Differentiated Thyroid Cancer

Optimization of Patient Well-being, Treatment and Prognosis

Evert F.S. van Velsen

Differentiated Thyroid Cancer: Optimization of Patient Well-being, Treatment and Prognosis

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optimalisatie van patiëntwelzijn,
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CONTENTS

Chapter 1

General Introduction

THYROID

The thyroid is an endocrine gland located in the lower part of the anterior neck, just below the larynx. It has a butterfly-shape consisting of two lobes connected by a strip that lays across the anterior surface of the trachea (see Figure 1). The main function of the thyroid is to produce thyroid hormone (TH), a hormone which is crucial for the normal development and metabolism regulation of all tissues. Several critical structures are located around the thyroid gland. The parathyroid glands are small glands located posterior to each thyroid pole. Also located along the left and right posterior part are the two recurrent laryngeal nerves through which the muscles of the vocal cords are innervated. The thyroid itself consists of follicular cells surrounding large follicles filled with colloid, and a smaller number of parafollicular cells, also called c-cells (see Figure 1).

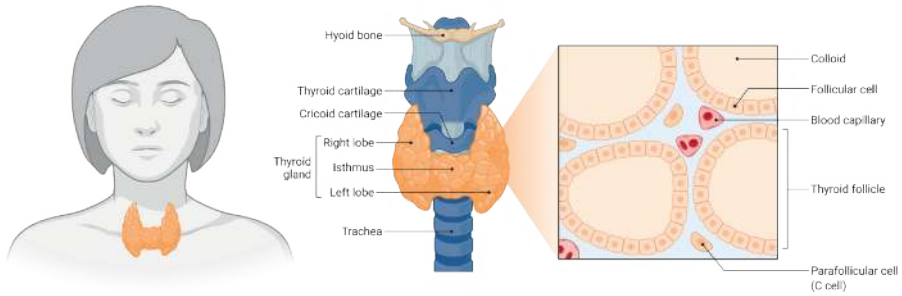

Figure 1. Anatomy (left and middle) and more detailed structure (right) of the thyroid gland. Created with BioRender.com.

The importance of normal functioning of an organ is shown by its dysfunction. Abnormal TH concentrations affect the functioning of several other organs, possibly resulting in a myriad of clinical symptoms (e.g. (1)). The clinical presentation of hypothyroidism is broad and reflects shortness of TH at the tissue level; symptoms include tiredness, depressiveness, cold tolerance, constipation, bradycardia, and weight gain. On the other hand, the clinical presentation of hyperthyroidism reflects an excess of TH; symptoms include palpitations, sweating, weight gain, and feeling rushed.

Development of abnormal density of thyroid cells could cause nodules, which in non-iodine deficient countries can be found in 4-7% of the population (2). A small proportion of these nodules are malignant (3).

In the remainder of this Introduction, I will focus first on thyroid hormone synthesis and action. Thereafter, hypothyroidism and thyroid cancer, including current knowledge gaps, will be discussed. Finally, the outline of this thesis will be given.

THYROID HORMONE SYNTHESIS, SECRETION AND ACTION

The synthesis of TH is a process consisting of multiple steps. Iodide is the principal component of TH, and is transported into the follicular cells by the sodium (Na)-iodine symporter (NIS). Thereafter, iodide is transported into the follicle by the SLC26A4 transporter (Pendrin). In the follicular lumen, iodide is oxidized (by thyroid peroxidase (TPO)), and incorporated into tyrosine residues present in the amino acid sequence of thyroglobulin (Tg); iodide is incorporated either as mono-iodinated tyrosine (MIT) or di-iodinated tyrosine (DIT). Coupling of two DIT residues leads to formation of 3,3',5,5'-tetraiodothyronine (thyroxine; T4), while coupling of one MIT and one DIT leads to formation of 3,3',5-triidothyronine (T3). These two compounds remain stored in the follicles until needed. About 85% of the daily thyroid gland output is T4. However, T4 is considered a pro-hormone, and T3 is the active hormone exerting all biological effects. T4 and T3 circulate around in serum mainly bound to thyroid transport proteins with a small fraction present in free form (free-T4 or free-T3). T4 is converted into T3 by deiodinases, which are selenium-containing enzymes. There are three forms of deiodinases, i.e. types 1 (D1), 2 (D2), and 3 (D3). T4 is converted into T3 using either D1 (in liver and kidney), or D2 (in brain, brown adipose tissue, skeletal muscle, and heart). D3 (in brain, skin and placenta) degrades T4 and T3 into either reverse-T3 (rT3; 3,3'5'-triiodothyronine) or 3,3'-diiodothronine (3,3'-T2). Further degradation of thyroid hormone metabolites (THM) is also supported by deiodinases (4) (see also Figure 2).

The hypothalamus pituitary thyroid (HPT)-axis controls TH concentrations by regulating both synthesis and secretion of T4 and T3. Thyroid Stimulation Hormone (TSH) is the main regulator, and is produced by the pituitary. TSH is under the regulation of 1) thyrotropin releasing hormone (TRH) that is produced by the hypothalamus, and 2) the circulating concentrations of TH. TSH is sensitive to small changes of peripheral TH levels. This mechanism is a classical feedback loop (see Figure 3). It has been shown that measuring serum TSH is the most sensitive method to screen for and to diagnose thyroid disease like hypo- and hyperthyroidism (5).

The uptake of T4 and T3 by target cells is mediated by several transporting proteins. These include the organic anion transporter (OATP) family members, monocarboxylate transporter (MCT) 8 and 10, and the L-type amino acid transporters LAT1 and LAT2 (6,7). TH acts via both genomic and non-genomic pathways. Genomic

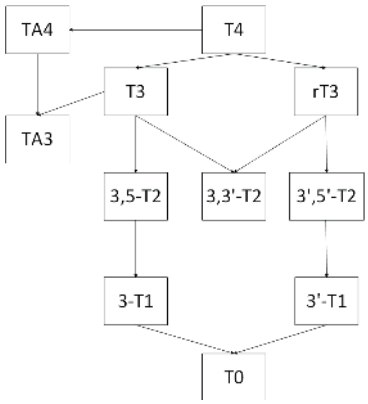

Figure 2. Structures of the principal thyroid hormones and their major pathways of deiodination.

Thyroxine (T4), 3,3',5-triiodothyronine (T3), 3,3',5'-triiodothyronine (rT3), 3,3'-diiodothyronine (3,3'-T2), 3,5-diiodothyronine (3,5-T2), 3',5'- diiodothyronine (3',5'-T2), 3-iodothyronine (3-T1), 3-iodothyronine (3'-T1), and L-thyronine (T0).

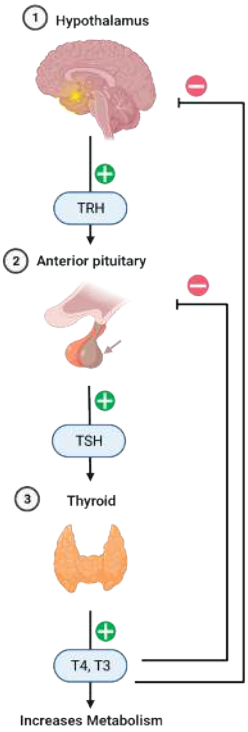

Figure 3. The hypothalamic-pituitary-thyroid-axis.

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actions are mediated by binding to specific nuclear T3 receptors, thereby regulating transcription of target genes (8). There are two forms of receptors, i.e. thyroid receptor alpha (TRα) and beta (TRβ). Isoforms of these receptors have tissue-specific expression and functions. There are three T3 binding isoforms, TRα1, TRβ1, and TRβ2. TRα1 is predominantly expressed in brain, heart, and bone, TRβ1 is mostly expressed in brain, liver, and kidney, while TRβ2 is mainly expressed in hypothalamus and the pituitary gland. Therewith, TRβ2 is the main player in the regulation of the HPT-axis (9). Non-genomic actions of TH have also been described, and these actions are mediated through a number of different membrane receptors (10).

HYPOTHYROIDISM

Hypothyroidism is a very common endocrine disorder (11). Primary hypothyroidism due to thyroid auto-immunity (Hashimoto's disease) is the most frequent cause. Replacing the deficient hormone is the basis for the treatment of hypothyroidism, and therefore levothyroxine (LT4) is given to restore euthyroidism. Although biochemical euthyroidism can be achieved by LT4, a substantial part of the patients (± 10-15%) show significant impairment of physical and psychological well-being compared to matched controls or the general population (12-17). In addition, thyroid cancer survivors after thyroidectomy being on LT4 therapy also continue to have a decreased quality of life (QoL) in different domains (18-23). Their QoL is at the same level of patients with other cancers with worse prognosis, and is even worse than QoL of breast cancer survivors (24).

Several possible explanations for these persistent symptoms exist. First, thyroid autoimmunity in itself could cause persisting symptoms (25-27). A second possibility would be the inability of LT4 replacement treatment to restore physiological T4 and T3 concentrations in serum and tissue (28,29). Although not all patients show significant impairment, in hypothyroid rats it was shown that LT4 monotherapy was not able to normalize concentrations of T4 and T3 in all tissues, and even supraphysiological serum T4 concentrations were needed to normalize their T3 concentrations (30). This argument has been used to advocate in favor of T4/T3 combination therapy. However, although more than ten randomized controlled trials (RCTs) and meta-analyses were conducted, there is still insufficient evidence for the benefit of routinely adding LT3 to LT4-treatment, and therefore, the current 2014 ATA Guidelines do not advise to treat patients with T4/T3 combination therapy outside a formal clinical trial or N-of-1 trial (31). Third, next to T3, THM, which may have physiological functions, might be altered in these patients as well. T3 independent effects have been reported for some of these metabolites such as 3,5-diiodo-L-thyronine (3,5-T2)

(32), and 3, 5, 3'-triiodothyroacetic acid (TA3) (33). Altered serum concentrations of different metabolites, like 3,3',5,5'-tetraiodothyroacetic acid (TA4), 3,5,3'-triiodothyroacetic acid (TA3), and 3,5-T2, rT3, L-thyronine (T0), 3-monoiodothyronine (3-T1), 3,3'-T2 have been reported in hypo and hyperthyroidism (4). Further, one study found higher 3,5-T2 concentrations in thyroid cancer patients after thyroidectomy compared to healthy controls (34), but in another study 3,5-T2 did not correlate with TSH, T4 or T3 (20). Besides, two earlier studies showed no correlation of T4, T3, rT3, and 3,5-T2 with QoL (20,22). Therefore, the possible relationship of THM with decreased QoL in patients on LT4 replacement therapy for hypothyroidism and in patients after thyroidectomy is still under debate, and further research is needed to solve this question.

THYROID CANCER

Epidemiology and Clinical Presentation

Thyroid cancer is the most common endocrine malignancy with approximately 700 new cases in The Netherlands in 2018 (35). The worldwide incidence has been steadily increasing over the last two decades (36,37). Different subtypes of thyroid cancer exist of which well differentiated thyroid cancer (DTC), comprising both papillary (PTC) and follicular thyroid cancer (FTC), to be the most frequent (80-85%) (38). Both DTC subtypes develop from the follicular cells, and therefore retain characteristic of normal thyroid cells, i.e. taking up iodine, producing Tg, and being under influence of TSH. For pathology images of both PTC and FTC see Figure 4. The other thyroid cancers are medullary (MTC), poorly differentiated (PDTC) and anaplastic carcinomas (ATC). MTC develops from the c-cells, while both PDTC and ATC also develop from follicular cells. However, these latter two are more aggressive due to genomic instability (39). For the remainder of this section I will focus on DTC unless otherwise specified.

The majority of the patients with thyroid cancer present with either one or more lumps in the neck region; this can be either a thyroid nodule or enlarged lymph nodes. Sometimes the presenting symptom is pain originating from distant metastases, e.g. due to pathological fractures from bone metastases. In general, neck ultrasonography combined with fine needle aspiration (FNA) is needed to make a diagnosis. Neck ultrasound is also very useful to determine the presence of suspicious lymph nodes. If the diagnosis is established, or very likely, further treatment is warranted (38).

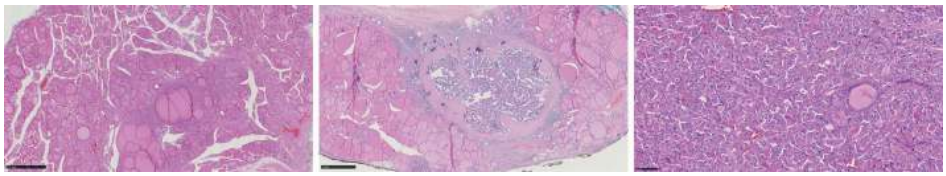

Figure 4. Pathology images of a normal thyroid (left), papillary thyroid carcinoma (middle) and follicular thyroid carcinoma (right).

Treatment

Initial treatment of DTC is multidisciplinary and is usually multistep wise (38). According to the current 2015 Dutch Thyroid Cancer Guidelines, a total thyroidectomy is recommended if the tumor is larger than 1cm, is multifocal, has extrathyroidal extension, or lymph node or distant metastases exist (38); otherwise a hemithyroidectomy is sufficient. Postoperatively, the tumor is staged according to the TNM system (40,41). After a total thyroidectomy, the Dutch Guidelines recommend to perform subsequent radioiodine (RAI) treatment with I-131 (38). To ensure optimal RAI uptake, treatment takes place under an iodine-deficient diet (during one week) and TSH stimulation. TSH stimulation can be achieved either after 3-4 weeks of TH withdrawal, or using two injections of recombinant human TSH (rhTSH). According to the Dutch Guidelines, rhTSH is only recommended in case of unifocal intrathyroidal small FTC or classical PTC without lymph node or distant metastases (pT1N0M0 or pT2N0M0), or in case of comorbidities. However international guidelines and experts are less strict regarding the indication for rhTSH (42-44). Goals for RAI therapy are to destroy any remaining thyroid remnants or thyroid cancer cells (local or distant). Besides, iodine uptake can be visualized using whole body scintigraphy in combination with a single-photon emission computer tomography (SPECT)-scan. After surgery in case of rhTSH, or after RAI therapy in case of TH withdrawal, patients are treated with TH replacement therapy with LT4. Due to the lack of TH producing thyroid cells after surgery, substitution is vital, and supraphysiological doses are commonly used to suppress TSH. The rationale behind this is that TSH can stimulate growth of thyroid cancer cells, and therefore lower TSH levels are thought to prevent re-growth of remaining cells (45,46). In contrast to the current 2015 Dutch Thyroid Cancer Guidelines, the 2015 American Thyroid Association (ATA) Guidelines are less aggressive (38,42). In the 2015 ATA Guidelines, the need for total thyroidectomy and need for subsequent RAI treatment is based on a combination of the TNM classification and the ATA Risk Stratification System (42). For example, in Low Risk tumors of <4cm there is no indication for completion thyroidectomy and subsequent RAI treatment (see Table 1). However, the approach stated in the 2015 ATA Guidelines regarding extent of surgery and need for RAI treatment has

Table 1. Overview of treatment differences between the 2015 Dutch and ATA Guidelines for Differentiated Thyroid Cancer.

	2015 Dutch Guidelines	2015 ATA Guidelines
pT1aN0Mx (unifocal)	HT	HT
pT1aN0Mx (multifocal)	TT + RAI	HT
pT1b/pT2 N0Mx	TT + RAI	HT
pT3a/b N0Mx	TT + RAI	TT + Consider RAI
pT1-3 N1aMX	TT + RAI	TT + Consider RAI
pT1-3 N1bMX	TT + RAI	TT + Consider RAI
pT4 Any N, Any M	TT + RAI	TT + RAI
M1, Any T, Any N	TT + RAI	TT + RAI
ATA Low Risk (pT1/pT2)	-	HT
ATA Low-Intermediate Risk (pT1-pT3 or N1a/1b)	-	TT + Consider RAI
ATA High Risk	-	TT + RAI

HT, hemithyroidectomy; TT, total thyroidectomy; RAI, radioactive iodine; ATA, American Thyroid Association.

been challenged by several experts in the field (47,48). Because one of the aims of RAI therapy is to treat any remaining unknown cancer tissue, omitting RAI therapy might therefore leave metastases unknown and untreated (47). One study showed that 3.6% of the ATA Low Risk patients had distant metastases that would have been missed when following the 2015 ATA Guidelines (49). However, they also included patients in whom distant metastases were detected during follow-up. Therefore, the impact of the 2015 ATA Guidelines on the possible proportion of undetected distant metastases is still unknown.

After initial treatment, follow-up is usually performed using Tg measurements during TSH-suppression (Tg-on), and an ultrasound of the neck. Tg is used as a tumor marker which is sensitive in case of absence of Tg-antibodies. After six to 18 months, response to initial therapy is assessed (i.e. Dynamic Risk Stratification) using rhTSH-stimulated Tg measurements (42). Response to initial therapy is classified (see also Table 2) as either excellent response (no evidence of disease), structural incomplete response (persistent structural disease) biochemical incomplete response (persistent elevated Tg), or indeterminate response (not one of the other three). In case of an excellent response, follow-up can be less frequent, while in both structural and biochemical incomplete response additional therapy can be considered. This can be either surgery and/or radioiodine therapy, but in case of extensive not curable disease, also external beam radiotherapy (EBRT) or tyrosine kinase inhibitors (TKI) can be considered (38,42).

Table 2. Overview of the Response to Therapy Definitions of the 2015 ATA Guidelines for Differentiated Thyroid Cancer.

Category	Definitions
Excellent Response	Negative imaging **and either**
	Suppressed Tg <0.2 ng/mL **or**
	TSH-stimulated Tg <1 ng/mL
Biochemical incomplete Response	Negative imaging **and**
	Suppressed Tg ≥1 ng/mL **or**
	Stimulated Tg ≥10ng/mL **or**
	Rising anti-Tg antibody levels
Structural incomplete Response	Structural or functional evidence of disease
	With any Tg level
	With or without anti-Tg antibodies
Indeterminate Response	Nonspecific findings on imaging studies
	Faint uptake in thyroid bed on RAI scanning
	Nonstimulated Tg detectable, but < 1ng/mL
	Stimulated Tg detectable, but < 10ng/mL
	or
	Anti-Tg antibodies stable or declining in the absence of structural or functional disease

Tg, thyroglobulin; TSH, Thyroid Stimulation Hormone.

Prognosis

The survival of patients with DTC is relatively good, with a 10-year disease specific survival (DSS) over 90% (42). Therefore, day-to-day treatment of patients is to a large extent based on the risk of recurrence. However, there are certain patients in which survival is worse, and it is important to identify them. Different systems have been proposed to predict the risk of recurrence and survival in patients with DTC to better determine the need for aggressive therapy and optimize follow-up strategies. The ATA and European Thyroid Association (ETA) risk stratification systems are designed to estimate the risk of disease recurrence (42,50), while the AJCC/TNM staging system is best used to predict DSS (40,51).

The 8th edition of the AJCC/TNM staging system for DTC has been developed to improve prediction of survival in patients with DTC, and was introduced in clinical practice in January 2018 (41). The most important differences with the previous 7th edition are 1) a raised age cutoff from 45 to 55 years, 2) removal of minor extrathyroidal extension from the definition of T3 tumors, and 3) N1 disease does no longer automatically result in staging into stage III or IV in older patients, but into stage II (51) (see also Table 3). After introduction, several studies showed superiority of the 8th to the 7th edition in predicting survival (52-56), but these studies comprised

Table 3. Overview of the 7th and 8th AJCC/TNM Staging Systems for Differentiated Thyroid Cancer.

Stage	7th edition		8th edition	
	Age	TNM	Age	TNM
	<45 years		<55 years	
I		AnyT/AnyN/M0		AnyT/AnyN/M0
II		AnyT/AnyN/M1		AnyT/AnyN/M1
	≥45 years		≥55 years	
I		T1/N0/M0		T1-2/N0/M0
II		T2/N0/M0		T1-2/N1/M0
				T3/AnyN/M0
III		T3/N0/M0		T4a/AnyN/M0
		T1-3/N1a/M0		
IVa		T4a/N0-1a/M0		T4b/AnyN/M0
		T1-4a/N1bM0		
IVb		T4b/AnyN/M0		AnyT/AnyN/M1
IVc		AnyT/AnyN/M1		

only patients with PTC (54,55), or had low numbers of patients with FTC and did not distinguish between PTC and FTC (52,53,56). Therefore, it is valuable to know if the 8th edition performs well in a population of patients with DTC, including a large set of FTC patients. Next to this, changing the age cutoff was one of the most important differences, but it is unclear if another age cutoff than 55 years may lead to further improvement of the prognostic value of the AJCC/TNM 8th edition.

The ATA Risk Stratification System (see Table 4 for 2015 edition) is widely used and several studies have shown its usefulness in predicting disease recurrence (55,57-62). The majority of these studies either evaluated the 2009 version or contained relatively few patients with ATA High Risk. Therefore, it is important to know how this Risk Stratification System performs in these High Risk patients, especially with respect to recurrence. In addition, earlier studies showed that patients with distantly metastasized DTC have a relative poor prognosis (63-66). However, no studies yet evaluated the 2015 ATA Risk Stratification System in DTC patients with distant metastases with respect to its ability to predict prognosis, recurrence and survival. In contrast to the AJCC/TNM staging system, age is not incorporated in the ATA Risk Stratification System. Recently, it was shown that the addition of age as a factor in the risk classification could improve this stratification system, especially for High Risk patients (67). However, this study comprised relative few High Risk patients and only cutoffs of 45 and 55 years were investigated.

