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Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

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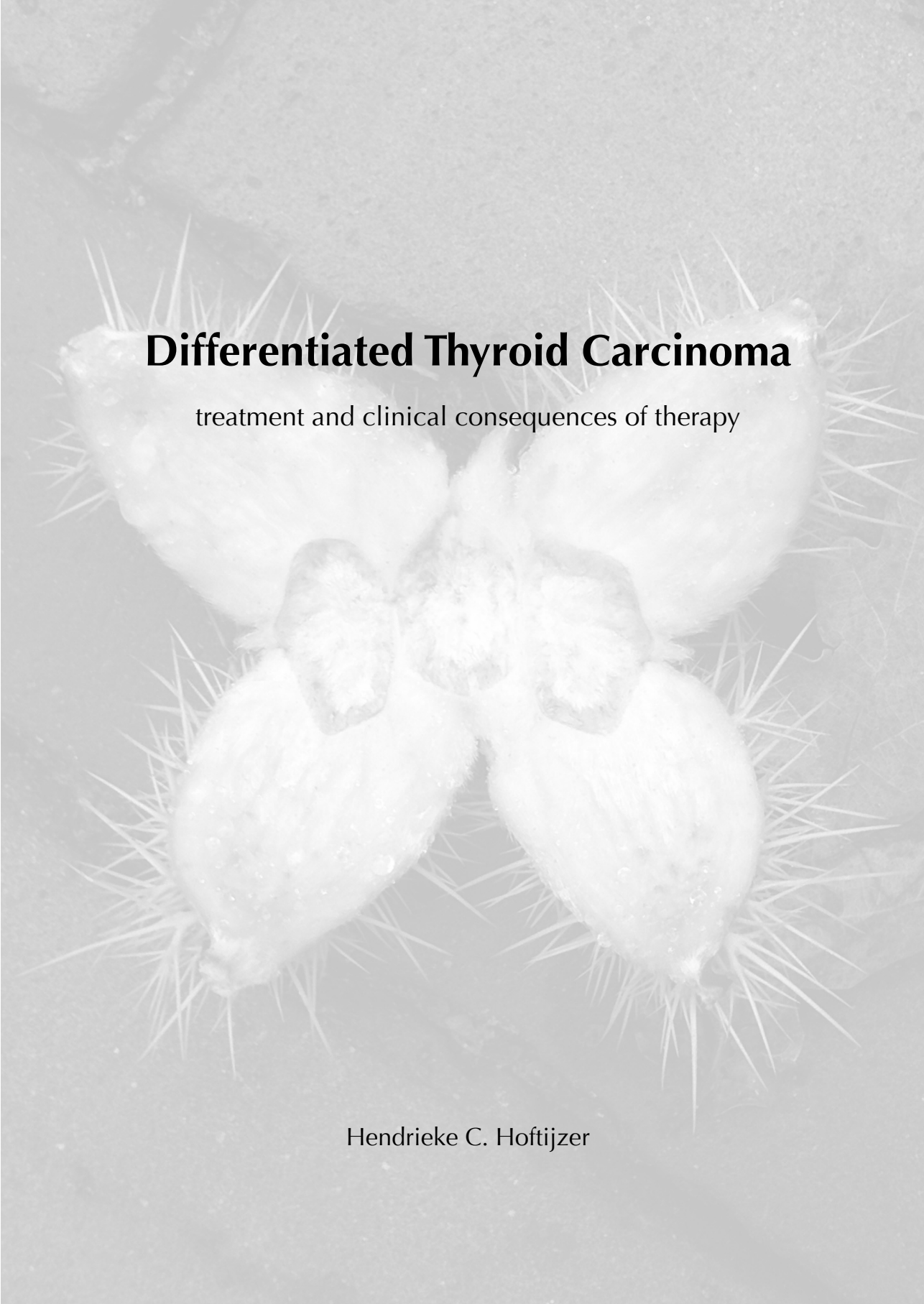
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Differentiated Thyroid Carcinoma

treatment and clinical consequences of therapy

Hendrieke C. Hoftijzer



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Differentiated Thyroid Carcinoma

treatment and clinical consequences of therapy

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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

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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**).

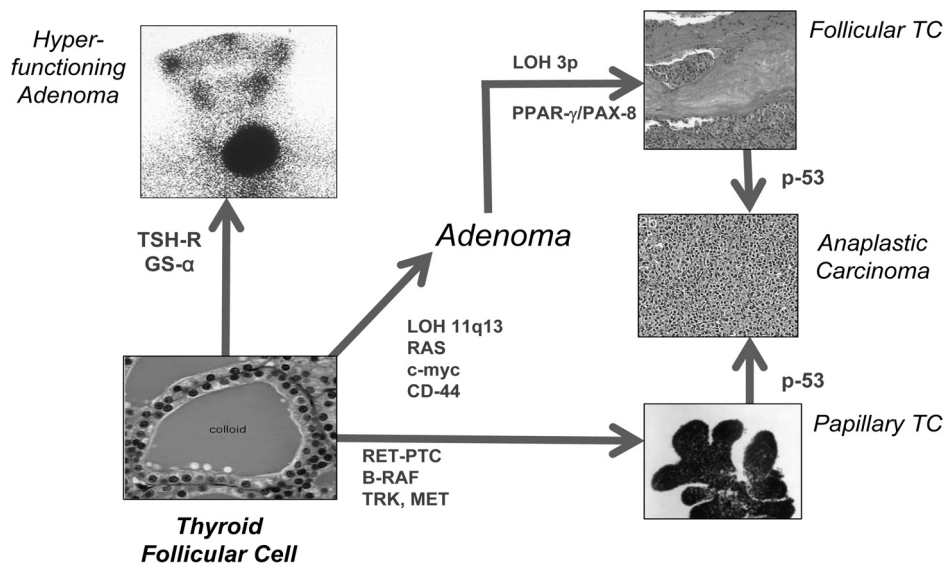


Figure 1
Pathogenesis of differentiated thyroid tumors

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 (EGFR) 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 Ral 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 Ral 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 Ral ablative therapy for thyroid remnants. There are three reasons for post-surgical Ral 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 Ral permits post ablative scanning to detect persistent carcinoma or metastatic disease (79,80). The third reason is that Ral 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 Ral 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 irradiated surgery (91). Moreover, various papers reported a relation between Ral therapy and non-thyroidal carcinoma, so the use of it should be applied carefully (92-97). Thus Ral 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 distant metastases as proposed by recent ATA and ETA guidelines (33,68).

The efficacy of Ral 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, Ral uptake is increased by depletion of plasma inorganic iodide before Ral therapy through a low iodide diet (100-102).

In addition, efficacy of Ral 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. Ral 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 Ral WBS has a much lower sensitivity than ultrasound and Tg measurement. Therefore the routine use of Ral 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 Ral 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, Ral ablation and Ral 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 Ral 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 Ral therapy will be given in patients that accumulate Ral. The remission rate in pulmonary metastases treated with Ral 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 Ral. 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 Ral retention. However, despite an increase in Ral uptake in tumor deposits, there are no data that demonstrate a better outcome of patients treated with lithium as an adjunct to Ral 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 beneficial response (153).

Retinoids are derivatives 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 Ral-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 Ral 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-PDGFR-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 Ral, 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 Ral uptake in FRTL-5 cells. We therefore hypothesized that treatment with a multiple kinase inhibitor not only reduces tumor progression, but may also restore Ral uptake in non-Ral 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 Ral 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 Ral 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.

