from an echocardiographic database containing this information and special care was taken to exclude those individuals with any cardiovascular comorbidity. Those individuals referred for echocardiographic evaluation of known valvular disease, murmur, congestive heart failure, or cardiac transplantation evaluation were excluded. Accordingly, the controls comprised of patients with curable breast cancer referred for examination of cardiac function before they undergo adjuvant chemotherapy, and of patients experiencing non-ischemic chest pain, palpitations or syncope without murmur.

Acquisition of echocardiographic data was performed by one experienced observer, whereas data analysis was performed by a single independent observer, both blinded with regard to the study subgroups (patients and controls). Intra-observer reproducibility of quantitative M-mode measurements assessed by linear regression and Bland–Altman analysis showed an excellent agreement with high Pearson's correlation coefficient ($r2$ = 0.99) and small bias (0.1 ± 2.4 mm). Similarly, the intra-observer reproducibility of quantitative Doppler measurements was also excellent, with an $r2$ value of 0.99 and small bias of 0.8 ± 3.6, with no significant trend for repeated measurements.

Assays

Serum FT4 concentration was measured with an IMx system (Abbott, Abbott Park, IL, USA) (intra-assay variability of 2.47–7.57% and interassay variability of 5.6–12.4%

at different levels). Serum TSH levels were determined with a Modular Analytics E-170 system (Roche Diagnostic Systems, Basel, Switzerland) (intra-assay variability of 0.88–10.66% and interassay variability of 0.91–12.05%). Serum T3 levels were measured by fluorescent polarization immunoassay using an Axsym system (Abbott) (intra-assay variability of 0.15–0.37% and interassay variability of 6.5–19%).

Statistical analysis

SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA), was used to perform data analysis. Data are expressed as mean ± standard deviation, unless mentioned otherwise. Outcomes of patients at the three visits were compared using analysis of variance for repeated measures, and post-hoc analysis if appropriate. Data from healthy controls were compared to data from the patients using Kruskal–Wallis non-parametric tests. Differences were considered statistically significant at $P < 0.05$.

Results

Patient characteristics

Patient characteristics are detailed in **Table 1**. Fifteen patients were included in this study. One patient was excluded from the analysis because follow-up data were not obtained. Accordingly, 14 patients completed this study (3 men and 11 women), with a mean age of 51.6 ± 14.5 years. Median duration of TSH suppression was 1 year (range 0.5–44.6 years). The dose of thyroxine replacement before withdrawal was 162 ± 42 µg/ day. The control group consisted of 21 women and 3 men, with a mean age of 45.4 ± 8.5 years ($P = 0.16$ *vs*. patients).

Clinical and laboratory parameters

Thyroid hormone levels

Thyroid hormone levels are summarized in **Table 2**. At visit 1, serum FT4 concentrations were above the upper limit of the normal range (reference range, 10–24 pmol/L), TSH levels (reference range, 0.4–4.8 mU/L) were below normal range, and T3 levels (reference range, 1.1–3.6 nmol/L) were within normal range. Seven days after thyroxine withdrawal, FT4 levels were already slightly below the lower limit of the reference values, and TSH levels had increased significantly, whereas T3 levels were still within normal range. At visit 3, at the end of the study, all patients had elevated TSH levels and decreased FT4 and T3 levels (**Table 2**).

Table 1: Patient characteristics

	Patients n=14
Age (years) mean ± SD (range)	51.6 ± 14.5 (24-69)
Sex (Male/Female)	3/11
Tumor stage	
T1 N0 M0	2
T2 N0 M0	8
T3 N0 M0	1
T3 N1 M0	2
T4 N1 M0	1
Histology tumor	
Papillary	7
Papillary-follicular	4
Follicular	2
Follicular Hürthle	1
Duration TSH suppression (years) median (range)	1.0 (0.5-44.6)
Dose Levo-thyroxine (µg/day) mean ± SD	162.5 ± 41.6
Dose I-131 (MBq) median (range)	8381 (1800-20690)

MBq: megabequerel; SD: standard deviation; TSH: thyroid stimulating hormone

Weight and body mass index

Weight and body mass index were significantly different at visits 2 and 3 compared to visit 1, and were also different between visits 2 and 3 (**Table 2**).

Table 2: Weight, body mass index, blood pressure, heart rate and thyroid hormone parameters.

	Healthy controls	**Visit 1 subclinical hyperthyroidism**	**Visit 2 7 days withdrawal**	**Visit 3 28 days withdrawal**
FT4 (pmol/l) (ref 10-24 pmol/l)	NA	26.4 ± 3.1	9.6 ± 1.9 **†**	2.2 ± 1.3 **†‡**
T3 (nmol/l) (ref 1.1-3.6 nmol/l))	NA	1.5 ± 0.5	1.1 ± 0.2 *	0.6 ± 0.2 **†‡**
TSH (mU/l) (ref 0.4-4.8 mU/l)	NA	0.3 ± 0.58	9.5 ± 15.5 *	105.2 ± 57.8 **†‡**
Weight (kg)	72.5 ± 11.0	78.9 ± 18.5	79.9 ± 18.5 *	81.6 ± 18.5 **†‡**
BMI (kg/m²)	24.9 ± 3.1	26.5 ± 6.1	26.9 ± 5.9 **†**	27.6 ± 6.0 **†‡**
Systolic blood pressure (mmHg)	124.8 ± 7.7	130.1 ± 23.2	130.2 ± 23.3	131.3 ± 20.1
Diastolic blood pressure (mmHg)	76.0 ± 6.9	81.7 ± 16.5	77.6 ± 12.9	85.5 ± 10.4 **#§**
Mean blood pressure (mmHg)	92.3 ± 6.3	97.8 ± 18.3	95.2 ± 15.4	100.8 ± 12.3
Heart rate (BPM)	70.4 ± 8.4	70.8 ± 8.0	65.2 ± 8.2 *	66.6 ± 6.7

Visit 2 reflects euthyroid to hypothyroid state, visit 3 reflects overt hypothyroidism. TSH= thyroid stimulating hormone, BMI= body mass index, BPM= beats per minute ***=** P< 0.05 compared to visit 1, **†=** P≤ 0.001 compared to visit 1, **# =** P< 0.05 compared to visit 2, **‡=** P≤ 0.001 compared to visit 2, §= P≤ 0.05 compared to controls

Blood pressure and heart rate

At baseline, six patients had hypertension, but only one patient was on antihypertensive treatment. No differences were observed in systolic blood pressure and mean arterial pressure 7 and 28 days after l-thyroxine withdrawal. Diastolic blood pressure increased significantly at visit 3 compared to visit 2. Heart rate was significantly decreased at visit 2 (**Table 2**).

Echocardiography

LV dimensions and systolic function

At baseline, LVM, LVMI, IVST and PWT were significantly higher in patients with subclinical hyperthyroidism as compared to control subjects. However, none of the patients met the criteria for LV hypertrophy (31). Echocardiography showed no significant changes in M-mode measurements of LV dimensions and systolic function during acute withdrawal of thyroid hormone (**Table 3**).

Table 3: Left ventricular dimensions and systolic function

	Healthy controls	**Visit 1 subclinical hyperthyroidism**	**Visit 2 7 days withdrawal**	**Visit 3 28 days withdrawal**
Left ventricular mass (g)	135.6 ± 27.2	157.8 ± 31.2	163.9 ± 32.4 *	168.6 ± 42.4 *
Left ventricular mass index (g/m²)	73.6 ± 9.3	81.6 ± 12.1 *	84.1 ± 12.2 *	85.2 ± 16.7 *
Inter-ventricular septum thickness (mm)	8.3 ± 1.0	w9.9 ± 1.3 *	9.3 ± 1.4 *	9.5 ± 1.5 *
Posterior wall thickness (mm)	8.2 ± 0.7	9.5 ± 1.9 *	9.4 ± 1.2 *	9.7 ± 1.5 *
Left ventricular end-diastolic diameter (mm)	48.5 ± 4.5	46.9 ± 5.3	49.4 ± 5.0	48.9 ± 5.0
Left ventricular end-systolic diameter (mm)	28.1 ± 4.1	28.7 ± 4.6	29.6 ± 3.5	29.1 ± 3.2
Fractional shortening (%)	38.2 ± 3.4	38.4 ± 7.4	39.8 ± 5.7	40.1 ± 5.8
Left ventricular ejection fraction (%)	68.0 ± 4.2	68.0 ± 8.8	70.4 ± 6.1	70.3 ± 6.7

Visit 2 reflects euthyroid to hypothyroid state, visit 3 reflects overt hypothyroidism. * P< 0.05 compared to healthy controls

Diastolic function

Control subjects had significantly higher mean values for E- and E′-wave as compared to patients at baseline. The E′/A′ ratio -was higher in controls when compared to the patients' baseline values. The values for A- and A′-wave were significantly lower in the patients at visit 3, 28 days after withdrawal, compared to visits 1 and 2. Baseline left atrium anteroposterior diameter was similar between patients and controls (**Table 4**). Twenty-eight days after withdrawal, E-wave was significantly lower compared to baseline. There were no changes in E/A ratio, E′/A′ ratio and left atrium anteroposterior diameter during the study (**Table 4**).

Table 4: Diastolic function

	Controls	Visit 1 subclinical hyperthyroidism	Visit 2 7 days withdrawal	Visit 3 28 days withdrawal
E (cm/sec)	68.8 ± 10.7	57.0 ± 19.2 *	55.6 ± 15.6 *	46.6 ± 15.1 *‡
A (cm/sec)	54.6 ± 12.0	50.6 ± 11.7	50.9 ± 9.9	40.6 ± 11.6 *†‡
E/A ratio	1.3 ± 0.2	1.2 ± 0.2	1.1 ± 0.4	1.2 ± 0.5
E′ (cm/sec)	-8.9 ± 1.6	-6.4 ± 2.6 *	-6.4 ± 2.4 *	-5.8 ± 1.6 *
A′ (cm/sec)	-6.5 ± 1.6	-6.9 ± 1.4	-6.8 ± 1.7	-5.7 ± 1.7 *‡
E′/A′ ratio	1.4 ± 0.5	1.0 ± 0.5 *	1.1 ± 0.7	1.2 ± 0.6 *
AP diameter of the LA (cm)	38.2 ± 4.1	37.2 ± 4.6	36.9 ± 5.1	36.1 ± 5.2

Visit 2 reflects euthyroid to hypothyroid state, visit 3 reflects overt hypothyroidism. E= peak flow of early filling phase, A= peak flow in atrial filling phase, E′= peak flow of early filling phase measured by Tissue Doppler Imaging, A′= peak flow in atrial filling phase measured by Tissue Doppler Imaging, LA= left atrium. *= P< 0.05 compared to healthy controls, †= P< 0.05 compared to visit 1, ‡= P< 0.05 compared to visit 2.

Discussion

The current study aimed at investigating the effects of overt acute hypothyroidism in DTC patients on cardiac function. At baseline, when patients were subclinically hyperthyroid, patients had higher LV size and mass and decreased diastolic function as compared to controls. Thyroxine withdrawal resulted in an additional subtle decrease in both E- and A-wave velocities, without an impact on E/A ratio, indicating discrete unfavorable effects on diastolic function as assessed by echocardiography. In line with this observation, diastolic function, when more specifically analyzed by TDI, decreased. This was reflected in decreased late diastolic velocity (A′) without impacting E′/A′ ratio. Overt hypothyroidism increased diastolic blood pressure significantly, but had no effect on systolic blood pressure. Therefore, long-term subclinical hyperthyroidism is accompanied by diastolic dysfunction. Subsequent acute hypothyroidism induces subtle changes in diastolic function.

The impact of acute hypothyroidism on cardiac function has been investigated in only a few studies. These studies were inconclusive and mainly showed decreased diastolic function (18,19,23–25) or decreased systolic function (21,24), measured by different techniques (conventional echocardiography and radionuclide imaging). In addition, none of these studies compared their outcomes to a control group without cardiovascular comorbidities or had their observers blinded with regards to treatment modalities. Moreover, none of these studies measured cardiac function using TDI, a new and sophisticated technique that permits quantification of diastolic parameters which are independent of cardiac loading conditions (28,34,35).

Our study is in line with only one other study that reported no impact on cardiac function after thyroxine withdrawal (20). In that study, conventional echocardiography without TDI was used and, in contrast to our study, the observers were not blinded with regards to treatment modalities.

After thyroxine withdrawal, early and late diastolic velocity (E and A, respectively) decreased mildly, whereas the E/A ratio was not affected. In addition, mean values for E, A and E/A ratio were still within the normal range of reference values when patients suffered from overt hypothyroidism (36). These findings imply that acute thyroxine withdrawal only minimally affects diastolic function.

In the present study, only six patients had an E/A ratio slightly below 1 during overt hypothyroidism. This is probably due to impaired ventricular relaxation associated with a delay in the energy-dependent reuptake of calcium by the sacroplasmatic reticulum, which in turn is under thyroid hormone control (26). This thyroid hormone control of cardiac function is mediated mainly by T3 (3), which in our study declined significantly during thyroxine withdrawal. Although the findings of the present study suggest minimal unfavorable cardiovascular effects of thyroxine withdrawal, the potential negative cardiovascular consequences of thyroxine withdrawal before diagnostic iodine-131 whole body scanning could be clinically relevant, especially in patients at cardiovascular risk (1,37,38). Therefore, recombinant TSH stimulation might be an attractive alternative in 'low-risk thyroid carcinoma patients' and/or high-risk cardiovascular patients.

At baseline, when patients had subclinical hyperthyroidism, echocardiography revealed decreased diastolic function. This is in line with a previous study in patients with exogenous subclinical hyperthyroidism (5). The clinical consequences of isolated diastolic dysfunction in subclinical hyperthyroidism are not entirely clear, but could be accompanied by increased morbidity and mortality when compared to isolated diastolic dysfunction in other conditions, especially in long-term subclinical hyperthyroidism (39). It has been suggested that diastolic dysfunction in subclinical hyperthyroidism results from an increased LVM (40,41). In our study, however, no patient fulfilled the criteria for LV hypertrophy, although there was a significant elevation in LVM at baseline compared to controls. Therefore, biochemical effects of thyroid hormone on cardiac function instead of increased LVM are more likely involved in the induction of diastolic dysfunction (1). Nonetheless, additional studies with longer follow-up are needed to elucidate the effects on LV dimensions and mass.

Systolic blood pressure did not change after thyroxine withdrawal, whereas diastolic blood pressure was higher at visit 3, when patients were overtly hypothyroid compared to visit 2, when patients were mildly hypothyroid. These findings are in line with those of previous studies (3, 10, 19, 42, 43). The increase in diastolic blood pressure in hypothyroidism is ascribed to increased peripheral vascular resistance (3, 43).

In conclusion, in the present study we demonstrated that long-term iatrogenically induced subclinical hyperthyroidism in patients with DTC induces diastolic dysfunction. Subsequently, overt acute hypothyroidism induces discrete decreases in diastolic parameters.

References

1. Fazio S, Palmieri EA, Lombardi G, Biondi B. Effects of thyroid hormone on the cardiovascular system. Recent Progress in Hormone Research 2004 59 31–50.
2. Forfar JC, Muir AL, Sawers SA, Toft AD. Abnormal left ventricular function in hyperthyroidism: evidence for a possible reversible cardiomyopathy. New England Journal of Medicine 1982 307 1165–1170.
3. Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. New England Journal of Medicine 2001 344 501–509.
4. Biondi B, Palmieri EA, Fazio S, Cosco C, Nocera M, Saccà L, Filetti S, Lombardi G, Perticone F. Endogenous subclinical hyperthyroidism affects quality of life and cardiac morphology and function in young and middle-aged patients. Journal of Clinical Endocrinology and Metabolism 2000 85 4701–4705.
5. Smit JW, Eustatia-Rutten CF, Corssmit EP, Pereira AM, Frölich M, Bleeker GB, Holman ER, van der Wall EE, Romijn JA, Bax JJ. Reversible diastolic dysfunction after long-term exogenous subclinical hyperthyroidism: a randomized, placebo controlled study. Journal of Clinical Endocrinology and Metabolism 2005 90 6041–6047.
6. Ladenson PW. Recognition and management of cardiovascular disease related to thyroid dysfunction. American Journal of Cardiology 1990 88 638–641.
7. Heeringa J, Hoogendoorn EH, van der Deure WM, Hofman A, Peeters RP, Hop WC, den Heijer M, Visser TJ, Witteman JC. High-normal thyroid function and risk of atrial fibrillation: the Rotterdam Study. Archives of Internal Medicine 2008 168 2219–2224.
8. Ochs N, Auer R, Bauer DC, Nanchen D, Gussekloo J, Cornuz J, Rodondi N. Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. Annals of Internal Medicine 2008 148 832–845.
9. Rodondi N, Bauer DC, Cappola AR, Cornuz J, Robbins J, Fried LP, Ladenson PW, Vittinghoff E, Gottdiener JS, Newman AB. Subclinical thyroid dysfunction, cardiac function, and the risk of heart failure. The Cardiovascular Health Study. Journal of the American College of Cardiology 2008 30 1152–1159.
10. Osman F, Gammage MD, Franklyn JA. Thyroid disease and its treatment: short-term and long-term cardiovascular consequences. Current Opinion in Pharmacology 2001 1 626–631.
11. Tielens ET, Pillay M, Storm C, Berghout A. Cardiac function at rest in hypothyroidism evaluated by equilibrium radionuclide angiography. Clinical Endocrinology 1999 50 497–502.
12. Santos AD, Miller RP, Mathew PK, Wallace WA, Cave WT Jr, Hinojosa L. Echocardiographic characterization of the reversible cardiomyopathy of hypothyroidism. American Journal of Cardiology 1980 68 675–682.
13. Kahaly GJ, Dillmann WH. Thyroid hormone action in the heart. Endocrine Reviews 2005 26 704–728.
14. Crowley WF Jr, Ridgway EC, Bough EW, Francis GS, Daniels GH, Kourides IA, Myers GS, Maloof F. Noninvasive evaluation of cardiac function in hypothyroidism. Response to gradual thyroxine replacement. New England Journal of Medicine 1977 296 1–6.
15. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. American Journal of Medicine 1994 97 418–428.
16. Schlumberger MJ, Torlantano M. Papillary and follicular thyroid carcinoma. Ballieres Best Practice and Research Clinical Endocrinology and Metabolism 2000 14 601– 613.