Table 4. Overview of the Risk Stratification System of the 2015 ATA Guidelines for Differentiated Thyroid Cancer.

Category	Definitions
ATA Low Risk	Papillary thyroid cancer (with all of the following): • No local or distant metastases • All macroscopic tumor has been resected • No tumor invasion of loco-regional tissues or structures • The tumor does not have aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma) • If I-131 is given, there are no RAI-avid metastatic foci outside the thyroid bed on the first posttreatment whole-body RAI scan • No vascular invasion • Clinical N0 or ≤ 5 pathologic N1 micrometastases (<0.2 cm in largest dimension)
	Intrathyroidal, encapsulated follicular variant of papillary thyroid cancer
	Intrathyroidal, well differentiated follicular thyroid cancer with capsular invasion and no or minimal (<4 foci) vascular invasion
	Intrathyroidal, papillary microcarcinoma, unifocal or multifocal, including BRAFV600E mutated (if known)
ATA Intermediate Risk	Microscopic invasion of tumor into the perithyroidal soft tissues
	RAI-avid metastatic foci in the neck on the first posttreatment whole-body RAI scan
	Aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma)
	Papillary thyroid cancer with vascular invasion
	Clinical N1 or >5 pathologic N1 with all involved lymph nodes <3 cm in largest dimension Multifocal papillary microcarcinoma with ETE and BRAFV600E mutated (if known)
ATA High Risk	Macroscopic invasion of tumor into the perithyroidal soft tissues (gross ETE)
	Incomplete tumor resection
	Distant metastases
	Postoperative serum thyroglobulin suggestive of distant metastases
	Pathologic N1 with any metastatic lymph node ≥3 cm in largest dimension
	Follicular thyroid cancer with extensive vascular invasion (> 4 foci of vascular invasion)

RAI, radioiodine; ETE, extrathyroidal extension.

Long-term impact of treatment

As earlier mentioned, the survival of patients with DTC is relatively good. For these patients, it is therefore very important to minimize adverse effects of (initial) therapy and preserve QoL. It is known from cross-sectional studies that QoL is decreased in different domains in thyroid cancer survivors compared to the general population (18-22). Their QoL is at the same level of patients with other cancers with worse prognosis, and is even worse than QoL of breast cancer survivors (24). Main drawback of these cross-sectional studies is that no conclusions can be drawn about QoL changes over time. Therefore, more recently several longitudinal studies were conducted (21,68-71), but their results were mixed, and also had one or more limitations such as relative short follow-up with a maximum of two years (69-71), or lack of knowledge of QoL before initial surgery (21,70). Further, one cross-sectional study showed that younger age, female sex, and lower education are related to lower QoL (72). Unfortunately, no factors were investigated longitudinally. Therefore, it is valuable to investigate long-term changes of QoL, including factors influencing this.

Adverse effects of (initial) therapy are for example hypoparathyroidism due to surgical damage of the parathyroid glands, voice alterations due to recurrent nerve damage caused by surgery, salivary gland dysfunction due to I-131, severe bone marrow dysfunction after high doses of I-131, and adverse cardiovascular effects or osteoporosis due to TSH-suppressive therapy (42,73). I-131 has probably also an effect on the gonadal system. In men, abnormalities in testicular function are common several months after one single therapeutic dose, while the risk of persistent gonadal dysfunction is increased after repeated or high RAI dose (74). Therefore, it is advised to cryopreserve semen (38). In women, a transient change of the menstrual cycle in 12-31%, and a temporary increase of Follicle Stimulation Hormone (FSH) during the first year after RAI therapy has been described (75-77). However, no increased infertility rates or adverse obstetric outcomes were seen in patients after RAI therapy (75,76). Recently, several studies evaluated Anti-Müllerian hormone (AMH) as a representative of ovarian reserve in patients with DTC receiving RAI therapy (78-81). AMH is relatively insensitive to inter- and intra-cycle variability and oral contraceptives use, is known to gradually decline with age, and is undetectable at menopause. Therefore, AMH seems to be a good marker for ovarian reserve (82-85). Earlier longitudinal studies showed a significant decrease of AMH concentrations when comparing concentrations just before RAI therapy and 12 months later (79,81), but no studies with follow-up longer than 12 months, or including patients with multiple RAI therapies exist.

AIMS AND OUTLINE OF THE THESIS

This thesis is subdivided in several parts. In the first part, we aimed to study the prognosis and prognostic factors of patients with DTC. In the second part, the long-term impact of treatment on patients with DTC is studied. Thereafter, we assessed THM in different thyroid states, and assessed their relationship with QoL to explore a potential cause of persistent complaints in patients with hypothyroidism. In the final part, I will discuss the results of the conducted research in light of each other.

In **Chapter 2**, the new 8th edition of the AJCC/TNM classification system is compared to the previous 7th in patients with DTC with respect to differences in staging and survival. Further, we assessed potential differences between subgroups, i.e. PTC and FTC, as literature on the performance of the AJCC/TNM classification system in FTC is scarce. In **Chapter 3**, we aimed to investigate if another age cutoff than 55 years leads to improvement of the prognostic value of the AJCC/TNM 8th edition; again with a focus on potential differences between patients with PTC and FTC. In **Chapter 4**, we assessed the performance of the 2015 ATA Guidelines with respect to its ability to predict prognosis, recurrence and survival in patients with High Risk DTC. As the majority of the present studies are conducted in patients with either Low or Intermediate Risk, studying High Risk patients is valuable. Thereafter, in **Chapter 5**, we again assessed the performance of het 2015 ATA Guidelines, but this time in patients having DTC with distant metastases. No earlier study assessed its ability to predict prognosis, recurrence and survival in patients with distant metastatic disease. In addition, we also evaluated the proportion of distant metastases that might be left unknown and untreated when applying the 2015 ATA Guidelines. In **Chapter 6**, we aimed to investigate the influence of age on recurrence and disease outcome in High Risk DTC patients, and whether the 2015 ATA Guidelines could be improved by adding an age cutoff to its Risk Stratification System.

In **Chapter 7** we investigated the long-term changes of QoL in patients treated for DTC, including factors (like age, sex, and surgical complications) that potentially influence these trajectories. In **Chapter 8**, the influence of RAI therapy on female gonadal reserve was studied, again in patients with DTC. We used AMH as a representative of gonadal reserve, since this was shown to be a relatively sensitive marker in earlier studies.

In **Chapter 9 and 10** we assessed THM in different thyroid states, i.e hypothyroidism, hyperthyroidism, and euthyroidism, using a newly developed and validated LC-MS/MS panel for nine thyroid hormones and thyroid hormone metabolites. First, we investigated if they change across the different thyroid states, and thereafter we assessed their possible relationship with QoL. In these studies, we used patients treated for DTC as a model because they experience different thyroid states during

treatment, i.e. euthyroid (before surgery), hypothyroid (before RAI therapy after TH withdrawal), and hyperthyroid on LT4 supplementation (during TSH-suppressive treatment).

In **Chapter 11**, results from the earlier studies will be discussed, and conclusions will be drawn.

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Part I

Prognosis and prognostic factors

Chapter 2

Comparing the Prognostic Value of the Eighth Edition of the American Joint Committee on Cancer/Tumor Node Metastasis Staging System Between Papillary and Follicular Thyroid Cancer

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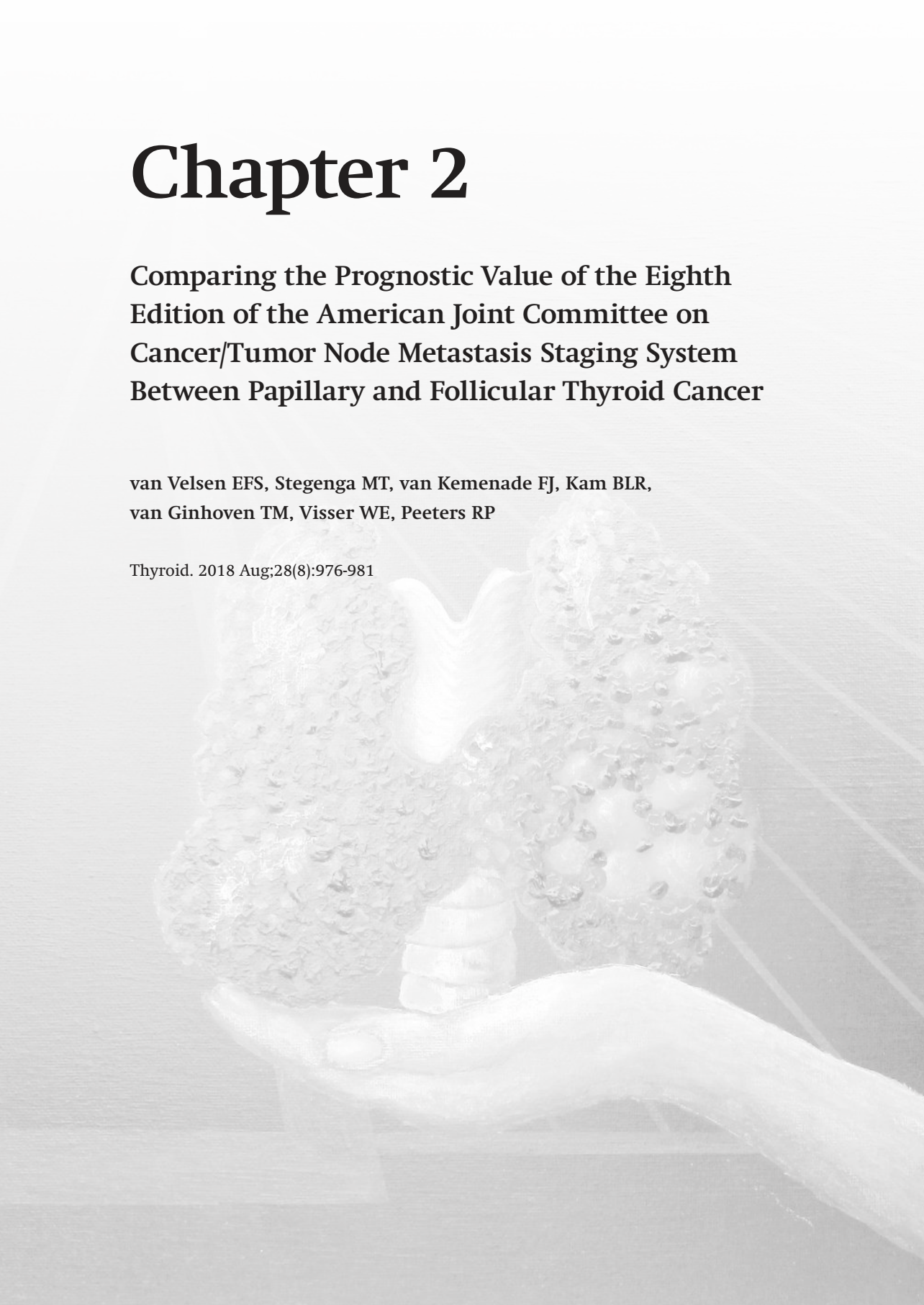

ABSTRACT

Background

Recently, the 8th edition of the AJCC/TNM staging system for differentiated thyroid cancer (DTC) was published. Studies evaluating this new edition so far only comprised patients with papillary thyroid cancer (PTC) or made no distinction between PTC and follicular thyroid cancer (FTC). Therefore, we evaluated the prognostic value of the AJCC/TNM 8th edition in a European population with DTC focusing on potential differences between PTC and FTC.

Methods

We retrospectively studied adult patients with DTC who were diagnosed and/or treated at a Dutch university hospital between January 2002 and April 2016. Overall survival (OS) and disease specific survival (DSS) were analyzed for DTC, and PTC and FTC separately, according to the 7th and 8th edition using the Kaplan-Meier method. The Cox proportional hazards model was used to compare the effect of PTC and FTC on survival. The statistical model performance was assessed using the C-index, AIC and BIC.

Results

We included 792 patients with DTC (79% PTC, 21% FTC) with mean age of 49 years. Median follow-up was 7.2 years. Reclassification using the 8th edition resulted in down-staging of 282 patients (36%), an increased number of patients in stage I and II, and an equivalent decrease in patients with stage III and IV. For DTC, as well as for PTC and FTC separately, stage at diagnosis was significantly related to both OS and DSS ($p<0.001$). When using the 7th edition, FTC patients had a significantly lower survival rate than PTC patients in stage I and IV for OS, and in stage IV for DSS. This difference in survival rates disappeared using the 8th edition. In general, the statistical model performance was better for the 8th than for the 7th edition.

Conclusions

In a European population of patients with DTC, the 8th edition of the AJCC/TNM staging system is a better predictor for both OS and DSS than the previous 7th edition for both PTC and FTC. Furthermore, differences in survival rates between PTC and FTC that were present using the 7th edition disappeared using the 8th edition, implicating that this new edition is predicting well regardless of DTC subtype.

INTRODUCTION

The worldwide incidence of differentiated thyroid cancer (DTC) has been steadily increasing over the last two decades, which is mainly caused by papillary micro carcinoma. In contrast, mortality has remained stable, which suggests overdiagnosis and overtreatment (1). Different systems have been proposed to predict the risk of recurrence and survival in patients with DTC to better determine the need for aggressive therapy and optimize follow-up strategies. The American Thyroid Association (ATA) and European Thyroid Association (ETA) risk stratification systems are designed to estimate the risk of disease recurrence in patients with DTC (2, 3), while the AJCC/TNM staging system is best used to predict disease-specific survival in thyroid cancer patients (4, 5). Recently, the 8th edition of the AJCC/TNM staging system for DTC has been developed, and was introduced in clinical practice in January 2018 (6). The most important differences with the 7th edition are 1) a raised age cut-off from 45 to 55 years, 2) removal of minor extrathyroidal extension from the definition of T3 tumors, and 3) N1 disease does no longer automatically result in staging into stage III or IV in older patients, but into stage II (4). These proposed changes were anticipated to result in downstaging of a significant number of patients and a better prediction of survival in patients with DTC (4). Recent studies from South Korea, Israel and the United States indeed showed superiority of the 8th compared to the 7th edition with respect to predicting survival (7-11). It is known that follicular thyroid cancer (FTC) can manifest differently from papillary thyroid cancer (PTC) as lymph node metastasis are uncommon, and patients are older and have more often distant metastasis at presentation (12). As the abovementioned studies only comprised patients with PTC (8, 9), or had low numbers of patients with FTC and did not distinguish between PTC and FTC (7, 10, 11), it is valuable to investigate if the 8th edition performs equally for both FTC and PTC. Our study evaluated the prognostic value of the AJCC/TNM 8th edition in a European population with a focus on potential differences in predictive value of the AJCC/TNM 8th edition between patients with PTC and FTC.

MATERIALS AND METHODS

Study population

We retrospectively included all patients, aged 18 years or above, who were diagnosed and/or treated for either PTC or FTC (including Hürthle Cell carcinoma) at the Erasmus Medical Center, Rotterdam, The Netherlands, between January 2002 and April 2016. From patient records, we obtained demographic, disease, treatment,

and mortality characteristics. Demographical variables included age at diagnosis, sex, and year of diagnosis. Disease characteristics included disease type, TNM-stage, presence/absence of multifocal disease, and minor/gross extrathyroidal extension. Data regarding treatment consisted of extent of surgery and use of radioactive iodine (RAI). Time to last follow-up, survival status, and date and cause of death were recorded. Cause of death was obtained from hospital or general practitioner records. Patients with extensive or rapidly progressive thyroid cancer and no clear other cause of death were classified as died from thyroid cancer. Survival was defined as the time of initial diagnosis to either last date of follow-up, death, or end of study (May 2017), whichever occurred first.

Patients were categorized according to the TNM classification of the 7th edition (5), and thereafter we reclassified them using the 8th edition (6) for the current study. Furthermore, we used the 8th edition's TNM classification together with the 7th edition's age cut-off to further analyze the reclassification patterns. Information on AJCC/TNM stage was registered for PTC and FTC separately, and combined as DTC. The study protocol was approved by the Institutional Review Board of the Erasmus Medical Center.

Statistical Analysis

For continuous variables, means and standard deviations (SD), or medians with interquartile ranges (IQR) were calculated. For categorical variables, absolute numbers with percentages were recorded. Differences in characteristics between PTC and FTC were assessed using the Student's t-test or $\chi 2$-test.

For DTC, using the 7th and 8th edition of the AJCC/TNM staging system, overall survival (OS) and disease specific survival (DSS) were analyzed using the Kaplan-Meier method, and compared across stages using the log-rank test. To assess the statistical model performance of both editions, we used the concordance index (Harrell's C-index) (13, 14), Akaike information criterion (AIC) (15), and the Bayesian information criterion (BIC) (16). The C-index measures the discriminative power of a model and is a measure of goodness-of-fit. It ranges from 0.5 to 1.0, with 0.5 meaning the model predicts as well as random chance, and 1.0 being the perfect prediction model. Further, the AIC and BIC measure the relative quality of a statistical model, and they provide the relative information lost when a statistical model is used to represent the true model. The model with the highest C-index and lowest AIC and BIC is considered to be the best model for predicting outcomes. The same analyses as for DTC were performed for both PTC and FTC separately. Thereafter, a Cox proportional hazards model was created to estimate and compare the effect of both disease types on survival.

P-values below 0.05 were considered significant. All analyses were performed using either SPSS Statistics for Windows (version 21.0) or R statistical software (version 3.4.1) with package survC1 for estimating the C-index.

RESULTS

Population characteristics

During the study period, a total of 801 patients were eligible for the study, of which nine were excluded because of insufficient information to determine the AJCC/TNM stage. Therefore, the analyses were performed in the remaining 792 patients. Table 1 lists the characteristics of the study population. Mean age was 48.7 years, and 545 (69%) were women. PTC was present in 628 (79%) patients. The remaining 164 patients (21%) had FTC including, 55 patients (34%) with Hürthle Cell carcinoma. The median follow-up time was 86 months, and during follow-up 106 patients (13%) died, of which 57 (54%) due to DTC. Comparing PTC with FTC revealed that patients with FTC were significantly older (46.7 years vs. 56.5 years; p<0.001), and had a significantly higher mortality rate (p<0.001). Also, patients with FTC had significantly

Table 1. Characteristics of the study population.

	DTC (n=792)[a]	PTC (n=628)[a]	FTC (n=164)[a]	p-value[b]
Age at baseline (years)	48.7 ± 16.6	46.7 ± 15.7	56.5 ± 17.4	**<0.001**
Male	247 (31.2)	189 (30.1)	58 (35.4)	0.229
Metastatic disease	87 (11.0)	53 (8.4)	34 (20.7)	**<0.001**
Surgery (TT or HT)	773 (97.6)	614 (97.8)	159 (97.0)	0.541
HT	25 (3.2)	23 (3.7)	2 (1.2)	0.111
TT	748 (94.4)	591 (94.1)	157 (95.7)	0.419
Neck dissection	239 (30.2)	215 (34.2)	24 (14.6)	**<0.001**
RAI treatment	736 (92.9)	581 (92.5)	155 (94.5)	0.374
Once	468 (59.1)	368 (58.6)	100 (61.0)	0.581
Twice	147 (18.6)	121 (19.3)	26 (15.9)	0.317
≥ 3	121 (15.3)	92 (14.6)	29 (17.7)	0.336
Cumulative dose (mCi)	146 (50 – 290)	148 (50 – 292)	100 (50 – 220)	0.883
Follow-up (months)	86 (40 – 131)	86 (40 – 132)	87 (50 – 125)	0.938
Dead	106 (13.4)	64 (10.2)	42 (25.6)	**<0.001**
Thyroid cancer	57 (7.2)	32 (5.1)	25 (15.2)	**<0.001**

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; TT, total thyroidectomy; HT, hemi-thyroidectomy; RAI, radioactive iodine; mCi, milliCurie

more often distant metastases at presentation (21% vs. 8%; p<0.001). Total or hemithyroidectomy was performed in 773 patients (98%), and 736 patients (93%) received radioiodine (468 (59%) once, 147 (19%) twice, and 121 (15%) with more than two therapies). Neck dissection was performed in 239 patients (30%). Afterwards patients were treated with thyroid-stimulation hormone (TSH)-suppressive therapy. Additional treatment modalities were applied in 41 (5%) patients (see Supplemental Table 1). An anticipated significant difference between treatment characteristics of PTC and FTC was that patients with PTC received significantly more often a neck dissection.