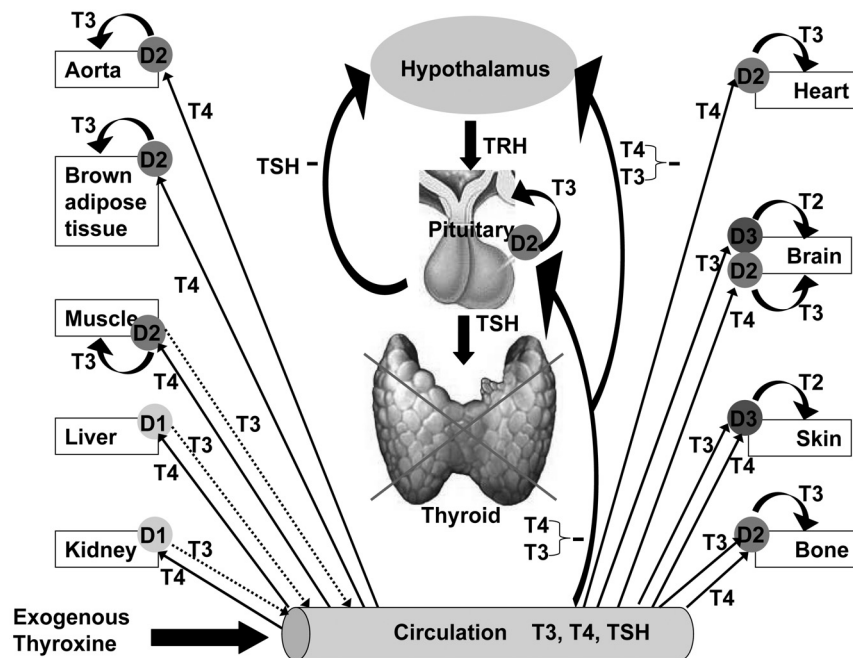


Figure 2

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 Ral 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.

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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 <i>et al.</i> (1998) (14)	58 samples 16 PTC 2 FTC 30 CTL 6 MNG 2 FA 2 toxic goiter	Immunohistochemistry Western blot	RAR β	Reduced RAR β expression in PTC compared to normal tissue Moderate RAR β expression in one FTC, none in the other
Schmutzler <i>et al.</i> (1998) (15)	4 cell lines 18 samples	RT-PCR Northern blot	RAR α RAR β RAR γ RXR α RXR β RXR γ	Expression RAR α , β , γ , RXR α and β on all carcinoma cell lines, however lower compared to goiterous cells. Lowest in FTC cells 9 of 12 tumor samples decreased or absent expression of RXR β
Takiyama <i>et al.</i> (2003) (16)	176 samples 3 cell lines	Immunohistochemistry RT-PCR and Western blot	RXR α RXR β RXR γ	Decreased nuclear expression of all RXR isoforms in carcinoma PTC and FTC low nuclear expression, moderate cytoplasmic expression ATC no expression RXR γ FA distinct nuclear staining RXR α and RXR β RXR γ undetectable in WRO, RXR α and RXR β detectable in all cell lines
Schmutzler <i>et al.</i> (2004) (17)	4 cell lines	RT-PCR and Northern blot	RAR α RAR β	Reduced level RAR β in FTC-238 Reduced level RAR α in HTh74 and C643

Table 1: Continued

Study	# tissue samples / cell lines	Method	Retinoid receptor investigated	Results
Haugen <i>et al.</i> (2004) (18)	10 samples 5 PTC 1 FTC 1 insular 1 ATC 2 FA 4 cell lines MRO-87 (FTC) WRO-82 (FTC) TAD-2 (CTL) DRO-90 (ATC)	RT-PCR and Western blot	RAR α RAR β RAR γ RXR α RXR β RXR γ	RAR α and γ expressed in all cell lines RAR β not expressed in FTC cells RXR α and β decreased expressed in ATC cells RXR γ expressed only in ATC RAR β expression decreased in malignant tissues RXR γ expression decreased in benign tissue and increased in malignant tissue
Elisei <i>et al.</i> (2005) (19)	24 samples 10 PTC 10 CTL 4 ATC	Immunohistochemistry	RAR β	Decreased RAR β in PTC and ATC compared to controls
Koh <i>et al.</i> (2006) (20)	3 cell lines SNU-80 (PTC) SNU-373 (PTC) SNU-790 (ATC)		RAR α RAR β RAR γ	RAR β not expressed RAR α detected in all three cell lines RAR γ 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 $\pm$ 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 $\pm$ 1.98	4.06 $\pm$ 1.46	4.88 $\pm$ 1.67	6.02 $\pm$ 3.71	1.12 $\pm$ 2.39	2.56 $\pm$ 2.43
Cytoplasmic RARα	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	2.43 $\pm$ 4.47	1.67 $\pm$ 2.42	1.21 $\pm$ 2.47
Nuclear RARβ	8.53 $\pm$ 2.20	8.62 $\pm$ 1.42	8.63 $\pm$ 2.21	5.62 $\pm$ 3.37	5.78 $\pm$ 3.12	6.21 $\pm$ 3.18
Nuclear RARγ	4.56 $\pm$ 2.59	3.17 $\pm$ 1.40	3.17 $\pm$ 2.26	1.61 $\pm$ 1.58	0.85 $\pm$ 1.92	2.00 $\pm$ 2.63
Cytoplasmic RARγ	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.55 $\pm$ 1.81	1.78 $\pm$ 3.54	0.54 $\pm$ 1.80	0.93 $\pm$ 2.58
Nuclear RXRα	2.29 $\pm$ 2.00	2.27 $\pm$ 2.03	2.64 $\pm$ 2.60	0.44 $\pm$ 0.12	0.00 $\pm$ 0.00	0.37 $\pm$ 1.10
Nuclear RXRβ	0.71 $\pm$ 1.62	0.36 $\pm$ 1.21	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.30 $\pm$ 0.95	0.32 $\pm$ 1.33
Cytoplasmic RXRβ	0.07 $\pm$ 0.53	0.00 $\pm$ 0.00	2.18 $\pm$ 4.85	5.99 $\pm$ 4.89	2.68 $\pm$ 4.54	6.80 $\pm$ 4.55
Membranous RXRβ	1.66 $\pm$ 2.06	3.34 $\pm$ 2.05	4.53 $\pm$ 2.69	1.88 $\pm$ 1.88	1.41 $\pm$ 2.84	0.92 $\pm$ 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 $\pm$ 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.