17. Vini L, Harmer C, McCready VR. Thyroid cancer: a review of treatment and follow-up. Annals of Nuclear Medicine 1996 10 1–7.
18. Aydin M, Reyhan M, Sukan A, Yapar AF, Aktas A. Gated SPECT findings revealing diastolic dysfunction in acute hypothyroidism. Clinical Nuclear Medicine 2007 32 94–100.
19. Botella-Carretero JI, Gómez-Bueno M, Barrios V, Caballero C, García-Robles R, Sancho J, Escobar-Morreale HF. Chronic thyrotropin-suppressive therapy with levothyroxine and short-term overt hypothyroidism after thyroxine withdrawal are associated with undesirable cardiovascular effects in patients with differentiated thyroid carcinoma. Endocrine Related Cancer 2004 11 345–356.
20. Chrisoulidou A, Pazaitou-Panayiotou K, Kaprara A, Platoyiannis D, Lafaras C, Boudina M, Georgiou E, Drimonitis A, Bischiniotis T, Vainas I. Effects of thyroxine withdrawal in biochemical parameters and cardiac function and structure in patients with differentiated thyroid cancer. Minerva Endocrinology 2006 31 173–178.
21. Di Paola PR, Alagona C, Pezzino V, Mangiameli S, Regalbuto C. Left ventricular function in acute hypothyroidism: a Doppler echocardiography study. Italian Heart Journal 2004 5 857–563.
22. Donaghue K, Hales I, Allwright S, Cooper R, Edwards A, Grant S, Morrow A, Wilmshurst E. Cardiac function in acute hypothyroidism. European Journal of Nuclear Medicine 1985 11 147–149.
23. Grossmann G, Wieshammer S, Keck FS, Goller V, Giesler M, Hombach V. Doppler echocardiographic evaluation of left ventricular diastolic function in acute hypothyroidism. Clinical Endocrinology 1994 40 227–233.
24. Grossmann G, Keck FS, Wieshammer S, Goller V, Schmidt A, Hombach V. Systolic ventricular function in acute hypothyroidism: a study using Doppler echocardiography. Experimental and Clinical Endocrinology 1994 102 104–110.
25. Kahaly G, Mohr-Kahaly S, Beyer J, Meyer J. Left ventricular function analyzed by Doppler and echocardiographic methods in short-term hypothyroidism. Am J Cardiol 1995 75 645–648.
26. Wieshammer S, Keck FS, Waitzinger J, Henze E, Loos U, Hombach V, Pfeiffer EF. Acute hypothyroidism slows the rate of left ventricular diastolic relaxation. Canadian Journal of Physiology and Pharmacology 1989 67 1007–1010.
27. Duntas LH, Biondi B. Short-term hypothyroidism after Levothyroxine-withdrawal in patients with differentiated thyroid cancer: clinical and quality of life consequences. European Journal of Endocrinology 2007 156 13–19.
28. Sohn DW, Chai IH, Lee DJ, Kim HC, Kim HS, Oh BH, Lee MM, Park YB, Choi YS, Seo JD, Lee YW. Assessment of mitral annulus velocity by Doppler tissue imaging in the evaluation of left ventricular diastolic function. Journal of the American College of Cardiology 1997 30 474–480.
29. Bruch C, Gradaus R, Gunia S, Breithardt G, Wichter T. Doppler tissue analysis of mitral annular velocities: evidence for systolic abnormalities in patients with diastolic heart failure. Journal of the American Society of Echocardiography 2003 16 1031–1036.
30. Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, Gutgesell H, Reichek N, Sahn D, Schnittger I. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. Journal of the American Society of Echocardiography 1989 2 358–367.

31. Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, Reichek N. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. American Journal of Cardiology 1986 57 450–458.
32. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. New England Journal Medicine 1990 322 1561–1566.
33. Park SH, Shub C, Nobrega TP, Bailey KR, Seward JB. Two dimensional echocardiographic calculation of left ventricular mass as recommended by the American Society of Echocardiography: correlation with autopsy and M-mode echocardiography. Journal of the American Society Echocardiography 1996 9 119–128.
34. Garcia MJ, Rodriguez L, Ares M, Griffin BP, Klein AL, Stewart WJ, Thomas JD. Myocardial wall velocity assessment by pulsed Doppler tissue imaging: characteristic findings in normal subjects. American Heart Journal 1996 132 648–656.
35. Kosar F, Sahin I, Aksoy Y, Uzer E, Turan N. Usefulness of pulsed wave tissue Doppler echocardiography for the assessment of the left and right ventricular function in patients with clinical hypothyroidism. Echocardiography 2006 23 471–477.
36. Cohen GI, Pietrolungo JF, Thomas JD, Klein AL. A practical guide to assessment of ventricular diastolic function using Doppler echocardiography. Journal of the American College of Cardiology 1996 27 1753–1760.
37. Fredlund BO, Olsson SB. Long QT interval and ventricular tachycardia of 'torsade de pointe' type in hypothyroidism. Acta Medica Scandinavica 1983 213 231–235.
38. Biondi B, Palmieri EA, Lombardi G, Fazio S. Effects of subclinical thyroid dysfunction on the heart. Annals of Internal Medicine 2002 137 904–914.
39. Zile MR, Brutsaert DL. New concepts in diastolic dysfunction and diastolic heart failure: Part I: diagnosis, prognosis, and measurements of diastolic function. Circulation 2002 105 1387–1393.
40. Bonow RO, Udelson JE. Left ventricular diastolic dysfunction as a cause of congestive heart failure. Mechanisms and management. Annals of Internal Medicine 1992 117 502–510.
41. Mandinov L, Eberli FR, Seiler C, Hess OM. Diastolic heart failure. Cardiovascular Research 2000 45 813–825.
42. Bengel FM, Nekolla SG, Ibrahim T, Weniger C, Ziegler SI, Schwaiger M. Effect of thyroid hormones on cardiac function, geometry, and oxidative metabolism assessed noninvasively by positron emission tomography and magnetic resonance imaging. Journal of Clinical Endocrinology and Metabolism 2000 85 1822–1827.
43. Kotsis V, Alevizaki M, Stabouli S, Pitiriga V, Rizos Z, Sion M, Zakopoulos N. Hypertension and hypothyroidism: results from an ambulatory blood pressure monitoring study. Journal of Hypertension 2007 25 993–999.

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Quality of life in cured patients with differentiated thyroid carcinoma

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Abstract

Objective

This study was performed to evaluate the impact of cured differentiated thyroid carcinoma (DTC) on quality of life. Previous studies on quality of life in patients with DTC were hampered by small patient numbers or limited quality-of-life parameters or were uncontrolled.

Design

This was a cross-sectional case-control study.

Method

We assessed quality of life in 153 cured DTC patients with a median duration of cure of 6.34 yr (range 0.3 – 41.8) and studied the contribution of disease-specific, biochemical, and social variables, focusing on the degree of TSH suppression. Four validated health-related questionnaires were used (Short Form-36, Multidimensional Fatigue Index-20, Hospital Anxiety and Depression Scale, and Somatoform Disorder Questionnaire), including multiple aspects of physical, psychological, and social functioning. Patients were compared with 113 controls selected by patients themselves (control group I) and 336 pooled age- and gender-matched controls from other Leiden quality-of-life studies (control group II).

Results

Patients had significantly decreased quality of life in 11 of 16 subscales when compared with control group I. In comparison with control group II, decreased scores in 13 of 16 items were observed. An important independent predictor for quality of life was duration of cure. Quality-of-life parameters were not influenced by serum TSH levels both measured at the time of quality-of-life assessment and measured over time since initial therapy.

Conclusions

Patients cured for DTC have impaired quality of life, independently of TSH level. Quality-of-life parameters were inversely affected by duration of cure and consequently may be restored after prolonged follow-up.

Introduction

Well-differentiated thyroid carcinoma (DTC) is associated with an excellent medical prognosis, with 10-yr survival rates reaching 90–95% (1). After initial therapy, usually consisting of total thyroidectomy and radioiodine thyroid remnant ablation therapy, most patients used to be treated with high doses of L-thyroxine to suppress TSH levels (1). On the one hand, the excellent prognosis and moderate invasiveness of the initial therapy may implicate that quality of life in cured DTC patients may be relatively normal. On the other hand, TSH-suppressive T4 replacement therapy may lead to a decreased quality of life (2–4). Only a few studies have evaluated quality of life in cured DTC patients (5–9). These studies are limited by small patient numbers (6, 7), limited number of quality-of-life questionnaires (5, 9) or the absence of a healthy control group (5, 6, 8).

Studies that focussed on the relation between the level of TSH suppression and quality of life in DTC patients are inconclusive because of small patient numbers, selection of patients with symptoms of hyperthyroidism, or selection of patients with a long duration of cure (2, 10). Therefore, the aim of the present study was to assess quality of life in a large cohort of cured DTC patients and investigate the determinants of quality of life, including serum TSH levels. We used four validated, health-related questionnaires and included controls matched for age, gender, and socioeconomic status.

Patients and Methods

Patients

Cured DTC patients, 18–70 yr old, were recruited from the outpatient clinic of the Department of Endocrinology of the Leiden University Medical Center. Other medical conditions or drugs that could influence quality of life were not permitted. Initial therapy consisted of near-total thyroidectomy, followed by postoperative radioiodine ablation therapy with I-131. Cure after initial therapy was defined as the absence of I-131 accumulation at diagnostic 185 MBq scintigraphy, serum thyroglobulin concentrations less than 2 μg/liter after TSH stimulation in absence of thyroglobulin antibodies, and no other evidence of disease (11). Patients with tumor relapse were included only if they were subsequently cured. Initially, 157 DTC patients who met these criteria were asked to participate. Four validated questionnaires were sent to their homes together with a list of general questions about level of education, country of origin, and marital state. Four patients specifically wished not to participate. Each patient was also asked to provide a control person of comparable sex, age, and socioeconomic status (friend, neighbor, relative) (control group I). We received 153

completed questionnaires from patients and 113 questionnaires from controls. To exclude bias in the selection of control group I, we also compared the patients with a larger cohort of age-, gender-, and socioeconomic status-matched healthy controls (n = 336) obtained from other quality-of-life studies performed in our center (12–15) (indicated as control group II). The study protocol was approved by the Medical Ethics Committee of the Leiden University Medical Center, and written consent was obtained from all patients.

Study parameters

Primary study parameters were the outcomes of the four health-related questionnaires and the contribution of patient characteristics (age, gender, educational level, marital status), disease-specific characteristics (initial tumor node metastasis stage, recurrent disease, duration of cure), treatment (extent of surgery, radioiodine therapy and additional treatments), and biochemical parameters (serum free T4, T3, and TSH levels) to quality of life. The influence of TSH on quality of life was investigated by both evaluation of serum TSH levels at time of the survey expressed as continuous variable or stratified as profoundly suppressed (<0.1 mU/liter), moderately suppressed (<0.4 mU/liter), and unsuppressed (<0.4 mU/liter) and summary TSH parameters over time since initial therapy for each patient. Summary TSH parameters over time were the mean, 25th, 50th, and 75th percentiles and the percentage of profoundly suppressed, suppressed, and unsuppressed TSH values from all available unstimulated TSH measurements since initial therapy.

Quality-of-life questionnaires

Short form-36 (SF-36)

The SF-36 questionnaire comprises 36 items and records general well-being during the previous 30 d (16), subdivided into six health concepts. Scores are expressed on a 0–100 scale, and higher scores are associated with a better quality of life.

Multidimensional Fatigue Index-20 (MFI-20)

The MFI-20 comprises 20 statements (five dimensions) to assess fatigue, which are measured on a 5-point scale (17). Scores vary from 0 to 20; higher scores indicate greater fatigue.

Hospital Anxiety and Depression Scale (HADS)

The HADS consists of 14 items pertaining to anxiety and depression. Scores for the anxiety and depression subscale range from 0 to 21, and values for the total score range from 0 to 42. Higher scores indicate more anxiety or depression (18).

Somatoform Disorders Questionnaire (SDQ)
All somatoform disorders mentioned in classification *Diagnostic and Statistical Manual of Mental Disorders*, third edition, were comprised in this questionnaire (19). The total score varies from 0 to 51 for women and 0 to 55 for men. The total score expresses the extent of physical complaints that were present in the previous week.

Assays

Serum free T4 (FT4; normal range 10–24 pmol/liter) and TSH levels (normal range 0.4–4.5 mU/liter) were measured by electrochemoluminescentic immunoassay using a Modular Analytics E-170 system (Roche, Almere, The Netherlands).

Statistical analysis

SPSS for Windows (version 12.0; SPSS Inc., Chicago, IL) was used to perform all analyses. Data are expressed as mean ± SD unless indicated otherwise. As dependent variables, we calculated delta-scores between each patient and age- and gender-matched Leiden controls by subtracting age- and gender-specific means of the controls from patient scores for all questionnaire subscales. Stepwise univariate linear regression analysis was used to identify independent variables for quality of life. Differences were considered statistically significant at $P < 0.05$.

Results

One hundred fifty-three patients (28 males, 125 females, aged 49 ± 13 yr, 127 papillary and 27 follicular carcinomas) were analyzed. Tumor stages were T1–3M0 in 131, T4 in 18 and M1 in four patients. Median duration of cure was 6.3 yr (range 0.3–41.8). At the time of the survey, median TSH was 0.1 mU/liter (range 0.005–6.8) and FT4 was 22.4 ± 4 pmol/liter. An average of 15 unstimulated TSH measurements per patient was obtained since initial therapy. Summary parameters of TSH over time per patient were: mean 0.4mU/ liter (range 0.1–3.4) and median 0.05 mU/liter (range 0.005 to 2.18); proportions of profoundly suppressed values: 58% (range 0–100) and moderately suppressed values: 80% (range 0–100%). The slope of TSH values was -0.0001 mU/yr (range -0.004 to 0.000 mU/yr), indicating that the TSH levels were reasonably stable. Mean dose of L-thyroxine was 183 ± 51 μg/d.

Quality of life in DTC patients and controls

Quality-of-life scores in patients were significantly reduced in 11 of the 16 items assessed when compared with control group I. According to the SF-36 questionnaire, patients had significantly worse scores on social functioning and general health perception. All MFI-20 subscales and HADS subscales were affected in DTC patients. The SDQ total score was also significantly worse than in control group I. Comparison of the patients with control group II (12–15) showed similar results: 13 of 16 quality-of-life parameters differed significantly between patients and controls (**Table 1**).

Table 1: Quality of life in patients treated for DTC compared with controls selected by patients themselves (Control Group I) and age and gender matched controls from other Leiden quality of life studies (Control Group II) (12, 13, 14, 15). Data shown are mean ± SD

Questionnaire	Patients (n=153)	Control group I (n=113)	P value (patients *vs.* control group I)	Control group II (n=336)	P value (patients *vs.* control group II)
Age	49.10	48.08	0.522	49.99	0.496
M/F	28/125	19/94	0.754	67/269	0.672
SF-36					
Physical functioning	83.70±21.02	88.27±16.78	0.052	87.77±17.14	0.040
Social functioning	81.09±24.90	87.39±20.01	0.037	88.06±19.28	0.007
Role limitations due to physical problems	75.35±40.04	81.42±34.36	0.194	83.38±32.43	0.035
Role limitations due to emotional problems	83.22±35.43	84.66±31.82	0.734	85.93±30.21	0.422
Bodily pain	82.74±21.70	84.78±18.93	0.426	85.17±19.24	0.216
General health perception	65.59±20.48	71.45±18.43	0.027	71.34±18.79	0.007
Change in health	52.15±18.37	55.18±18.19	0.185	54.77±18.64	0.105
MFI-20					
General fatigue	11.03±4.72	8.11±3.35	<0.001	8.60±4.01	<0.001
Physical fatigue	9.95±4.93	6.65±2.64	<0.001	7.60±3.69	<0.001
Reduced activity	8.79±4.15	6.85±3.30	<0.001	7.18±3.57	<0.001
Reduced motivation	8.64±3.76	6.67±2.79	<0.001	7.26±3.53	<0.001
Mental fatigue	9.53±4.50	7.93±3.60	0.002	7.92±3.31	<0.001
HADS					
Anxiety	5.69±3.95	4.14±3.15	<0.001	4.21±3.21	<0.001
Depression	3.61±3.08	2.37±2.52	<0.001	2.86±2.99	0.011
Total	9.30±6.30	6.51±4.92	<0.001	7.07±5.39	<0.001
SDQ					
SDQ total	5.92±6.20	1.66±2.51	<0.001	1.65±2.50	<0.001

Determinants of quality of life

Marital status, country of birth, initial tumor node metastasis stage, total activity of I-131, tumor recurrence, L-thyroxine dose, postsurgical hypoparathyroidism, and serum FT4 level did not affect any of the questionnaire items. TSH levels measured at the time of the assay (both continuous and stratified) and summary TSH values over time appeared not to be a significant independent predictor for quality of life. *Post hoc* power calculation revealed sufficient power (all items > 0.9) to draw this conclusion. A longer duration of cure was correlated with better scores on SF-36 social functioning (standardized β=0.21, *P*=0.030), role limitations due to physical problems (β=0.17, *P*=0.049), general health perception (β=0.32, *P*=0.001), MFI-20 general fatigue (β=-0.17, *P*=0.035), physical fatigue (β=-0.24, *P*=0.003), and mental fatigue scores (β=-0.17, *P*=0.038). We calculated the duration of cure needed for the quality-of-life scores to reach the normal range of all healthy subjects (**Figure 1**). The 95% confidence intervals of quality-of-life scores included only 0 (no difference between quality of life of patients and controls) after a relatively long duration of cure (~12–20 years for SF-36 and MFI-20, respectively).

Discussion

The purpose of this study was to evaluate quality of life in a large cohort of cured DTC patients using multiple quality-of-life parameters and a matched healthy control group. We found that quality-of-life scores assessed by the majority of subscales are reduced in patients previously treated for DTC, compared with controls. Although our observations are in line with other studies on quality of life in DTC patients (5–9), our study includes a higher number of patients, uses more quality-of-life questionnaires, and uses matched control groups.

Longer duration of cure was associated with better scores on different quality-of-life items. This finding is in line with studies by Dagan *et al.* (7) and Crevenna *et al.* (5), but this is the first study to quantify the predicted duration of affected quality of life in relation to duration of cure. After a long duration of cure, approximately 12–20 yr (MFI-20 and SF-36, respectively) the 95% confidence intervals of 6 of the 16 quality of life subscales included a normal score (**Figure 1**).