Patient stage migration

Using the 7th edition, 431 patients (54%) were classified as stage I, 82 (10%) as stage II, 96 (12%) as stage III, and 183 (23%) as stage IV. Applying the 8th edition, 282 patients (36%) were reclassified into a lower stage. Patients with FTC were more

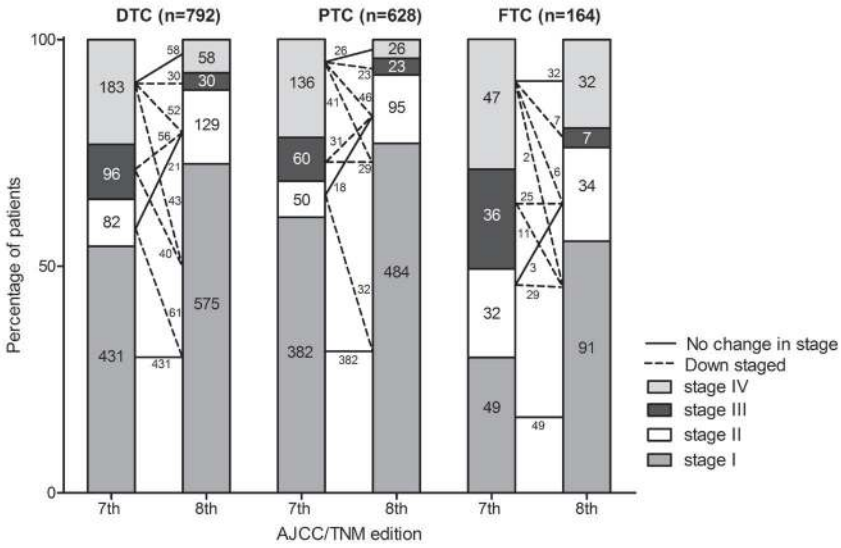

Figure 1. Distribution of patients per disease type and stage according to the 7th and 8th edition of the AJCC/TNM staging systems.

often reclassified than those with PTC (49% vs. 32%). All patients in stage I of the 7th edition remained in stage I using the 8th edition. In addition, reclassification led to increased numbers in stage I (431 => 575) and II (82 => 129), while stage III (96 => 30) and IV (183 => 58) showed an equivalent decrease (see Figure 1). This reclassification pattern of increased numbers in stage I and II, and an equivalent decrease in stage III and IV was also seen for PTC and FTC separately. Using the new TNM classification

together with the 7th edition's age cut-off, still 271 (34%) of the DTC patients were reclassified into a lower stage, however fewer were downstaged into stage I (63 vs. 144), and more into II (172 vs. 108) and III (36 vs. 30). So, the 8th edition's age cut-off point further reclassifies patients into a lower stage.

Survival Prediction

Disregarding stage at diagnosis, 10-year OS and DSS were significantly higher for PTC than FTC in both the 7th and 8th edition ($p<0.001$ in both editions). For DTC, as well as PTC and FTC separately, stage at diagnosis was significantly related to both OS and DSS, using either the 7th or 8th edition (both $p<0.001$). However, compared with the 7th edition, the 8th showed a better distinction between the stages with regard to survival, and a worse prognosis of patients in stage II (10-year DSS: 100% vs. 85%), III (96% vs. 46%), and IV (59% vs. 28%). The same pattern was seen for PTC and FTC separately (see Table 2 and 3). Examination of the Kaplan-Meier survival curves (see Supplemental Figures 1 and 2) shows a better separation of the stage curves for the 8th compared with the 7th edition. Again, the same pattern was seen for PTC and FTC separately (see Figure 2 and 3). Using Cox proportional hazards models, FTC is a significant prognostic factor in stage I and IV for OS (HR 2.91 ($p<0.05$) and HR 2.53 ($p<0.001$) respectively), and in stage IV for DSS (HR 2.84 ($p<0.001$)) according to the 7th edition. In contrast, there were no significant differences in both OS and DSS between PTC and FTC according to the 8th edition (see Supplemental Table 2 and Table 4). For DTC, the 8th edition had a higher C-index (0.910 vs. 0.898), and lower

Table 2. Ten-year overall survival (OS).

	DTC		PTC		FTC	
Stage	7th edition	8th edition	7th edition	8th edition	7th edition	8th edition
I	95.8%	94.5%	97.0%	95.5%	88.5%	90.1%
II	87.7%	71.7%	84.0%	75.0%	90.8%	64.5%
III	86.2%	33.2%	88.3%	34.1%	83.2%	-
IV	47.5%	19.7%	58.7%	23.5%	15.6%	16.8%

Table 3. Ten-year disease-specific survival (DSS).

	DTC		PTC		FTC	
Stage	7th edition	8th edition	7th edition	8th edition	7th edition	8th edition
I	99.4%	99.3%	100%	99.6%	95.6%	97.5%
II	100%	85.2%	100%	87.8%	100%	79.7%
III	96.2%	45.6%	96.3%	44.8%	96.0%	-
IV	59.2%	27.6%	69.7%	30.8%	27.8%	26.4%

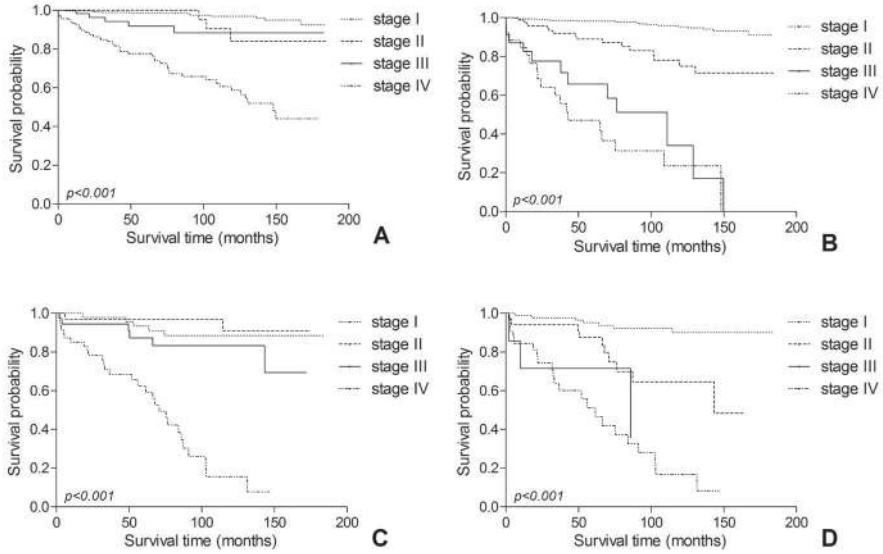

Figure 2. Kaplan-Meier curves for OS in PTC patients using the (A) 7th and (B) 8th edition, and in FTC patients using the (C) 7th and (D) 8th edition.

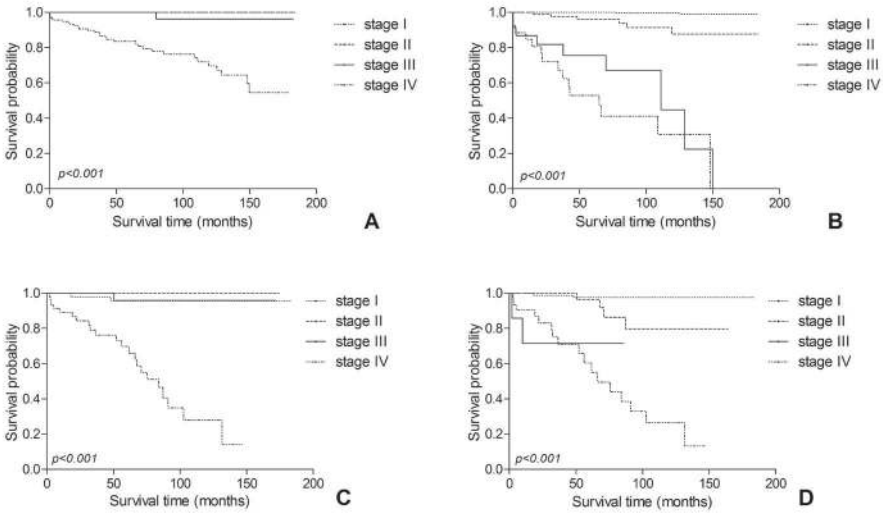

Figure 3. Kaplan-Meier curves for DSS in PTC patients using the (A) 7th and (B) 8th edition, and in FTC patients using the (C) 7th and (D) 8th edition.

Table 4. Effect of cancer subtype within stages on DSS.

Stage	HR (95% CI)	p-value[a]
7th edition		
I	-	-
II	-	-
III	1.47 (0.09 – 23.5)	0.790
IV	2.84 (1.61 – 4.99)	**<0.001**
8th edition		
I	4.74 (0.67 – 33.6)	0.120
II	1.31 (0.51 – 6.43)	0.360
III	1.11 (0.23 – 5.49)	0.900
IV	0.98 (0.48 – 1.99)	0.950

[a] p-value comparing FTC with PTC (PTC as reference group).
HR, hazard ratio; CI, confidence interval.

AIC (531 vs. 558) and BIC (532 vs. 560) compared to the 7th edition for DSS, indicating a better model performance (see Table 5). This was also seen for both PTC and FTC separately for DSS, and for DTC, PTC and FTC for OS (see Supplemental Table 3).

Table 5. Measures of model performance of both staging systems for DSS.

	C-index	AIC	BIC
DTC			
7th edition	0.898	558.3	560.4
8th edition	0.910	530.8	532.8
PTC			
7th edition	0.916	280.1	281.5
8th edition	0.917	266.8	268.3
FTC			
7th edition	0.855	189.2	190.4
8th edition	0.865	187.0	188.2

DISCUSSION

We showed that, in a European population of patients with DTC, the AJCC/TNM 8th edition is a better predictor of both overall and disease specific survival than the previous 7th edition regardless of DTC subclass.

Applying the new 8th edition resulted in reclassification into a lower stage in 36% of the total population, which is in line with other recent studies in DTC and PTC patients (7-11, 17). We observed a worsened prognosis (both 10-year OS and DSS) of

patients in stage II , III, and IV for the 8th compared with the 7th edition for DTC and PTC. This is in line with the results of the 10-year OS of Pontius et al. (8) in patients with PTC, and the 10-year DSS of Kim et al. (10) and Shteinshnaider et al. (11) in patients with DTC. Kim et al. (7), using 10-year DSS in patients with DTC, found a worsened prognosis in stage III and IV. However, difference between the 10-year DSS of the 7th and 8th edition in stage II in their study is minimal, i.e. 1.5%. Our 10-year DSS of 46% for the 8th edition's stage III DTC patients is lower than found by others (7, 10), which might be caused by older patients with T4 tumors with a poor prognosis in our study, and who are downstaged from stage IV in the 7th to stage III in the 8th edition

The new edition reclassified 49% of the FTC patients into a lower stage, which was mainly caused by downstaging of T2 and T3 tumors into stage I and II respectively. Using the 7th edition, comparing PTC and FTC using Cox proportional hazards models showed a significant effect of FTC on OS in stage I and IV, and on DSS in stage IV. However, no conclusions can be drawn for stage I and II due to the small number of events. Importantly, using the 8th edition, no significant differences between both OS and DSS between PTC and FTC in the different stages of PTC and FTC were observed.

Together, our data show that the 8th edition has better performance than the 7th with respect to predicting both OS and DSS. Similar results were found by others for DSS (7, 8, 10) and for disease-free-survival (9). In these studies, no comparison between PTC and FTC was made, while we are the first, to our knowledge, showing that the 8th edition also has a better predictive performance than the 7th for PTC and FTC separately.

Strengths of our study include the relative large proportion of patients with advanced disease stages compared to other studies (7-11, 17) as 11% of our patients had distant metastasis at presentation. Furthermore, the relative high proportion of FTC patients enabled us, for the first time in the 8th edition to our knowledge, to compare PTC with FTC patients. A possible limitation of our study is the fact that patients were recruited from a single tertiary university hospital, which might attracts patients with more aggressive DTC, especially FTC, because of availability of certain treatments. However, based on the staging, there was more a balanced mix of patients of different stages compared to other studies(7-11, 17).

CONCLUSIONS

In conclusion, our study shows that in a European population of patients with DTC harboring a large subset of FTC patients, applying the AJCC/TNM 8th edition leads to reclassification of 36% of the patients into a lower stage. Furthermore, using this 8th edition, there is no significant difference between PTC and FTC anymore, regarding survival rates per stage, implicating that AJCC/TNM stage predicts well for both DTC subtypes. Therefore, the 8th edition of the AJCC/TNM staging system is a better predictor of both overall and disease specific survival than the previous 7th edition for both PTC and FTC.

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Supplemental Table 1. Additional treatment modality characteristics.

	DTC (n=792)[a]	PTC (n=628)[a]	FTC (n=164)[a]	p-value[b]
Radiotherapy	22 (2.8)	21 (3.3)	1 (0.6)	0.056
TKI	14 (1.8)	9 (1.4)	5 (3.0)	0.167
Lutetium octreotate therapy	13 (1.6)	-	13 (7.9)	**< 0.001**

[a] Values are numbers (percentages).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; TKI, Tyrosine-kinase inhibitors.

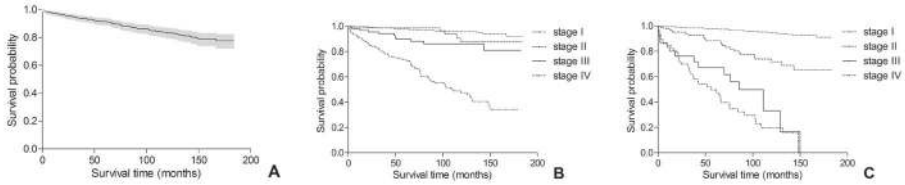
Supplemental Table 2. Effect of cancer subtype within stages on OS.

Stage	HR (95% CI)	p-value[a]
7th edition		
I	2.91 (1.01 – 8.41)	**0.048**
II	0.70 (0.12 – 4.23)	0.070
III	1.90 (0.56 – 6.23)	0.290
IV	2.53 (1.57 – 4.08)	**<0.001**
8th edition		
I	1.79 (0.75 – 4.28)	0.215
II	1.71 (0.77 – 3.77)	0.199
III	1.10 (0.30 – 4.06)	0.891
IV	1.04 (0.55 – 1.96)	0.906

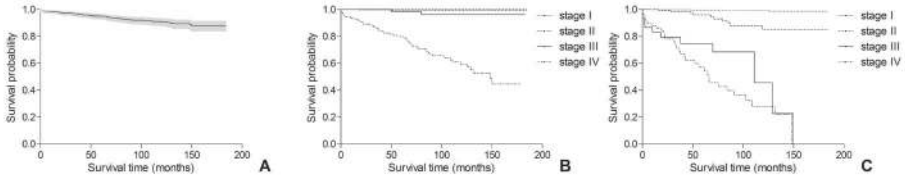
[a] p-value comparing FTC with PTC (PTC as reference group).
HR, hazard ratio; CI, confidence interval.

Supplemental Table 3. Measures of model performance of both staging systems for OS.

	C-index	AIC	BIC
DTC			
7th edition	0.775	1167.9	1170.6
8th edition	0.787	1131.7	1134.4
PTC			
7th edition	0.759	669.7	671.9
8th edition	0.759	646.7	648.9
FTC			
7th edition	0.781	347.5	349.2
8th edition	0.794	343.2	344.9



Supplemental Figure 1. Kaplan-Meier curves for OS in DTC patients for (A) the total population, and according to the (B) 7th and (C) 8th edition.



Supplemental Figure 2. Kaplan-Meier curves for DSS in DTC patients for (A) the total population, and according to the (B) 7th and (C) 8th edition.

Chapter 3

Finding the Optimal Age Cutoff for the UICC/AJCC TNM Staging System in Patients with Papillary or Follicular Thyroid Cancer

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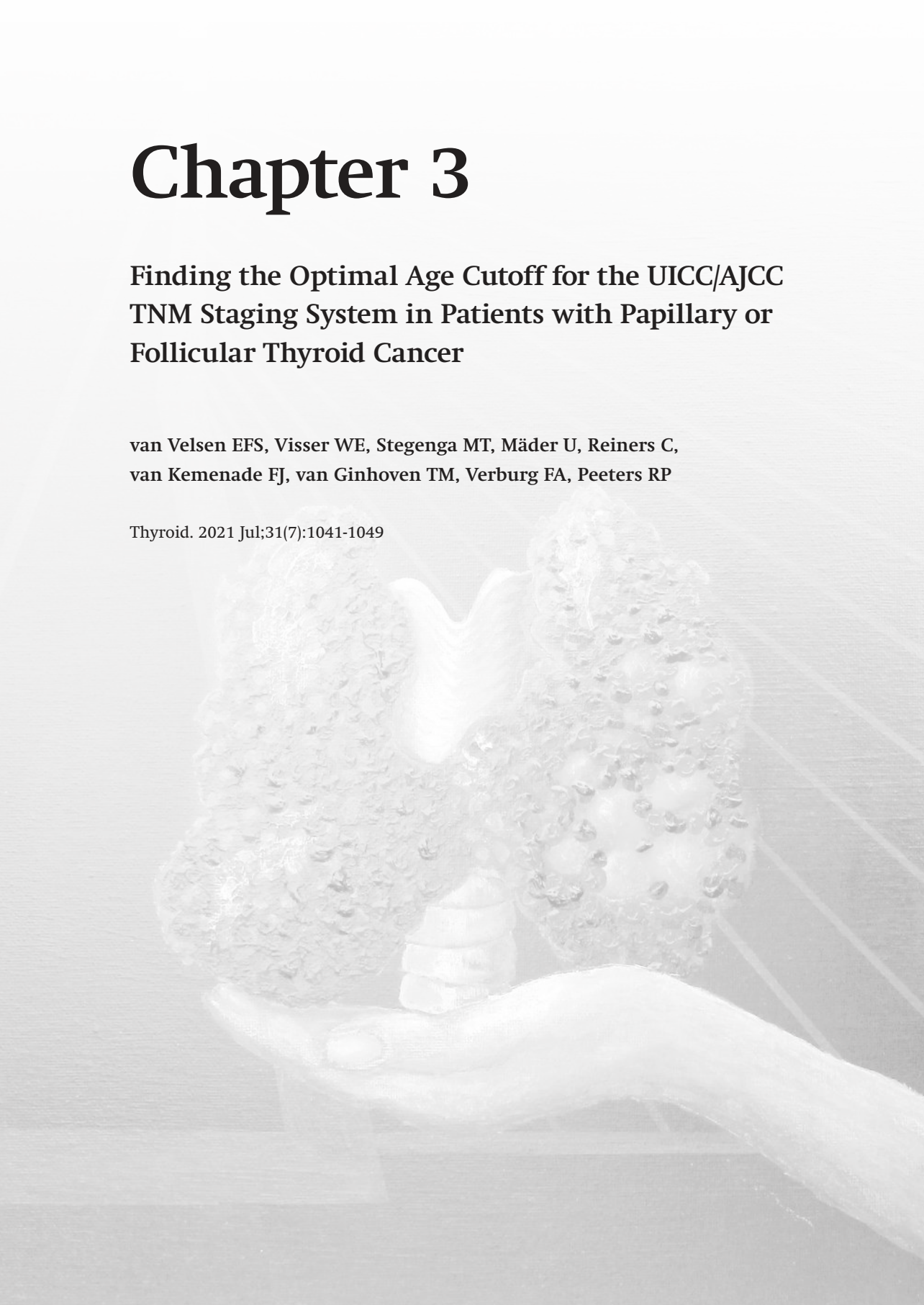

ABSTRACT

Background

Differentiated thyroid cancer (DTC) is the only cancer entity for which the UICC/AJCC TNM staging system involves an age cutoff as a prognostic criterion. However, the optimal age cutoff has not yet been determined in detail. The aim of our study was therefore to investigate the optimal age cutoff for the TNM staging system to predict disease specific survival (DSS) with a focus on differences between patients with papillary (PTC) and follicular (FTC) thyroid cancer.

Methods

We retrospectively studied two large, well-described cohorts of adult DTC patients from a Dutch and a German university hospital. DSS was analyzed for DTC overall, and for PTC and FTC separately, using several age cutoffs (per 5-years increment between 20 and 85 years, and subsequently 1-year increments between 35 and 55 years) employing the histopathological criteria from the TNM staging system (8th edition).

Results

We included 3074 DTC patients (77% PTC, 23% FTC; mean age at diagnosis 49 years). Median follow-up was 7 years. For DTC, and for PTC and FTC separately, the majority of the age cutoffs had a better statistical model performance than a model with no age cutoff. For DTC overall and for PTC, an age cutoff of 50 years had the best statistical model performance, while this was 40 years for FTC.

Conclusions

In this large European population of DTC patients, when employing the histopathological criteria of the TNM system (8th edition), the optimal age cutoff to predict DSS is 50 years rather than the 55 years currently in use. With the optimal age cutoff being 50 years for PTC and 40 years for FTC, there was a substantial difference in age cutoff for the respective histological entities. Therefore, the implementation of different age cutoffs for PTC and FTC could improve the predictive value of the TNM staging system.

INTRODUCTION

The joint Union International Contre le Cancer and American Joint Committee on Cancer (UICC/AJCC) Tumor, Node, Metastasis (TNM) staging system in its various iterations is consistently among the best systems used to predict disease-specific survival (DSS) in differentiated thyroid cancer (DTC) (1, 2). For DTC, a unique feature of the TNM staging system is the fact that age plays a major role in the classification of patients into different prognostic groups, which is in contrast to other malignancies (1). In January 2018, the 8th edition of the UICC/AJCC TNM staging system for DTC was introduced in clinical practice (3). Besides a changed definition of T3 tumors and a downstaging of N1 disease, a raised age cutoff from 45 to 55 years was a major difference compared to the 7th edition (1). Several studies compared the performance of the 8th to the 7th edition in patients with DTC (4-18), and the majority of these showed a slight superiority of the 8th edition.