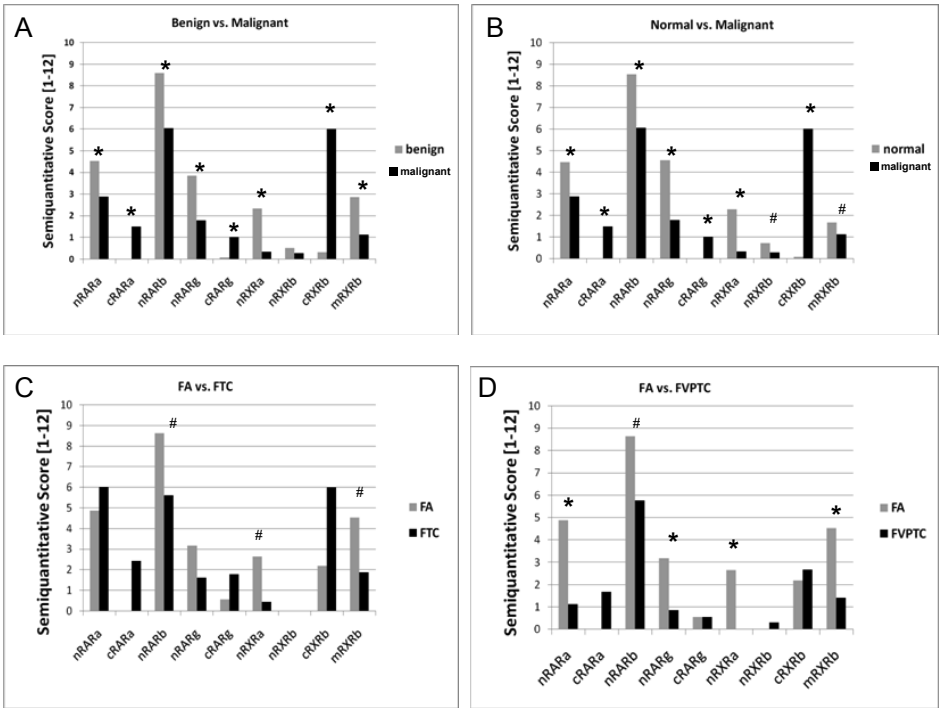


Figure 1 Immunostaining of RAR and RXR subtype antibodies in normal, benign, and malignant thyroid lesions. Immunohistochemistry scores were expressed semi-quantitatively (for explanation, see text). Data are expressed as mean $\pm$ S.D. Comparisons between (A) benign and malignant, (B) normal and malignant, 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 mRXR β - and cRXR β +	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 nRXR α + and cRXR β -	Combination not present	Total
(b) Diagnostic value of nRXR α and cRXR β 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 cRAR α , cRAR γ , cRXR β , and a decreased expression of nRAR β , nRAR γ , and nRAR α 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 nRAR α and the highest specificity for nRAR β . In the comparison between FA and FVPTC, the highest sensitivity for FVPTC was found for nRXR α and the highest specificities for nRAR α and nRAR γ . 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 RAR β expression in PTC, compared with normal tissue. Rochaix *et al.* (14) only investigated two FTC samples of which one sample showed moderate RAR β expression and the other did not. Our finding of higher nuclear and lower cytoplasm expression of RAR γ in malignant thyroid tissues was not reported before. Nuclear RXR α 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 RXR γ staining in thyroid tissues, which is unexpected, given the results of Haugen *et al.* by western blot (18).

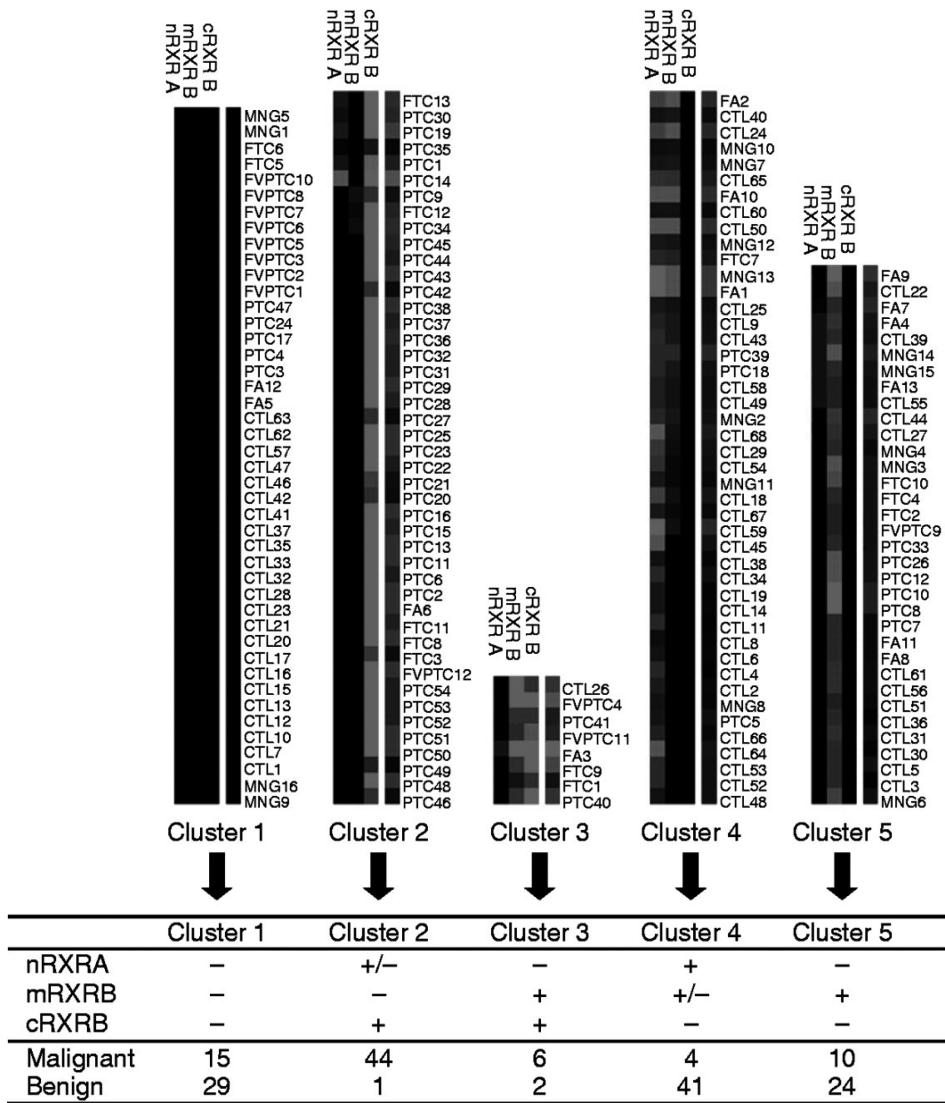


Figure 2 Hierarchical cluster analyses using RAR and RXR subtype antibodies in thyroid tissues. Nuclear RXRalpha, cytoplasmic RXRbeta, and membranous RXRbeta were identified as the best predictors of benign or malignant thyroid lesions. The absence of membranous (m) RXRbeta and the presence of cytoplasmic (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.

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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 (EGFR) 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 Ral, 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 Ral uptake in FRTL-5 cells. We therefore hypothesized that treatment with a multiple kinase inhibitor not only reduces tumor progression, but may also restore Ral uptake in non-Ral 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 Ral 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 Ral 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 Ral 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 Ral 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 Ral 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 Ral 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 Ral 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 $\leq 10\%$ or $\geq 40\%$. 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 $\pm$ 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).

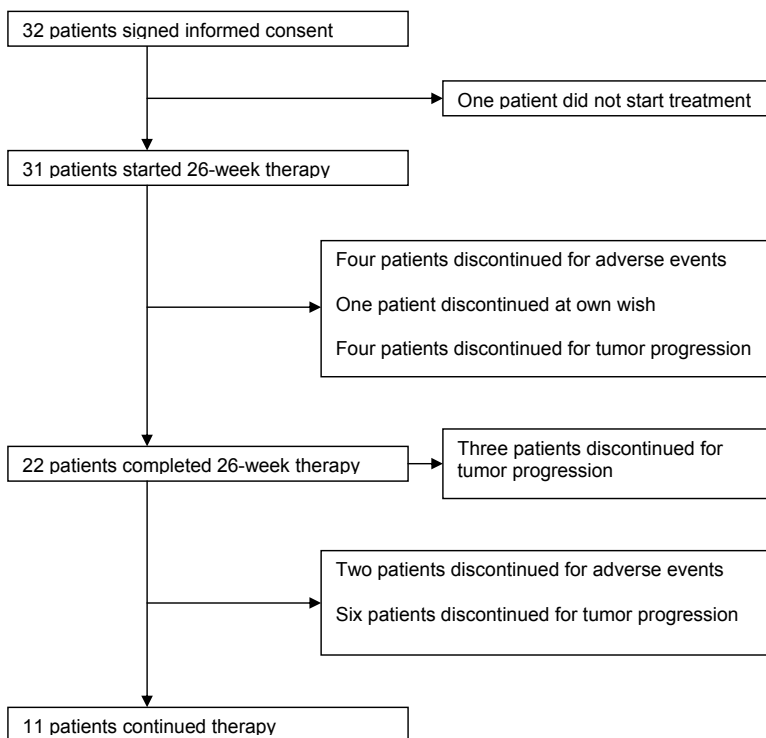


Figure 1
Study flowchart.

