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Differentiated thyroid carcinoma : treatment and clinical consequences of therapy

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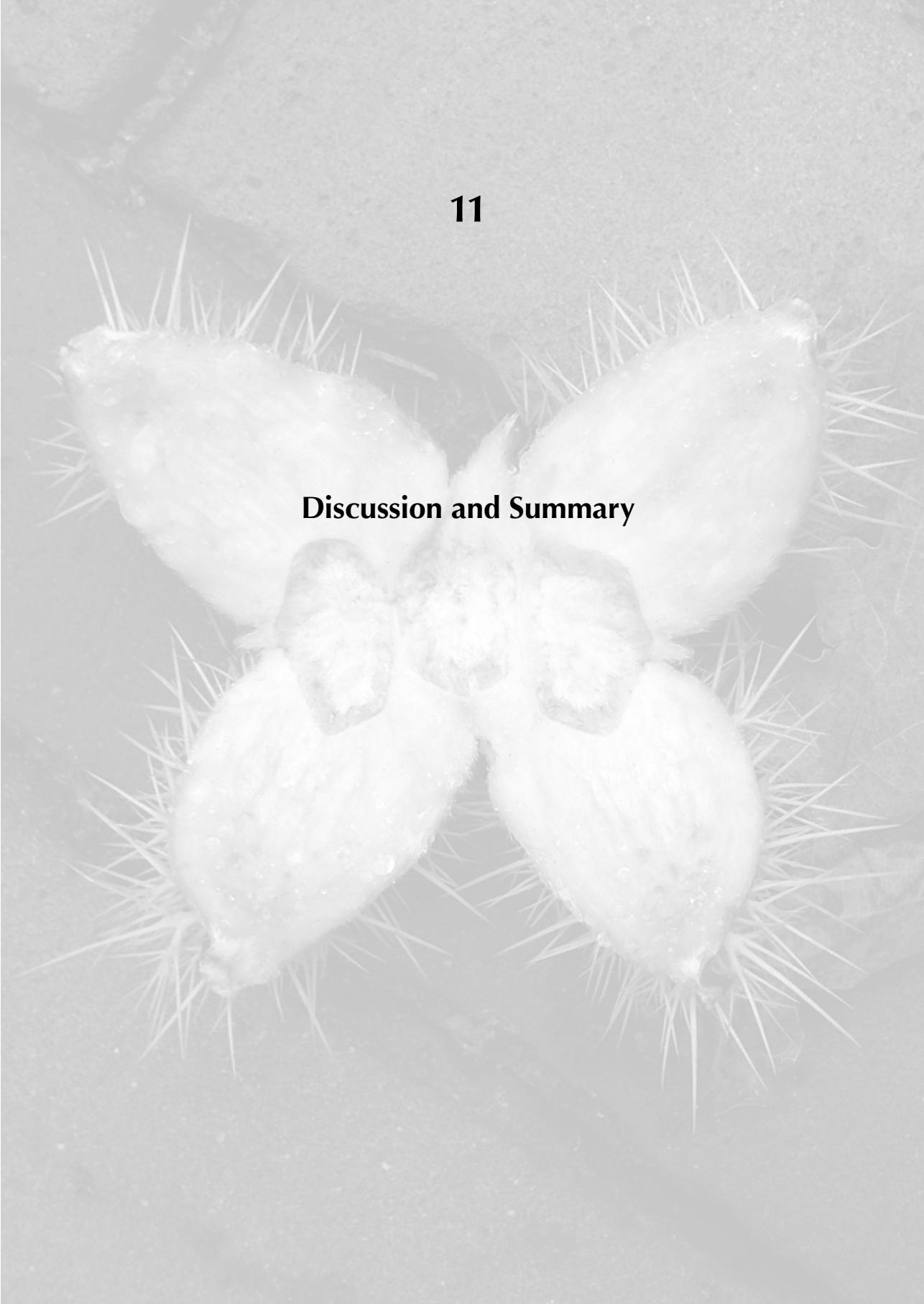
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Discussion and Summary



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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 (IC₅₀nM), whereas sorafenib inhibits RET (IC 50 47nM), RET/PTC3 (IC₅₀ 50nM) and BRAF (IC₅₀ 22 nM). However comparison of the results of phase II studies with different tyrosine kinase inhibitors in DTC is hampered by differences in patient categories (including histologies, tumor stages, sites of metastases, and tumor extent), study design, and analytical methods.

We decided to study the effects of the multitarget tyrosine kinase inhibitor sorafenib on the reinduction of 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 thyroxin substitution after treatment for thyroid carcinoma (26,28,42,43). Therefore, the mechanisms of hypothyroidism may include alterations in thyroid hormone metabolism as well. Stepwise deiodination is the major route of thyroid hormone degradation and is mediated by iodothyronine deiodinases (D1, D2, and D3) (44) and by hepatic conjugating enzymes (45).

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

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

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

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

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

Clinical implications

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

IV. Consequences of treatment of thyroid carcinoma

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

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

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

1. Insights in thyroid hormone metabolism

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

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

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

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

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

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

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

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

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

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

Our observations are in contrast with the findings of Coppotelli *et al.* (62) who found an increased D2 activity of the D2-ORFa-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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