An issue in DTC staging concerns clinical differences between its histological subtypes. It is well-established that follicular thyroid cancer (FTC) has a different clinical manifestation than papillary thyroid cancer (PTC) as lymph node metastasis are uncommon, patients are in general older and more often have distant metastasis at initial presentation (19). Two studies showed superiority of the 8th edition compared to the 7th edition of the TNM system in patients with FTC (9, 11), while another study showed no differences (13). To the best of our knowledge, however, it has not yet been firmly established whether the age cutoff for FTC and PTC are, in fact, similar.

The raised age cutoff for the 8th edition of the TNM system was based on three earlier studies showing that an age cutoff of 55 years lead to better predictability of DSS in patients with DTC (20-22). However, these studies were performed using the histopathological criteria of the 7th edition of the TNM staging system; an extrapolation of these results to the different histopathological staging criteria of the 8th edition may therefore not necessarily be correct. Consequently, the aim of the present study was to investigate the optimal age cutoff for the TNM system to predict DSS, employing the histopathological criteria of the current 8th edition. The secondary aim of our study was to examine whether differences with regard to age cutoff exist between patients with PTC and FTC.

MATERIALS AND METHODS

Study population

For the current study we combined two established, well-described databases from The Netherlands and Germany (9, 10, 23-27). These databases were earlier used to investigate, among others, the predictive value of the 8th edition of the TNM system in patients with DTC (9, 10).

From the Erasmus Medical Center (Erasmus MC), Rotterdam, The Netherlands, we retrospectively obtained data from patients, aged 18 years or above, who were diagnosed and/or treated for either PTC or FTC (including Hürthle Cell carcinoma) between January 2002 and December 2016. All patients underwent thyroid surgery, and were treated according to the previous and current Dutch Guidelines (28). Demographic, disease, treatment, and mortality characteristics were obtained from patient records. Cause of death was obtained from hospital or general practitioner records. Survival was defined as the time from the date of the initial diagnosis to either the date of the last-known follow-up, death, or end of study (December 2017), whichever occurred first. The study protocol was approved by the Institutional Review Board of the Erasmus MC.

From the University of Würzburg thyroid cancer database, we retrospectively obtained data on patients, aged 18 years or above, who were treated for either PTC or FTC between January 1980 and December 2015. All patients underwent thyroid surgery and were treated further in accordance with the standards of the respective time period, as described previously (25, 26). Demographic, disease, treatment, and mortality data were immediately recorded in the database at each patient visit. Cause of death was obtained from hospital or general practitioner records or public registration offices. Survival was defined as the time of initial diagnosis to either last date of follow-up, death, or end of study data collection (December 2016), whichever occurred first. The Würzburg Thyroid Cancer Database was maintained with approval of and continuous monitoring by the local medical ethical committee.

Patients from both cohorts were reclassified using the histopathological criteria from the 8th edition of the TNM system, but applying different age cutoffs. For this purpose, we investigated age cutoffs at five-year increments from 20 up to and including 85 years. Additionally, we also investigated one-year increments between 35 and 55 for sensitivity analysis. These analyses were performed separately for both PTC and FTC, as well as combined for the overall DTC patient population.

Statistical Analysis

For DTC, using the previously described age cutoffs for the TNM staging system, DSS was analyzed using the Kaplan-Meier method, and compared across stages using

the log-rank test. To assess the statistical model performance of the TNM staging system with different age cutoffs, we used the concordance index (Harrell's C-index) (29, 30), Akaike information criterion (AIC) (31), and the Bayesian information criterion (BIC) (32). The C-index measures the discriminative power of a model and is a measure of goodness-of-fit. It ranges from 0.5 to 1.0, with 0.5 meaning the model predicts no better than random chance, and 1.0 being the perfect prediction model. Furthermore, the AIC and BIC measure the relative quality of a statistical model, and they provide the relative information lost when a statistical model is used to represent the true model. The model with the highest C-index and lowest AIC and BIC is considered to be the best model for predicting outcomes. Therefore, using these three criteria, we aimed to find the age cutoff that optimizes the statistical performance. The same analyses as for DTC were performed for both PTC and FTC separately. Thereafter, a Cox proportional hazards model was created to estimate and compare the effect of both disease types on DSS. These analyses were performed unadjusted, and adjusted for age, sex, and cohort.

P-values below 0.05 were considered significant. All analyses were performed using either SPSS Statistics for Windows (version 25.0) or the open source statistical software R (version 3.4.1) with package survC1 for estimating the C-index (33).

RESULTS

Population characteristics

A total of 3074 patients fulfilled the inclusion criteria and had sufficient information to adequately determine their TNM stage. Table 1 lists the characteristics of the study population. PTC was present in 2355 patients (77%), whereas the remaining 719 patients (23%) had FTC. The median available follow-up time was 84 months. Of the 3074 patients, the majority (2254; 73%) were included from the University of Würzburg. These characteristics can be found in Supplemental Table 1.

TNM Stage

Using the 8th edition's original age cutoff, 2430 patients (79%) were classified as stage I, 384 (13%) as stage II, 88 (3%) as stage III, and 172 (6%) as stage IV. Lowering the age cutoff resulted in a lower number of patients in stage I; these patients were redistributed over the other three stages. Increasing the age cutoff resulted in a higher number of patients being classified as stage I and fewer patients as having higher stage disease (Table 2). This same pattern was also seen for PTC and FTC separately.

Table 1. Characteristics of the study population.

	DTC (n=3074)[a]	PTC (n=2355)[a]	FTC (n=719)[a]	p-value[b]
Age at baseline (years)	48.7 ± 15.7	47.1 ± 15.3	54.2 ± 16.0	**<0.001**
Sex				**<0.001**
Male	939 (30.5)	672 (28.5)	267 (37.1)	
Female	2135 (69.5)	1683 (74.5)	452 (62.9)	
Disease				
HCC	98 (3.2)	-	98 (13.6)	
Cohort				**0.007**
Erasmus MC	820(26.7)	656 (27.9)	164 (22.8)	
Würzburg	2254 (73.3)	1699 (72.1)	555(77.2)	
T-stage				**<0.001**
T0	10 (0.3)	8 (0.3)	2 (0.3)	
T1	1487 (48.4)	1324 (56.2)	163 (22.7)	
T2	796 (25.9)	540 (22.9)	256 (35.6)	
T3	509 (16.6)	297 (12.6)	212 (29.5)	
T4	246 (8.0)	176 (7.5)	70 (9.7)	
Lymph node metastases				**<0.001**
Not present	2364 (76.9)	1711 (82.7)	653 (90.8)	
Present	710 (23.1)	644 (27.3)	66 (9.2)	
Distant metastases				**<0.001**
Not present	2802 (91.2)	2214 (94.0)	588 (81.8)	
Present	272 (8.8)	141 (6.0)	131 (18.2)	
Follow-up (months)	84 (37 – 154)	81 (36 – 149)	91 (43 – 169)	**0.033**
Vital status at end of follow-up				
Alive	2607 (84.8)	2076 (88.2)	531 (73.9)	**<0.001**
Died (All causes)	467 (15.2)	279 (11.8)	188 (26.1)	**<0.001**
Died (Thyroid cancer)	133 (4.3)	71 (3.0)	62 (8.6)	**<0.001**
Survival				
10-year OS (%)	84.9 ± 0.8	88.4 ± 0.9	75.1 ± 1.9	**<0.001**
10-year DSS (%)	94.7 ± 0.5	96.5 ± 0.5	89.5 ± 1.4	**<0.001**

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers of patients (percentage of the respective population).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; HCC, Hurthle Cell Carcinoma; OS, overall survival; DSS, disease specific survival

Table 2. Distribution of patients across stages employing different age cutoffs in addition to the established histopathological staging criteria of the 8th edition of the TNM system.

	DTC (n=3074)[a]	PTC (n=2355)[a]	FTC (n=719)[a]
No age cutoff			
Stage I	1799 (57.9%)	1418 (60.2%)	361 (50.2%)
Stage II	845 (27.5%)	661 (28.1%)	184 (25.6%)
Stage III	167 (5.4%)	129 (5.5%)	38 (5.3%)
Stage IV	283 (9.2%)	147 (6.2%)	136 (18.9%)
40 years cutoff			
Stage I	2123 (69.1%)	1730 (73.5%)	393 (54.7%)
Stage II	590 (19.2%)	430 (18.3%)	160 (22.3%)
Stage III	134 (4.4%)	97 (4.1%)	37 (5.1%)
Stage IV	227 (7.4%)	98 (4.2%)	129 (17.9%)
45 years cutoff			
Stage I	2225 (72.4%)	1818 (77.2%)	407 (56.6%)
Stage II	516 (16.8%)	361 (15.3%)	155 (21.6%)
Stage III	118 (3.3%)	83 (3.5%)	35 (4.9%)
Stage IV	215 (7.0%)	93 (3.9%)	122 (17.0%)
50 years cutoff			
Stage I	2329 (75.8%)	1902 (80.8%)	427 (59.4%)
Stage II	452 (14.7%)	305 (13.0%)	147 (20.4%)
Stage III	106 (3.4%)	73 (3.1%)	33 (4.6%)
Stage IV	187 (6.1%)	75 (3.2%)	112 (15.6%)
55 years cutoff			
Stage I	2430 (79.1%)	1977 (83.9%)	453 (63.0%)
Stage II	384 (12.5%)	250 (10.6%)	134 (18.6%)
Stage III	88 (2.9%)	60 (2.5%)	28 (3.9%)
Stage IV	172 (5.6%)	68 (2.9%)	104 (14.5%)
60 years cutoff			
Stage I	2521 (82.0%)	2041 (86.7%)	480 (66.8%)
Stage II	320 (10.4%)	200 (8.5%)	120 (16.7%)
Stage III	77 (2.5%)	50 (2.1%)	27 (3.8%)
Stage IV	156 (5.1%)	64 (2.7%)	92 (12.8%)

[a] Values are numbers (percentages).
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer

Survival Prediction

The 10-year DSS was significantly higher for PTC than FTC (p<0.001). After adjusting for age, sex, and cohort, the difference between PTC and FTC remained significant (p<0.001; Table 3). The 10-year DSS per stage for DTC overall, and for PTC and FTC separately are shown in Table 4.

Table 3. Effect of DTC histological subtype on Survival.

		HR (95% CI)	p-value[d]
OS			
	Model I [a]	1.97 (1.64 - 2.37)	**<0.001**
	Model II [b]	1.37 (1.13 - 1.65)	**<0.001**
	Model III [c]	1.37 (1.13 - 1.65)	**<0.001**
DSS			
	Model I [a]	2.63 (1.87 - 3.70)	**<0.001**
	Model II [b]	1.76 (1.25 - 2.48)	**0.001**
	Model III [c]	1.77 (1.26 - 2.51)	**0.001**

[a] Model I; unadjusted.
[b] Model II; adjusted for age and sex.
[c] Model III; adjusted for age, sex and cohort.
[d] p-value comparing FTC with PTC (PTC as reference group).
OS, Overall Survival; DSS, Disease Specific Survival; HR, hazard ratio; CI, confidence interval.

Table 4. Ten-year disease specific survival for DTC, PTC, and FTC employing different age cutoffs in addition to the established histopathological staging criteria of the 8th edition of the TNM system.

Stage	None	40 years	45 years	50 years	55 years	60 years
DTC						
I	99.1 ± 0.3%	99.3 ± 0.2%	99.1 ± 0.3%	99.1 ± 0.3%	98.9 ± 0.3%	98.3 ± 0.4%
II	96.2 ± 1.0%	94.2 ± 1.4%	92.8 ± 1.6%	90.8 ± 1.9%	88.7 ± 2.2%	89.1 ± 2.1%
III	84.7 ± 3.3%	80.2 ± 4.2%	78.4 ± 4.7%	74.8 ± 5.5%	71.7 ± 6.3%	72.7 ± 6.4%
IV	70.1 ± 3.3%	62.9 ± 4.0%	62.2 ± 4.1%	59.6 ± 4.7%	59.1 ± 4.9%	56.4 ± 5.5%
PTC						
I	99.4 ± 0.3%	99.5 ± 0.2%	99.5 ± 0.2%	99.5 ± 0.2%	99.2 ± 0.3%	98.7 ± 0.4%
II	96.7 ± 1.1%	94.8 ± 1.6%	93.6 ± 2.0%	91.7 ± 2.4%	90.2 ± 2.7%	91.8 ± 2.4%
III	90.3 ± 3.0%	86.7 ± 4.0%	84.3 ± 4.7%	81.2 ± 5.6%	79.1 ± 6.7%	82.4 ± 6.4%
IV	75.7 ± 4.2%	63.9 ± 5.9%	62.4 ± 6.0%	57.4 ± 7.1%	55.0 ± 7.6%	52.8 ± 8.1%
FTC						
I	98.1 ± 0.9%	98.3 ± 0.8%	97.7 ± 0.9%	97.8 ± 0.9%	97.5 ± 0.9%	96.7 ± 1.0%
II	94.8 ± 2.1%	92.6 ± 2.7%	91.3 ± 3.0%	88.9 ± 3.2%	86.3 ± 3.6%	85.2 ± 3.9%
III	55.7 ± 12.2%	52.8 ± 12.9%	55.6 ± 13.2%	52.2 ± 14.1%	50.8 ± 13.9%	49.5 ± 13.9%
IV	63.3 ± 5.3%	62.0 ± 5.5%	62.6 ± 5.7%	60.9 ± 6.2%	61.9 ± 6.5%	59.2 ± 7.4%

DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer

For DTC, and PTC and FTC separately, the majority of the age cutoffs had a better statistical model performance than the model without any age cutoff (Figure 1). Furthermore, using 5-year increments for the age cutoffs, the highest C-index, and lowest AIC and BIC were identified for an age cutoff of 50 years for DTC overall as well as for PTC separately, while this was 40 years for FTC (Figure 2 for Kaplan-Meier curves). From the original age cutoff towards the best identified cutoffs, 3.7% (DTC), 3.4% (PTC), and 11.7% (FTC) of the patients migrated to a higher stage (Figure 3).

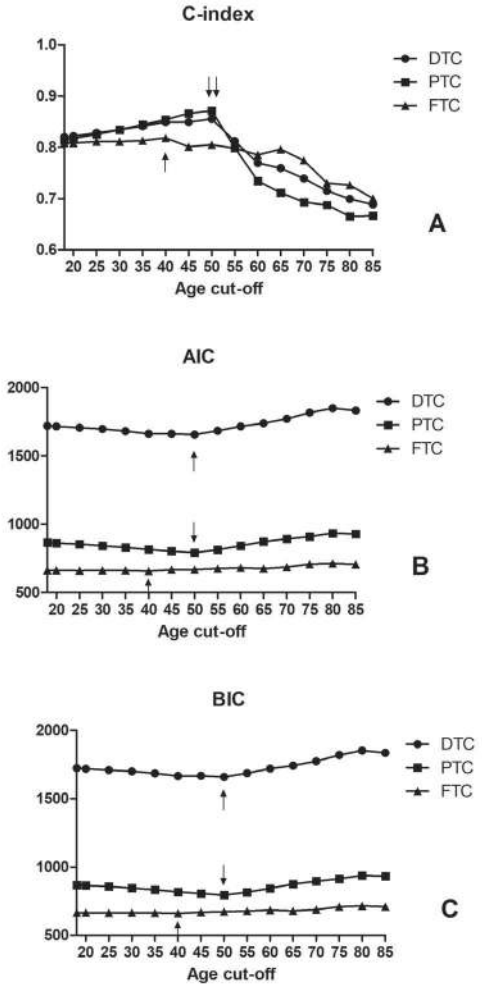

Figure 1. Statistical Model Performance for (A) C-index, (B) AIC, and (C) BIC. Using 5-year increments. Arrows showing the optimal age cutoffs.

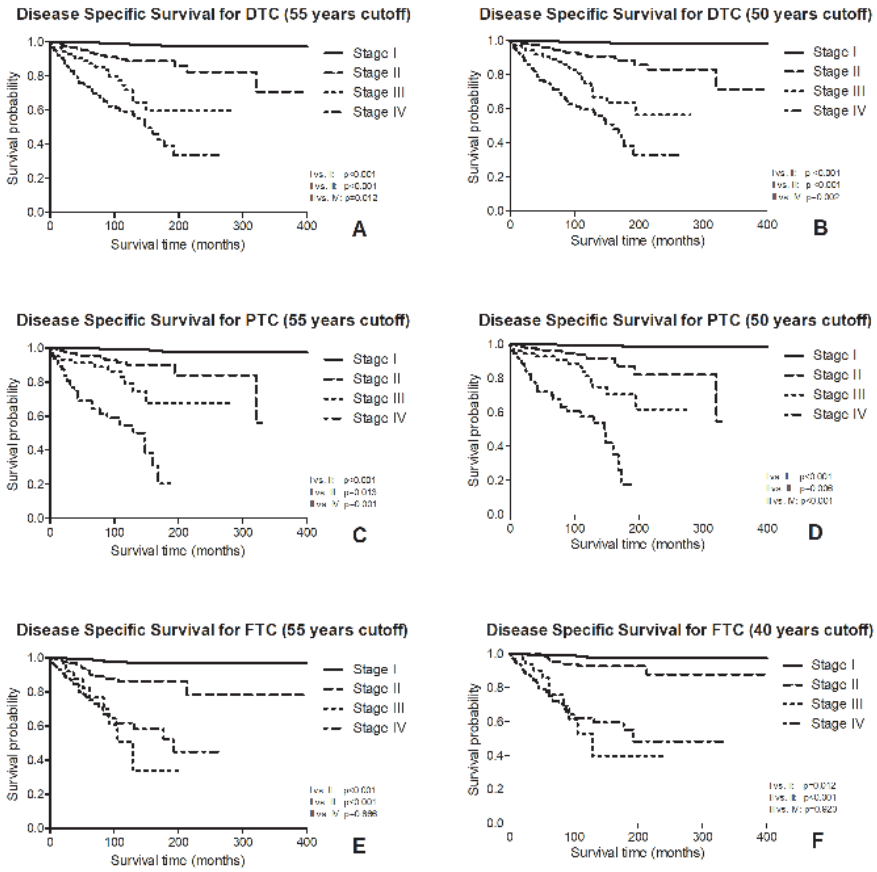

Figure 2. Kaplan-Meier curves for DSS in (A, B) DTC, (C, D) PTC, and (E, F) FTC for either the original 55 years age cutoff or the cutoffs with the best statistical performance.

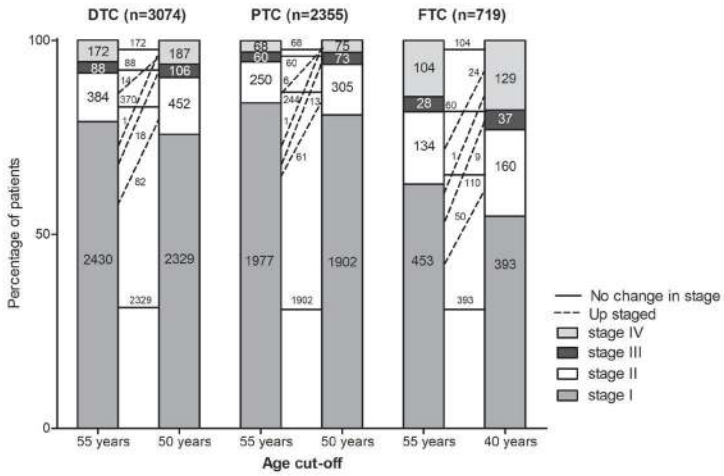

Figure 3. Distribution of patients per disease type and stage according to the original 55 years age cutoff and best identified age cutoffs for the TNM staging system.

Using 1-year increments, for DTC overall, the highest C-index was established with an age cutoff of 50 years (lowest AIC and BIC at 46 years). For PTC, the highest C-index was identified at 48 years (lowest AIC and BIC at 50 years), while for FTC the highest C-index, and lowest AIC and BIC were all identified at 41 years (Supplemental Figure 1). C-index, AIC and BIC values for the patients from the University of Würzburg and the Erasmus MC separately are shown in Supplemental Figure 2, 3 and 4.

Using Cox proportional hazards models, unadjusted, significant differences between PTC and FTC were seen for all age cutoffs, except 65 and 70 years. Adjusting for age, sex and cohort, there were significant differences between PTC and FTC for age cutoffs 45, 50, 75 and 85 years (Supplemental Table 2).

DISCUSSION

In the present study combining two well-described large DTC patient cohorts from two different European countries, we showed that, when employing the histopathological staging criteria from the 8th edition of the TNM system, the optimal age cutoff for classification of disease stage with respect to disease specific survival is 50 years rather than the defined 55 years. In addition, there was a 10-years difference in age cutoff for patients with PTC versus FTC.