Baseline characteristics are given in **Table 1**. All patients had undergone prior Ral treatments with a median activity of 15.2 (range 3.7–30.0) GBq. The number of Ral 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, Ral WBS revealed uptake at the thyroid bed, but not at the sites of metastases. In 20 patients, there was no Ral uptake at metastatic sites after 26 weeks of sorafenib therapy. In one patient with an occipital skeletal metastasis that did not accumulate Ral at baseline, very slight Ral uptake was observed at 26 weeks. However, a post-therapeutic WBS performed after subsequent 7600 MBq Ral therapy did not reveal any uptake, and consequently it was concluded that no clinically relevant reinduction of Ral therapy had occurred in this patient.

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 Ral 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 Ral uptake as studied by diagnostic WBS was not observed in any patient.

Ral therapy is the only available conventional therapy for patients with metastases of DTC. Hürthle cell carcinomas respond less favorably to Ral, 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-Ral 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 Ral uptake. We did not find reinduction of Ral 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 Ral uptake (39,45), it may well be that these additional mechanisms have prevented a beneficial effect of sorafenib on Ral uptake in our study. Another explanation for the lack of an effect of sorafenib on Ral 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 Ral 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 Ral is an important determinant of tumor response, another important factor is radio sensitivity. Indeed, a subgroup of thyroid carcinomas does not respond to Ral therapy, despite Ral 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-Ral 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 Ral uptake in these patients. Future phase III studies should confirm the efficacy of sorafenib for DTC.

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**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 Ral 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: $(\text{FT4 (pmol/liter)} \times \text{weight (kg)}) / \text{levothyroxine dose (}\mu\text{g)}$.

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 $\pm$ 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 μ g/kg before vs. 2.71 μ g/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/ μ g) 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

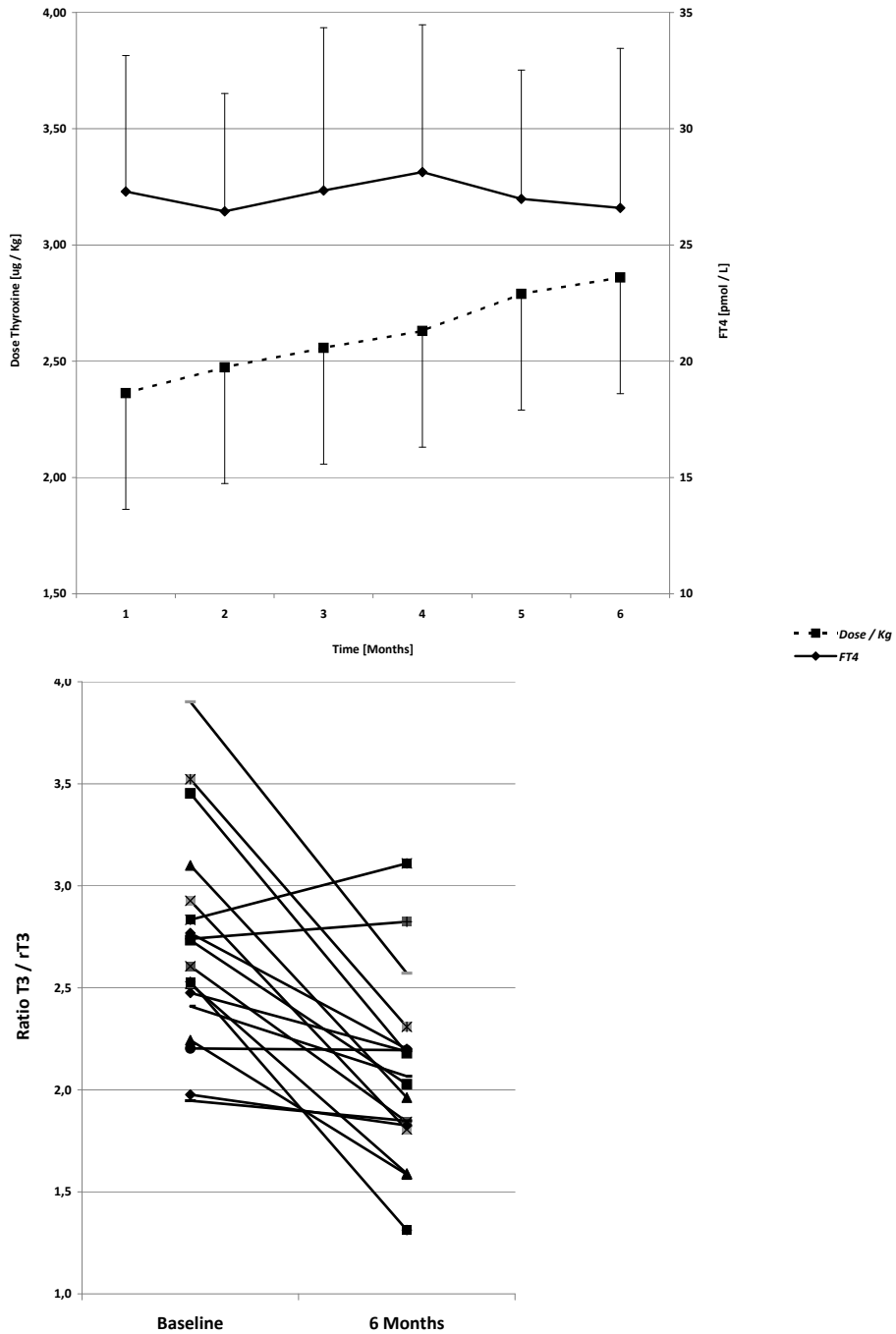
	N	Baseline	26-weeks sorafenib	P-value
Weight (kg)	21	77.3 ± 13.5	71.0 ± 14.2	<0.001
Sorafenib dose (mg/kg/day)	21	10.4 ± 2.4	8.4 ± 2.7	0.003
Levothyroxine dose (µg/kg/day)	21	2.48 ± 0.67	2.71 ± 0.61	0.008
T4 (nmol/L)	21	149.4 ± 31.9	152.9 ± 37.7	0.664
T3 (nmol/L)	20	1.90 ± 0.33	1.60 ± 0.34	0.007
FT4 (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 * 10³	13	2.73 ± 0.33	2.93 ± 0.31	0.279
FT4/T4 * 10³	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.

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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 $\mu\text{g/l}$ 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 $\times$ 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 $\pm$ 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 $\mu\text{g/day}$. Mean T4 dose was 2.2 ± 1.0 $\mu\text{g/kg}$ 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 $\mu\text{g/day}$.

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 $\ln\text{TSH}$ 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/m ²)	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	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	NS	0.046	0.026	NS	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.