In our study, quality of life was not influenced by TSH levels at the time of the survey and by TSH levels over time since initial therapy; although it can be objected that generic questionnaires were used. Other studies on the effects of subclinical hyperthyroidism on well being yielded inconclusive results. Most of these studies have been performed in patients with endogenous subclinical hyperthyroidism (3)

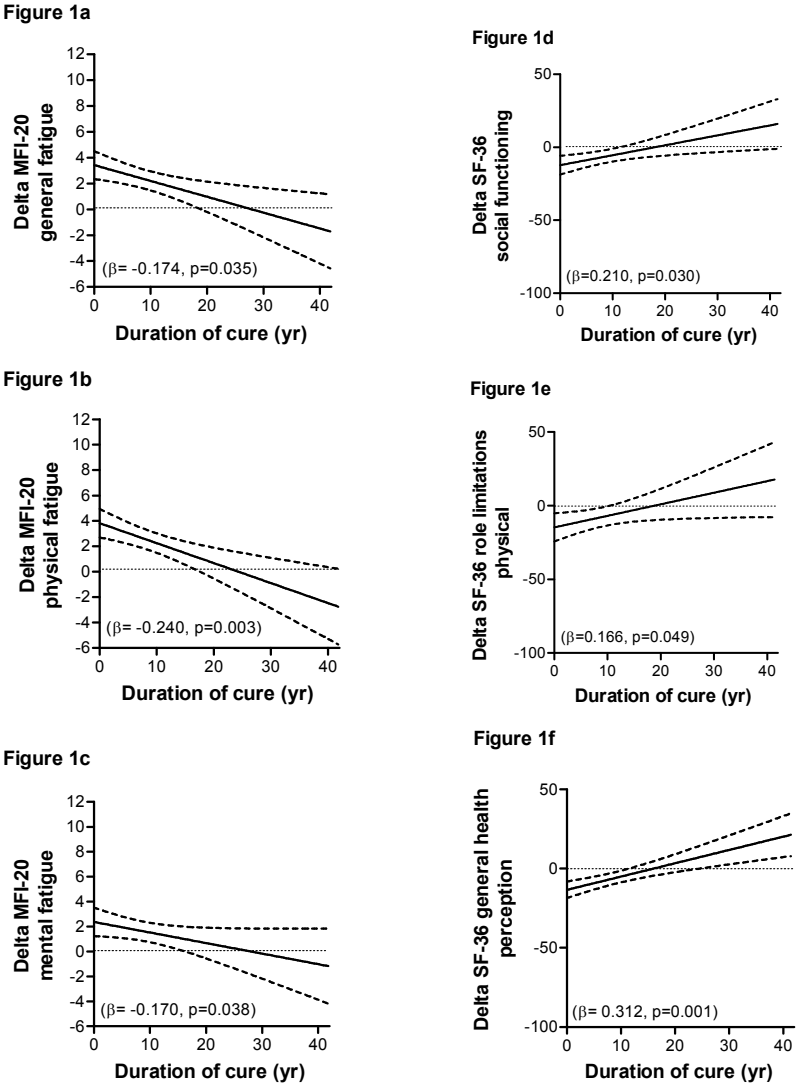

Figure 1

Differences between age- and gender-matched controls and patients for the quality-of-life parameters plotted against duration of cure; linear regression line and 95% confidence interval are shown (standardized Beta and significance of linear regression analysis). The horizontal line represents the value for quality-of-life parameters where there is no difference between patients and the means of age- and gender-matched controls.

who cannot easily be compared with DTC patients or contained selected patients with DTC (2).

Comparison of DTC survivors to survivors of other cancer types is complicated because of the many differences between the several cancer types. A large study (20)

revealed that DTC survivors had similar quality of life as patients with breast cancer, worse than survivors of melanoma or colorectal cancer, but better than hematological malignancies. Despite cure, excellent prognosis, and moderate aggressive treatment, DTC patients have an evident decrease in quality of life that may be restored only after years of follow-up. The findings of our study have therefore implications for the approach of the cured DTC patients: attention for the psychological well-being of the patient and availability of professional support may be important aspects in follow-up.

References

1. Schlumberger MJ, Torlantano M. Papillary and follicular thyroid carcinoma. Baillieres Best Practice and Research Clinical Endocrinology and Metabolism 2000 14 601–613
2. Biondi B, Fazio S, Carella C, Sabatini D, Amato G, Cittadini A, Bellastella A, Lombardi G, Sacca L. Control of adrenergic overactivity by _-blockade improves the quality of life in patients receiving long term suppressive therapy with levothyroxine. Journal of Clinical Endocrinology and Metabolism 1994 78 1028–1033
3. Biondi B, Palmieri EA, Fazio S, Cosco C, Nocera M, Sacca L, Filetti S, Lombardi G, Perticone F. Endogenous subclinical hyperthyroidism affects quality of life and cardiac morphology and function in young and middle-aged patients. Journal of Clinical Endocrinology and Metabolism 2000 85 4701–4705
4. Gulseren S, Gulseren L, Hekimsoy Z, Cetinay P, Ozen C, Tokatlioglu B. Depression, anxiety, health-related quality of life, and disability in patients with overt and subclinical thyroid dysfunction. Archives of Medical Research 2006 37 133–139
5. Crevenna R, Zettinig G, Keilani M, Posch M, Schmidinger M, Pirich C, Nuhr M, Wolzt M, Quittan M, Fialka-Moser V, Dudczak R. Quality of life in patients with non-metastatic differentiated thyroid cancer under thyroxine supplementation therapy. Support Care Cancer 2003 11 597–603
6. Giusti M, Sibilla F, Cappi C, Dellepiane M, Tombesi F, Ceresola E, Augeri C, Rasore E, Minuto F. A case-controlled study on the quality of life in a cohort of patients with history of differentiated thyroid carcinoma. Journal of Endocrinology Investment 2005 28 599–608
7. Dagan T, Bedrin L, Horowitz Z, Chaushu G, Wolf M, Kronenberg J, Talmi YP. Quality of life of well-differentiated thyroid carcinoma patients. Journal of Laryngology and Otology 2004 118 537–542
8. Schultz PN, Stava C, Vassilopoulou-Sellin R. Health profiles and quality of life of 518 survivors of thyroid cancer. Head and Neck 2003 25 349–356
9. Tan LG, Nan L, Thumboo J, Sundram F, Tan LK. Health-related quality of life in thyroid cancer survivors. Laryngoscope 2007 117 507–510
10. Eustatia-Rutten CF, Corssmit EP, Pereira AM, Frolich M, Bax JJ, Romijn JA, Smit JW. Quality of life in long-term exogenous subclinical hyperthyroidism and the effects of restoration of uthyroidism, a randomized controlled trial. Clinical Endocrinology (Oxford) 2006 64 284–291
11. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Sherman SI, TuttleRM. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2006 16 109–142
12. Dekkers OM, Biermasz NR, Smit JW, Groot LE, Roelfsema F, Romijn JA, Pereira AM. Quality of life in treated adult craniopharyngioma patients. European Journal of Endocrinology 2006 154 483–489
13. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, Hoftijzer HC, Smit JW, Roelfsema F, Lamberts SW, Romijn JA. Quality of life in patients after long-term biochemical cure of Cushing's disease. Journal of Clinical Endocrinology and Metabolism 2005 90 3279–3286
14. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. Journal of Clinical Endocrinology and Metabolism 2004 89 5369–5376

15. Dekkers OM, van der Klaauw AA, Pereira AM, Biermasz NR, Honkoop PJ, Roelfsema F, Smit JW, Romijn JA. Quality of life is decreased after treatment for nonfunctioning pituitary macroadenoma. Journal of Clinical Endocrinology and Metabolism 2006 91 3364–3369
16. VanderZee KI, Sanderman R, Heyink J. A comparison of two multidimensional measures of health status: the Nottingham Health Profile and the RAND 36-Item Health Survey 1.0. Quality of Life Research 1996 5 165–174
17. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. Journal of Psychosomatic Research 1995 39 315–325
18. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatrica Scandinavica 1983 67 361–370
19. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 1980 3rd ed. Arlington, VA: American Psychiatric Association
20. Schultz PN, Beck ML, Stava C, Vassilopoulou-Sellin R. Health profiles in 5836 long-term cancer survivors. International Journal of Cancer 2003 104 488–495

11

Discussion and Summary

Contents

I. Introduction

Differentiated thyroid carcinoma (DTC) has a low incidence and a relative good prognosis with a 10-year survival of approximately 90-95%. The incidence has increased during the last years and this trend appears to be continuing (1-4).

Despite the low incidence, many medical centers treat patients with thyroid carcinoma. This decentralized approach does not contribute to optimal treatment, since not all clinicians are fully familiar with the optimal treatment strategy. Moreover, DTC is a unique malignant disease in which fascinating biological phenomena, like the physiology of iodine transport, are present. This makes that general principles of clinical oncology cannot always be extrapolated to DTC. However, publications of consensus and guideline papers (5,6) have improved the implementation of uniform protocols for diagnosis, treatment and follow-up.

Regardless of these guidelines, still many uncertainties exist with respect to the optimal strategy for diagnosis, treatment and follow-up of DTC. An example of an unresolved diagnostic dilemma is that the diagnosis of DTC is still largely dependent on conventional histological staining procedures. Particularly the distinction between follicular adenoma (benign) and follicular thyroid carcinoma (malignant) is difficult to make. As a consequence, many patients will undergo surgery although they do not have DTC.

In this thesis, we tried to contribute to improve diagnostic markers for the distinction between benign and malignant thyroid disease. Furthermore, we performed a phase II trial with the tyrosine kinase inhibitor sorafenib in order to optimize treatment for non-RaI avid metastatic DTC. In the other part of this thesis, we focused on the clinical consequences of initial therapy consisting of thyroidectomy and RaI ablative therapy. The subsequent treatment with TSH suppressive thyroxine replacement therapy and regular withdrawal of thyroxine for TSH stimulated whole body scanning makes DTC patients an interesting model to study the metabolic effects of thyroid hormone on various organ systems and quality of life.

II. Diagnosis of differentiated thyroid carcinoma

Despite increasing standards of imaging techniques like FDG-PET and ultrasound, fine needle aspiration (FNA) is the procedure of choice in patients presenting with a thyroid nodule (5). However, the diagnosis of DTC and in particular the differentiation between follicular adenoma (FA), follicular thyroid carcinoma (FTC) and follicular variant of papillary thyroid carcinoma (FVPTC) is difficult to make on cytology. The consequence is that 70-80% of the patients with suspicious results from FNA, who

undergo surgery, have a benign tumor (5,7). The use of molecular markers (e.g. galectin-3, PAX8-PPARgamma, BRAF, RAS or RET/PTC), may be considered for patients with indeterminate cytology on FNA to help guide management (8-13).

After hemithyroidectomy the microscopical distinction between benign and malignant neoplastic thyroid nodules by conventional histology remains difficult as these lesions may share overlapping histological characteristics. It is therefore important to identify new markers to distinguish benign from malignant thyroid tumors. In recent years, several immunohistochemical markers have been studied to improve the differential diagnosis of thyroid lesions, using both candidate markers and unbiased approaches (14-19).

Differential expression of retinoic acid receptor (RAR) subtypes between benign and malignant thyroid tissues has been described; their diagnostic value has not been reported yet. In **Chapter 2**, we describe the diagnostic accuracy of RAR and retinoid X receptor (RXR) subtype protein expression for the differential diagnosis of thyroid neoplasms. We used a tissue array containing 93 benign thyroid tissues (normal thyroid, multinodular goiter, and FA) and 77 thyroid carcinomas (papillary thyroid carcinoma (PTC), FTC and FVPTC). Immunostaining was performed for RAR and RXR subtypes. Staining was analyzed semiquantitatively, based on receiver operating curve (ROC) analyses and using hierarchical cluster analysis.

We found increased expression of cytoplasmic (c) RARalpha, cRARgamma, cRXRbeta and decreased expression of nuclear (n) RARbeta, nRARgamma, and nRXRalpha in thyroid carcinomas compared with benign tissues. We found three proteins expressed differently between FA and FTC and five proteins differentially expressed between FA and FVPTC, with high diagnostic accuracies. Using cluster analysis, the combination of negative staining of membranous RXRbeta and positive staining for cRXRbeta had a high positive predictive value (98%) for malignant thyroid disease, whereas the combination of positive nRXRalpha and negative cRXRbeta staining had a high predictive value (91%) for benign thyroid lesions.

We conclude that differences in RAR and RXR subtype protein expression may be valuable for the differential diagnosis of thyroid neoplasms. The results of this study and especially the value of cluster analysis have to be confirmed in subsequent studies.

Perspective

The findings of chapter 2 have some limitations before they can be implemented in standard diagnostic strategies. Although we were able to distinguish between follicular lesions, the number of follicular lesions was relatively small. Therefore, additional studies should be performed with larger numbers of follicular lesions, also including histological subtypes of follicular lesions. Moreover, the findings of our study and the

clinical usefulness of hierarchical cluster analysis have to be validated in subsequent studies and most importantly in cytological preparations. Also, other difficult-to-classify thyroid neoplasms such as minimally invasive follicular carcinomas as well as FA subclasses should be included in subsequent studies. The biological mechanisms responsible for the differential expression of RAR and RXR between thyroid tissues also remain to be elucidated.

III. Novel treatment strategies for non-RaI avid metastatic disease

1. Sorafenib for RaI non-avid DTC

Distant metastases, usually in the bones and lungs, occur in approximately 10-15% of patients with differentiated thyroid carcinoma (DTC). The major problem in this category of patients is the dedifferentiation of thyroid cancer and with that the diminished or lost ability to accumulate RaI. Treatment options for patients with RaI refractory metastases of DTC are limited. Metastatic thyroid cancer that has become inoperable or refractory to radioiodine therapy is associated with a poor 10 year survival of 5-10%.

The extensive characterization in recent years of the molecular pathways involved in the pathogenesis of DTC has revealed potential targets for new therapies. The identification of tyrosine kinase activated pathways in DTC together with the introduction of novel classes of tyrosine kinase inhibitors has provided new therapeutic perspectives for patients with non-RaI avid DTC. In DTC, a relationship has been identified between genetic alterations in the RET, RAS, RAF cascade and loss of NIS expression (21,22). Interestingly, in an *in-vitro* study, a multikinase inhibitor sunitinib was able to reinduce NIS expression in RET/PTC transformed thyroid cells (22). In addition, sunitinib also increased RaI uptake in FRTL-5 cells (23).

The anti-EGFR compound gefitinib was not successful in 27 patients with DTC, medullary or anaplastic thyroid carcinoma (24). In a phase II study in 60 thyroid carcinoma patients with various histologies, the VEGFR inhibitor axitinib showed a partial response of 30% (25). Motesanib diphosphate, a multitarget kinase inhibitor induced a partial response in 14% of 93 DTC patients (median PFS 40 weeks) (28). Two studies have been published using sorafenib. Sorafenib (BAY 43-9006) is an inhibitor of RET, C-RAF, wild-type and mutant (V600E) BRAF, VEGFR1, -2, -3, Flt3, and c-KIT. In the first study, including DTC, anaplastic and medullary thyroid carcinoma patients, sorafenib induced a partial response in 23% (median PFS 79 weeks) (26). The second study included patients with DTC but also with anaplastic thyroid

carcinoma. As a result of that, the response rate was probably significantly lower, 11% (median PFS 4.5–16 weeks) (27).

The mechanism behind the difference in response rate between the two multikinase inhibitors motesanib (14%) and sorafenib (23%) is probably based on the difference in IC50 for the tyrosine kinases which the compound inhibits. Motesanib has stronger inhibitory effects on VEGFR1 (IC50 2 nM), VEGFR2 (IC50 3 nM) and VEGFR3 (IC50nM), whereas sorafenib inhibits RET (IC 50 47nM), RET/PTC3 (IC50 50nM) and BRAF (IC50 22 nM). However comparison of the results of phase II studies with different tyrosine kinase inhibitors in DTC is hampered by differences in patient categories (including histologies, tumor stages, sites of metastases, and tumor extent), study design, and analytical methods.

We decided to study the effects of the multitarget tyrosine kinase inhibitor sorafenib on the reinduction of RaI uptake and tumor progression (**Chapter 3**). This was an open, single center, single arm, 26-week prospective phase II study with open-ended extension. We hypothesized that treatment with a multitarget tyrosine kinase inhibitor not only reduces tumor progression, but may also restore RaI uptake in non-RaI avid DTC. We treated 31 patients with progressive metastatic or locally advanced RaI refractory DTC with sorafenib 400 mg b.i.d. The primary endpoint was reinduction of RaI uptake at 26 weeks. Additional endpoints were the radiological response and the influence of bone metastases.

RaI therapy is the only available conventional therapy for patients with metastases of DTC. Hürthle cell carcinomas tend to respond less favorably to RaI, which is compatible with the fact that the most prevalent histology in our study was Hürthle cell metaplasia. The fact that 10/13 PTC harbored BRAFV600E mutations also illustrates the unfavorable prognostic characteristic of our patient group (29).

At 26 weeks of sorafenib therapy, unfortunately no reinduction of RaI uptake at metastatic sites was observed. However, 19 patients (59%) had a clinical beneficial response, eight of whom had a partial response (25%) and 11 had stable disease (34%). Seven patients had progressive disease (22%). The estimated median progression free survival was 58 weeks (95% confidence interval, CI, 47–68). In general, thyroglobulin (Tg) response (both unstimulated and TSH stimulated) reflected radiological response. The median time of the nadir of Tg levels was 3 months. Responses were not influenced by histological subtype, mutational status or other variables. No unusual side effects were observed. Sorafenib was significantly less effective in patients with bone metastases.

Although a clear relation has been found *in-vitro* between genetic alterations in DTC and decreased NIS gene expression (20,21), multiple mechanisms may be involved in decreased NIS functionality, including impaired NIS membrane trafficking (14,30), epigenetic changes in NIS and/or NIS promoter genes (31). Although

in-vitro studies have shown that multitarget tyrosine kinase inhibitors may lead to reinduction of RaI uptake (22,23), it may well be that these additional mechanisms have prevented a beneficial effect of sorafenib on RaI uptake in our study.

Perspective

The results of our study are comparable with the results of previous studies. The results obtained with sorafenib in different studies, including our own, suggest that sorafenib is a successful and promising compound for metastatic DTC. However we found that patients with bone metastases respond less favorably and that diagnostic WBS did not reveal an effect of sorafenib on the reinduction of RaI uptake in these patients. Future phase III studies should confirm the efficacy of sorafenib for DTC. At the moment a large multicenter phase III study is being performed internationally and the decision whether sorafenib will become part of regular treatment for non-RaI avid thyroid carcinoma will depend on the results of this trial.