Nixon et al. showed that when employing the histopathological criteria of the 7th edition of the TNM system in DTC patients from the United States, an age cutoff of

55 years outperformed the standard cutoff of 45 years (20). Later on, they confirmed this result in DTC patients from centers across the world, though no groups from Europe or Asia were included (21). Kim et al. also confirmed this result in DTC patients from Asia as ROC analysis revealed an optimal cutoff 55.4 years employing the histopathological criteria of the 7th edition of the TNM system (22). Ito et al. compared, the original 7th edition of the TNM system with a slightly altered version in Japanese PTC patients. The altered version also included a changed age cutoff of 55 years (34). They showed that their altered system performed better than the original 7th edition. Unfortunately, they did not investigate different age cutoffs. Except for the study of Kim et al, no other age cutoffs than 45 and 55 years were reported, and therefore it is unknown if in these three studies another age cutoff would have given an optimized result. Besides, these four studies employed the histopathological staging criteria of the 7th instead of the 8th TNM edition.

Recently, there were several other studies that investigated the influence of age on prognosis without further consideration of staging factors (35-37). Based on these results, it was argued that an age cutoff does not do justice to the continuous effect age likely has on prognosis, and that it should therefore be considered to remove a dichotomizing age criterion from the TNM staging system (38). Nonetheless, in the present study it was clearly shown that the addition of an age-based cutoff increases the prognostic power of the TNM system, as the majority of the age cutoffs significantly outperform a model based on histopathological criteria alone. However, this still does not necessarily imply that using a dichotomic age classification is optimal; it still remains a dichotomization of what most likely is a sliding scale. Therefore, further research is needed to investigate whether there is a better method to incorporate age into the TNM staging system, e.g. adjusting for relative survival rates (25, 39), combining histopathological staging criteria with age (continuously or e.g. per decade) in a risk calculator, or considering employing multiple age cutoffs. Next to this, it is also important to not only focus on statistical performance, but also on feasibility in clinical practice.

Our results showed that for FTC, the optimal age cutoff was 41 years (40 years for 5-year increments). For PTC, the C-index showed 48 years (50 years for 5-year increments), while 50 years also was the optimal cutoff using the AIC or BIC. For DTC overall, the C-index showed 50 years which is in agreement with the 5-years increments analysis, while 46 years was the optimal cutoff using the AIC or BIC – which is remarkably close to the cutoff of 45 years employed in previous iterations of the TNM system up to and including the 7th edition. Overall, as there were only minor differences between the one- and five-year increment analyses, it is more convenient to use a multiple of five in prognostic systems aimed for clinical practice. We feel

that the results of the 5-year increment analysis represent a good, clinically easily usable compromise between the minor differences of the various statistical analysis.

To the best of our knowledge, the present study is the first one to investigate the optimal age cutoff specifically for patients with FTC. Compared to PTC, patients with FTC in our population were older and had more advanced disease in terms of both local disease and distant metastases, which is in accordance with literature (19). The DSS for stage III FTC patients is relatively low, and its Kaplan-Meier curve overlaps with the curve of stage IV patients (p=0.923). This might be caused by older patients with T4 tumors having a poor prognosis in this study. This phenomenon was also seen in our earlier study (9). Therefore, one might consider to combine stage III and IV disease in patients with FTC into a single staging category resulting into three instead of four stages for FTC. However, further research on this topic is needed.

Furthermore, two earlier studies, in partially overlapping populations, compared PTC and FTC using either the 7th or 8th edition of the TNM system regarding prognosis and showed no significant differences between the two histological subtypes (9, 40). In the present study, these earlier results from both our centers cannot be confirmed in their entirety. When combining the two populations to a single large cohort, the optimal overall DTC cutoff of 50 years results in a survival difference between PTC and FTC in stage I. This is likely explained by the difference in optimal age cutoff observed between these two distinct histological entities in the present study, with a misclassification of a number of patients over 40 years with higher risk FTC erroneously as having stage I disease. Consequently, this results in an optimal age cutoff of 40 years for FTC, which is markedly different from the optimal age cutoff for PTC. As stated earlier, it is well-established that FTC and PTC have different clinical manifestations (19), and our study further emphasizes this. Hence, the present study implies that PTC and FTC should be staged as separate entities.

In the present study we included patients from two large university medical centers from Europe. With respect to the statistical model performance for FTC, both populations showed an optimal age cutoff of 40 years. For PTC there was a minor discrepancy, as for the Erasmus MC the optimal age cutoff was 45 years while this was 50 years for the University of Würzburg. However, when using the 1-year increments analysis, the difference between both populations was minor with only 3 years, i.e. 47 years for the Erasmus MC and 50 years for the University of Würzburg. These differences might in part be attributable to differences in baseline characteristics at diagnosis as patients from the Erasmus MC more often had local and/or distant metastases and a higher DTC related mortality, which is possibly due to the fact that the Erasmus MC is a reference center for thyroid cancer in The Netherlands, and therefore has a comparatively high proportion of patients with advanced disease. Combining populations with minor differences, but both treated

in high-quality healthcare systems, may increase the robustness of results, resulting a more generalizable conclusion. Additionally, because differences between the optimal age cutoffs were marginal, we consider our findings to be valid. Nonetheless, further research in populations from around the globe is needed to further confirm and/or refine our results.

Strengths of our study include the substantial proportion of patients with advanced disease stages compared to other studies as 9% of our patients had distant metastasis at presentation. Furthermore, the relatively high proportion (23%) of FTC patients enabled us to be, to our knowledge, the first to investigate the optimal age cutoff in patients with FTC, and additionally, compare PTC with FTC patients. A possible limitation of our study is the fact that it is retrospective, and therefore patients were reclassified using the histopathological criteria from the 8th edition of the TNM system, it might be that in some cases the reclassification differs from direct classification of a pathologist using the 8th edition's criteria. Additionally, it is possible that an (unknown) proportion of DTC patients would in the current day be classified otherwise. Unfortunately, the retrospective nature of the our study leaves no way of ascertaining this. Therefore, longitudinal studies are needed to confirm our results. Furthermore, we used three statistical measures (C-index, AIC, and BIC) to be able to define the age cutoff that optimizes statistical performance. These three measures in the various analyses occasionally showed only minor discrepancies, allowing us to weigh the purely statistical analyses with pragmatic clinical considerations to balance the various results.

CONCLUSIONS

The present study shows that in a large European population of patients with DTC harboring a large subset of FTC patients, the overall optimal age cutoff employing the histopathological staging criteria for the 8th edition of the TNM system to predict disease specific survival is 50 years for patients with PTC, but not for those with FTC, as in these patients 40 years was the optimal cutoff. Therefore, our study implies that for an optimal estimate of prognosis, PTC and FTC should be staged as separate entities.

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Supplemental Table 1. Characteristics of the study population as well as for each of the separate cohorts and tests for differences between the two cohorts.

	Total Population (n=3074)[a]	Erasmus MC (n=820)[a]	Würzburg (n=2254)[a]	p-value[b]
Age at baseline (years)	48.7 ± 15.7	48.7 ± 16.2	48.8 ± 15.5	0.488
Sex				0.966
Male	939 (30.5)	250 (30.5)	689 (30.6)	
Female	2135 (69.5)			
Disease				**0.007**
PTC	2355 (76.6)	656 (80.0)	1699 (75.4)	
FTC	719 (23.4)	164 (20.0)	555 (24.6)	
HCC	98 (13.6)	57 (34.8)	41 (7.4)	**<0.001**
T-stage				**<0.001**
T0	10 (0.3)	10 (1.2)	-	
T1	1487 (48.4)	313 (39.2)	1174 (52.1)	
T2	796 (25.9)	222 (27.1)	574 (25.5)	
T3	509 (16.6)	205 (25.0)	304 (13.5)	
T4	246 (8.0)	62 (7.6)	184 (8.2)	
Lymph node metastases				**<0.001**
Not present	2364 (76.9)	551 (67.2)	1813 (80.4)	
Present	710 (23.1)	269 (32.8)	441 (19.6)	
Distant metastases				**0.038**
Not present	2802 (91.2)	733 (89.4)	2069 (91.8)	
Present	272 (8.8)	87 (10.6)	185 (8.2)	
Follow-up (months)	84 (37 – 154)	86 (42 – 136)	83 (36 – 166)	0.271
Vital status at end of follow-up				
Alive	2607 (84.8)	724 (88.3)	1883 (83.5)	**0.001**
Died (All causes)	467 (15.2)	96 (11.7)	371 (16.5)	**0.001**
Died (Thyroid cancer)	133 (4.3)	51 (6.2)	82 (3.6)	**0.002**
Survival				
10-year OS (%)	84.9 ± 0.8	84.9 ± 1.6	85.0 ± 1.0	0.648
10-year DSS (%)	94.7 ± 0.5	91.9 ± 1.2	95.8 ± 0.5	**<0.001**

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
[b] p-value comparing Erasmus MC and Würzburg.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; HCC, Hurthle Cell Carcinoma; OS, overall survival; DSS, disease specific survival

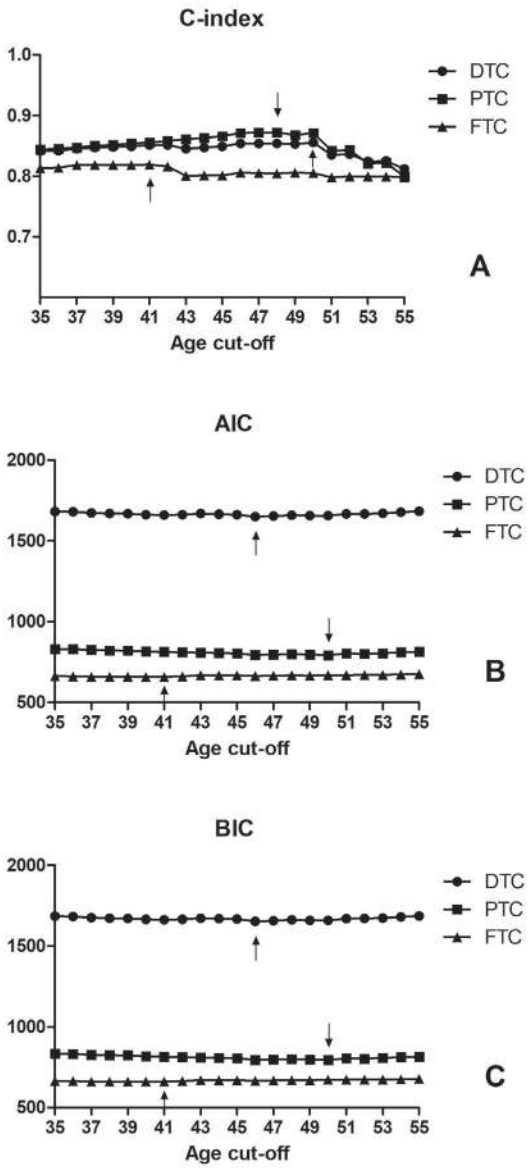
Supplemental Table 2. Effect of cancer subtype within stages on disease specific survival.

Stage		HR (95% CI)	p-value[a]
No age cutoff			
	Stage I	2.57 (0.92 - 7.15)	0.071
	Stage II	0.74 (0.29 - 1.88)	0.523
	Stage III	2.39 (0.95 - 6.01)	0.065
	Stage IV	2.39 (0.95 - 6.01)	0.793
20 years cutoff			
	Stage I	2.58 (0.93 - 7.17)	0.070
	Stage II	0.74 (0.29 - 1.88)	0.530
	Stage III	2.40 (0.95 - 6.04)	0.063
	Stage IV	0.94 (0.58 - 1.52)	0.788
25 years cutoff			
	Stage I	2.58 (0.93 - 7.17)	0.070
	Stage II	0.74 (0.29 - 1.88)	0.530
	Stage III	2.40 (0.95 - 6.04)	0.063
	Stage IV	0.94 (0.58 - 1.52)	0.795
30 years cutoff			
	Stage I	2.59 (0.93 - 7.21)	0.068
	Stage II	0.90 (0.37 - 2.20)	0.825
	Stage III	2.42 (0.96 - 6.08)	0.060
	Stage IV	0.89 (0.55 - 1.44)	0.629
35 years cutoff			
	Stage I	2.61 (0.94 - 7.26)	0.066
	Stage II	0.90 (0.37 - 2.20)	0.822
	Stage III	2.50 (0.99 - 6.32)	0.054
	Stage IV	0.87 (0.54 - 1.42)	0.582
40 years cutoff			
	Stage I	2.65 (0.95 – 7.36)	0.066
	Stage II	0.88 (0.36 – 2.14)	0.822
	Stage III	2.51 (0.99 – 6.34)	0.054
	Stage IV	0.86 (0.54 – 1.40)	0.582
45 years cutoff			
	Stage I	3.42 (1.31 - 8.94)	**0.012**
	Stage II	0.96 (0.41 - 2.25)	0.926
	Stage III	2.04 (0.79 - 5.26)	0.140
	Stage IV	0.82 (0.50 - 1.34)	0.426
50 years cutoff			
	Stage I	3.12 (1.23 - 7.91)	**0.017**
	Stage II	1.11 (0.52 - 2.39)	0.784
	Stage III	2.17 (0.84 - 5.63)	0.112

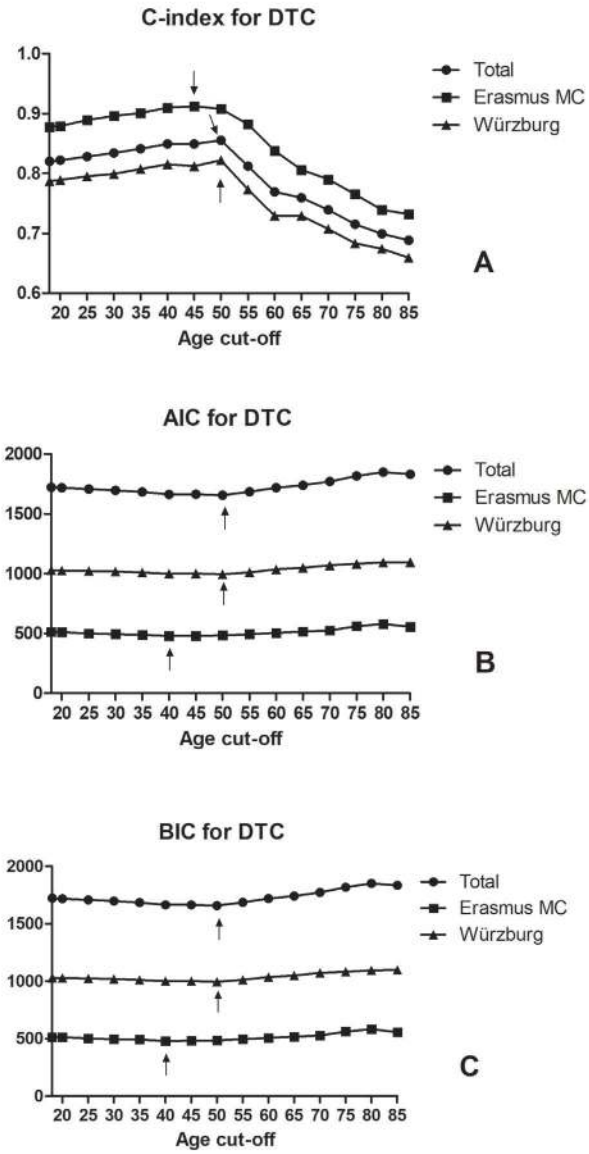
Supplemental Table 2. Effect of cancer subtype within stages on disease specific survival. (*continued*)

Stage		HR (95% CI)	p-value[a]
	Stage IV	0.72 (0.43 - 1.19)	0.199
55 years cutoff			
	Stage I	1.95 (0.88 - 4.31)	0.100
	Stage II	1.55 (0.73 - 3.27)	0.251
	Stage III	2.19 (0.83 - 5.82)	0.115
	Stage IV	0.68 (0.40 - 1.16)	0.155
60 years cutoff			
	Stage I	1.72 (0.89 - 3.33)	0.109
	Stage II	1.71 (0.78 - 3.76)	0.179
	Stage III	2.34 (0.82 - 6.70)	0.112
	Stage IV	0.72 (0.41 - 1.27)	0.155
65 years cutoff			
	Stage I	1.42 (0.75 - 2.71)	0.285
	Stage II	1.42 (0.70 - 2.90)	0.332
	Stage III	1.99 (0.71 - 5.58)	0.193
	Stage IV	0.91 (0.47 - 1.73)	0.764
70 years cutoff			
	Stage I	1.53 (0.85 - 2.77)	0.160
	Stage II	1.16 (0.63 - 2.11)	0.636
	Stage III	1.26 (0.37 - 4.28)	0.715
	Stage IV	0.73 (0.33 - 1.62)	0.442
75 years cutoff			
	Stage I	1.79 (1.05 - 3.05)	**0.034**
	Stage II	1.19 (0.69 - 2.06)	0.525
	Stage III	3.59 (0.33 - 38.83)	0.293
	Stage IV	0.81 (0.25 - 2.57)	0.717
80 years cutoff			
	Stage I	1.55 (0.93 - 2.59)	0.092
	Stage II	1.17 (0.71 - 1.94)	0.535
	Stage III	-	-
	Stage IV	-	-
85 years cutoff			
	Stage I	1.68 (1.02 - 2.75)	**0.040**
	Stage II	1.00 (0.61 - 1.64)	0.999
	Stage III	-	-
	Stage IV	-	-

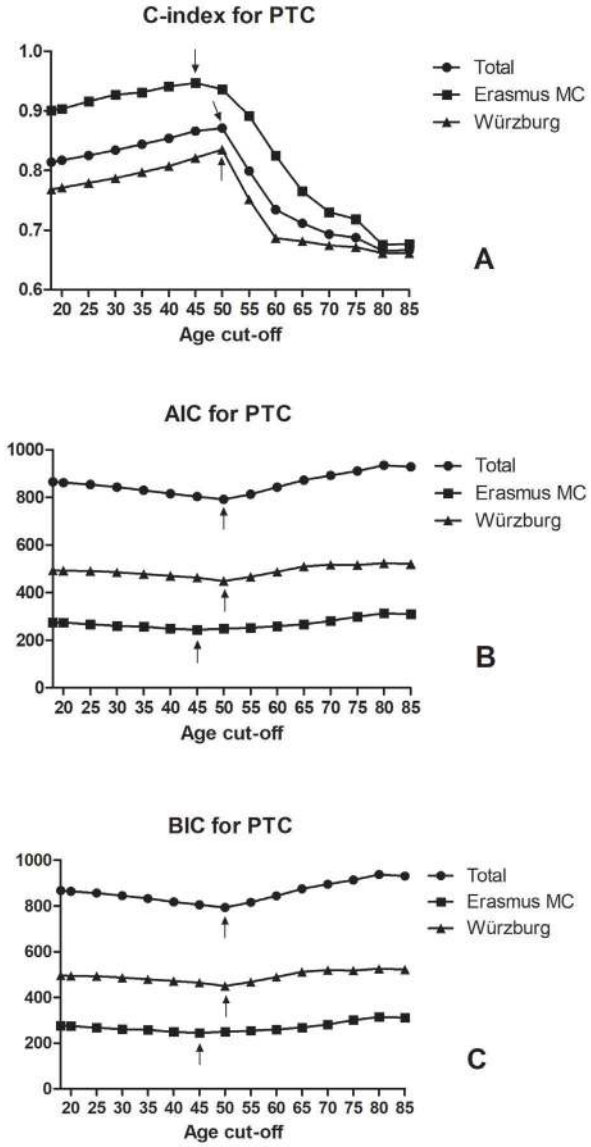
[a] p-value comparing FTC with PTC (PTC as reference group); adjusted for age, sex, and cohort.
HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer



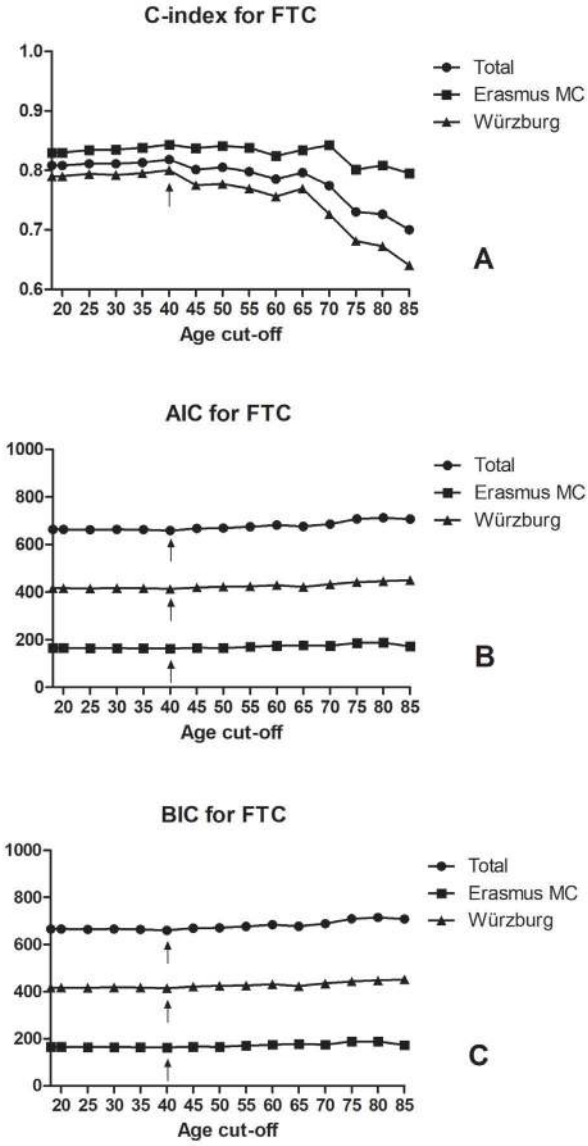
Supplemental Figure 1. Statistical Model Performance for (A) C-index, (B) AIC, and (C) BIC. Using 1-year increments between 35 to 55 years. Arrows showing the optimal age cutoffs.