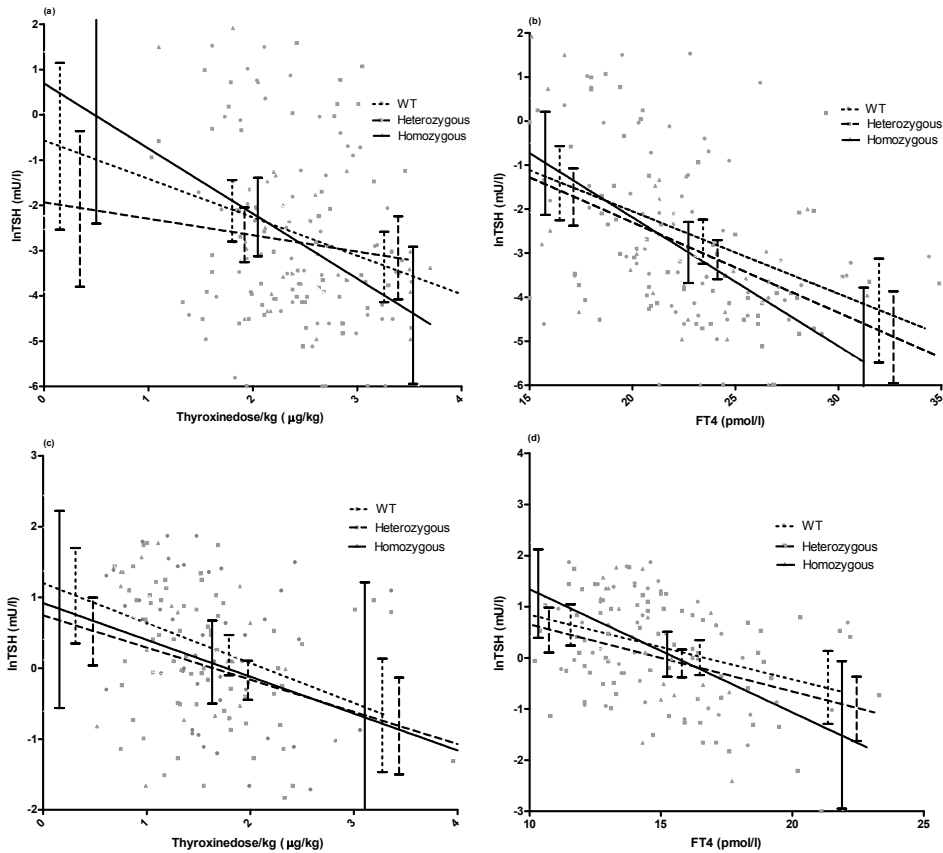


Figure 1

(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 it 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.

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**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 ($\ln[\text{TSH mU/l}]/[\text{FT4 pmol/l}]$), the intercept 4.95. For heterozygous patients the slope was -0.30 ± 0.028 ($\ln[\text{TSH mU/l}]/[\text{FT4 pmol/l}]$), the intercept 4.23. The slope of homozygous patients was -0.35 ± 0.026 ($\ln[\text{TSH mU/l}]/[\text{FT4 pmol/l}]$) 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 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 $\pm$ standard deviation (SD), median (range) or as numbers or proportions of patients. Deviation from the Hardy-Weinberg Equilibrium was analysed using a χ^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 $\ln\text{TSH} = \beta \cdot \text{FT4} + \alpha$, 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 $\pm$ SD)	49.10 $\pm$ 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 $\pm$ SD)	11.45 $\pm$ 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 $\pm$ 5.49 (range 4-32)
L-thyroxin dose (μ g/day) (mean $\pm$ SD)	182.87 $\pm$ 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 $\ln$ TSH 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 $\pm$ SD)	21.17 $\pm$ 5.63	21.27 $\pm$ 6.26	22.08 $\pm$ 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 $\pm$ SD)	-0.269 $\pm$ 0.023	-0.275 $\pm$ 0.024	-0.296 $\pm$ 0.021
Intercept (mean $\pm$ SD)	5.82 $\pm$ 0.58	5.53 $\pm$ 0.58	5.08 $\pm$ 0.82
Cross-sectional data 2007			
TSH (mU/l)	0.63 $\pm$ 1.36	0.28 $\pm$ 0.64	0.41 $\pm$ 1.01
T4 (nmol/l)	138.33 $\pm$ 33.67	141.78 $\pm$ 45.07	133.04 $\pm$ 41.51
T3 (nmol/l)	1.47 $\pm$ 0.31	1.50 $\pm$ 0.36	1.37 $\pm$ 0.33
T3/T4 ratio	0.011 $\pm$ 0.002	0.011 $\pm$ 0.002	0.011 $\pm$ 0.004
rT3 (nmol/l)	0.57 $\pm$ 0.24	0.51 $\pm$ 0.19	0.54 $\pm$ 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 $\pm$ SD)	21.61 $\pm$ 5.83	21.27 $\pm$ 5.73	22.96 $\pm$ 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 $\pm$ SD)	-0.338 $\pm$ 0.061	-0.323 $\pm$ 0.023	-0.317 $\pm$ 0.008
Intercept (mean $\pm$ SD)	4.96 $\pm$ 1.48	5.70 $\pm$ 0.55	5.52 $\pm$ 0.67
Cross-sectional data 2007			
TSH (mU/l)	0.44 $\pm$ 1.01	0.63 $\pm$ 1.23	0.09 $\pm$ 0.13
T4 (nmol/l)	137 $\pm$ 37.64	151.70 $\pm$ 50.05	115.37 $\pm$ 19.09
T3 (nmol/l)	1.46 $\pm$ 0.33	1.46 $\pm$ 0.40	1.55 $\pm$ 0.43
T3/T4 ratio	0.011 $\pm$ 0.003	0.010 $\pm$ 0.002	0.013 $\pm$ 0.003
rT3 (nmol/l)	0.56 $\pm$ 0.23	0.52 $\pm$ 0.14	0.41 $\pm$ 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 $\pm$ SD)	21.73 $\pm$ 5.93	21.63 $\pm$ 5.79	21.10 $\pm$ 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 $\pm$ SD)	-0.313 $\pm$ 0.022	-0.319 $\pm$ 0.022	-0.315 $\pm$ 0.018
Intercept (mean $\pm$ SD)	5.64 $\pm$ 0.54	5.47 $\pm$ 0.53	5.29 $\pm$ 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

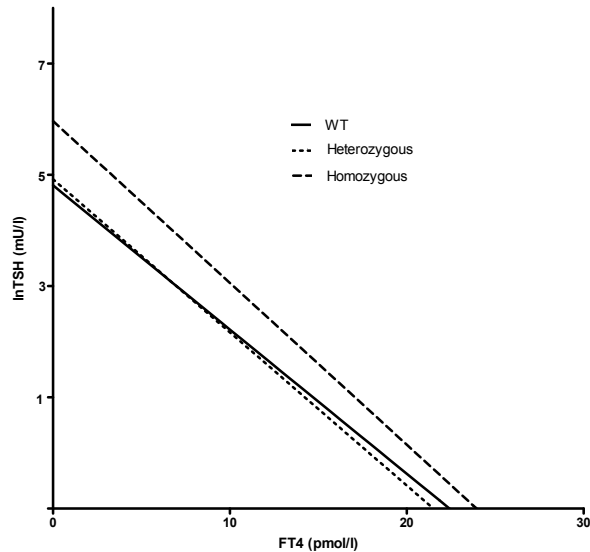


Figure 1

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.