2. Tyrosine kinase therapy and thyroid hormone metabolism

Therapy with tyrosine kinase inhibitors is associated with thyroid dysfunction. Sunitinib has been associated with hypothyroidism in 14–85% of the patients (32-37) and in some patients it even induced hyperthyroidism (37-39). Both sunitinib induced hypothyroidism and hyperthyroidism may be caused by destructive thyroiditis, but other mechanisms, such as interference of sunitinib with thyroid peroxidase (37) or inhibition of thyroidal vascularization leading to thyroid atrophy (40,41), have been proposed. Decreased serum thyroid hormone levels during tyrosine kinase inhibitor therapy are also observed in athyreotic patients on thyroxin substitution after treatment for thyroid carcinoma (26,28,42,43). Therefore, the mechanisms of hypothyroidism may include alterations in thyroid hormone metabolism as well. Stepwise deiodination is the major route of thyroid hormone degradation and is mediated by iodothyronine deiodinases (D1, D2, and D3) (44) and by hepatic conjugating enzymes (45).

In our study (**Chapter 4**), we assessed the relationship between treatment with the multitarget kinase inhibitor sorafenib and alterations in thyroid hormone parameters in athyreotic DTC patients. We hypothesized that sorafenib may influence thyroid hormone metabolism through the activities of iodothyronine deiodinases, which had not been studied in humans so far. The design included a prospective open, single-center, single-arm 26-week study. We measured serum thyroxine (T4), free T4, 3,5,3-triiodothyronine (T3), free T3, reverse T3 (rT3), and TSH concentrations at baseline and after 26 wk in 21 patients with progressive non-medullary thyroid

carcinoma treated with sorafenib. Ratios of T3/T4 and T3/rT3, which are independent of substrate availability and reflect iodothyronine deiodination, were calculated.

We found that a higher substitution dose of thyroxine was needed to maintain serum FT4 levels and T3 levels. Adjusted for levothyroxine dose per kilogram body weight, the FT4 and T3 levels decreased by 11% and 18%, respectively, whereas TSH levels increased. In addition, we found a clear decrease in serum T3/T4, T3/rT3, and T4/rT3 ratios. These ratios reflect alterations in the peripheral metabolism of thyroid hormone, being positively influenced by deiodinases D1 and D2 and negatively by D3 (46).

The decreased T3/T4 and T3/rT3 ratios may be caused by a decrease in D1 and/or D2 activity. However, this would be associated with a decreased rather than an increased metabolism of T4. Therefore, the decreased T3/T4 and T3/rT3 ratios are best explained by an increased D3 activity. It is unlikely that the increased D3 activity reflects a state of non thyroidal illness because serum TSH increased during sorafenib, whereas in non thyroidal illness, decreased rather than increased TSH levels would be expected.

Although it can be hypothesized that decreased absorption of T4 could also have played a role, the interval between sorafenib and thyroxine intake was approximately 12 hours. In addition, decreased T4 absorption would not affect T3/T4 and T3/rT3 ratios. No changes in thyroxine binding globulin levels and the ratios between free and bound thyroid hormones were observed, ruling out effects of sorafenib on thyroid hormone binding proteins, which again, even if present, would not have affected the T3/rT3 ratio. It may be hypothesized that sorafenib may also influence conjugation of thyroid hormone with glucuronates and sulfates by hepatic microsomal enzymes. However, altered conjugation would not influence T3/rT3 ratios.

Clinical implications

This study shows that, in addition to direct effects of tyrosine kinase inhibitors on the thyroid gland, enhanced peripheral metabolism of thyroid hormone, likely by activity of type 3 deiodinase, may contribute to hypothyroidism during therapy with these drugs. It is worthwhile to further elucidate the effects of sorafenib on D3 in *in-vitro* studies. Also, it is important to analyze thyroid hormone parameters regularly during treatment with tyrosine kinase inhibitors. Well-being of patients can be seriously decreased in case of hypothyroidism, which can be easily treated with exogenous T4 or in case of thyroidectomized patients, an increase in thyroxine dose.

IV. Consequences of treatment of thyroid carcinoma

Patients with DTC who are treated with total thyroidectomy and radioiodine ablative therapy become completely dependent on exogenous thyroid replacement therapy. Because of the favorable effects on tumor recurrence, patients used to be treated with TSH suppressive doses of thyroxine for approximately 15 years. Whereas this long-term TSH suppression is associated with an overall better prognosis (46,47), this subclinical hyperthyroid state is also associated with deleterious effects on multiple organ systems and on well-being. For this reason, recent guidelines recommend initial TSH suppression below 0.1 mU/L for high risk and intermediate risk thyroid cancer, while maintenance of TSH at or slightly below the lower limit of normal (0.1-0.5 mU/L) is appropriate for low-risk patients.

During follow-up, DTC patients used to be regularly withdrawn from thyroxine therapy to evaluate recurrence and disease state with TSH stimulated whole body scintigraphy and thyroglobulin measurement. This creates a state of controlled hypothyroidism.

The long term subclinical hyperthyroidism combined with episodes of short-term hypothyroidism make DTC patients an interesting model to study the effects of thyroid hormone. Also, there is no interference by endogenous thyroid hormone, because patients are treated with total thyroidectomy.

1. Insights in thyroid hormone metabolism

Peripheral thyroid metabolism is mainly regulated by the iodothyronine deiodinases D1, D2, and D3 (44,48). D1 converts T4 to T3, and is involved in serum T3 production. In addition, it plays a role in the breakdown of rT3 (49,50). D2 catalyzes local T3 production in various tissues (49,51,52). D2 in skeletal muscle may also contribute to plasma T3 production. D3 inactivates T3 and T4 and thus regulates the clearance of T3 and T4. It is thought that it contributes to thyroid hormone metabolism by protecting tissues from excess thyroid hormone. The deiodinases adjust the thyroid hormone levels of individual tissues in response to various conditions.

Several polymorphisms in the deiodinases have been described of which some are associated with alterations of serum levels of TSH, T3 and T4 (53-57). Most studies investigate the consequences of the D2-Thr92Ala (rs225014) polymorphism. Patients treated for DTC are ideal to investigate thyroid hormone metabolism, because they have been treated with total thyroidectomy and radioiodine ablation therapy. Because of this treatment they have no intrinsic T3 production. Therefore T3 levels are dependent on production at the tissue level through deiodination of exogenous T4 by D1 and D2. The negative feedback regulation of pituitary TSH secretion by T3, which

in DTC patients is completely produced outside the thyroid, is mainly dependent on pituitary D2.

The D2-Thr92Ala polymorphism has been associated with decreased D2 activity in some *in-vitro* experiments (53), but not in others (54,57). So far no association between the D2-Thr92Ala polymorphism and serum thyroid hormone levels has been observed in humans (49,53,54,57). However, in a recent study in athyroid patients, it was suggested that patients homozygous for the 92Ala allele need higher T4 doses to achieve TSH suppression (58). We therefore performed a study to reconfirm these findings (**Chapter 5**) in order to elucidate the association between the D2-Thr92Ala polymorphism, thyroid hormone levels and T4 dosage in patients treated for DTC and Hashimoto thyroiditis. We studied 154 patients with DTC treated with TSH suppressive thyroid hormone replacement therapy for longer than 3 years and 141 patients with Hashimoto thyroiditis treated for at least 6 months with thyroxine. In all patients, serum levels of TSH, free T4, T3 and reverse T3 were measured and genotypes of the D2-Thr92Ala polymorphism were determined by Taqman assay. Univariate regression analysis was performed to determine the relation between T4 dosages and the D2-Thr92Ala polymorphism corrected for age, gender, BMI and serum TSH levels.

Both in DTC patients and Hashimoto patients, no association was observed between serum thyroid hormone levels or T4 dosages in the presence of the D2-Thr92Ala polymorphism. Categorization of DTC patients according to degree of TSH suppression did not change these results. We concluded that the D2-Thr92Ala polymorphism was not associated with thyroid hormone levels or T4 dose in patients treated for neither DTC nor for Hashimoto thyroiditis.

Intraindividual variation in serum T4, T3 and TSH is narrow; however there is a considerable interindividual variability (59). A large body of evidence suggests that every individual has a unique thyroid function setpoint, compatible with a genetic influence on the regulation of the pituitary-thyroid axis (59-61). We hypothesized that polymorphisms in D1 and D2 could influence the setpoint of the hypothalamus-pituitary-thyroid axis **(Chapter 6)**.

We therefore performed a study on the effect of the following D1 and D2 polymorphism on this axis: D1-C785T (rs11206244), D1-A1814G (rs12095080), D2-Thr92Ala (rs225014) and D2-ORFa-Gly3Asp (rs12885300). Effects of these polymorphisms on the setpoints were analyzed with regression analysis using a general mixed model with a unique series 1905 serum measurements of TSH and FT4 of 151 patients treated and cured for DTC. These serum samples were collected as routine laboratory measurements during follow-up of the disease.

Our study demonstrates that thyroidectomised DTC patients on thyroxine substitution who are homozygous for the D2-ORFa-Gly3Asp polymorphism have an altered setpoint of the hypothalamus-pituitary-thyroid axis. The mixed model analysis

of the TSH/FT4 ratios is a precise approach to determine differences in individual setpoints. Our data suggest that the negative feedback of T4 on TSH is weaker in patients homozygous for the D2-ORFa-Gly3Asp than in wild-type and heterozygous subjects. We did not find any other differences in pituitary-thyroid axis for the other polymorphisms.

Although we have found a clear difference in the setpoint of the hypothalamus-pituitary-thyroid axis for the different D2-ORFa-Gly3Asp polymorphisms, there are some unknown factors that could have also influenced TSH/FT4 ratios. Unfortunately, because samples were collected as routine clinical follow-up, only TSH and FT4 levels were available, hence T3 and rT3 are only measured at one time point. Therefore we are not able to speculate about the serum values of T3 and rT3, and with that not the complete metabolic cycle of thyroid hormones during the entire period of the sample collection.

Our observations are in contrast with the findings of Coppotelli *et al*. (62) who found an increased D2 activity of the D2-ORFa-GlyAsp polymorphism in an *in-vitro* study and with the results of the study by Peeters *et al*. (56), who found that healthy blood donors with a D2-ORFa-Gly3Asp mutation needed less T4 to produce local T3 for the negative feedback action on the pituitary. These results were not confirmed in a group of healthy elderly men (56). However their observations in healthy blood donors with intrinsic thyroid function cannot be easily compared to DTC patients on TSH-suppressive thyroxine therapy.

Another factor could be that long term subclinical hyperthyroidism may result in downregulation of D1 and D2 and/or upregulation of D3 (48). However, we did not find a significant contribution of follow-up time and age at presentation to the observed effects of the D2-ORFa-Gly3Asp polymorphism on the setpoint of the hypothalamus-pituitary-thyroid axis.

Perspective

In our study no association was found between the Thr92Ala polymorphism and thyroxine dose. However, not many studies have been performed on this subject and results are discordant. Future studies are necessary to elucidate any major clinical implication of the Thr92Ala polymorphism.

In our second study we concluded that patients homozygous for the D2-ORFa-Gly3Asp polymorphism have an altered setpoint of the hypothalamus-pituitary-thyroid axis. However, it is unknown what the clinical significance of this altered setpoint will be. In the future, it would be interesting to investigate the proof of functionality of this D2 polymorphism and differences in biological variability in cell lines containing the different alleles of the D2-ORFa-Gly3Asp polymorphism.

2. Bone Metabolism

Although clinical observations suggest a clear involvement of thyroid hormone in bone metabolism, the molecular mechanisms by which thyroid hormone acts on bone are only partially uncovered so far. It is however an important subject since patients treated for thyroid carcinoma are treated with a TSH suppressive thyroxine dose during a long period of time.

T3 promotes osteoblastic proliferation, differentiation and apoptosis, and by induction of IL-6, prostaglandins and RANKL, and probably also promotes osteoclast formation and activation. This suggests that osteoblasts are the primary target cells for T3 in the regulation of bone remodeling (63-68). A functional role of TSH on skeletal development and metabolism has also been proposed on the basis of data obtained in animal studies (69-71) and in humans (72). This was however disputed by data obtained in thyroid hormone receptor (TR) deficient mice, which indicated that bone remodeling was predominantly mediated by T3 (64,72). It has also been reported recently in humans that there is a significant association between BMD and serum thyroid hormone concentrations rather than TSH (73).

Also the role of type 2 deiodinase (D2) in the human skeleton remains unclear. The D2 polymorphism Thr92Ala has been associated with lower TSH and lower enzymatic activity, which could result in lower local triiodothyronine (T3) availability in bone (53). We therefore performed a study to investigate a potential role for the deiodinase D2 in bone metabolism in humans by studying the relationship between the D2-Thr92Ala polymorphism, BMD, and bone turnover (**Chapter 7**). We studied this relationship in a human model of thyroidectomized patients cured from differentiated thyroid carcinoma receiving thyroid hormone substitution. The advantage of this model is that study subjects have uniform FT4 levels.

BMD and bone turnover markers [bone-specific alkaline phosphatase (BAP), cross-linking terminal C-telopeptide of type I collagen (CTX), procollagen type 1 aminoterminal propeptide (P1NP), and cross-linked N-telopeptide of type I collagen (NTX)] were measured. Sixty patients were wild type (Thr/Thr), 66 were heterozygous (Thr/Ala), and 28 were homozygous (Ala/Ala) for the D2 polymorphism.

In support of the involvement of D2 in bone metabolism was the observation of a 6% decrease in femoral neck BMD and increased levels of P1NP (32%), CTX (27%), and NTX/creatinine (54%) in the Ala/Ala subgroup compared with wild-type subgroup. Furthermore, these increased levels of bone formation (P1NP) and indicators of bone resorption (CTX and NTX) were independent of other determinants of bone metabolism, such as age, gender, BMI, estrogen status, calcium, vitamin D, PTH and most importantly independent of T3 and TSH. This may indicate a true effect of the D2-Thr92Ala polymorphism.

The effect the D2-Thr92Ala polymorphism on bone turnover markers is not easy to explain. It is conventionally accepted that higher rather than lower circulating thyroid hormone levels result in higher bone turnover and decreased bone mass. However, the model we used is unique in the sense that circulating T3 levels were similar among the three D2 genotypes, allowing us to specifically study the consequences of the polymorphism for local T3 availability in the bone microenvironment. Williams and colleagues (74) showed D2 activity in mature osteoblasts, but not in osteoclasts. The effects of the polymorphism on the markers of bone degradation (NTX/creatinine and CTX) therefore may not be explained by direct effects on osteoclasts but are more likely to result from changes in the interaction between osteoblasts and osteoclasts, possibly by alterations in the RANK/RANKL/OPG signaling pathway, which potentially can be modulated by local T3 availability in the bone microenvironment.

In the context of conflicting data on the functional role for TSH rather than T3 in skeletal metabolism, we performed a second study in order to dissect the effects of increased TSH levels from those of decreased thyroid hormone levels on bone (**Chapter 8**). We therefore studied the effects of recombinant human TSH (rhTSH) in 11 athyroid DTC patients on thyroxine substitution. In addition, we compared them with 11 age-, gender- and BMI-matched athyroid patients previously treated for differentiated thyroid carcinoma (DTC), who were studied after 4 weeks of thyroxine withdrawal and during thyroxine replacement therapy. We measured plasma levels of PTH, 25-OH-vitamin D, P1NP, CTX, RANKL and osteoprotegerin.

No differences were observed on parameters of bone turnover after rhTSH administration. During thyroxine withdrawal, levels of CTX were significantly lower, whereas levels of osteoprotegerin were significantly higher compared to thyroxine replacement therapy, indicating decreased bone resorption. Our findings suggest that acute changes in TSH in the presence of stable thyroid hormone levels obtained by rhTSH administration do not significantly affect skeletal metabolism. Moreover, it can be suggested that hypothyroidism results in decreased bone turnover rather by decreased plasma thyroid hormone concentrations than by increased TSH concentrations, because rhTSH had no impact on bone turnover in DTC patients.

In summary our data suggest that a decrease in local availability of T3 potentially owing to a D2 polymorphism may result in increased bone turnover and decreased bone mass at the predominantly cortical femoral neck. We believe that our study provides additional information on the role of D2 in bone metabolism and the functional consequences of the D2-Thr92Ala polymorphism, supporting a role for D2 in mature bone cells.

The data of the second study concluded that bone turnover is decreased during hypothyroidism due to thyroxine withdrawal in DTC patients. As rhTSH had no impact on bone turnover, it can be suggested that low thyroid hormone levels instead

of the increased TSH levels are responsible for the decreased bone resorption during hypothyroidism in DTC patients. We believe therefore that alterations in thyroid hormone levels are of more importance for bone turnover then TSH levels.1

Perspective

Although the observations of our studies suggest a clear involvement of thyroid hormone in bone metabolism, the molecular mechanisms by which thyroid hormone acts on bone has not been completely discovered. It is an important subject though, in patients treated for thyroid carcinoma on a TSH suppressive thyroxine dose. These patients may be at risk for osteoporosis, which is however mainly reported in postmenopausal women. In these patients screening at baseline and during TSH suppressive therapy is advised to allow timely intervention with bone protective agents.

3. Cardiac function

Thyroid hormone has profound effects on the cardiovascular system. Hyperthyroidism induces cardiac arrhythmias, left ventricular (LV) hypertrophy and diastolic dysfunction, and enhances systolic function (75-78). Subclinical hyperthyroidism, resulting from TSH suppressive thyroxine therapy, is associated with increased heart rate and supraventricular arrhythmias, increased LV mass (LVM) with a slightly enhanced systolic function, and diastolic dysfunction. Diastolic dysfunction is at least partly reversible after restoration of euthyroidism (78-80). Conversely, hypothyroidism is associated with bradycardia, hypertension, increased peripheral cardiovascular resistance, heart failure (75,78,81), decreased cardiac output and diastolic dysfunction (75,77,81). Hypothyroidism is also associated with coronary artery disease, presumably because of associated hypercholesterolaemia, hypertriglyceridaemia and hypertension (75,77,82).

The consequences of episodes of acute hypothyroidism on cardiac function have been investigated in only a few studies, and their results are inconclusive (83-90). We therefore performed a study aimed at the investigation of the effects of overt hypothyroidism on cardiac function in patients with iatrogenically induced subclinical hyperthyroidism after treatment for differentiated thyroid carcinoma (**Chapter 9**). Fourteen patients with a history of differentiated thyroid carcinoma on thyroid stimulating hormone (TSH)-suppressive thyroxine replacement therapy were studied. We assessed cardiac function before, and 1 and 4 weeks after withdrawal of thyroxine substitution. We measured serum levels of free thyroxin, triiodothyronine and TSH and used a new sophisticated Doppler echocardiography technique, tissue Doppler imaging (TDI), to assess detailed and quantitative assessment of systolic and diastolic

cardiac function. Echocardiographic parameters in patients were compared to controls without cardiac disease.

At baseline, when patients had subclinical hyperthyroidism, echocardiography revealed decreased diastolic function, higher LV size and LV mass. The clinical consequences of isolated diastolic dysfunction in subclinical hyperthyroidism are not entirely clear, but could be accompanied by increased morbidity and mortality, especially in long-term subclinical hyperthyroidism (74).