Supplemental Figure 2. Statistical Model Performance for DTC per cohort for (A) C-index, (B) AIC, and (C) BIC. Using 5-year increments separately for each cohort. Arrows showing the optimal age cutoffs.



Supplemental Figure 3. Statistical Model Performance for PTC per cohort for (A) C-index, (B) AIC, and (C) BIC. Using 5-year increments separately for each cohort. Arrows showing the optimal age cutoffs.



Supplemental Figure 4. Statistical Model Performance for FTC per cohort for (A) C-index, (B) AIC, and (C) BIC. Using 5-year increments separately for each cohort. Arrows showing the optimal age cutoffs.

Chapter 4

Evaluating the 2015 American Thyroid Association Risk Stratification System in High Risk Papillary and Follicular Thyroid Cancer Patients

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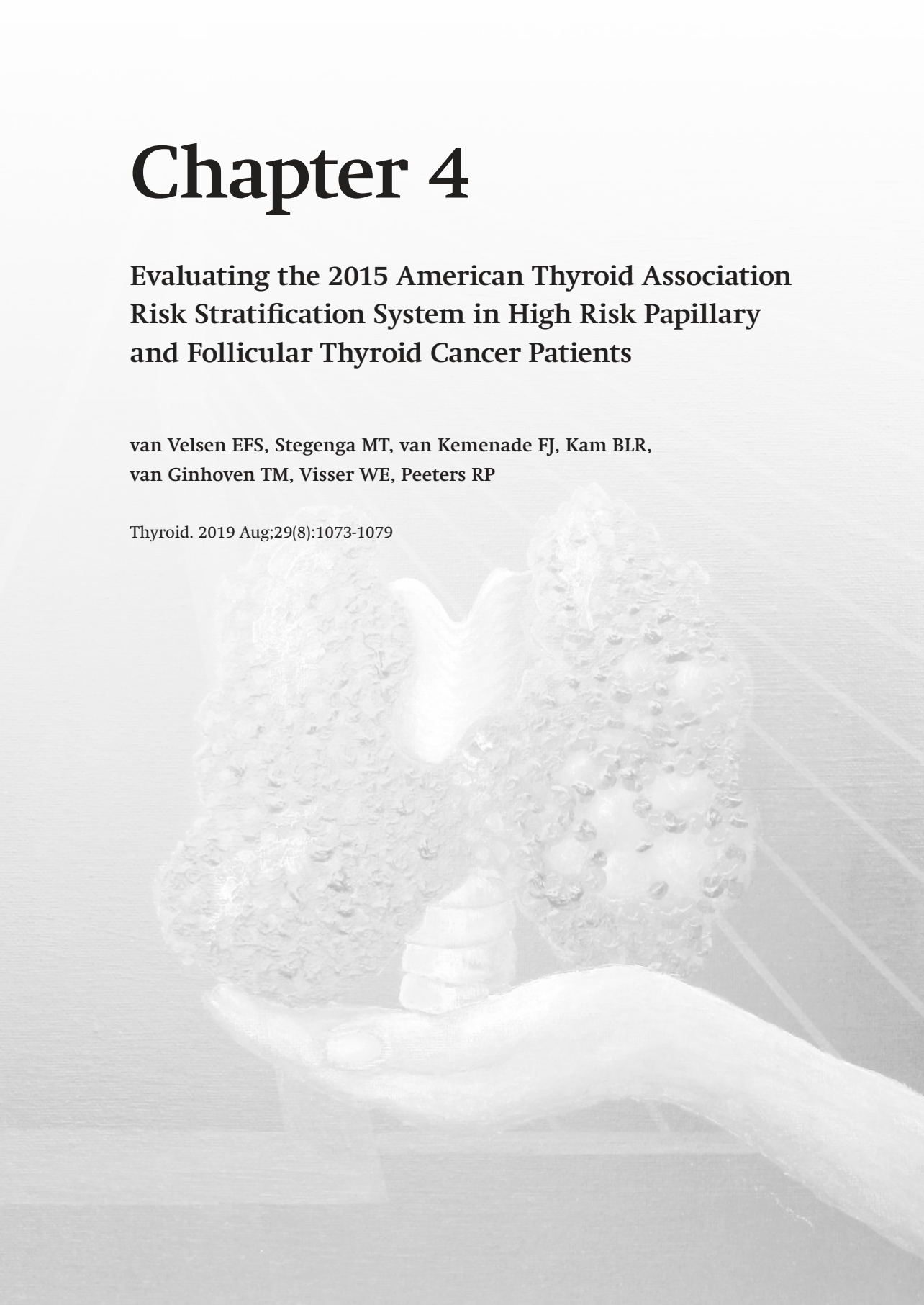

ABSTRACT

Background

The 2015 American Thyroid Association (ATA) Risk Stratification System for differentiated thyroid cancer (DTC) is designed to predict recurring/persisting disease but not survival. Earlier studies evaluating this System either evaluated the 2009 edition, comprised low number of patients with ATA High Risk, had low number of patients with follicular thyroid cancer (FTC), or did not distinguish between papillary and follicular thyroid cancer. Therefore, we evaluated the prognostic value of the 2015 ATA Risk Stratification System in a large population of High Risk thyroid cancer patients which included a substantial proportion of FTC patients.

Methods

We retrospectively studied adult patients with DTC who were diagnosed and/or treated at a Dutch university hospital between January 2002 and December 2015. All patients fulfilled the 2015 ATA High Risk criteria. Overall survival (OS) and disease specific survival (DSS) were analyzed using the Kaplan-Meier method. Logistic regression and Cox proportional hazards models were used to estimate the effects of DTC subtype and ATA High Risk criteria on response to therapy, recurrence as well as survival.

Results

We included 236 patients with High Risk DTC (32% FTC) with a mean age of 56 years. Median follow-up was 6 years. At final follow-up, 69 patients (29%) had excellent response, while 120 (51%) had structural disease. All High Risk criteria, except large pathologic lymph node, were inversely related to excellent response and positively related to structural disease at final follow-up. During follow-up, 11 of the 79 patients (14%) that achieved excellent response developed a recurrence. Finally, 10-year DSS was much higher in the initial excellent response than in the initial structural disease group (100% vs. 61% respectively).

Conclusions

In a population of High Risk DTC patients harboring a large subset of FTC patients, the 2015 ATA Risk Stratification System is not only an excellent predictor of persisting disease, but also of survival. As much as 14% of the High Risk patients that had an excellent response upon dynamic risk stratification experienced a recurrence during follow-up. Clinicians should thus be aware of the relatively high recurrence risk in these patients, even after an excellent response to therapy.

INTRODUCTION

The worldwide incidence of differentiated thyroid cancer (DTC) has been steadily increasing over the last two decades (1, 2). This seems partly due to increased diagnosis of indolent tumors, and since mortality has remained stably low for DTC, a less aggressive therapeutic approach seems more appropriate (1-3). To optimize the need for aggressive therapy and follow-up strategies, different systems that predict the risk of recurrence and survival in patients with DTC have been proposed. While the American Joint Committee on Cancer (AJCC)/Tumor Node Metastasis (TNM) Staging System has been designed to predict disease specific survival (4-8), the American Thyroid Association (ATA) and European Thyroid Association (ETA) Risk Stratification Systems have been designed to estimate the risk of disease recurrence (3, 9). The ATA Risk Stratification System is widely used and several studies have shown its usefulness in predicting disease recurrence (10-15) and even disease specific survival (11, 16). However, these studies either comprised relatively small proportions of ATA High Risk patients (14, 15), evaluated the previous 2009 edition (10-13), only comprised patients with papillary thyroid cancer (PTC) (13-15), or had low numbers of patients with follicular thyroid cancer (FTC). Furthermore, these studies did not distinguish between PTC and FTC (10-16) although FTC can manifest very differently from PTC in several ways, i.e. lymph node metastases are less common in FTC, and patients are usually older with more often distant metastases at initial presentation (17). The aim of our study was therefore to evaluate the prognostic value of the 2015 ATA Risk Stratification System in a large population of ATA High Risk thyroid cancer patients and to compare PTC and FTC.

MATERIALS AND METHODS

Study population and Clinical Outcomes

We retrospectively included all patients, aged 18 years or above, who were diagnosed and/or treated for either PTC or FTC (including Hürthle Cell carcinoma (HCC)) at the Erasmus Medical Center, Rotterdam, The Netherlands, between January 2002 and December 2015. All patients fulfilled the 2015 ATA High Risk criteria (3), i.e. macroscopic invasion of the tumor into the perithyroidal soft tissues (gross extrathyroidal extension (ETE)), incomplete tumor resection, distant metastases or postoperative serum thyroglobulin level (Tg) suggestive for distant metastatic disease, any metastatic lymph node larger than 3cm in size, or follicular thyroid cancer with extensive vascular invasion. Besides, all patients underwent thyroid surgery. From patient records, we obtained demographic, disease, treatment, response to therapy,

recurrence, and mortality characteristics. Demographical variables included age at diagnosis, sex, and year of diagnosis. Disease characteristics included disease type, TNM-stage (8th edition), presence/absence of multifocal disease, and minor/gross ETE. Data regarding treatment consisted of extent of surgery, use of radioactive iodine (RAI), and use of other treatment modalities (e.g. external beam radiotherapy).

Response to therapy was defined according to the four 2015 ATA response to therapy categories and was continually assessed during follow-up (i.e. dynamic risk stratification (DRS)) (3). Patients were considered to have an excellent response to therapy, i.e. No Evidence of Disease (NED), if they had a suppressed Tg <0.2ng/mL or TSH-stimulated Tg <1ng/mL, no detectable antibodies, and no evidence of structural disease on imaging. Patients were considered to have biochemical incomplete response if they had a suppressed Tg ≥1ng/mL or stimulated Tg ≥10ng/mL or rising anti-Tg antibody levels, and no evidence of structural disease on imaging. Patients were considered to have structural incomplete response if they had structural evidence of disease on imaging. And finally, patients were considered to have indeterminate response if they had a nonstimulated Tg <1ng/mL or a stimulated Tg <10ng/mL, declining or stable anti-Tg antibody levels. Persistent disease was defined as either structural or biochemical incomplete response. Response to therapy was recorded for the first time at six to 18 months after the first therapy; thereafter during and at end of follow-up. A recurrence was defined as new biochemical, structural, or functional disease after longer than twelve months of NED. Time to last follow-up, survival status, and date and cause of death were recorded. Survival was defined as the time of initial diagnosis to either last date of follow-up, death, or end of study (December 2017), whichever occurred first. Cause of death was obtained from hospital or general practitioner records. Patients with extensive or rapidly progressive thyroid cancer and no clear other cause of death were classified as death from thyroid cancer. The study protocol was approved by the Institutional Review Board of the Erasmus Medical Center.

Statistical Analysis

For continuous variables, means and standard deviations (SD), or medians with interquartile ranges (IQR) were calculated. For categorical variables, absolute numbers with percentages were recorded. Differences in characteristics between PTC and FTC were assessed using the Student's t-test or $\chi 2$-test. For DTC, overall survival (OS) and disease specific survival (DSS) were analyzed using the Kaplan-Meier method, and compared across response to therapy categories using the log-rank test. The same analyses were also performed for PTC and FTC separately. Additionally, we compared 'regular' FTC and HCC based on evidence suggesting that HCC is not a subtype of FTC (18, 19). Univariate and multivariate logistic regression or Cox

proportional hazards models were used to examine the effect of DTC subtype and the different ATA High Risk criteria on either response to therapy, developing NED, recurrence, or survival. Data on ATA High Risk criteria was missing in 3% of the values. Due to this low percentage, a patient was left out from the corresponding analysis if a value was missing. P-values below 0.05 were considered significant. All analyses were performed using SPSS Statistics for Windows (version 24.0).

RESULTS

Population characteristics

During the study period, a total of 255 patients were eligible for the study. Nineteen patients were excluded because they had insufficient follow-up information. Therefore, the analyses presented here were performed in the remaining 236 patients.

Table 1 lists the characteristics of the study population. Mean age was 56.3 years, and 148 (63%) were women. PTC was present in 160 (68%) patients, and the remaining 76 patients (32%) had FTC, including 29 patients (38%) with Hürthle Cell carcinoma. Median follow-up time was 72 months, and during follow-up 70 patients (30%) died, of which 49 (70%) due to thyroid cancer. Total or hemi-thyroidectomy was performed in all patients, and 227 patients (96%) received radioiodine therapy (68 (29%) once, 76 (32%) twice, and 82 (35%) received more than two therapies). Neck dissection was performed in 105 patients (45%). Patients with FTC were significantly older (64.1 years vs. 52.6 years; p<0.001), had significantly larger tumors at presentation (5.0cm vs. 3.0cm; p<0.001), and received a lower cumulative RAI dose (195 mCi vs. 298 mCi; p=0.019) than those with PTC. Additionally, patients with HCC were significantly more often male than those with 'regular' FTC (see Supplemental Table 1).

At diagnosis, 78 patients (33%) had distant metastases, with lung and bone as most common sites. PTC patients had significantly more often gross ETE and large

Table 1. Characteristics of the study population.

		DTC (n=236)[a]	PTC (n=160)[a]	FTC (n=76)[a]	p-value[b]
Age at diagnosis (years)		56.3 ± 17.6	52.6 ± 17.8	64.1 ± 14.7	**<0.001**
Women		148 (63%)	102 (64%)	46 (61%)	0.632
AJCC/TNM Staging system (8th edition)					**0.001**
	I	84 (36%)	63 (39%)	21 (28%)	
	II	79 (34%)	56 (35%)	23 (30%)	
	III	24 (10%)	19 (12%)	5 (7%)	
	IV	49 (21%)	22 (14%)	27 (36%)	

Table 1. Characteristics of the study population. (*continued*)

	DTC (n=236)[a]	PTC (n=160)[a]	FTC (n=76)[a]	p-value[b]
Hürthle Cell	29 (12%)	-	29 (38%)	-
Tumor Size (cm)	3.4 (2.0 – 5.0)	3.0 (1.6 – 4.2)	5.0 (3.1 – 7.9)	**<0.001**
Metastatic disease	78 (33%)	47 (29%)	31 (41%)	0.082
Pulmonary	58 (25%)	39 (24%)	19 (25%)	
Bone	26 (11%)	10 (6%)	16 (21%)	
Surgery (TT or HT)	236 (100%)	160 (100%)	76 (100%)	-
HT	3 (1%)	2 (1%)	1 (1%)	0.996
TT	233 (99%)	157 (99%)	75 (99%)	
Neck dissection	105 (45%)	88 (55%)	17 (22%)	**<0.001**
RAI treatment	227 (96%)	156 (98%)	71 (93%)	0.126
Once	68 (29%)	38 (24%)	30 (40%)	
Twice	76 (32%)	57 (36%)	19 (25%)	**0.017**
≥ 3	82 (35%)	61 (38%)	21 (28%)	
Cumulative dose (mCi)	295 (150 – 450)	298 (150 – 450)	195 (142 – 400)	**0.019**
Other treatments				
Radiotherapy	41 (17%)	23 (14%)	18 (24%)	0.078
TKI	19 (8%)	11 (7%)	8 (11%)	0.335
Follow-up (months)	72 (44 – 120)	75 (44 – 128)	66 (42 – 103)	0.329
Dead	70 (30%)	39 (24%)	31 (41%)	**0.010**
Thyroid cancer	49 (21%)	28 (18%)	21 (28%)	0.073

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; TT, total thyroidectomy; HT, hemi-thyroidectomy; RAI, radioactive iodine; cm, centimeter; mCi, milliCurie; TKI, Tyrosine Kinase Inhibitor.

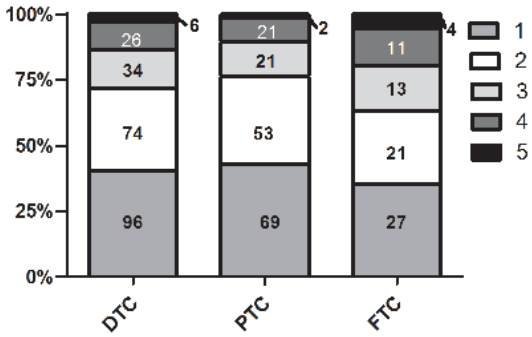

Figure 1. Number of ATA High Risk Criteria per disease type.

pathological lymph nodes than FTC patients (see Supplemental Table 2). Further, no differences between HCC and 'regular' FTC were seen. Finally, the majority of patients had either one (41%) or two (31%) High Risk factors (see Figure 1).

Response to therapy and Survival

Seven patients (3%) died within six months after initial therapy, precluding assessment of initial response to therapy in these patients. After initial therapy, the majority of the remaining 229 patients continued to have structural disease (51%), while an excellent response was seen in only 38 patients (17%). The other patients had either biochemical incomplete (7%) or an indeterminate (26%) response. These percentages were similar for PTC and FTC separately (see Table 2), and also for HCC and 'regular' FTC (see Supplemental Table 3). For DTC in general, as well as for PTC and FTC separately, the initial response to therapy category was significantly related to OS and DSS (both $p<0.001$). Patients with an initial excellent response had the best prognosis, followed by indeterminate, biochemical incomplete and structural

Table 2. Response to Therapy after first therapy.

	DTC (n=229)[a, b]	PTC (n=157)[a]	FTC (n=72)[a]	p-value[c]
Excellent	38 (17%)	27 (17%)	11 (15%)	0.717
Indeterminate	59 (26%)	44 (28%)	15 (21%)	0.250
Biochemical Incomplete	15 (7%)	11 (7%)	4 (6%)	0.681
Structural Incomplete	117 (51%)	75 (48%)	42 (58%)	0.139
Persistent Disease	132 (58%)	86 (55%)	46 (64%)	0.196

[a] Values are numbers (percentages).
[b] Seven patients were excluded due to death precluding initial response to therapy assessment.
[c] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

incomplete response (see Figure 2). This same pattern was seen for PTC and FTC (see Supplemental Figures 1 and 2), and also for HCC and 'regular' FTC separately. None of the patients with an initial excellent response died from thyroid cancer during follow-up.

At the end of follow-up, 69 patients (29%) had an excellent response, while 120 patients (51%) still had structural disease (see Table 3). The other patients had either biochemical incomplete (4%) or indeterminate (16%) response. In the majority of patients, structural disease was confined to either the neck region, or present as lung or bone metastases. Patients with FTC had more often structural disease than those with PTC (63% vs. 45%; $p=0.01$), while no differences between FTC and PTC were seen in the excellent response group. No differences between HCC and 'regular' FTC were

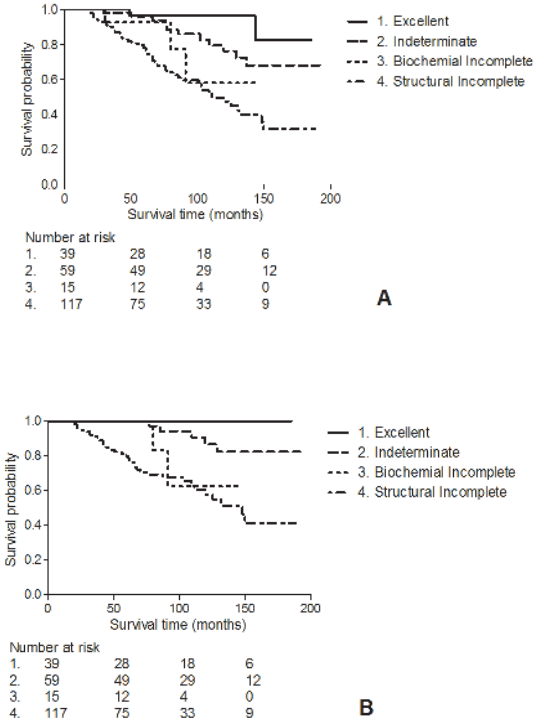

Figure 2. Kaplan-Meier curves for (A) OS and (B) DSS for DTC regarding Initial Response To Therapy.

Table 3. Response to Therapy at end of follow-up.

	DTC (n=236)[a]	PTC (n=160)[a]	FTC (n=76)[a]	p-value[b]
Excellent	69 (29%)	49 (31%)	20 (26%)	0.497
Indeterminate	38 (16%)	30 (19%)	8 (11%)	0.113
Biochemical Incomplete	9 (4%)	9 (6%)	-	0.997
Structural Incomplete	120 (51%)	72 (45%)	48 (63%)	**0.010**
Local	69 (57%)	48 (67%)	21 (44%)	**0.014**
Distant	89 (74%)	49 (68%)	40 (83%)	0.065
Both	38 (32%)	25 (35%)	13 (27%)	0.379
Persistent Disease	129 (55%)	81 (51%)	48 (63%)	0.072

[a] Values are numbers (percentages).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

seen (see Supplemental Table 4). Of the patients with structural disease after initial therapy, 74% still had structural disease at final follow-up, while 12 patients (10%) had an excellent response at final follow-up.