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**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 TR α (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 $\pm$ 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 r^2 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 μ g/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

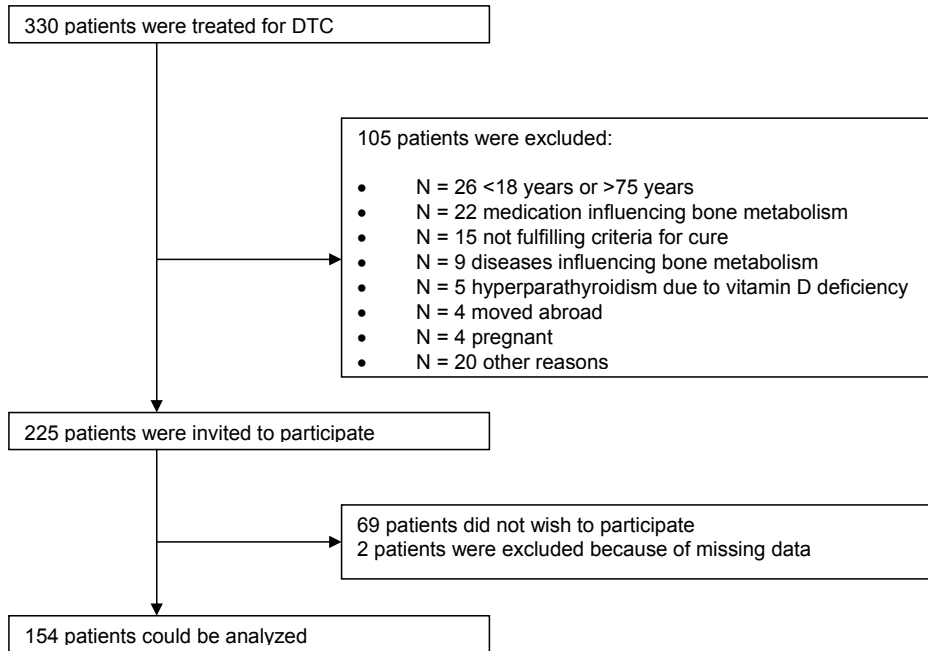


Figure 1
Flowchart of the study

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/cm²)	0.90 ± 0.02	0.84 ± 0.01	0.85 ± 0.03	0.028 /0.015 ²
BMD total hip (g/cm²)	0.97 ± 0.02	0.92 ± 0.02	0.92 ± 0.03	0.064 /0.049 ²
BMD lumbar spine (g/cm²)	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 Amino-terminal 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.

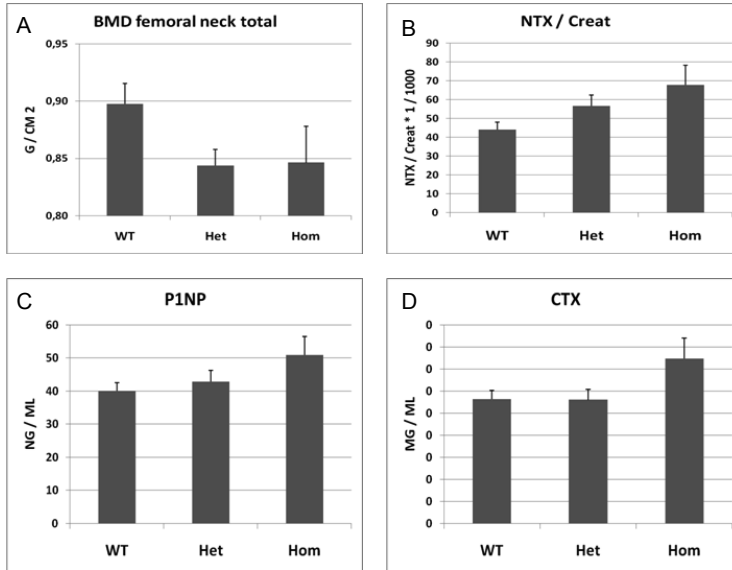
Physical activity and smoking habits did not differ either. Biochemical covariables for bone metabolism [ie, serum calcium, 25(OH)-vitamin D and PTH] were not different, as were serum FT4 and T3 levels, serum T3/T4 ratio, and TSH levels.

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 25th and 75th 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 TR α and - β , concluded that TR α 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).

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

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 ^a = 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 Ral 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) $>35 \text{ kg/m}^2$ 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 ($\text{weight [kg]} / \text{lenght}^2 \text{ [m]}$) 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 $\pm$ 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 ^s	Thyroxine replacement therapy	rhTSH day 1	rhTSH day 2
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
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
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
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
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
25(OH)Vit D (nmol/L)	59 ± 6	57 ± 6	0.52	68 ± 8	67 ± 8	65 ± 9
PINP (ng/ml)	28 ± 5	29 ± 6	0.27	38 ± 4	36 ± 4	37 ± 4
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
OPG (pg/ml)	193 ± 17	246 ± 22	0.00	174.4 ± 11.8	210.4 ± 21.9	198.7 ± 16.5
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
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
Alk. Phosphates (U/L)	66 ± 5	66 ± 5	0.81	76 ± 8	77 ± 8	75 ± 7

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 significantly 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 stimulation 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 at 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 significantly 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.

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**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 thyroxine, 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): $0.8 \times \{1.04[(LVEDD + PWT + IVST)^3 - (LVEDD)^3]\} + 0.6$, 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 $> 120 \text{ g m}^2$ for men and $> 116 \text{ g m}^2$ 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 ($r^2 = 0.99$) and small bias ($0.1 \pm 2.4 \text{ mm}$). Similarly, the intra-observer reproducibility of quantitative Doppler measurements was also excellent, with an r^2 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 $\pm$ 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 $\mu\text{g}/\text{day}$. 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 $\pm$ SD (range)	51.6 $\pm$ 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 (μ g/day) mean $\pm$ SD	162.5 $\pm$ 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 $\pm$ 3.1	9.6 $\pm$ 1.9 †	2.2 $\pm$ 1.3 †‡
T3 (nmol/l) (ref 1.1-3.6 nmol/l))	NA	1.5 $\pm$ 0.5	1.1 $\pm$ 0.2 *	0.6 $\pm$ 0.2 †‡
TSH (mU/l) (ref 0.4-4.8 mU/l)	NA	0.3 $\pm$ 0.58	9.5 $\pm$ 15.5 *	105.2 $\pm$ 57.8 †‡
Weight (kg)	72.5 $\pm$ 11.0	78.9 $\pm$ 18.5	79.9 $\pm$ 18.5 *	81.6 $\pm$ 18.5 †‡
BMI (kg/m ²)	24.9 $\pm$ 3.1	26.5 $\pm$ 6.1	26.9 $\pm$ 5.9 †	27.6 $\pm$ 6.0 †‡
Systolic blood pressure (mmHg)	124.8 $\pm$ 7.7	130.1 $\pm$ 23.2	130.2 $\pm$ 23.3	131.3 $\pm$ 20.1
Diastolic blood pressure (mmHg)	76.0 $\pm$ 6.9	81.7 $\pm$ 16.5	77.6 $\pm$ 12.9	85.5 $\pm$ 10.4 #§
Mean blood pressure (mmHg)	92.3 $\pm$ 6.3	97.8 $\pm$ 18.3	95.2 $\pm$ 15.4	100.8 $\pm$ 12.3
Heart rate (BPM)	70.4 $\pm$ 8.4	70.8 $\pm$ 8.0	65.2 $\pm$ 8.2 *	66.6 $\pm$ 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 $\leq$ 0.001 compared to visit 1, # = P< 0.05 compared to visit 2, ‡= P $\leq$ 0.001 compared to visit 2, §= P $\leq$ 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	9.9 ± 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 sarcoplasmic 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.