Thyroxine withdrawal resulted in an additional subtle decrease in both E- and A-wave velocities, without an impact on E/A ratio, indicating discrete unfavorable effects on diastolic function as assessed by echocardiography. When more specifically analyzed by TDI, diastolic function decreased, with a decrease in late diastolic velocity (A′) without impact on the E′/A′ ratio. Overt hypothyroidism increased diastolic blood pressure significantly, but had no effect on systolic blood pressure. Therefore, long-term subclinical hyperthyroidism is accompanied by diastolic dysfunction. Subsequent acute overt hypothyroidism induces subtle unfavorable changes in diastolic function.

Only six patients had an E/A ratio below 1 during overt hypothyroidism. This is probably due to impaired ventricular relaxation associated with a delay in the energy-dependent reuptake of calcium by the sacroplasmatic reticulum, which in turn is under thyroid hormone control. This thyroid hormone control of cardiac function is mediated mainly by T3, which in our study declined significantly during thyroxine withdrawal (77).

Perspective

We demonstrated that long-term iatrogenically induced subclinical hyperthyroidism in patients with DTC induces diastolic dysfunction and increases LV mass and size. It is therefore not recommended to treat all patients with TSH suppressive thyroxine replacement unconditionally.

Acute overt hypothyroidism induced only minimal unfavorable cardiovascular effects, but significantly increased diastolic blood pressure during thyroxine withdrawal. The potential negative cardiovascular consequences of thyroxine withdrawal before diagnostic iodine-131 whole body scanning could be clinically relevant, especially in patients at cardiovascular risk. Therefore, recombinant TSH stimulation might be an attractive alternative in low-risk thyroid carcinoma patients and/or high-risk cardiovascular patients.

4. Quality of life

Quality of life may be affected in DTC patients by either the diagnosis of having a malignant disease, with the impact of the initial therapy, or by the consequences of TSH suppressive therapy. A few studies have investigated this subject, but results are inconclusive (91-95). For that reason, we studied quality of life in a large cohort of cured DTC patients. For this we used multiple quality of life questionnaires and compared the results to those of a large group of healthy controls, who weren matched for age, gender and socioeconomic status (**Chapter 10**). Longer duration of cure was associated with better scores on different quality-of-life items. After a long duration of cure, approximately 12–20 yr, 6 of the 16 quality of life subscales were comparable with the quality of life of healthy controls. Our findings indicate decreased quality of life in DTC patients, which may restore after a long period of follow-up.

The consequences of long duration of subclinical hyperthyroidism are less clear (78,91,92). Studies investigating this subject included selected groups of DTC patients or patients with endogenous subclinical hyperthyroidism in which duration and course of subclinical hyperthyroidism were not known. In our study, quality of life was not affected by alterations in TSH during the complete period of follow-up.

Perspective

Despite cure, excellent prognosis, and moderate aggressive treatment, DTC patients have an evident decrease in quality of life that may be restored only after years of follow-up. The findings of our study have therefore implications for the approach of the cured DTC patients: attention for the psychological well-being of the patient and availability of professional support may be important aspects during follow-up.w

References

1. Davies L and Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA 2006 295 2164-2167
2. Davies L and Welch HG. Thyroid cancer survival in the United States: observational data from 1973 to 2005. Archives of Otolaryngology: Head and Neck Surgery 2010 136 440-444
3. Hundahl SA, Fleming ID, Fremgen AM, and Menck HR. A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995 [see comments]. Cancer 1998 83 2638-2648
4. Jemal A, Siegel R, Ward E, Hao Y, Xu J, and Thun MJ. Cancer statistics, 2009. CA: Cancer Journal for Clinicians 2009 59 225-249
5. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, and Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009 19 1167-1214
6. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, and Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. European Journal of Endocrinology 2006 154 787-803
7. Haugen BR, Woodmansee WW, and McDermott MT. Towards improving the utility of fine-needle aspiration biopsy for the diagnosis of thyroid tumours. Clinical Endocrinology (Oxf) 2002 56 281-290
8. Bartolazzi A, Orlandi F, Saggiorato E, Volante M, Arecco F, Rossetto R, Palestini N, Ghigo E, Papotti M, Bussolati G, Martegani MP, Pantellini F, Carpi A, Giovagnoli MR, Monti S, Toscano V, Sciacchitano S, Pennelli GM, Mian C, Pelizzo MR, Rugge M, Troncone G, Palombini L, Chiappetta G, Botti G, Vecchione A, and Bellocco R. Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study. Lancet Oncology 2008 9 543-549
9. Kim SW, In LJ, Kim JW, Ki CS, Oh YL, Choi YL, Shin JH, Kim HK, Jang HW, and Chung JH. BRAFV600E Mutation Analysis in Fine-Needle Aspiration Cytology Specimens for Evaluation of Thyroid Nodule: A Large Series in a BRAFV600E-Prevalent Population. Journal of Clinical Endocrinology and Metabolism 2010
10. Kwak JY, Kim EK, Kim JK, Han JH, Hong SW, Park TS, and Choi JR. Dual priming oligonucleotide-based multiplex PCR analysis for detection of BRAFV600E mutation in FNAB samples of thyroid nodules in BRAFV600E mutation-prevalent area. Head and Neck 2010 32 490-498
11. Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, and Nikiforova MN. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. Journal of Clinical Endocrinology and Metabolism 2009 94 2092-2098
12. Sapio MR, Posca D, Raggioli A, Guerra A, Marotta V, Deandrea M, Motta M, Limone PP, Troncone G, Caleo A, Rossi G, Fenzi G, and Vitale M. Detection of RET/PTC, TRK and BRAF mutations in preoperative diagnosis of thyroid nodules with indeterminate cytological findings. Clinical Endocrinology (Oxf) 2007 66 678-683
13. Segev DL, Clark DP, Zeiger MA, and Umbricht C. Beyond the suspicious thyroid fine needle aspirate. A review. Acta Cytology 2003 47 709-722
14. Liu YY, Morreau H, Kievit J, Romijn JA, Carrasco N, and Smit JW. Combined immunostaining with galectin-3, fibronectin-1, CITED-1, Hector Battifora mesothelial-1, cytokeratin-19,

peroxisome proliferator-activated receptor-{gamma}, and sodium/iodide symporter antibodies for the differential diagnosis of non-medullary thyroid carcinoma. European Journal of Endocrinology 2008 158 375-384

15. Zhao J, Leonard C, Gemsenjager E, Heitz PU, Moch H, and Odermatt B. Differentiation of human follicular thyroid adenomas from carcinomas by gene expression profiling. Oncology Reports 2008 19 329-337
16. Zhao J, Leonard C, Brunner E, Gemsenjager E, Heitz PU, and Odermatt B. Molecular characterization of well-differentiated human thyroid carcinomas by cDNA arrays. International Journal of Oncology 2006 29 1041-1051
17. Lubitz CC and Fahey TJ, III. Gene expression profiling of thyroid tumors--clinical applicability. National Clinical Practice in Endocrinoogy nadl Metabolism 2006 2 472-473
18. Stolf BS, Abreu CM, Mahler-Araujo MB, Dellamano M, Martins WK, de Carvalho MB, Curado MP, Diaz JP, Fabri A, Brentani H, Carvalho AF, Soares FA, Kowalski LP, Hirata R, Jr., and Reis LF. Expression profile of malignant and non-malignant diseases of the thyroid gland reveals altered expression of a common set of genes in goiter and papillary carcinomas. Cancer Letters 2005 227 59-73
19. Finley DJ, Lubitz CC, Wei C, Zhu B, and Fahey TJ, III. Advancing the molecular diagnosis of thyroid nodules: defining benign lesions by molecular profiling. Thyroid 2005 15 562-568
20. De Vita G, Zannini M, Cirafici AM, Melillo RM, Di Lauro R, Fusco A, and Santoro M. Expression of the RET/PTC1 oncogene impairs the activity of TTF-1 and Pax-8 thyroid transcription factors. Cell Growth and Differentation 1998 9 97-103
21. Riesco-Eizaguirre G, Rodriguez I, De l, V, Costamagna E, Carrasco N, Nistal M, and Santisteban P. The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. Cancer Research 2009 69 8317-8325
22. Fenton MS, Marion K, Salem AK, Hogen R, Naeim F, and Hershman. Induction of cell cycle arrest by sunitinib through inhibition of the MEK/ERK pathway in RET/PTC1 papillary thyroid cancer cells. 90th Annual Meeting of the Endocrine Society, San Francisco (abstract) 1990
23. Salem AK, Fenton MS, Marion KM, and Hershman JM. Effect of sunitinib on growth and function of FRTL-5 thyroid cells. Thyroid 2008 18 631-635
24. Pennell NA, Daniels GH, Haddad RI, Ross DS, Evans T, Wirth LJ, Fidias PH, Temel JS, Gurubhagavatula S, Heist RS, Clark JR, and Lynch TJ. A phase II study of gefitinib in patients with advanced thyroid cancer. Thyroid 2008 18 317-323
25. Cohen EE, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, Kane MA, Sherman E, Kim S, Bycott P, Tortorici M, Shalinsky DR, Liau KF, and Cohen RB. Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. Journal of Clinical Oncology 2008 26 4708-4713
26. Gupta-Abramson V, Troxel AB, Nellore A, Puttaswamy K, Redlinger M, Ransone K, Mandel SJ, Flaherty KT, Loevner LA, O'Dwyer PJ, and Brose MS. Phase II trial of sorafenib in advanced thyroid cancer. Journal of Clinical Oncology 2008 26 4714-4719K
27. Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, Liang J, Wakely PE, Jr., Vasko VV, Saji M, Rittenberry J, Wei L, Arbogast D, Collamore M, Wright JJ, Grever M, and Shah MH. Phase II trial of sorafenib in metastatic thyroid cancer. Journal of Clinical Oncology 2009 27 1675-1684

28. Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, Licitra L, Eschenberg MJ, Sun YN, Juan T, Stepan DE, and Schlumberger MJ. Motesanib diphosphate in progressive differentiated thyroid cancer. New England Journal of Medicine 2008 359 31-42
29. Fagin JA. How thyroid tumors start and why it matters: kinase mutants as targets for solid cancer pharmacotherapy. Journal of Endocrinology 2004 183 249-256
30. Wapnir IL, van de RM, Nowels K, Amenta PS, Walton K, Montgomery K, Greco RS, Dohan O, and Carrasco N. Immunohistochemical profile of the sodium/iodide symporter in thyroid, breast, and other carcinomas using high density tissue microarrays and conventional sections. Journal of Clinical Endocrinology and Metabolism 2003 88 1880-1888
31. Furuya F, Shimura H, Suzuki H, Taki K, Ohta K, Haraguchi K, Onaya T, Endo T, and Kobayashi T. Histone deacetylase inhibitors restore radioiodine uptake and retention in poorly differentiated and anaplastic thyroid cancer cells by expression of the sodium/iodide symporter thyroperoxidase and thyroglobulin. Endocrinology 2004 145 2865-2875
32. Desai J, Yassa L, Marqusee E, George S, Frates MC, Chen MH, Morgan JA, Dychter SS, Larsen PR, Demetri GD, and Alexander EK. Hypothyroidism after sunitinib treatment for patients with gastrointestinal stromal tumors. Annals of Internal Medicine 2006 145 660-664
33. Mannavola D, Coco P, Vannucchi G, Bertuelli R, Carletto M, Casali PG, Beck-Peccoz P, and Fugazzola L. A novel tyrosine-kinase selective inhibitor, sunitinib, induces transient hypothyroidism by blocking iodine uptake. Journal of Clinical Endocrinology and Metabolism 2007 92 3531-3534
34. Rini BI, Tamaskar I, Shaheen P, Salas R, Garcia J, Wood L, Reddy S, Dreicer R, and Bukowski RM. Hypothyroidism in patients with metastatic renal cell carcinoma treated with sunitinib. Journal of the National Cancer Instute 2007 99 81-83
35. Wong E, Rosen LS, Mulay M, Vanvugt A, Dinolfo M, Tomoda C, Sugawara M, and Hershman JM. Sunitinib induces hypothyroidism in advanced cancer patients and may inhibit thyroid peroxidase activity. Thyroid 2007 17 351-355
36. Chu TF, Rupnick MA, Kerkela R, Dallabrida SM, Zurakowski D, Nguyen L, Woulfe K, Pravda E, Cassiola F, Desai J, George S, Morgan JA, Harris DM, Ismail NS, Chen JH, Schoen FJ, Van den Abbeele AD, Demetri GD, Force T, and Chen MH. Cardiotoxicity associated with tyrosine kinase inhibitor sunitinib. Lancet 2007 370 2011-2019
37. Wolter P, Stefan C, Decallonne B, Dumez H, Bex M, Carmeliet P, and Schoffski P. The clinical implications of sunitinib-induced hypothyroidism: a prospective evaluation. British Journal of Cancer 2008 99 448-454
38. Grossmann M, Premaratne E, Desai J, and Davis ID. Thyrotoxicosis during sunitinib treatment for renal cell carcinoma. Clinical Endocrinology (Oxf) 2008 69 669-672
39. Faris JE, Moore AF, and Daniels GH. Sunitinib (sutent)-induced thyrotoxicosis due to destructive thyroiditis: a case report. Thyroid 2007 17 1147-1149
40. Kamba T, Tam BY, Hashizume H, Haskell A, Sennino B, Mancuso MR, Norberg SM, O'Brien SM, Davis RB, Gowen LC, Anderson KD, Thurston G, Joho S, Springer ML, Kuo CJ, and McDonald DM. VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. American Journal of Physiology – Heart and Circulatory Physiology 2006 290 H560-H576
41. Kamba T and McDonald DM. Mechanisms of adverse effects of anti-VEGF therapy for cancer. British Journal of Cancer 2007 96 1788-1795

42. de Groot JW, Zonnenberg BA, Plukker JT, Der Graaf WT, and Links TP. Imatinib induces hypothyroidism in patients receiving levothyroxine. Clinical Pharmacology and Therapy 2005 78 433-438
43. de Groot JW, Links TP, and van der Graaf WT. Tyrosine kinase inhibitors causing hypothyroidism in a patient on levothyroxine. Annals of Oncology 2006 17 1719-1720
44. Bianco AC, Salvatore D, Gereben B, Berry MJ, and Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocrine Reviews 2002 23 38-89
45. Eelkman Rooda SJ, Kaptein E, and Visser TJ. Serum triiodothyronine sulfate in man measured by radioimmunoassay. Journal of Clinical Endocrinology and Metabolism 1989 69 552-556
46. Cooper DS, Specker B, Ho M, Sperling M, Ladenson PW, Ross DS, Ain KB, Bigos ST, Brierley JD, Haugen BR, Klein I, Robbins J, Sherman SI, Taylor T, and Maxon HR, III. Thyrotropin suppression and disease progression in patients with differentiated thyroid cancer: results from the National Thyroid Cancer Treatment Cooperative Registry. Thyroid 1998 8 737-744
47. Hovens GC, Stokkel MP, Kievit J, Corssmit EP, Pereira AM, Romijn JA, and Smit JW. Associations of serum thyrotropin concentrations with recurrence and death in differentiated thyroid cancer. Journal of Clinical Endocrinology and Metabolism 2007 92 2610-2615
48. Bianco AC and Kim BW. Deiodinases: implications of the local control of thyroid hormone action. Journal of Clinical Investigation 2006 116 2571-2579
49. de Jong FJ, Peeters RP, den Heijer T, van der Deure WM, Hofman A, Uitterlinden AG, Visser TJ, and Breteler MM. The association of polymorphisms in the type 1 and 2 deiodinase genes with circulating thyroid hormone parameters and atrophy of the medial temporal lobe. Journal of Clinical Endocrinology and Metabolism 2007 92 636-640
50. Kohrle J. Local activation and inactivation of thyroid hormones: the deiodinase family. Mol Cell Endocrinol 1999 151 103-119
51. Maeda A, Toyoda N, Yasuzawa-Amano S, Iwasaka T, and Nishikawa M. Type 2 deiodinase expression is stimulated by growth factors in human vascular smooth muscle cells. Molecular and Cellular Endocrinology 2003 200 111-117
52. Salvatore D, Bartha T, Harney JW, and Larsen PR. Molecular biological and biochemical characterization of the human type 2 selenodeiodinase. Endocrinology 1996 137 3308-3315
53. Canani LH, Capp C, Dora JM, Meyer EL, Wagner MS, Harney JW, Larsen PR, Gross JL, Bianco AC, and Maia AL. The type 2 deiodinase A/G (Thr92Ala) polymorphism is associated with decreased enzyme velocity and increased insulin resistance in patients with type 2 diabetes mellitus. Journal of Clinical Endocrinology and Metabolism 2005 90 3472-3478
54. Mentuccia D, Proietti-Pannunzi L, Tanner K, Bacci V, Pollin TI, Poehlman ET, Shuldiner AR, and Celi FS. Association between a novel variant of the human type 2 deiodinase gene Thr92Ala and insulin resistance: evidence of interaction with the Trp64Arg variant of the beta-3-adrenergic receptor. Diabetes 2002 51 880-883
55. Peeters RP, van Toor H, Klootwijk W, de Rijke YB, Kuiper GG, Uitterlinden AG, and Visser TJ. Polymorphisms in thyroid hormone pathway genes are associated with plasma TSH and iodothyronine levels in healthy subjects. Journal of Clinical Endocrinology and Metabolism 2003 88 2880-2888
56. Peeters RP, van den Beld AW, Attalki H, Toor H, de Rijke YB, Kuiper GG, Lamberts SW, Janssen JA, Uitterlinden AG, and Visser TJ. A new polymorphism in the type II deiodinase gene is associated with circulating thyroid hormone parameters. American Journal of Physiology, Endocrinology and Metabolism 2005 289 E75-E81