During follow-up, 79 patients (35%) achieved NED after a median of 22 months. In 11 patients (14%) who achieved NED, a recurrence occurred during follow-up after a median of 47 months. Both these percentages were similar for PTC and FTC, and also no differences were seen taking time into account (see Figure 3 for recurrence). Further, also no differences between HCC and 'regular' FTC were seen. Of the 11 patients with a recurrence, there were two patients with elevated Tg-levels, while two patients had local, three had distant, and four had both local and distant disease. Further, two out of these 11 patients with a recurrence died from thyroid cancer.

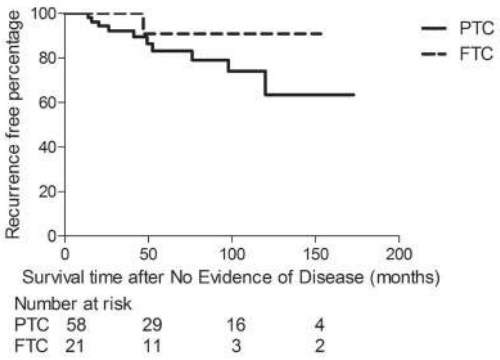

Figure 3. Kaplan-Meier curves for recurrence for PTC and FTC separately.

Risk factors

In a univariate analysis, the presence of distant metastases or an elevated postoperative Tg increased the risk of having persistent disease and not having an excellent response after initial therapy (see Supplemental Table 5a). All ATA High Risk criteria, except any metastatic lymph node larger than 3cm in size, increased the risk of having persistent disease and not having excellent response at end of follow-up (see Supplemental Table 6a). Presence of gross ETE or distant metastases resulted into an increased all-cause and thyroid cancer specific mortality (see Supplemental Table 7a). Distant metastases or an elevated postoperative Tg also resulted in a lower risk of developing NED during follow-up (see Supplemental Table 8), while gross ETE led to a higher risk of recurrence (see Supplemental Table 9).

Also after adjusting for age, sex and the other ATA High Risk criteria, the presence of distant metastases or an elevated postoperative Tg increased the risk of having persistent disease and not having an excellent response after initial therapy (see Supplemental Table 5b). Gross ETE and elevated postoperative Tg still increased the risk of having persistent disease and not having excellent response at end of follow-up in the multivariate analysis, while the other criteria were not significantly associated anymore (see Supplemental Table 6b). None of the ATA High Risk criteria influenced OS or DSS in the multivariate analysis (see Supplemental Table 7b). Distant metastases and elevated postoperative Tg still resulted in a lower risk of developing NED during follow-up (see Supplemental Table 8). Because of insufficient events, no multivariate analysis with respect to recurrence was performed.

DISCUSSION

This study shows that, in a population of patients with High Risk differentiated thyroid cancer, the 2015 ATA Risk Stratification System is an excellent predictor for persisting disease as well as for survival. At the end of follow-up, half of the population still had structural disease, while one-third showed excellent response. Fourteen percent of the patients with an excellent response experienced a recurrence later during follow-up.

We observed that the majority of the patients still had structural disease (51%) after initial therapy. This percentage is similar to the study of Pitoia et al. (13), who used the 2009 version of the ATA Risk Stratification System in patients with PTC. However, two other studies found lower percentages of structural disease using the 2015 version (14, 16). We showed that particularly initial distant metastases at presentation increases the risk of having persistent structural disease. This might be the reason for these differences as the percentage of patients with distant metastases in our study was more than twice the percentage of the other two studies (33% vs. 15%) (14, 16). An excellent response after initial therapy was seen in 17% of our patients, which is similar to earlier studies using the 2009 version (10, 11). We found no differences between PTC and FTC, and between HCC and 'regular' FTC, regarding the initial response to therapy categories.

One-third of the patients had no evidence of disease at final follow-up, whereas half of them still had structural disease. These percentages are similar as the studies of Shah et al. (16) using the 2015 version, and Pitoia et al. (13) using the 2009 version. On the other hand, both Vaisman et al. (11) and Tuttle et al. (10) showed lower numbers of NED and higher numbers of structural disease using the 2009 version. Explanations for these differences might be the use of different versions of the ATA

Risk Stratification System in combination with disease characteristics, e.g. gross ETE or elevated postoperative Tg. We showed that these two factors increase the risk of having persistent disease and not having an excellent response. However, data on these factors was unfortunately unavailable for the other studies.

We had a recurrence rate of 14% in the High Risk patients that achieved an excellent response according to the DRS defined in the 2015 ATA Guideline. In contrast, the 2015 ATA Guideline states a recurrence rate of 1-4% in DTC patients with an excellent response (3). However, this is predominantly based on recurrence rates in ATA Low and Intermediate risk patients, and previous studies in High Risk patients showed much higher rates of 14-30% (10, 13, 14, 16). This indicates that the recurrence rates after excellent response are much higher in High Risk patients than in those with Low or Intermediate Risk, illustrating the importance of careful follow-up in ATA High Risk patients. Further, in a univariate analysis, we showed that the presence of gross ETE at initial presentation increases the risk of a recurrence; this might have been caused by the aggressive and invasive nature of these kinds of tumors. Due to the low number of events, we were unable to confirm this in a multivariate analysis.

The risk of dying from thyroid cancer in this High Risk population was 21%, which is in line with earlier studies (reporting 15-18%) (10, 11, 16). We also showed that the response to therapy category determined after initial therapy is a strong predictor of survival in High Risk patients. Our 10-year DSS of 100% in excellent responders is the same as found by Shah et al. (16). However, our 10-year DSS was 61% in patients with structural incomplete response, whereas Shah et al. reported a DSS of 28% (16). This difference might be due to the fact that 21% of their patients had poorly differentiated thyroid cancer; those patients had a significantly higher mortality rate than the other patients in their study.

One of the main strengths of this study is the large number of patients with High Risk thyroid cancer compared to previous studies ((10-12, 20)); only the recent study of Shah et al. (16) had a similar number of patients with High Risk DTC, but they included poorly differentiated thyroid cancer as well. Furthermore, the relatively high number of FTC patients enabled us to compare PTC with FTC. Additionally, our follow-up of 72 months was comparable to earlier studies (10, 13, 14, 16, 21). A possible limitation of the study is that patients were recruited from a single tertiary university hospital, which might attract patients with more aggressive disease, especially FTC, because of the availability of advanced treatments. Another limitation might be the inability to perform a multivariate analysis for recurrence because of the low number of events. Finally, because of the retrospective character of the study, our dataset was incomplete in 3% of the ATA High Risk criteria values. Further, only 16 patients had insufficient information to determine their ATA Risk category, and

19 patients had insufficient follow-up information. It is therefore highly unlikely that such a small proportion would have altered the overall results.

CONCLUSIONS

In conclusion, this study shows that, in a population of High Risk patients with DTC harboring a large subset of FTC patients, the 2015 ATA Risk Stratification System is not only an excellent predictor of persisting disease, but also of survival. In addition, as 14% of the High Risk patients with an excellent response to therapy experienced a recurrence during follow-up, clinicians should be aware of this substantial high recurrence risk when treating and following up on these patients.

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Supplemental Table 1. Characteristics of the study population regarding 'regular' FTC and HCC.

	'regular' FTC (n=47)[a]	HCC (n=29)[a]	p-value[b]
Age at diagnosis (years)	63.4 ± 16.4	65.2 ± 11.5	0.588
Women	34 (72%)	12 (41%)	**0.007**
AJCC/TNM Staging system (8th edition)			0.304
I	13 (28%)	8 (28%)	
II	11 (23%)	12 (41%)	
III	3 (6%)	2 (7%)	
IV	20 (43%)	7 (24%)	
Hürthle Cell	-	29 (100%)	-
Tumor Size (cm)	4.5 (3.0 – 7.4)	5.3 (4.0 – 8.0)	0.511
Metastatic disease	23 (49%)	8 (28%)	0.066
Pulmonary	13 (28%)	6 (21%)	
Bone	6 (28%)	1 (3%)	
Surgery (TT or HT)	47 (100%)	29 (100%)	-
HT	1 (2%)	-	0.429
TT	46 (98%)	29 (100%)	
Neck dissection	10 (21%)	7 (24%)	0.771
RAI treatment	44 (94%)	27 (93%)	0.930
Once	17 (36%)	13 (45%)	
Twice	10 (21%)	9 (31%)	0.231
≥ 3	17 (36%)	4 (14%)	
Cumulative dose (mCi)	225 (142 – 555)	150 (109 – 295)	0.472
Other treatments			
Radiotherapy	10 (21%)	8 (28%)	0.530
TKI	3 (6%)	5 (17%)	0.134
Follow-up (months)	83 (46 – 125)	62 (35 – 82)	0.174
Dead	17 (36%)	14 (48%)	0.297
Thyroid cancer	11 (23%)	10 (35%)	0.294

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
[b] p-value comparing 'regular' FTC and HCC.
HCC, hürthle cell carcinoma; FTC, follicular thyroid cancer; TT, total thyroidectomy; HT, hemi-thyroidectomy; RAI, radioactive iodine; cm, centimeter; mCi, milliCurie; TKI, Tyrosine Kinase Inhibitor.

Supplemental Table 2. Reason ATA High Risk.

	DTC (n=236)[a]	PTC (n=160)[a]	FTC (n=76)[a]	p-value[b]
Gross ETE	75 (32%)	60 (38%)	15 (20%)	**0.008**
Incomplete tumor resection	118 (50%)	85 (53%)	33 (43%)	0.243
Distant Metastases	78 (33%)	47 (29%)	31 (41%)	0.082
Postoperative Tg suggestive for distant metastases	108 (46%)	75 (47%)	33 (43%)	0.131
Pathologic N1 with lymph node ≥ 3cm	45 (19%)	41 (26%)	4 (5%)	**<0.001**
FTC with extensive vascular invasion (> 4 foci)	57 (24%)	-	57 (75%)	-

[a] Values are numbers (percentages).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 3. Response to Therapy after first therapy regarding 'regular' FTC and HCC.

	'regular' FTC (n=44)[a]	HCC (n=28)[a]	p-value[b]
Excellent	8 (18%)	3 (11%)	0.396
Indeterminate	8 (18%)	7 (25%)	0.489
Biochemical Incomplete	3 (7%)	1 (4%)	0.564
Structural Incomplete	25 (57%)	17 (61%)	0.744
Persistent Disease	28 (64%)	18 (64%)	0.955

[a] Values are numbers (percentages).
[b] p-value comparing 'regular' FTC and HCC.
HCC, hürthle cell carcinoma; FTC, follicular thyroid cancer.

Supplemental Table 4. Response to Therapy at end of follow-up regarding 'regular' FTC and HCC.

	'regular' FTC (n=47)[a]	HCC (n=29)[a]	p-value[b]
Excellent	14 (30%)	6 (21%)	0.384
Indeterminate	5 (11%)	3 (10%)	0.968
Biochemical Incomplete	-	-	-
Structural Incomplete	28 (60%)	20 (69%)	0.411
Local	11 (39%)	10 (50%)	0.462
Distant	24 (86%)	16 (80%)	0.602
Both	7(25%)	6 (30%)	0.701
Persistent Disease	28 (60%)	20 (69%)	0.411

[a] Values are numbers (percentages).
[b] p-value comparing 'regular' FTC and HCC.
HCC, hürthle cell carcinoma; FTC, follicular thyroid cancer.

Supplemental Table 5a. Effect of ATA Risk Criteria on Initial Response To Therapy.

	Excellent Response		Structural Incomplete Response		Persistent disease	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Gross ETE	0.44 (0.18 – 1.05)	0.064	1.50 (0.85 – 2.64)	0.164	1.46 (0.82 – 2.61)	0.196
Incomplete tumor resection	0.52 (0.26 – 1.08)	0.078	1.33 (0.79 – 2.24)	0.288	1.29 (0.76 – 2.18)	0.347
Distant Metastases	0.26 (0.10 – 0.70)	**0.008**	11.09 (5.39 – 22.80)	**<0.001**	8.44 (4.04 – 17.66)	**<0.001**
Postoperative Tg suggestive for distant metastases	0.22 (0.09 – 0.52)	**0.001**	8.15 (4.14 – 16.07)	**<0.001**	11.48 (5.58 – 23.64)	**<0.001**
Pathologic N1 with lymph node ≥ 3cm	2.18 (0.99 – 4.78)	0.053	0.81 (0.42 – 1.58)	0.544	1.01 (0.52 – 1.98)	0.967
FTC with extensive vascular invasion (> 4 foci)	0.75 (0.13 – 4.13)	0.736	1.46 (0.42 – 5.10)	0.558	2.00 (0.56 – 7.09)	0.283

[a] univariate analysis.
[b] p-value for presence of risk factor.
OR, odds ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 5b. Effect of ATA Risk Criteria on Initial Response To Therapy.

	Excellent Response		Structural Incomplete Response		Persistent disease	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Gross ETE	0.74 (0.19 – 2.79)	0.652	1.60 (0.56 – 4.59)	0.379	2.10 (0.63 – 7.02)	0.228
Incomplete tumor resection	0.97 (0.36 – 2.64)	0.955	0.94 (0.39 – 2.27)	0.895	0.97 (0.38 – 2.44)	0.942
Distant Metastases	0.24 (0.06 – 0.91)	**0.037**	10.15 (3.51 – 29.31)	**<0.001**	4.92 (1.64 – 14.77)	**0.005**
Postoperative Tg suggestive for distant metastases	0.34 (0.12 – 0.93)	**0.035**	7.94 (3.28 – 19.24)	**<0.001**	16.18 (5.99 – 43.72)	**<0.001**
Pathologic N1 with lymph node ≥ 3cm	3.49 (0.99 – 12.36)	0.053	0.65 (0.21 – 2.02)	0.460	1.39 (0.40 – 4.89)	0.605
FTC with extensive vascular invasion (> 4 foci)	2.22 (0.13 – 37.84)	0.582	0.41 (0.02 – 8.80)	0.568	0.51 (0.02 – 11.56)	0.670

[a] multivariate analysis adjusted for age, sex, disease type and other factors
[b] p-value for presence of risk factor.
OR, odds ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 6a. Effect of ATA Risk Criteria on Response To Therapy at End of Follow-up.

	Excellent Response		Structural Incomplete Response		Persistent disease	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Gross ETE	0.26 (0.12 – 0.54)	**<0.001**	2.49 (1.41 – 4.41)	**0.002**	2.57 (1.43 – 4.59)	**0.001**
Incomplete tumor resection	0.51 (0.29 – 0.90)	**0.021**	1.80 (1.07 – 3.04)	**0.026**	1.74 (1.03 – 2.93)	**0.038**
Distant Metastases	0.36 (0.19 – 0.72)	**0.004**	2.88 (1.63 – 5.10)	**<0.001**	2.58 (1.46 – 4.57)	**0.001**
Postoperative Tg suggestive for distant metastases	0.21 (0.11 – 0.43)	**<0.001**	2.14 (1.18 – 3.88)	**0.012**	2.49 (1.40 – 4.12)	**0.002**
Pathologic N1 with lymph node ≥ 3cm	0.82 (0.39 – 1.70)	0.585	1.03 (0.54 – 1.99)	0.921	1.21 (0.62 – 2.34)	0.578
FTC with extensive vascular invasion (> 4 foci)	0.27 (0.07 – 0.98)	**0.046**	4.33 (1.15 – 16.28)	**0.030**	4.33 (1.15 – 16.28)	**0.030**

[a] univariate analysis.
[b] p-value for presence of risk factor.
OR, odds ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 6b. Effect of ATA Risk Criteria on Response To Therapy at End of Follow-up.

	Excellent Response		Structural Incomplete Response		Persistent disease	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Gross ETE	0.14 (0.03 – 0.57)	**0.006**	2.68 (1.01 – 7.11)	**0.047**	3.42 (1.24 – 9.43)	**0.017**
Incomplete tumor resection	0.84 (0.34 – 2.09)	0.703	1.24 (0.55 – 2.82)	0.610	1.29 (0.58 – 2.90)	0.535
Distant Metastases	0.42 (0.15 – 1.16)	0.095	2.87 (1.17 – 7.04)	**0.022**	1.90 (0.79 – 4.57)	0.155
Postoperative Tg suggestive for distant metastases	0.14 (0.05 – 0.38)	**<0.001**	1.87 (0.83 – 4.21)	0.129	3.07 (1.36 – 6.94)	**0.007**
Pathologic N1 with lymph node ≥ 3cm	0.40 (0.11 – 1.47)	0.166	1.19 (0.42 – 3.39)	0.740	1.84 (0.64 – 5.35)	0.261
FTC with extensive vascular invasion (> 4 foci)	2.41 (0.11 – 54.85)	0.582	1.18 (0.14 – 10.18)	0.878	1.18 (0.14 – 10.18)	0.878

[a] multivariate analysis adjusted for age, sex, disease type and other factors
[b] p-value for presence of risk factor.
OR, odds ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 7a. Effect of ATA Risk Criteria on Survival.

	Overall Survival		Disease Specific Survival	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Gross ETE	1.78 (1.11 – 2.87)	**0.018**	1.87 (1.05 – 3.32)	**0.033**
Incomplete tumor resection	1.33 (0.81 – 2.16)	0.258	1.83 (0.99 – 3.36)	0.051
Distant Metastases	2.27 (1.42 – 3.64)	**0.001**	3.81 (2.14 – 6.78)	**<0.001**
Postoperative Tg suggestive for distant metastases	1.68 (0.96 – 2.95)	0.072	1.89 (0.96 – 3.72)	0.066
Pathologic N1 with lymph node ≥ 3cm	0.68 (0.34 – 1.38)	0.286	0.63 (0.27 – 1.50)	0.297
FTC with extensive vascular invasion (> 4 foci)	2.38 (0.56 – 2.38)	0.242	2.94 (0.39 – 22.41)	0.298

[a] univariate analysis.
[b] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 7b. Effect of ATA Risk Criteria on Survival.

	Overall Survival		Disease Specific Survival	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Gross ETE	1.09 (0.56 – 2.13)	0.802	1.14 (0.53 – 2.47)	0.737
Incomplete tumor resection	1.30 (0.69 – 2.46)	0.425	1.68 (0.79 – 3.58)	0.179
Distant Metastases	1.24 (0.62 – 2.50)	0.546	2.27 (0.99 – 5.18)	0.051
Postoperative Tg suggestive for distant metastases	1.22 (0.61 – 2.45)	0.575	1.03 (0.44 – 2.38)	0.953
Pathologic N1 with lymph node ≥ 3cm	0.71 (0.29 – 1.77)	0.467	0.62 (0.20 – 1.92)	0.408
FTC with extensive vascular invasion (> 4 foci)	3.85 (0.44 – 33.57)	0.223	3.41 (0.38 – 30.53)	0.273

[a] multivariate analysis adjusted for age, sex, disease type and other factors
[b] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 8. Effect ATA Risk Criteria on developing Excellent Response.

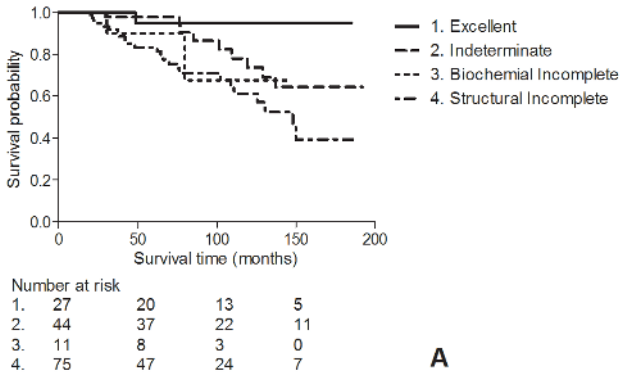
	HR (95% CI)[a]	p-value[c]	HR (95% CI)[b]	p-value[c]
Gross ETE	0.58 (0.35 – 1.01)	0.055	0.55 (0.23 – 1.32)	0.179
Incomplete tumor resection	0.70 (0.45 – 1.09)	0.117	0.68 (0.37 – 1.26)	0.219
Distant Metastases	0.34 (0.19 – 0.62)	**<0.001**	0.47 (0.23 – 0.96)	**0.038**
Postoperative Tg suggestive for distant metastases	0.27 (0.15 – 0.47)	**<0.001**	0.29 (0.15 – 0.54)	**<0.001**
Pathologic N1 with lymph node ≥ 3cm	0.90 (0.50 – 1.61)	0.713	0.92 (0.42 – 2.03)	0.839
FTC with extensive vascular invasion (> 4 foci)	0.41 (0.16 – 1.10)	0.078	1.81 (0.39 – 8.42)	0.450

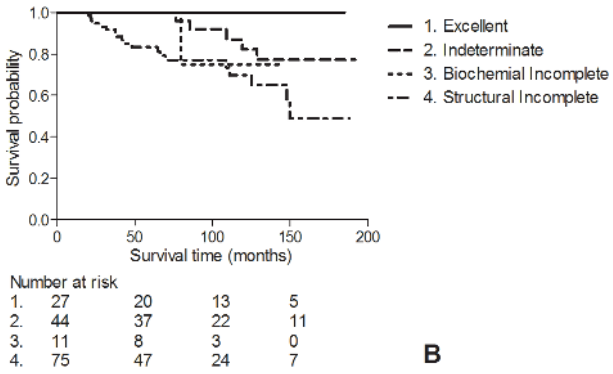
[a] univariate analysis.
[b] multivariate analysis adjusted for age, sex, disease type and other factors
[c] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.