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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 electrochemoluminescent 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 $\pm$ 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 0.4 mU/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 μ g/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 $\pm$ 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 $\pm$ 21.02	88.27 $\pm$ 16.78	0.052	87.77 $\pm$ 17.14	0.040
Social functioning	81.09 $\pm$ 24.90	87.39 $\pm$ 20.01	0.037	88.06 $\pm$ 19.28	0.007
Role limitations due to physical problems	75.35 $\pm$ 40.04	81.42 $\pm$ 34.36	0.194	83.38 $\pm$ 32.43	0.035
Role limitations due to emotional problems	83.22 $\pm$ 35.43	84.66 $\pm$ 31.82	0.734	85.93 $\pm$ 30.21	0.422
Bodily pain	82.74 $\pm$ 21.70	84.78 $\pm$ 18.93	0.426	85.17 $\pm$ 19.24	0.216
General health perception	65.59 $\pm$ 20.48	71.45 $\pm$ 18.43	0.027	71.34 $\pm$ 18.79	0.007
Change in health	52.15 $\pm$ 18.37	55.18 $\pm$ 18.19	0.185	54.77 $\pm$ 18.64	0.105
MFI-20					
General fatigue	11.03 $\pm$ 4.72	8.11 $\pm$ 3.35	<0.001	8.60 $\pm$ 4.01	<0.001
Physical fatigue	9.95 $\pm$ 4.93	6.65 $\pm$ 2.64	<0.001	7.60 $\pm$ 3.69	<0.001
Reduced activity	8.79 $\pm$ 4.15	6.85 $\pm$ 3.30	<0.001	7.18 $\pm$ 3.57	<0.001
Reduced motivation	8.64 $\pm$ 3.76	6.67 $\pm$ 2.79	<0.001	7.26 $\pm$ 3.53	<0.001
Mental fatigue	9.53 $\pm$ 4.50	7.93 $\pm$ 3.60	0.002	7.92 $\pm$ 3.31	<0.001
HADS					
Anxiety	5.69 $\pm$ 3.95	4.14 $\pm$ 3.15	<0.001	4.21 $\pm$ 3.21	<0.001
Depression	3.61 $\pm$ 3.08	2.37 $\pm$ 2.52	<0.001	2.86 $\pm$ 2.99	0.011
Total	9.30 $\pm$ 6.30	6.51 $\pm$ 4.92	<0.001	7.07 $\pm$ 5.39	<0.001
SDQ					
SDQ total	5.92 $\pm$ 6.20	1.66 $\pm$ 2.51	<0.001	1.65 $\pm$ 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 $\beta=0.21$, $P=0.030$), role limitations due to physical problems ($\beta=0.17$, $P=0.049$), general health perception ($\beta=0.32$, $P=0.001$), MFI-20 general fatigue ($\beta=-0.17$, $P=0.035$), physical fatigue ($\beta=-0.24$, $P=0.003$), and mental fatigue scores ($\beta=-0.17$, $P=0.038$). 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 1a

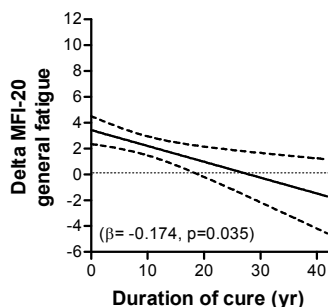


Figure 1d

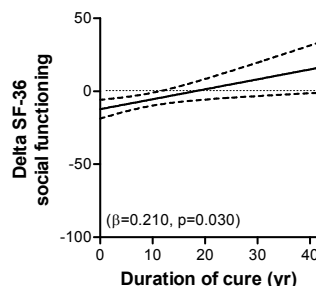


Figure 1b

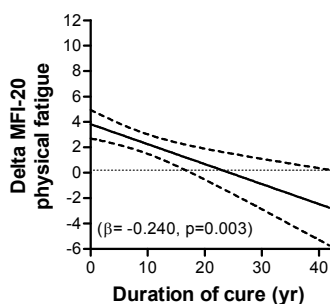


Figure 1e

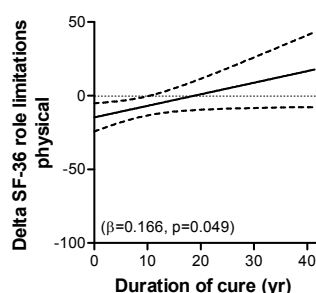


Figure 1c

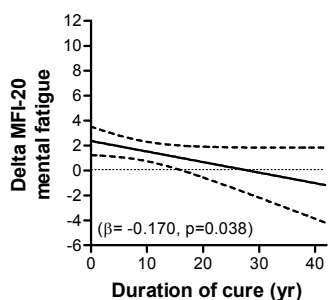


Figure 1f

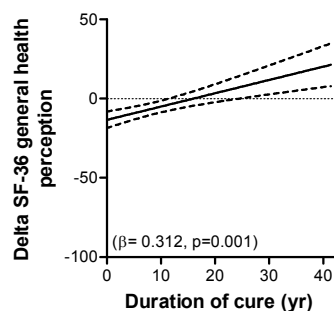


Figure 1

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.

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Discussion and Summary



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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-Ral avid metastatic DTC. In the other part of this thesis, we focused on the clinical consequences of initial therapy consisting of thyroidectomy and Ral 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-Ral avid metastatic disease

1. Sorafenib for Ral 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 Ral. Treatment options for patients with Ral 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-Ral 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 Ral 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 (IC₅₀ nM), whereas sorafenib inhibits RET (IC₅₀ 47 nM), RET/PTC3 (IC₅₀ 50 nM) 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 Ral 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 Ral uptake in non-Ral avid DTC. We treated 31 patients with progressive metastatic or locally advanced Ral refractory DTC with sorafenib 400 mg b.i.d. The primary endpoint was reinduction of Ral uptake at 26 weeks. Additional endpoints were the radiological response and the influence of bone metastases.

Ral therapy is the only available conventional therapy for patients with metastases of DTC. Hürthle cell carcinomas tend to respond less favorably to Ral, 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 Ral 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 Ral uptake (22,23), it may well be that these additional mechanisms have prevented a beneficial effect of sorafenib on Ral 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 Ral 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-Ral 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 thyroxine 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-Gly3Asp 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 than 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 thyroxine, 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 sarcoplasmic 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 were 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.