57. Peeters RP, van der Deure WM, and Visser TJ. Genetic variation in thyroid hormone pathway genes; polymorphisms in the TSH receptor and the iodothyronine deiodinases. European Journal of Endocrinology 2006 155 655-662
58. Torlontano M, Durante C, Torrente I, Crocetti U, Augello G, Ronga G, Montesano T, Travascio L, Verrienti A, Bruno R, Santini S, D'Arcangelo P, Dallapiccola B, Filetti S, and Trischitta V. Type 2 deiodinase polymorphism (threonine 92 alanine) predicts L-thyroxine dose to achieve target thyrotropin levels in thyroidectomized patients. Journal of Clinical Endocrinology and Metabolism 2008 93 910-913
59. Hansen PS, Brix TH, Sorensen TI, Kyvok KO, Hegedus L. Major genetic influence on the regulation of the pituitary-thyroid-axis: a study of healthy Danish twins. Journal of Clinical Endocrinology and Metabolism 2004 89:1181-1187
60. Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. Journal of Clinical Endocrinology and Metabolism 2002 87:1068-1072
61. Benhadi N, Fliers E, Visser TJ, Reitsma JB, Wiersinga WM. Pilot study on the assessment of the setpoint of the hypothalamus-pituitary-thyroid axis in healthy volunteers. European Journal of Endocrinology 2010 162:323-329
62. Coppotelli G, Summers A, Chidakel A, Ross JM, Celi FS. Functional characterization of the 258 A/G (D2-ORFa-Gly3Asp) human type-2 deiodinase polymorphism: a naturally occurring variant increases the enzymatic activity by removing a putative repressor site in the 5′ UTR of the gene. Thyroid 2006 16 625-632
63. Bassett JH and Williams GR. The molecular actions of thyroid hormone in bone. Trends in Endocrinology and Metabolism 2003 14 356-364
64. Bassett JH, O'Shea PJ, Sriskantharajah S, Rabier B, Boyde A, Howell PG, Weiss RE, Roux JP, Malaval L, Clement-Lacroix P, Samarut J, Chassande O, and Williams GR. Thyroid hormone excess rather than thyrotropin deficiency induces osteoporosis in hyperthyroidism. Molecular Endocrinology 2007 21 1095-1107
65. Bassett JH and Williams GR. Critical role of the hypothalamic-pituitary-thyroid axis in bone. Bone 2008 43 418-426
66. Britto JM, Fenton AJ, Holloway WR, and Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994 134 169-176
67. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, and Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. J Cell Physiology 2004 201 17-25
68. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, and Nakao K. A novel interaction between thyroid hormones and 1,25(OH)(2)D(3) in osteoclast formation. Biochemical and Biophysical Research Communications 2002 291 987-994
69. Abe E, Marians RC, Yu W, Wu XB, Ando T, Li Y, Iqbal J, Eldeiry L, Rajendren G, Blair HC, Davies TF, and Zaidi M. TSH is a negative regulator of skeletal remodeling. Cell 2003 115 151-162
70. Galliford TM, Murphy E, Williams AJ, Bassett JH, and Williams GR. Effects of thyroid status on bone metabolism: a primary role for thyroid stimulating hormone or thyroid hormone? Minerva Endocrinology 2005 30 237-246
71. Morimura T, Tsunekawa K, Kasahara T, Seki K, Ogiwara T, Mori M, and Murakami M. Expression of type 2 iodothyronine deiodinase in human osteoblast is stimulated by thyrotropin. Endocrinology 2005 146 2077-2084

72. Bassett JH, Nordstrom K, Boyde A, Howell PG, Kelly S, Vennstrom B, and Williams GR. Thyroid status during skeletal development determines adult bone structure and mineralization. Molecular Endocrinology 2007 21 1893-1904
73. van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, and Visser TJ. Effects of serum TSH and FT4 levels and the TSHR-Asp727Glu polymorphism on bone: the Rotterdam Study. Clinical Endocrinology (Oxf) 2008 68 175-181
74. Williams AJ, Robson H, Kester MH, van Leeuwen JP, Shalet SM, Visser TJ, and Williams GR. Iodothyronine deiodinase enzyme activities in bone. Bone 2008 43 126-134
75. Fazio S, Palmieri EA, Lombardi G, and Biondi B. Effects of thyroid hormone on the cardiovascular system. Recent Progress in Hormone Research 2004 59 31-50
76. Forfar JC, Muir AL, Sawers SA, and Toft AD. Abnormal left ventricular function in hyperthyroidism: evidence for a possible reversible cardiomyopathy. New England Journal of Medicine 1982 307 1165-1170
77. Klein I and Ojamaa K. Thyroid hormone and the cardiovascular system. New England Journal of Medicine 2001 344 501-509
78. Biondi B, Palmieri EA, Fazio S, Cosco C, Nocera M, Sacca L, Filetti S, Lombardi G, and Perticone F. Endogenous subclinical hyperthyroidism affects quality of life and cardiac morphology and function in young and middle-aged patients. Journal of Clinical Endocrinology and Metabolism 2000 85 4701-4705
79. Heeringa J, Hoogendoorn EH, van der Deure WM, Hofman A, Peeters RP, Hop WC, den Heijer M, Visser TJ, and Witteman JC. High-normal thyroid function and risk of atrial fibrillation: the Rotterdam study. Archives of Internal Medicine 2008 168 2219-2224
80. Ladenson PW. Recognition and management of cardiovascular disease related to thyroid dysfunction. American Journal of Medicine 1990 88 638-641
81. Rodondi N, Bauer DC, Cappola AR, Cornuz J, Robbins J, Fried LP, Ladenson PW, Vittinghoff E, Gottdiener JS, and Newman AB. Subclinical thyroid dysfunction, cardiac function, and the risk of heart failure. The Cardiovascular Health study. Journal of the American College of Cardiology 2008 52 1152-1159
82. Kahaly GJ and Dillmann WH. Thyroid hormone action in the heart. Endocr Rev 2005 26 704-728
83. Botella-Carretero JI, Gomez-Bueno M, Barrios V, Caballero C, Garcia-Robles R, Sancho J, and Escobar-Morreale HF. Chronic thyrotropin-suppressive therapy with levothyroxine and short-term overt hypothyroidism after thyroxine withdrawal are associated with undesirable cardiovascular effects in patients with differentiated thyroid carcinoma. Endocrine Related Cancer 2004 11 345-356
84. Chrisoulidou A, Pazaitou-Panayiotou K, Kaprara A, Platoyiannis D, Lafaras C, Boudina M, Georgiou E, Drimonitis A, Bischiniotis T, and Vainas I. Effects of thyroxine withdrawal in biochemical parameters and cardiac function and structure in patients with differentiated thyroid cancer. Minerva Endocrinology 2006 31 173-178
85. Di Paola R, Alagona C, Pezzino V, Mangiameli S, and Regalbuto C. Left ventricular function in acute hypothyroidism: a Doppler echocardiography study. Italian Heart Journal 2004 5 857-863
86. Donaghue K, Hales I, Allwright S, Cooper R, Edwards A, Grant S, Morrow A, and Wilmshurst E. Cardiac function in acute hypothyroidism. European Journal of Nuclear Medicine 1985 11 147-149

87. Grossmann G, Wieshammer S, Keck FS, Goller V, Giesler M, and Hombach V. Doppler echocardiographic evaluation of left ventricular diastolic function in acute hypothyroidism. Clinical Endocrinology (Oxf) 1994 40 227-233
88. Grossmann G, Keck FS, Wieshammer S, Goller V, Schmidt A, and Hombach V. Systolic ventricular function in acute hypothyroidism: a study using Doppler echocardiography. Experimental Clinical Endocrinology 1994 102 104-110
89. Kahaly G, Mohr-Kahaly S, Beyer J, and Meyer J. Left ventricular function analyzed by Doppler and echocardiographic methods in short-term hypothyroidism. American Journal of Cardiology 1995 75 645-648
90. Wieshammer S, Keck FS, Waitzinger J, Henze E, Loos U, Hombach V, and Pfeiffer EF. Acute hypothyroidism slows the rate of left ventricular diastolic relaxation. Canadian Journal of Physiology and Pharmacology 1989 67 1007-1010
91. Biondi B, Fazio S, Carella C, Sabatini D, Amato G, Cittadini A, Bellastella A, Lombardi G, and Sacca L. Control of adrenergic overactivity by beta-blockade improves the quality of life in patients receiving long term suppressive therapy with levothyroxine. Journal of Clinical Endocrinology and Metabolism 1994 78 1028-1033
92. Giusti M, Sibilla F, Cappi C, Dellepiane M, Tombesi F, Ceresola E, Augeri C, Rasore E, and Minuto F. A case-controlled study on the quality of life in a cohort of patients with history of differentiated thyroid carcinoma. Journal of Endocrinology Investigation 2005 28 599-608
93. Gulseren S, Gulseren L, Hekimsoy Z, Cetinay P, Ozen C, and Tokatlioglu B. Depression, anxiety, health-related quality of life, and disability in patients with overt and subclinical thyroid dysfunction. Archives of Medical Research 2006 37 133-139
94. Schultz PN, Stava C, and Vassilopoulou-Sellin R. Health profiles and quality of life of 518 survivors of thyroid cancer. Head & Neck 2003 25 349-356
95. Tan LG, Nan L, Thumboo J, Sundram F, and Tan LK. Health-related quality of life in thyroid cancer survivors. Laryngoscope 2007 117 507-510

12

Nederlandse samenvatting

Inhoud

I. Introductie

Gedifferentieerd schildkliercarcinoom is een aandoening met een lage incidentie van 1/100.000 personen per jaar en een relatief gunstige prognose met een 10-jaars overleving van 90-95%. De incidentie is de afgelopen decennia gestegen, en deze trend lijkt zich te continueren. De prevalentie is echter relatief hoog door de gunstige prognose als gevolg van een relatief langzaam groeiende tumor en een zeer effectieve initiële behandeling. Deze initiële behandeling bestaat uit totale thyreoïdectomie en ablatieve therapie met radioactief jodium (RaJ). De prognose wordt aanzienlijk slechter indien er metastasen op afstand bestaan. Deze metastasen ontstaan voornamelijk in longen en bot en kunnen door dedifferentiatie minder jodium opnemen en raken daardoor vaak ongevoelig voor RaJ therapie. RaJ therapie is de enige curatieve behandelingsoptie. Metastasen zijn niet onmiddellijk levensbedreigend, maar geven vaak wel aanzienlijke klachten en beperken daarmee de kwaliteit van leven. Helaas zijn in het geval van gemetastaseerde ziekte conventionele behandelingen als chemotherapie en radiotherapie vaak weinig effectief.

Recent is veel onderzoek gedaan naar de pathogenese van schildkliercarcinoom, waarbij veel nieuwe inzichten zijn ontdekt in de genetische ontwikkeling van schildklierkanker. Hierbij zijn nieuwe potentiële aangrijpingspunten ontdekt voor de behandeling van gemetastaseerde ziekte die ongevoelig is geworden voor RaJ. Tyrosinekinaseremmers, zoals sorafenib, zijn hierbij veelbelovende nieuwe medicamenten.

Ondanks de lage incidentie, wordt de behandeling van schildkliercarcinoom in verscheidene ziekenhuizen verricht. Deze gedecentraliseerde aanpak draagt waarschijnlijk niet bij aan een optimale behandeling. Wel dragen recente publicaties van artikelen met Europese en Amerikaanse richtlijnen voor de behandeling van schildkliercarcinoom bij aan de verbetering van de behandeling door de implementatie van uniforme behandelingsprotocollen en follow-up strategieën. Daarbij is schildkliercarcinoom een bijzondere kankersoort met unieke biologische fenomenen zoals het bijvoorbeeld het jodiumtransport. Dit zorgt ervoor dat standaard oncologische behandelingen niet zomaar geëxtrapoleerd kunnen worden naar schildkliercarcinoom.

Ongeacht deze richtlijnen, zijn er nog steeds onzekerheden en blijven er nog steeds verschillende opinies bestaan over de optimale strategie voor diagnose, behandeling en follow-up van schildkliercarcinoom. Een voorbeeld hiervan is dat de optimale strategie voor de diagnose van schildkliercarcinoom nog steeds afhankelijk is van conventionele histologische kleuringen. Hiermee is met name het onderscheid tussen folliculair adenoom (goedaardig) en folliculair carcinoom (kwaadaardig) moeilijk te maken.

Na de initiële behandeling voor schildkliercarcinoom zijn patiënten afhankelijk van de substitutie van schildklierhormoon door middel van thyroxine. Zij worden

ingesteld op een hoge dosering schildklierhormoon om het thyroid stimulerend hormoon (TSH) te onderdrukken. TSH wordt geproduceerd door de hypofyse en stimuleert normaal gesproken de schildklier tot het maken van schildklierhormoon. Het is echter ook een groeifactor voor schildkliercellen en dus kan de remming ervan de potentiële groei van eventuele achtergebleven tumorcellen onderdrukken. Met de onderdrukking van TSH ontstaat een status van milde hyperthyreoïdie. Dit kan gevolgen hebben voor de functie van multipele orgaansystemen en kan van invloed zijn op hartfunctie, botmetabolisme, glucosemetabolisme en de kwaliteit van leven. Tijdens de follow-up van schildkliercarcinoom werden patiënten met name in het begin van de therapie regelmatig onttrokken van schildklierhormoon teneinde een TSH gestimuleerde meting van thyroglobuline en RaJ scintigrafie te bewerkstelligen. Hiermee kan gecontroleerd worden of er nog ziekteactiviteit is, of dat er in de tussentijd metastasen zijn ontstaan. Gedurende een aantal weken ontstaat er dan een gecontroleerde status van hypothyreoïdie. Er wordt tegenwoordig echter steeds vaker gebruik gemaakt van recombinant humaan TSH (rhTSH), waarbij patiënten hun standaard dosis schildklierhormoon blijven gebruiken. Deze laatste methode heeft veel minder negatieve gevolgen voor de kwaliteit van leven en wordt inmiddels aanbevolen in de internationale richtlijnen.

De behandeling met TSH supressieve dosering van schildklierhormoon en de gecontroleerde onttrekking van thyroxine substitutie vormen een uniek model om het effect van exogeen schildklierhormoon en schildklierhormoonovermaat en -deficiëntie te onderzoeken op de verschillende orgaansystemen.

In dit proefschrift wordt een aantal studies beschreven die kunnen bijdragen aan de verbetering van de diagnostiek en behandeling van schildkliercarcinoom. Ook hebben we studies verricht die meer inzicht geven in de lokale werking en beschikbaarheid van schildklierhormoon. We hebben een studie verricht naar potentiële nieuwe markers die kunnen bijdragen aan de differentiatie tussen benigne en maligne schildklierweefsel. Daarnaast hebben we een fase II studie verricht naar het effect van de multitarget tyrosinekinaseremmer sorafenib, met het doel de behandeling van RaJ-resistent schildkliercarcinoom te verbeteren. In de rest van dit proefschrift focussen we op de klinische consequenties van de behandeling van schildkliercarcinoom. Hierbij wordt gefocust op de effecten van exogene milde hyperthyreoïdie en gecontroleerde hypothyreoïdie op verschillende orgaansystemen alsmede op de kwaliteit van leven.

II. Diagnose van gedifferentieerd schildkliercarcinoom

Ondanks verbeterde beeldvormende technieken als 18-F fluorodeoxyglucose-positron emission tomography (FDG-PET) en echografie blijft fijne naaldaspiratie (FNA)

de procedure van eerste keus bij patiënten die zich presenteren met een zwelling in de schildklier. Hiermee blijft de diagnose van schildkliercarcinoom en in het bijzonder de differentiatie tussen het benigne follicular adenoom en maligne follicular carcinoom lastig. Als gevolg hiervan zal tussen de 70-80% van de patiënten met een verdachte FNA uitslag, een onnodige operatie ondergaan. Het gebruik van moleculaire markers als galectin-3, PAX8-PPARgamma, BRAF, RAS of RET/PTC kan bijdragen aan een verbeterde diagnostiek.

Ook na (hemi-)thyreoïdectomie kan het microscopische onderscheid tussen benigne en maligne schildklierzwellingen moeilijk te maken zijn met conventionele histologie, aangezien de verschillende laesies histologische karakteristieke kenmerken kunnen delen. Het is daarom belangrijk nieuwe markers te ontwikkelen die dit onderscheid vergemakkelijken. In het verleden zijn verscheidene immunohistochemische markers geïdentificeerd, maar geen van deze is succesvol geïmplementeerd in de routine diagnostiek.

Verschillen in de expressie van de retinoïdezuur receptor (RAR) subtypes zijn beschreven tussen benigne en maligne schildklierweefsels. De diagnostische waarde is echter nog niet eerder beschreven. In **Hoofdstuk 2** van dit proefschrift beschrijven wij de diagnostische waarde van de expressie van de retinoïdezuur receptor (RAR) en de retinoïde X receptor (RXR) subtype eiwitexpressie voor de differentiatie tussen verschillende schildklierneoplasmata. Hiervoor gebruikten we een tissue microarray met 93 benigne schildklierweefsels (normaal schildklierweefsel, multinodulair struma en follicular adenoom) en 77 schildkliercarcinomen (papillair schildkliercarcinoom (PTC), follicular schildkliercarcinoom (FTC) en follicular variant van PTC (FVPTC)).

De immunohistochemische kleuring werd verricht voor verschillende RAR en RXR subtypes. De kleuringen werden semikwantitatief geanalyseerd met behulp van receiver operating curves (ROC-curves) en hiërarchische cluster analyse.

Wij vonden hiermee een verhoogde expressie van cytoplasma (c) RARalpha, cRARgamma, cRXRbeta en verminderde expressie van nucleaire (n) RARbeta, nRARgamma, en nRXRalpha in schildkliercarcinomen vergeleken met goedaardige weefsels. Drie eiwitten vertoonden significant verschillende expressie tussen FA (goedaardig) en FTC (kwaadaardig). Tussen FA (goedaardig) en FVPTC (kwaadaardig) kwamen vijf eiwitten verschillend tot expressie. Met behulp van cluster analyse gaf de combinatie van een negatieve kleuring van membraneus RXRbeta en een positieve kleuring voor cRXRbeta en zeer hoge positief voorspellende waarde voor maligne schildklierziekte (98%). Terwijl de combinatie van een positieve kleuring van nRXRalpha en een negatieve kleuring van cRXRbeta een hoge positief voorspellende waarde had voor benigne schildklierlaesies (91%).

Uit deze studie kan geconcludeerd worden dat de verschillen in expressie van eiwitten van RAR en RXR subtypes kunnen bijdragen aan de differentiaal diagnose van

schildklierzwellingen. De resultaten van deze studie, en dan met name de waarde van de clusteranalyse zal bevestigd moeten worden in andere studies.

Perspectief
De bevindingen van hoofdstuk 2 moeten eerst bevestigd worden voordat ze geïmplementeerd kunnen worden in de standaard diagnostiek. Aanvullende studies zouden verricht moeten worden met grotere aantallen weefsels en dan voornamelijk met verschillende folliculaire weefsels. Bovendien moet het klinisch nut van de hiërarchische cluster analyse eerst gevalideerd worden, waarbij het gebruik van door FNA verkregen materiaal essentieel is. Ook moet het biologische mechanisme dat verantwoordelijk is voor de verschillen in eiwitexpressie van RAR en RXR in de verschillende schildklierweefsels nog opgehelderd worden.

III. Nieuwe behandelingsstrategieën voor gemetastaseerd schildklier-carcinoom dat ongevoelig is voor radioactief jodium (RaJ)

1. Sorafenib en de behandeling van RaJ-ongevoelig schildkliercarcinoom

Metastasen op afstand komen meestal voor in botten en longen en ontstaan in 10-15% van de patiënten met schildkliercarcinoom. Het grootste probleem in deze categorie van patiënten is dat als gevolg van dedifferentiatie van schildklierkankercellen, de RaJ opname afgenomen of afwezig is. RaJ is bij schildkliercarcinoom de enige curatieve behandelingsoptie. Andere behandelingsopties, zoals conventionele chemotherapie of radiotherapie hebben meestal maar beperkt effect. Gemetastaseerde ziekte is daarom ook geassocieerd met een slechte 10 jaars overleving van 5-10%

De laatste jaren is veel onderzoek verricht naar de moleculaire routes die betrokken zijn bij de pathogenese van gedifferentieerd schildkliercarcinoom. Dit heeft geleid tot de ontdekking van nieuwe aangrijpingspunten voor nieuwe therapieën. De identificatie van tyrosinekinase geactiveerde routes en de ontwikkeling van nieuwe tyrosinekinaseremmers bieden nieuwe perspectieven voor de behandeling van schildkliercarcinoom dat ongevoelig is geworden voor RaJ. Daarnaast is er bij schildkliercarcinoom een relatie ontdekt tussen genetische veranderingen in de RET-RAS-RAF-cascade, die betrokken is bij de maligne ontaarding en het verlies van expressie van de jodiumtransporter NIS. Een *in-vitro* studie met de multi-kinaseremmer sunitinib liet zelfs hernieuwde NIS expressie zien bij verschillende schildklierkankercellen.

Er zijn al enkele studies verricht met tyrosinekinaseremmers bij patiënten met schildkliercarcinoom. Hierbij was gefitinib niet succesvol en axitinib wel succesvol

bij patiënten met verscheidene soorten schildkliercarcinoom. De multi-tyrosinekinaseremmer motesanib (remt RET- PDGF –VEGFR-KIT) gaf een partiële respons bij 14% van de patiënten met gedifferentieerd schildkliercarcinoom. De multi-tyrosinekinaseremmer sorafenib (remt RET, C-RAF, wild-type and mutant (V600E) BRAF, VEGFR1, -2, -3, Flt3, and c-KIT) gaf in twee studies een respons (partiële respons of stabiele ziekte) van respectievelijk 11% en 23%, waarbij in de eerste studie ook patiënten met het agressievere anaplastisch carcinoom waren geïncludeerd.

Het mechanisme achter het verschil in respons tussen de multi-kinaseremmers motesanib en sorafenib zou kunnen berusten op het verschil in IC50 voor de tyrosinekinases van de doeleiwitten die geremd worden. Motesanib heeft hierbij een sterkere remming op VEGFR1 (IC50 2 nM), VEGFR2 (IC50 3 nM) en VEGFR3 (IC50nM), terwijl sorafenib remmend werkt op RET (IC 50 47nM), RET/PTC3 (IC50 50nM) en BRAF (IC50 22 nM). Desondanks zijn de resultaten van de verschillende studies niet zomaar te vergelijken in verband met verschillen in patiëntcategorieën, soorten schildkliercarcinoom, studieontwerp en analytische methoden.

Wij hebben ervoor gekozen een studie te verrichten naar het effect van de multi-tyrosinekinaseremmer sorafenib op tumorprogressie, maar daarnaast ook naar het effect op de opname van RaJ (**Hoofdstuk 3**). Dit was een open, singlecenter, 26-weekse prospectieve fase II studie. De hypothese was dat behandeling met sorafenib niet alleen effect zou hebben op tumorprogressie, maar ook de RaJ opname zou kunnen herstellen bij patiënten met schildkliercarcinoom dat ongevoelig is voor RaJ. We behandelden daarvoor 31 patiënten met progressieve, gemetastaseerde of lokaal doorgroeiende ziekte. Zij kregen tweemaal daags 400 mg sorafenib. Het primaire eindpunt was hernieuwde RaJ opname. Additionele eindpunten waren radiologische respons van tumor of metastasen gemeten met CT scans.

Na 26 weken behandeling was er helaas geen nieuwe opname te zien van RaJ. Wel hadden 19 patiënten (59%) een klinisch gunstige respons, waarbij 8 (25%) een partiële respons hadden en 11 (34%) stabiele ziekte. Zeven patiënten (22%) hadden onder de behandeling progressie van hun ziekte. De mediane progressievrije overleving was 58 weken (95% betrouwbaarheidsinterval 47-68 weken). De tumormarker thyroglobuline daalde gedurende de behandeling en kwam overeen met het kleiner worden van de tumorgrootte op de CT scans. De respons werd niet beïnvloed door histologisch carcinoomsubtype, mutatiestatus of andere variabelen. Behalve al bekende bijwerkingen van sorafenib deden zich geen andere bijwerkingen voor. Sorafenib bleek minder effectief bij patiënten met botmetastasen. Er is hiervoor geen duidelijke verklaring.

Perspectief

De resultaten van onze studie zijn vergelijkbaar met de resultaten van eerdere studies met sorafenib. De resultaten van alle studies lijken erop te wijzen dat sorafenib een succesvol en veelbelovend product is voor patiënten met gemetastaseerd of progressief schildkliercarcinoom. Helaas blijkt sorafenib minder effectief bij botmetastasen. Ook konden de *in-vitro* studies waarbij tyrosinekinaseremming de RaJ opname verbeterde niet bevestigd worden. Op dit moment wordt een grote multicenter fase III studie verricht naar de effectiviteit van sorafenib. Indien deze studie gunstige resultaten laat zien, zou sorafenib onderdeel kunnen worden van de standaard behandeling van schildkliercarcinoom dat ongevoelig is geworden voor RaJ. Het is mogelijk dat combinatiebehandeling met meerdere tyrosinekinaseremmers in de toekomst een nieuwe behandelingsstrategie gaat worden.

2. Tyrosinekinase remmende therapie en schildklierhormoon-metabolisme

Behandeling met tyrosinekinaseremmers is geassocieerd met schildklierdysfunctie. Sunitinib is geassocieerd met hypothyreoïdie bij 14-85% van de patiënten en bij enkele patiënten werd ook hyperthyreoïdie geobserveerd. Er zijn hiervoor verschillende hypothesen over het onderliggende mechanisme, zoals destructieve thyreoïditis, interferentie met thyroid peroxidase of schildklieratrofie als gevolg van vaatdestructie. Ook bij patiënten met status na thyreoïdectomie werden schildklierfunctiestoornissen waargenomen. Zodoende zou de remming van perifere conversie van schildklierhormoon het mechanisme achter de hypothyreoïdie kunnen zijn. Stapsgewijze deiodinatie is normaal gesproken de route van schildklierhormoondegradatie en deze wordt gemedieerd door iodothyronine deiodinases D1, D2 en D3 en leverenzymen.

D1 zet T4 om in T3, en draagt bij aan de serumproductie van T3. Daarnaast speelt het een rol in de afbraak van rT3. D2 katalyseert de lokale T3 productie in verschillende weefsels. D2 in het skeletspierweefsel draagt mogelijk ook bij aan de serum T3 productie. D3 inactiveert T3 en T4. Het beschermen van weefsels tegen een overmaat van schildklierhormoon is waarschijnlijk de belangrijkste rol van D3. De deiodinases passen het niveau van schildklierhormoon aan aan de behoefte van lokale weefsels en aan diverse condities.

Om het mechanisme achter de schildklierfunctiestoornissen bij het gebruik van tyrosinekinaseremmers te ontrafelen, hebben we een studie verricht bij schildklierkankerpatiënten die behandeld werden met sorafenib (**Hoofdstuk 4**). De hypothese was dat sorafenib het schildklierhormoonmetabolisme zou beïnvloeden via de activiteit van iodothyronine deiodinases. Dit was niet eerder in mensen onderzocht. Hiervoor hebben we een prospectieve 26 weken durende studie verricht waarbij patiënten tweemaal daags 400 mg sorafenib kregen. Vervolgens werden de serum

thyroxine (T4), vrij T4, 3,5,3-triiodothyronine (T3), vrij T3, reverse T3 (rT3), en TSH concentraties gemeten op baseline en na 26 weken bij 21 patiënten met progressief gedifferentieerd schildkliercarcinoom. Ook werden de ratios van T3/T4, T3/rT3 en T4/rT3 berekend. Deze zijn namelijk afhankelijk van het substraataanbod en reflecteren iodothyronine deiodinatie.

We vonden dat er een hogere dosis thyroxine nodig was om vrij T4 en T3 concentraties binnen de normaalwaarden te houden. Gecorrigeerd voor thyroxinedosis per kilogram lichaamsgewicht daalden de vrij T4 en T3 concentraties met 11% en 18%. Daarbij stegen de TSH concentraties. Daarnaast werden er duidelijke dalingen in de serum T3/T4, T3/rT3 en T4/rT3 ratios gevonden. Deze ratios reflecteren veranderingen in het perifere metabolisme van schildklierhormoon, dat positief beïnvloed wordt door D1 en D2 en negatief door D3. De daling van de T3/T4 en T3/rT3 ratios zou een gevolg kunnen zijn van een daling in D1 en/of D2 activiteit. Echter, dit zou ook geassocieerd kunnen zijn met een verlaagd metabolisme van T4, maar dit was juist verhoogd. Daarom zouden de verlaagde ratios het best verklaard kunnen worden door een verminderde activiteit van D3.

Klinische implicaties

De resultaten van onze studie tonen aan dat tyrosinekinaseremmers naast directe effecten op de schildklier, ook het perifere schildklierhormoonmetabolisme beïnvloeden. Dit is waarschijnlijk het gevolg van een verminderde activiteit van deiodinase type 3. Het zou van waarde zijn om deze theorie te testen met *in-vitro* studies. Daarnaast is het van belang om tijdens de behandelingen met tyrosinekinaseremmers de schildklierfunctie regelmatig te testen en zonodig de dosering thyroxine aan te passen.

IV. Consequenties van de behandeling van schildkliercarcinoom

Patiënten met gedifferentieerd schildkliercarcinoom die behandeld zijn met totale thyreoïdectomie en radioactief jodium ablatietherapie worden volledig afhankelijk van de toediening van exogeen schildklierhormoon. Omdat TSH een groeifactor is voor schildklierkankercellen, werden de patiënten voorheen standaard behandeld met een TSH supressieve dosis thyroxine gedurende een periode van 15 jaar. Waar deze TSH supressieve dosis een gunstige invloed heeft op de prognose, heeft de zo verkregen milde hyperthyreoïdie een negatieve invloed op diverse orgaansystemen en op de kwaliteit van leven. Daarom wordt tegenwoordig in de internationale richtlijnen aanbevolen dat alleen patiënten met een hoog of intermediair risico schildkliercarcinoom moeten worden gesubstitueerd tot een gesupprimeerde TSH concentratie

beneden 0.1 mU/L. Bij laagrisico patiënten wordt aanbevolen dat het TSH ingesteld wordt tussen 0.1-0.5 mU/L, net iets onder de normaalwaarde.

Gedurende de follow-up van schildkliercarcinoom werden patiënten voorheen regelmatig onttrokken van schildklierhormoon om de ziektestaat en ziekteprogressie te beoordelen door middel van TSH gestimuleerde thyroglubulinemeting en schildklierscintigrafie. Dit creëert een staat van gecontroleerde, maar wel acute hypothyreoïdie. Tegenwoordig wordt steeds vaker gebruik gemaakt van recombinant humaan TSH tijdens follow-up, wat veel minder impact heeft op de kwaliteit van leven. De patiënten blijven dan namelijk hun gewone dosering schildklierhormoon gebruiken.

De afhankelijkheid van exogeen schildklierhormoon, de langdurige milde hyperthyreoïdie in combinatie met episodes van acute hypothyreoïdie maken schildkliercarcinoompatiënten een interessant model om de effecten van schildklierhormoon op diverse orgaansystemen te onderzoeken.

1. Inzicht in het schildklierhormoonmetabolisme

Het perifere schildklierhormoonmetabolisme wordt onder andere gereguleerd door de iodothyronine deiodinases D1, D2 en D3. Er zijn verschillende polymorfismen beschreven in D1 en D2, waarvan sommigen serumwaarden van TSH, T3 en T4 lijken te beïnvloeden. Patiënten behandeld voor schildkliercarcinoom zijn ideaal om het schildklierhormoonmetabolisme in te onderzoeken. De meeste studies focussen op het D2-Thr92Ala polymorfisme.

Het D2-Thr92Ala polymorfisme wordt geassocieerd met een verminderde D2 activiteit in sommige *in-vitro* experimenten, maar in anderen juist weer niet. Er was voorheen nog geen associatie gevonden tussen het D2-Thr92Ala polymorfisme en circulerende schildklierhormoonconcentraties bij mensen. Wel heeft een recente studie gesuggereerd dat patiënten die homozygoot zijn voor het Ala 92 allel in het deiodinase type 2 een hogere dosis thyroxine nodig hebben om het TSH te onderdrukken.

Om deze bevindingen (**Hoofdstuk 5**) te bevestigen hebben wij een studie uitgevoerd om de associatie tussen het D2-Thr92Ala polymorfisme, schildklierhormoonconcentraties en de thyraxdosering te onderzoeken in patiënten behandeld voor schildkliercarcinoom en patiënten met Hashimoto thyreoïditis. Daarvoor hebben we 154 patiënten met gedifferentieerd schildkliercarcinoom en 141 patiënten met M.Hashimoto die allen behandeld werden met schildklierhormoonsubstitutie, onderzocht. Bij alle patiënten werden de serumconcentraties van TSH, vrij T4, T3 en reverse T3 gemeten. Ook werd het D2-Thr92Ala polymorfisme bepaald. In beiden groepen patiënten werd geen verband gevonden tussen schildklierhormoonconcentraties, thyroxinedosering en de aanwezigheid van het D2-Thr92Ala polymorfisme.

Wij hebben uit onze studie geconcludeerd dat de aanwezigheid van het D2-Thr92Ala polymorfisme niet geassocieerd is met serum schildklierhormoonspiegels bij patiënten die behandeld waren voor schildkliercarcinoom en Hashimoto thyreoïditis.

De intra-individuele variatie in serum TSH, T3 en T4 is erg klein, terwijl de inter-individuele variatie juist erg groot is. In verschillende studies wordt aangetoond dat elk individu een eigen uniek setpoint heeft van zijn of haar schildklierfunctie. Dit wordt beïnvloed door genetische verschillen in de regulatie van de hypothalamus-hypofyse-schildklier as. Wij hebben onderzocht of polymorfismen in D1 en D2 dit setpoint kunnen beïnvloeden.

We hebben daarom een studie uitgevoerd **(Hoofdstuk 6)** naar het effect van de volgende polymorfismen: D1-C785T, D1-A184G, D2-Thr92Ala en D2-ORFa-Gly3Asp. De effecten van deze polymorfismen op het setpoint van de hypothalamus-hypofyse-schildklier as werden onderzocht met behulp van een general mixed model. We gebruikten daarvoor 1905 bloedmonsters waar tegelijk FT4 en TSH bepaald waren tijdens routine follow-up van 151 patiënten behandeld voor en genezen van schildklierkanker.

Onze studie laat zien dat patiënten behandeld voor schildkliercarcinoom homozygoot voor het D2-ORFa-Gly3Asp polymorfisme een veranderd setpoint hebben van de hypothalamus-hypofyse-schildklier as. De negatieve feedback van T4 op TSH is zwakker in homozygote patiënten in vergelijking met wildtype en heterozygote patiënten. De andere polymorfismen bleken het setpoint niet te beïnvloeden..

Hoewel we een duidelijk effect van het D2-ORFa-Gly3Asp polymorfisme zagen op het setpoint van de hypothalamus-hypofyse-schildklier as, is het nog niet makkelijk om te bepalen wat de klinische consequentie hiervan is. Ook zijn er onbekende factoren die eventueel de TSH/FT4 ratio zouden kunnen beïnvloeden. Helaas hebben we geen informatie over T3 en rT3, omdat de bloedmonsters tijdens routine follow-up zijn afgenomen en deze bepalingen niet standaard gedaan worden. We hebben daarom geen inzicht in de gehele cyclus van het schildklierhormoonmetabolisme. Ook verschilden onze resultaten van een eerdere studie bij gezonde vrijwilligers. Echter, het is ook niet duidelijk of onze patiënten zonder intrinsieke schildklierfunctie die afhankelijk zijn van een vaste dosis schildklierhormoon wel te vergelijken zijn met patiënten met een eigen schildklierhormoonproductie. Daarnaast zou het feit dat onze patiënten behandeld worden met een TSH onderdrukkende dosis schildklierhormoon, nog van invloed kunnen zijn op onze uitkomst. Het zou kunnen dat de langdurige subklinische hyperthyreoidie resulteert in downregulatie van D1 en D2 en/of upregulatie van D3. Hier valt tegenin te brengen dat we geen effect zagen van de duur van de follow-up op het setpoint.

Perspectief

In onze eerste studie werd geen verband gevonden tussen het D2-Thr92Ala polymorfisme en thyroxine dosis. Tot op heden zijn hier nog maar weinig studies naar verricht en zijn de resultaten tegenstrijdig. Eventuele toekomstige studies zouden hier doorslag in kunnen geven, hoewel een grote klinische consequentie niet verwacht wordt.

Na het verrichten van onze tweede studie kunnen we concluderen dat patiënten homozygoot voor het D2-ORFa-Gly3Asp polymorfisme een ander setpoint hebben voor hun hypothalamus-hypofyse-schildklier as. Het is echter ook niet duidelijk wat de klinische consequentie hiervan is. In de toekomst zou het interessant zijn om verschillen in biologisch werking van de verschillende polymorfismes in cellijnen te testen.

2. Botmetabolisme

Klinische observaties suggereren een belangrijke bijdrage van schildklierhormoon aan het botmetabolisme, echter het moleculaire mechanisme is nog maar gedeeltelijk ontrafeld. Het is wel een belangrijk onderwerp, aangezien patiënten met schildkliercarcinoom gedurende een lange periode behandeld worden met een TSH supressieve thyroxinedosering en dus langdurig milde hyperthyreoïdie hebben.