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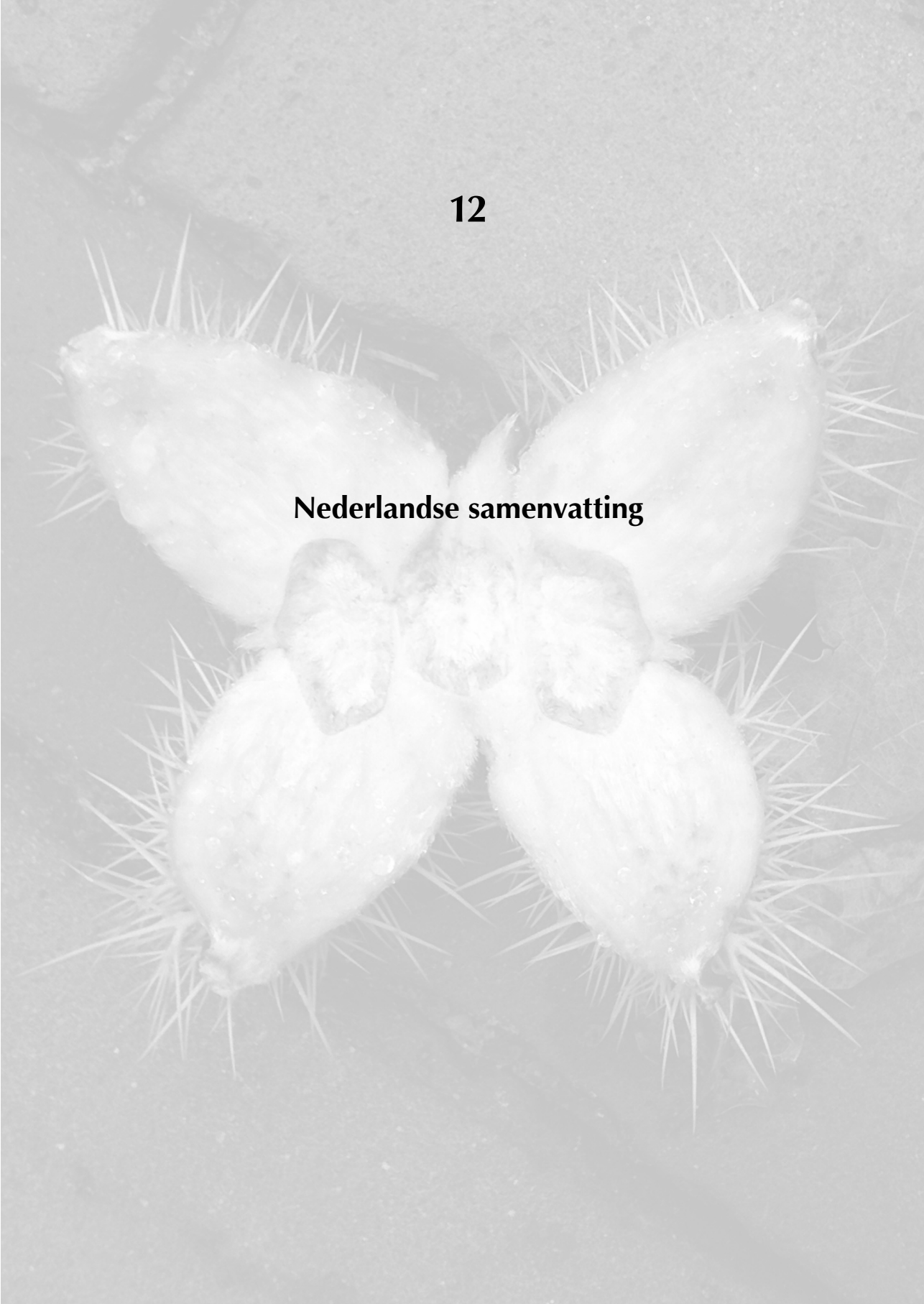
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Nederlandse samenvatting



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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 aangrijppingspunten 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 multiële orgaansystemen en kan van invloed zijn op hartfunctie, botmetabolisme, glucosemetabolisme en de kwaliteit van leven. Tijdens de follow-up van schildklier carcinoom 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 schildklier carcinoom. 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 schildklier carcinoom te verbeteren. In de rest van dit proefschrift focussen we op de klinische consequenties van de behandeling van schildklier carcinoom. 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 schildklier carcinoom

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 folliculair adenoom en maligne folliculair 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-)threoïdectomie 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 folliculair adenoom) en 77 schildkliercarcinomen (papillair schildkliercarcinoom (PTC), folliculair schildkliercarcinoom (FTC) en folliculaire 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-curve) 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 membraaneus 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 nR-XRalpha 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 geïnccludeerd.

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 inactieveert 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 suppressieve dosis thyroxine gedurende een periode van 15 jaar. Waar deze TSH suppressieve 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 thyroxinedosering te onderzoeken in patiënten behandeld voor schildkliercarcinoom en patiënten met Hashimoto thyreoïditis. Daarvoor hebben we 154 patiënten met gediifferentieerd 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-schilddklier 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 schildklier-carcinoom 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 schildklier-car-

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 suppressieve 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 suppressieve 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 coronariaalijden, 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 suppressieve 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 (vroeg 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 schildklierhormoonsschommelingen 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.

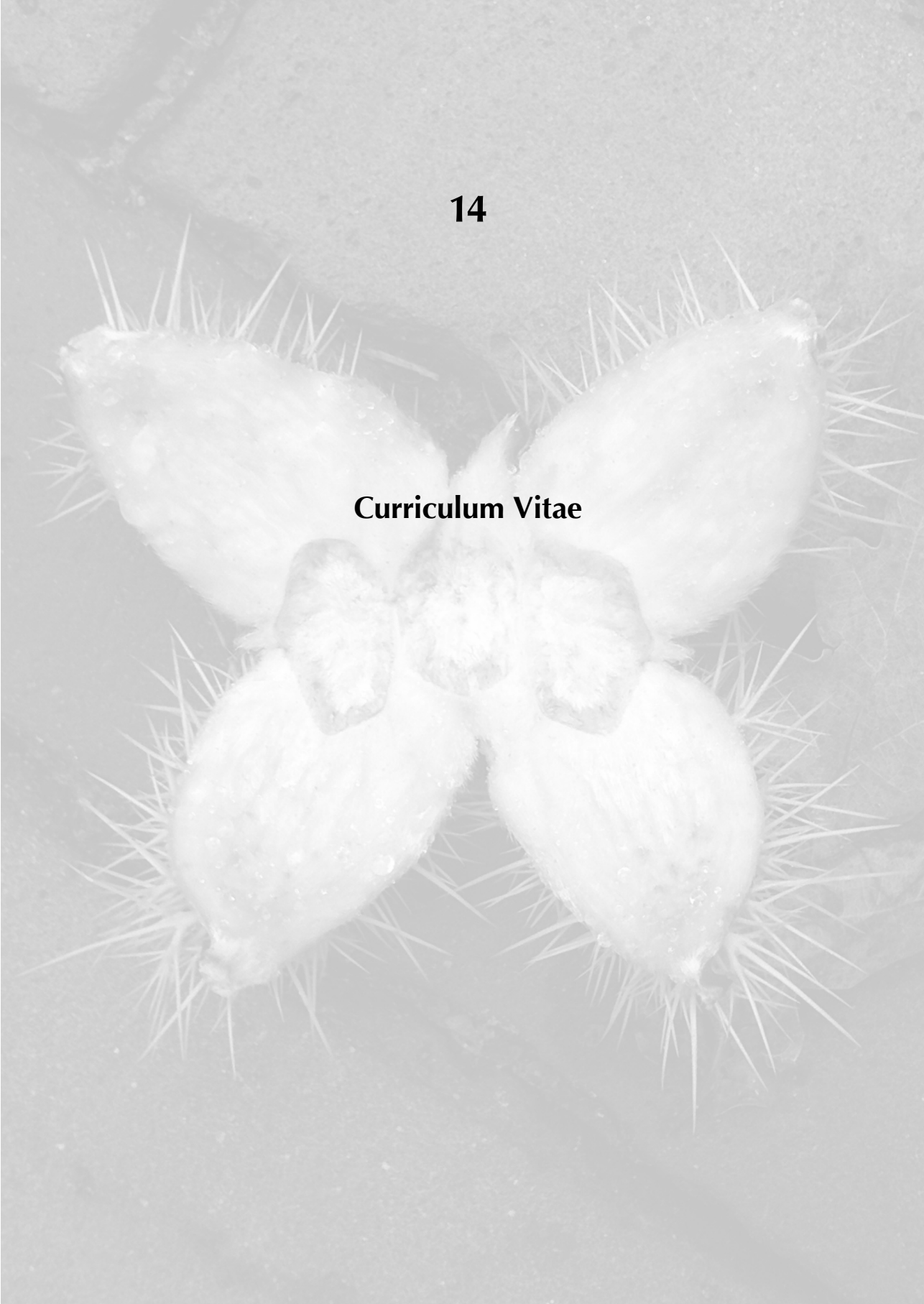
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Curriculum Vitae



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).