T3 stimuleert proliferatie, differentiatie en apoptose van osteoblasten. Dit suggereert dat osteoblasten het belangrijkste doel zijn van T3 in de regulatie van botmetabolisme. Echter, er is ook een functionele rol toebedeeld aan TSH bij de regulatie van botontwikkeling, dit op basis van resultaten uit dierexperimenten, maar ook uit experimenten bij mensen. Dit werd daarentegen weer betwist door resultaten van andere studies waarbij bij schildklierhormoonreceptor deficiënte muizen ernstige skeletafwijkingen ontwikkelden, wat de suggestie wekt dat botremodelling voornamelijk gemedieerd wordt door T3. Ook werd in mensen al aangetoond dat er een significante relatie is tussen schildklierhormoonconcentraties en botdichtheid, maar niet tussen TSH en botdichtheid.

Ook de rol van het deiodinase type 2 bij de skeletontwikkeling is niet geheel opgehelderd. Het D2-Thr92Ala polymorfisme is geassocieerd met een lagere TSH concentratie en een lagere enzymatische activiteit, wat zou kunnen leiden tot een lagere T3 beschikbaarheid op lokaal niveau.

Om de rol van het deiodinase D2 bij botmetabolisme te onderzoeken hebben we een studie verricht naar de relatie tussen het D2-Thr92Ala polymorfisme, botdichtheid en botturnover (**Hoofdstuk 7**). We hebben deze relatie onderzocht bij patiënten die behandeld waren met een totale thyreoïdectomie in verband met schildkliercar-

cinoom en vervolgens behandeld werden met schildklierhormoon substitutie. Een voordeel van dit model is dat T4 en TSH serum spiegels vrij uniform zijn.

De botdichtheid en markers van botmetabolisme [bot-specifiek alkalisch fosfatase (BAP), cross-linking terminal C-telopeptide van type I collageen (CTX), procollageen type 1 aminoterminal propeptide (P1NP) en cross-linked N-telopeptide van type I collageen (NTX)] werden gemeten. Zestig patiënt hadden wildtype (Thr/Thr), 66 waren heterozygoot (Thr/Ala), en 28 waren homozygote (Ala/Ala) dragers van het D2 polymorfisme.

In deze studie werd geobserveerd dat patiënten die homozygoot zijn voor het D2 polymorfisme (Ala/Ala) een 6% lagere botdichtheid hadden, gemeten bij het femur. Daarnaast waren er verhoogde concentraties van P1NP (32%), CTX (27%) en de NTX/creatinine ratio (54%) in de Ala/Ala subgroep vergeleken met de wild-type subgroep. Tevens waren de verhoogde concentraties van botformatie (P1NP) en indicatoren van botresorptie (CTX en NTX) onafhankelijk van andere indicatoren die botmetabolisme kunnen beïnvloeden (leeftijd, geslacht, BMI, oestrogeenstatus, calcium, vitamine D, PTH), maar belangrijker nog, onafhankelijk van T3 en TSH. De verhoogde botresorptie kan waarschijnlijk verklaard worden door veranderingen in interactie tussen osteoclasten en osteoblasten. Onze studie laat zien dat de relatie tussen de lokale T3 beschikbaarheid en D2 activiteit complex is en niet volledig verklaard kan worden door traditioneel waargenomen directe effecten van T3 op bot. Waarschijnlijk zijn hier vele onbekende componenten van het micromillieu van het bot bij betrokken.

In een poging om onderscheid te maken tussen de effecten op bot gemedieerd door TSH of door schildklierhormoon, hebben we een tweede studie verricht (**Hoofdstuk 8**). Hiervoor hebben we 11 patiënten met gedifferentieerd schildklier-carcinoom tijdens onttrekking van schildklierhormoon (hoge TSH concentratie, lage vrij T4 concentratie) vergeleken met 11 patiënten na toediening van recombinant TSH (hoge TSH concentratie, normale vrij T4 concentratie). De eerste groep hebben we vergeleken na 4 weken onttrekking van schildklierhormoon en acht weken na herstarten van schildklierhormoon. De patiënten van de twee groepen waren gelijk qua leeftijd, geslacht en BMI. Voor de bestudering van het botmetabolisme hebben we plasmaspiegels gemeten van PTH, 25-OH-vitamine D, P1NP, CTX, RANKL en osteoprotegerin.

Tijdens hypothyreoïdie werden er significant lagere CTX spiegels en hogere osteoprotegerin concentraties gevonden, hetgeen wijst op verminderde botresorptie. Na injectie van recombinant TSH werden er geen verschillen gevonden in de bepalingen van botmetabolisme.

Onze bevindingen suggereren dat acute veranderingen van TSH bij stabiele schildklierhormoonspiegels geen significant effect hebben op skeletmetabolisme.

Daarnaast laten onze resultaten zien dat hypothyreoïdie resulteert in verminderde botturnover door de lage vrij T4 spiegel en niet door de hoge TSH spiegel.

Concluderend suggereren onze data dat een vermindering in lokale T3 beschikbaarheid als gevolg van polymorfisme in het deiodinase type 2 leidt tot vermindering van botturnover en verminderde botmassa.

De tweede studie leert dat de botturnover is verminderd tijdens acute hypothyreoïdie, waarbij de belangrijkste bevinding lijkt te zijn dat het botmetabolisme meer afhankelijk is van T4 dan van TSH.

Perspectief

Hoewel de resultaten van onze studies een duidelijke relatie aantonen tussen schildklierhormoon en botmetabolisme, is het moleculaire mechanisme dat ten grondslag ligt aan deze relatie nog niet volledig opgehelderd. Het is wel een belangrijk onderwerp voor patiënten die langdurig behandeld worden met een TSH supressieve thyroxinedosering, aangezien zij mogelijk een verhoogd risico hebben op osteoporose. Dit is echter voornamelijk geobserveerd bij postmenopausale vrouwen. Bij deze patiënten is screening op baseline en tijdige interventie met medicijnen noodzakelijk in geval van optredende osteoporose.

3. Hartfunctie

Schildklierhormoon heeft uitgesproken effecten op het cardiovasculaire systeem. Hyperthyreoïdie induceert cardiale ritmestoornissen, linker ventrikel hypertrofie en diastolische dysfunctie, maar tast de systolische functie niet aan. Ook milde hyperthyreoïdie als gevolg van een TSH supressieve thyroxinedosis is geassocieerd met tachycardie en supraventriculaire ritmestoornissen, een vergrote linker ventrikel massa en diastolische dysfunctie. Deze diastolische dysfunctie is gedeeltelijk herstelbaar na herstel van euthyreoidie. Hypothyreoïdie is daarentegen geassocieerd met bradycardie, hypertensie, een verhoogde perifere vasculaire weerstand, hartfalen, verminderde cardiale output en diastolische dysfunctie. Ook is hypothyreoïdie geassocieerd met coronarialijden, waarschijnlijk als gevolg van hypercholesterolaemie, hypertriglyceridemie en hypertensie.

De gevolgen van episodes van acute hypothyreoïdie op de hartfunctie zijn in slechts enkele studies onderzocht. De resultaten hiervan waren echter niet conclusief. Daarom hebben wij een studie verricht naar de effecten van acute hypothyreoïdie op de hartfunctie bij patiënten die behandeld zijn voor schildkliercarcinoom (**Hoofdstuk 9**).

Hiervoor werden 14 patiënten gerekruteerd die in het verleden behandeld waren voor schildkliercarcinoom en vervolgens gesuppleerd werden met een TSH supressieve dosering schildklierhormoon. De hartfunctie werd beoordeeld voor, één week

na en vier weken na staken van schildklierhormoonsubstitutie. Voor de beoordeling van de hartfunctie werd een nieuwe geavanceerde echotechniek gebruikt, namelijk Tissue Doppler imaging (TDI). Hiermee kan een gedetailleerde en kwantitatieve meting van de systolische en diastolische hartfunctie verricht worden. De echoresultaten werden vergeleken op de verschillende tijdstippen na staken van schildklierhormoon en werden daarnaast vergeleken met de resultaten van gezonde vrijwilligers met normale hartfunctie.

Op het eerste tijdstip, tijdens milde hyperthyreoïdie, liet echocardiografie zien dat patiënten een verminderde diastolische functie en hogere linker ventrikelmassa hadden in vergelijking met gezonde controlepersonen. De klinische consequenties van geïsoleerde diastolische dysfunctie zijn bij milde hyperthyreoïdie niet geheel duidelijk, maar zou gepaard kunnen gaan met verhoogde morbiditeit en mortaliteit.

Onttrekking van schildklierhormoon resulteerde in een subtiele additionele daling van de E wave (vroege diastolische instroomsnelheid over de mitralisklep, representeert de passieve vulling van de linker ventrikel) en A wave (late instroomsnelheid over de mitralisklep, representeert de actieve vulling van de linker ventrikel en dus de atriale systole), zonder impact op E/A ratio. Dit wijst op discrete onvoordelige effecten op de diastolische functie. Ook bij de evaluatie met TDI werd een subtiele vermindering gevonden van de diastolische functie. Daarnaast werd tijdens overte hypothyreoïdie een significante stijging gevonden van de diastolische bloeddruk.

Uit onze studie kan geconcludeerd worden dat langdurige milde hyperthyreoïdie gepaard gaat met diastolische dysfunctie. Vervolgens zorgt acute overte hypothyreoïdie voor een subtiele verslechtering van de diastolische functie.

Perspectief

In onze studie werd aangetoond dat langdurige iatrogene milde hyperthyreoïdie bij patiënten behandeld voor gedifferentieerd schildkliercarcinoom diastolische dysfunctie induceert. Daarnaast geeft subklinische hyperthyreoïdie een stijging van de linkerventrikelmassa. Hoewel de klinische consequenties voor de patiënt niet geheel duidelijk zijn, is het niet aanbevolen alle patiënten met schildkliercarcinoom onvoorwaardelijk in te stellen op TSH suppressie.

Acute hypothyreoïdie induceerde bij onze patiënten vervolgens minimale nadelige effecten op de diastolische functie van het hart, met daarbij een duidelijke verhoging van de diastolische bloeddruk. De potentiële negatieve cardiovasculaire consequenties van thyroxineonttrekking voor een RaJ scan zouden klinisch relevant kunnen zijn met name bij patiënten die al een verhoogd cardiovasculair risico hebben. Daarom is recombinant humaan TSH een aantrekkelijk alternatief.

4. Kwaliteit van leven

De kwaliteit van leven kan veranderd zijn bij patiënten met gedifferentieerd schildkliercarcinoom, enerzijds doordat ze gediagnosticeerd zijn met een kwaadaardige ziekte met de bijbehorende initiële behandeling. Anderzijds zou de TSH supressieve behandeling en de eventuele onttrekking van schildklierhormoon tijdens follow-up ook de kwaliteit van leven kunnen beïnvloeden. Er zijn eerdere studies verschenen over kwaliteit van leven bij patiënten met schildkliercarcinoom, maar de resultaten hiervan zijn niet eenduidig. Daarom hebben wij de kwaliteit van leven onderzocht in een grote groep patiënten die genezen is van gedifferentieerd schildkliercarcinoom. We hebben hiervoor gebruik gemaakt van vier verschillende kwaliteit van leven vragenlijsten en hebben de uitkomsten van patiënten vergeleken met de uitkomsten van een groep vrijwilligers gematcht voor geslacht, leeftijd en socio-economische status (**Hoofdstuk 10**).

Onze bevindingen lieten zien dat patiënten die genezen zijn van gedifferentieerd schildkliercarcinoom nog langdurig een verminderde kwaliteit van leven hebben. Dit kan echter wel herstellen na langdurige follow-up. Na langdurige genezing, circa 12-20 jaar waren 6 van de 16 subschalen van kwaliteit van leven vergelijkbaar met gezonde controles.

De gevolgen van langdurige milde hyperthyreoïdie op de kwaliteit van leven waren ook niet geheel duidelijk. Studies die dit onderwerp eerder onderzochten includeerden geselecteerde groepen patiënten met gedifferentieerd schildkliercarcinoom of patiënten met endogene hyperthyreoïdie waarbij de duur en het beloop van schildklierhormoonschommelingen niet bekend zijn. In onze studie bleek dat de kwaliteit van leven niet beïnvloed werd door TSH spiegels.

Perspectief

Ondanks genezing, een excellente prognose en een matig agressieve initiële behandeling is de kwaliteit van leven van patiënten met schildkliercarcinoom evident verminderd. De kwaliteit van leven kan uiteindelijk wel verbeteren na een lange periode van genezing. De bevindingen van deze studie hebben implicaties voor de aanpak van de behandeling van schildkliercarcinoompatiënten. Er moet naast medisch noodzakelijke ingrepen ook aandacht zijn voor de psychosociale gevolgen voor de patiënt en eventueel kan professionele ondersteuning aangeboden worden tijdens de follow-up.

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List of publications

1. **Hoftijzer HC**, Kapiteijn E, Schneider T, Hovens GC, Morreau H, Gelderblom H, Smit JW. Tyrosine kinase inhibitors in differentiated thyroid carcinoma: review of the clinical evidence. Clinical Investigation 2011 Volume 1 Issue 2

2. Abdulrahman RM, Verloop H, **Hoftijzer HC**, Verburg E, Hovens GC, Corssmit EP, Reiners C, Gelderblom H, Pereira AM, Kapiteijn E, Romijn JA, Visser TJ, Smit JW. Sorafenib-Induced Hypothyroidism Is Associated with Increased Type 3 Deiodination. Journal of Clinical Endocrinology and Metabolism 2010 95 3758-3762

3. Heemstra KA, **Hoftijzer HC**, van der Deure WM, Peeters RP, Hamdy NA, Pereira A, Corssmit EP, Romijn JA, Visser TJ, Smit JW. The type 2 deiodinase Thr92Ala polymorphism is associated with increased bone turnover and decreased femoral neck bone mineral density. Journal of Bone and Mineral Research 2010 25 1385-91.

4. **Hoftijzer HC**, Heemstra KA, Morreau H, Stokkel MP, Corssmit EP, Gelderblom H, Weijers K, Pereira AM, Huijberts M, Kapiteijn E, Romijn JA, Smit JW. Beneficial effects of sorafenib on tumor progression, but not on radioiodine uptake, in patients with differentiated thyroid carcinoma. European Journal of Endocrinology 2009 161 923-931.

5. **Hoftijzer HC**, Bax JJ, Heemstra KA, Bleeker GB, Delgado V, van der Klaauw AA, Romijn JA, Smit JW, Corssmit EP. Short-term overt hypothyroidism induces discrete diastolic dysfunction in patients treated for differentiated thyroid carcinoma. European Journal of Clinical Investigation 2009 39 204-210.

6. **Hoftijzer HC**, Liu YY, Morreau H, van Wezel T, Pereira AM, Corssmit EP, Romijn JA, Smit JW. Retinoic acid receptor and retinoid X receptor subtype expression for the differential diagnosis of thyroid neoplasms. European Journal of Endocrinology 2009 160 631-638.

7. **Hoftijzer HC**, Heemstra KA, van der Deure WM, Peeters RP, Fliers E, Appelhof BC, Wiersinga WM, Corssmit EP, Visser TJ, Smit JW. Thr92Ala polymorphism in the type 2 deiodinase is not associated with T4 dose in athyroid patients or patients with Hashimoto thyroiditis. Clinical Endocrinology (Oxford) 2009 71 279-283.

8. Havekes B, van der Klaauw AA, **Hoftijzer HC**, Jansen JC, van der Mey AG, Vriends AH, Smit JW, Romijn JA, Corssmit EP. Reduced quality of life in patients

with head-and-neck paragangliomas. European Journal of Endocrinology 2008 158 247-253.

9. van der Klaauw AA, Biermasz NR, **Hoftijzer HC**, Pereira AM, Romijn JA. Previous radiotherapy negatively influences quality of life during 4 years of follow-up in patients cured from acromegaly. Clinical Endocrinology (Oxford) 2008 69 123-128.

10. **Hoftijzer HC**, Heemstra KA, Corssmit EP, van der Klaauw AA, Romijn JA, Smit JW. Quality of life in cured patients with differentiated thyroid carcinoma. Journal of Clinical Endocrinology and Metabolism 2008 93 200-203.

11. van Aken MO, Pereira AM, Biermasz NR, van Thiel SW, **Hoftijzer HC**, Smit JW, Roelfsema F, Lamberts SW, Romijn JA. Quality of life in patients after long-term biochemical cure of Cushing's disease. Journal of Clinical Endocrinology and Metabolism 2005 90 3279-3286.

12. Biermasz NR, van Thiel SW, Pereira AM, **Hoftijzer HC**, van Hemert AM, Smit JW, Romijn JA, Roelfsema F. Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. Journal of Clinical Endocrinology and Metabolism 2004 89 5369-5376

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Curriculum Vitae

Hendrieke Catherijn Hoftijzer werd op 20 juni 1980 geboren te Schiedam. Zij behaalde in 1998 haar Atheneumdiploma aan het Rotterdams Montessori Lyceum te Rotterdam. Hierna studeerde zij Biomedische Wetenschappen aan de Universiteit Leiden in verband met uitloting bij de numerus fixus voor de studie Geneeskunde. Na twee jaar Biomedische Wetenschappen heeft zij haar studie onderbroken om een jaar als voorzitter van de studievereniging van de Medische Faculteit der Leidse Studenten te fungeren. Hierna werd zij opnieuw uitgeloot, maar besloot gebruik te maken van de hardheidsclausule van de IB-groep om toegelaten te worden tot de studie Geneeskunde. Met behulp van een aanbevelingsbrief van wijlen Professor B.J. Vermeer, toenmalig decaan van de Medische Faculteit, werd zij uiteindelijk in 2002 toegelaten tot de studie Geneeskunde. In 2003 begon zij als student met onderzoek op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum. In 2004 deed zij haar afstudeerstage Biomedische Wetenschappen op de afdeling Neonatologie in het Royal Infirmary, te Edinburgh. Vervolgens begon zij haar co-schappen in 2005, waarna het doctoraal Biomedische Wetenschappen werd behaald in 2006 en het artsexamen volgde in 2007. Daarna startte zij in maart 2007 met promotieonderzoek op de afdeling Endocrinologie van het Leids Universitair Medisch Centrum onder begeleiding van Prof. Dr. J.W.A. Smit, Prof. Dr. J.A. Romijn en Dr. E.P.M. van der Kleij-Corssmit. Zij verrichtte onderzoek naar de behandeling en de klinische consequenties van de behandeling van schildkliercarcinoom. Van mei 2008 tot mei 2010 deed zij het perifere deel van de opleiding tot internist in het Bronovo Ziekenhuis te Den Haag (Opleider Dr. J.W. van 't Wout). Tijdens deze periode kreeg zij een ZonMw-AGIKO stipendium toegekend. Sinds 1 mei 2010 heeft zij haar promotieonderzoek op de afdeling Endocrinologie voortgezet. Per 1 juli 2011 zal zij starten met de opleiding tot cardioloog in het Onze Lieve Vrouwe Gasthuis te Amsterdam (Opleider Dr. G.A. Somsen).