Supplemental Table 9. Effect of ATA Risk Criteria on Recurrence.

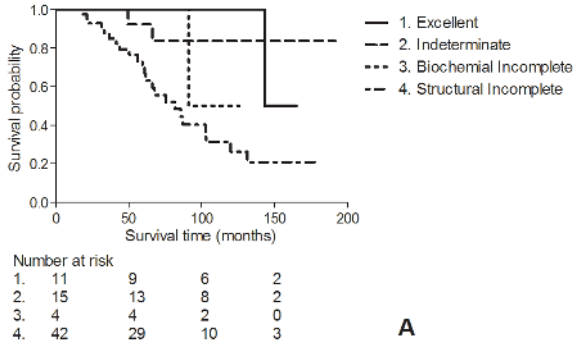
	HR (95% CI)[a]	p-value[b]
Gross ETE	10.04 (2.65 – 38.00)	**0.001**
Incomplete tumor resection	2.24 (0.65 – 7.66)	0.201
Distant Metastases	0.04 (0.00 – 79.67)	0.403
Postoperative Tg suggestive for distant metastases	1.12 (0.20 – 6.32)	0.897
Pathologic N1 with lymph node ≥ 3cm	2.03 (0.52 – 7.88)	0.308
FTC with extensive vascular invasion (> 4 foci)	-	-

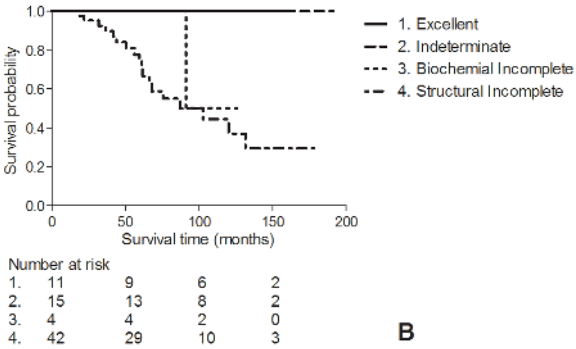
[a] univariate analysis.
[b] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extra thyroidal extension; Tg, thyroglobulin.





Supplemental Figure 1. Kaplan-Meier curves for (A) OS and (B) DSS for PTC regarding Initial Response To Therapy.





Supplemental Figure 2. Kaplan-Meier curves for (A) OS and (B) DSS for FTC regarding Initial Response To Therapy.

Chapter 5

Evaluation of the 2015 ATA Guidelines in Patients with Distant Metastatic Differentiated Thyroid Cancer

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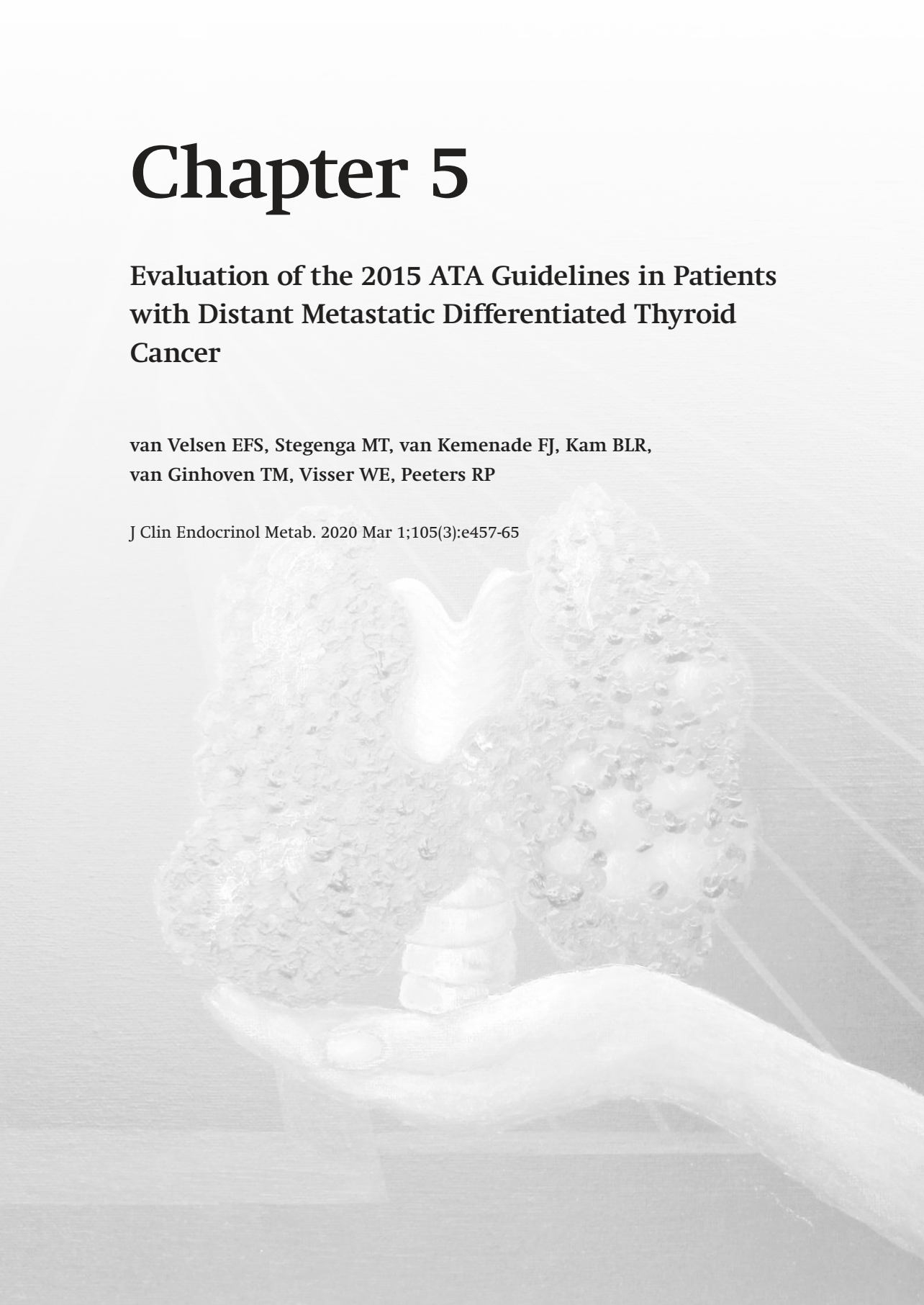

ABSTRACT

Context

Current ATA Guidelines for differentiated thyroid cancer (DTC) stratify patients to decide on additional radioiodine (RAI) therapy after surgery, and to predict recurring/persisting disease. However, studies evaluating the detection of distant metastases and how these Guidelines perform in patients with distant metastases are scarce.

Objective

To evaluate the 2015 ATA Guidelines in DTC patients with respect to 1) the detection of distant metastases, and 2) the accuracy of its Risk Stratification System in patients with distant metastases.

Patients and Main Outcome Measures

We retrospectively included 83 DTC patients who were diagnosed with distant metastases around initial therapy, and a control population of 472 patients (312 Low,160 Intermediate Risk) who did not have a certain indication for RAI therapy. We used the control group to assess the percentage of distant metastases that would have been missed if no RAI therapy was given.

Results

Two hundred-forty-six patients had no routine indication for RAI therapy of which four (1.6%) had distant metastases. Further, of the 83 patients with distant metastases, 14 patients (17%) had excellent response, while 55 (67%) had structural disease after a median follow-up of 62 months. None of the 14 patients that achieved an excellent response had a recurrence.

Conclusions

In patients without a routine indication for RAI therapy according to the 2015 ATA Guidelines, distant metastases would initially have been missed in 1.6% of the patients. Further, in patients with distant metastases upon diagnosis, the 2015 ATA Guidelines are an excellent predictor of both persistent disease and recurrence.

INTRODUCTION

The worldwide incidence of differentiated thyroid cancer (DTC) has been steadily increasing over the last two decades (1,2). As mortality has remained stable to slightly increasing, a less aggressive therapeutic approach seems appropriate (1-4). To optimize the need for de-escalation of therapy and follow-up strategies, different systems that predict the risk of recurrence and survival in patients with DTC have been proposed and evaluated (3,5-9).

The current 2015 American Thyroid Association (ATA) Guidelines use their own Risk Stratification System to determine the need for radioiodine (RAI) therapy after surgery (3). However, this approach has been challenged by several experts in the field (10-12). Because one of the aims of RAI therapy is to treat any remaining unknown cancer tissue, omitting RAI therapy might therewith leave metastases unknown and untreated (11). Using the criteria defined in the 2015 ATA Guidelines, one study claimed that a substantial proportion of patients with distant metastatic disease would not have been treated with RAI and thereby would have been missed (13). However, this study also included patients in whom metastatic disease was diagnosed during follow-up. Another study showed, in Low and Intermediate Risk patients defined by the 2009 ATA Guidelines, that 1% of the distant metastases would have been missed with omission of RAI therapy (14).

Several studies show that patients with distantly metastasized DTC have a relative poor prognosis (15-21). Risk factors such as age, RAI avidity, tumor size, and follicular type influence this prognosis (15-23). However, to our knowledge, no studies yet evaluated the 2015 ATA Risk Stratification System in DTC patients with distant metastases with respect to its ability to predict prognosis, recurrence and survival.

We initiated the current study because 1) very few studies evaluating risk factors in distant metastatic disease identified before or during initial therapy, 2) just two studies, having limitations, so far evaluated the consequences of omitting RAI therapy according to the new ATA Guidelines on the proportion of possible undetected distant metastases (13,14), and 3) no studies evaluated the 2015 ATA Risk Stratification System in DTC patients with distant metastases. The aim of our study was to evaluate the 2015 ATA Guidelines in DTC patients with respect to, 1) the proportion of possible distant metastases, and 2) the performance of the ATA Risk Stratification System in patients with distant metastases.

MATERIALS AND METHODS

Study population and Clinical Outcomes

We retrospectively identified all patients, aged 16 years or above, who were diagnosed and/or treated for either papillary (PTC) or follicular (FTC) thyroid carcinoma (including Hürthle Cell carcinoma (HCC)) at the Erasmus Medical Center, Rotterdam, The Netherlands, between January 2002 and December 2016. For the current study we included all patients who underwent thyroid surgery followed by RAI therapy, which is in line with the 2015 Dutch Thyroid Cancer Guidelines (24). Patients included were 1) diagnosed with distant metastases either before first RAI therapy based on pathology or imaging such as CT (pre-RAI group), or direct afterwards using the post-therapy whole-body scan (post-RAI group), or 2) had DTC classified as either ATA Low or Intermediate Risk (according to the 2015 ATA Guidelines). We used the patients with distant metastases (pre- and post-RAI group) to assess the performance of the Risk Stratification System. The ATA Low and Intermediate Risk groups served as control groups for the calculation of the proportion of undetected distant metastases when omitting RAI therapy. In a previous publication (25), we evaluated the Risk Stratification System of the 2015 ATA Guidelines in 236 High Risk DTC patients, also including patients with metastatic disease treated in our institute (n=78). In the current study, which only focuses on the metastatic group and studies this in much greater detail, we included 74 of the patients from this previous publication plus some additional metastatic patients.

From patient records, we obtained demographic, disease, treatment, response to therapy, recurrence, and mortality characteristics. Demographic variables included age at diagnosis, sex, and year of diagnosis. Disease characteristics included disease type, TNM-stage (8th edition), tumor size, presence/absence of multifocal disease, presence/absence of vascular invasion, presence/absence of lymph node metastases, and minor/gross extrathyroidal extension (ETE). Data regarding treatment consisted of extent of surgery, use of RAI, and use of other treatment modalities (e.g. external beam radiation therapy (EBRT)).

Indication for RAI therapy was retrospectively re-assessed using the 2015 ATA Guidelines (3) in 1) the post-RAI group, ignoring knowledge about the found distant metastases, and 2) the control groups. RAI therapy is routinely recommended in ATA High Risk patients, should be considered in ATA Intermediate Risk patients, not routinely recommended in ATA Low Risk patients with tumors larger than one and smaller than four centimeters, and not given in ATA Low Risk patients with tumors of one centimeter or smaller. The current and past Dutch Guidelines recommend to always treat patients with RAI therapy after a total thyroidectomy; a total thyroidectomy is always indicated in tumors ≥ 1cm.

Response to therapy was defined according to the four 2015 ATA response to therapy categories and was continually assessed during follow-up (i.e. dynamic risk stratification (DRS)) (3). Patients were considered to have an excellent response to therapy, i.e. No Evidence of Disease (NED), if they had a suppressed Tg <0.2ng/mL or TSH-stimulated Tg <1ng/mL, no detectable antibodies, and no evidence of structural disease on imaging. Patients were considered to have biochemical incomplete response if they had a suppressed Tg ≥1ng/mL or stimulated Tg ≥10ng/mL or rising anti-Tg antibody levels, but no evidence of structural disease on imaging. Patients were considered to have structural incomplete response if they had structural evidence of disease on imaging. And finally, patients were considered to have indeterminate response if they had a suppressed Tg <1ng/mL or a stimulated Tg <10ng/mL, declining or stable anti-Tg antibody levels. Persistent disease was defined as either structural or biochemical incomplete response. Response to therapy was recorded for the first time six to 18 months after the first therapy (i.e. initial DRS); thereafter during and at end of follow-up. A recurrence was defined as new biochemical or structural disease after longer than twelve months of NED.

RAI refractory disease was defined according to 2015 ATA Guidelines (3), i.e. 1) the malignant/metastatic tissue never concentrated RAI, 2) the tumor tissue loses the ability to concentrate RAI after previous evidence of RAI-avid disease, 3) RAI is concentrated in some lesions but not in others, 4) metastatic disease progresses despite significant concentration of RAI. Additionally, we also considered patients with a stimulated Tg ≥30ng/mL without significant concentration of RAI as having RAI refractory disease.

Time to last follow-up, survival status, and date and cause of death were recorded. Survival was defined as the time of initial diagnosis to either last date of follow-up, death, or end of study (December 2017), whichever occurred first. Cause of death was obtained from hospital or general practitioner records. Patients with extensive or rapidly progressive thyroid cancer and no clear other cause of death were classified as death from thyroid cancer. The study protocol was approved by the Institutional Review Board of the Erasmus Medical Center.

Statistical Analysis

For continuous variables, means and standard deviations (SD), or medians with interquartile ranges (IQR) were calculated. For categorical variables, absolute numbers with percentages were recorded. To assess the influence of the 2015 ATA Guidelines on the occurrence of possible undetected distant metastases, we compared the post-RAI and control group. Thereafter, differences in characteristics between the pre- and post-RAI groups were assessed using the Student's t-test or $\chi 2$-test. Overall survival (OS) and disease specific survival (DSS) were analyzed using the

Kaplan-Meier method for the patients with distant metastases. The same analyses were also performed for the pre- and post-RAI groups, and for the control population separately. In the pre- and post-RAI groups, univariate and multivariate logistic regression or Cox proportional hazards models were used to examine the effect of different (potential) risk factors on either response to therapy (at first DRS and at final follow-up), developing NED, recurrence, or survival. Data on these (potential) risk factors were missing in 4% of the values; due to this low percentage, a patient was left out from the corresponding analysis if a value was missing. P-values below 0.05 were considered significant. All analyses were performed using SPSS Statistics for Windows (version 24.0).

RESULTS

Population characteristics

During the study period, a total of 85 patients with distant metastases, 312 with ATA Low Risk, and 160 with ATA Intermediate Risk were eligible for the study. Two of the patients with distant metastases were excluded because of insufficient data on follow-up, leaving 83 patients available for analyses

Table 1 lists the characteristics of the study population with distant metastases. Mean age was 56.3 years, and 57 (69%) were women. Distant metastatic disease was identified before RAI therapy (pre-RAI group) in 33 (40%) patients. In these 33 patients, these metastases were discovered either because of symptoms (30%; e.g. pain), during pre-operative staging because of large tumor burden in the neck (27%), or incidentally discovered on a CT or FDG-PET made for another reason (21%). On the other hand, in the remaining 50 (60%) patients, the distant metastases were detected directly after RAI therapy by the post-therapy whole-body scan (post-RAI group). PTC was present in 53 (64%) patients (including ten (19%) with follicular variant of PTC), and the remaining 30 patients (36%) had FTC, including 7 patients (8%) with HCC. Median follow-up time was 62 months, and during follow-up 30 patients (36%) died, of which 26 were due to thyroid cancer. Total thyroidectomy was performed in all patients except one who received a hemithyroidectomy because of presence of one sided recurrent nerve paralysis. All patients received RAI therapy (19 (23%) once, 21 (25%) twice, and 43 (52%) received more than two therapies). Neck dissection was performed in 40 (48%) patients (central in six (7%), lateral in five (6%), and both in 29 (35%)). Patients in the pre-RAI group were significantly older (62.5 years vs. 52.3 years; $p<0.001$), had significantly more often FTC (58% vs. 22%; $p=0.001$), and received more often EBRT (46% vs. 18%; $p=0.008$) than those in the post-RAI group. There were no differences between the pre- and post-RAI groups regarding elevated Tg, presence

Table 1. Characteristics of the study population.

	Total population (n=83)[a]	Pre-RAI group (n=33)[a]	Post-RAI group (n=50)[a]	p-value[b]
Age at diagnosis (years)	56.3 ± 20.0	62.5 ± 17.9	52.3 ± 20.4	**<0.001**
Women	57 (69%)	20 (61%)	37 (74%)	0.198
Histopathological subtype				
Papillary	53 (64%)	14 (42%)	39 (78%)	**0.001**
Follicular	30 (36%)	19 (58%)	11 (22%)	
Hürthle Cell	7 (8%)	5 (15%)	2 (4%)	0.074
AJCC/TNM Staging system (8th)				
I	-	-	-	0.051
II	36 (43%)	10 (30%)	26 (52%)	
III	-	-	-	
IV	47 (57%)	23 (70%)	24 (48%)	
Tumor Size (cm)	3.5 (2.0 – 5.2)	4.1 (1.7 – 6.6)	3.5 (2.1 – 5.0)	0.668
Metastatic disease	83 (100%)	34 (100%)	50 (100%)	-
Pulmonary	64 (77%)	22 (67%)	42 (84%)	0.071
Bone	24 (29%)	12 (36%)	12 (24%)	0.145
Pulmonary and Bone	12 (15%)	4 (12%)	8 (16%)	0.902
Surgery (TT or HT)	83 (100%)	33 (100%)	50 (100%)	-
HT	1 (1%)	1 (3%)	-	0.216
TT	82 (99%)	32 (97%)	50 (100%)	
Neck dissection	40 (48%)	14 (42%)	24 (48%)	0.226
Central	6 (7%)	2 (6%)	4 (8%)	0.131
Lateral	5 (6%)	4 (12%)	1 (2%)	
Both	29 (35%)	8 (24%)	21 (42%)	
RAI treatment	83 (100%)	33 (100%)	50 (100%)	-
Once	19 (23%)	11 (33%)	8 (16%)	0.115
Twice	21 (25%)	9 (27%)	12 (24%)	
≥ 3	43 (52%)	13 (39%)	30 (60%)	
Cumulative dose (mCi)	387 (193 – 599)	294 (146 – 598)	446 (271 – 599)	0.295
Other treatments				
EBRT	24 (29%)	15 (46%)	9 (18%)	**0.008**
TKI	11 (13%)	6 (18%)	5 (10%)	0.299
Follow-up (months)	62 (34 – 103)	56 (31 – 76)	75 (42 – 122)	0.088
Death	30 (36%)	16 (49%)	14 (28%)	0.057
Thyroid cancer	26 (31%)	13 (39%)	13 (26%)	0.198

[a] Values are means (± standard deviation), medians (25-75-IQR), or numbers (percentages).
[b] p-value comparing metastases pre- and post-RAI groups.
TT, total thyroidectomy; HT, hemi-thyroidectomy; RAI, radioactive iodine; cm, centimeter; mCi, milliCurie; TKI, Tyrosine Kinase Inhibitor; EBRT, External Beam Radiation Therapy.

of lymph node metastases or gross ETE. The only difference was that patients in the post-RAI group more often had multifocal disease (see Supplemental Table 1 (26)).

Influence of the 2015 ATA Guidelines

We retrospectively re-evaluated the indication for RAI therapy in the 50 post-RAI patients. Unfortunately, for one patient insufficient information was available to assess the initial Risk Category. Of the remaining 49 patients, 39 (80%) were ATA High Risk, six (12%) were Intermediate Risk, and four (8%) were Low Risk. These four patients with Low Risk would not have been treated with RAI therapy according to the 2015 ATA Guidelines, while for the six Intermediate Risk patients RAI therapy should have been considered (see Table 2). The 10-year DSS of these 49 patients was 100% in the Low, 80% in the Intermediate, and 68% in the High Risk group (p=0.607).

As earlier mentioned, the control population of ATA Low and Intermediate Risk patients consisted of 472 patients who all received total thyroidectomy followed by RAI therapy according to 2015 Dutch Thyroid Cancer Guidelines (see Supplemental Table 2 for their characteristics (26)). According to the 2015 ATA Guidelines, 54 (11%) should not, and 188 (40%) would not routinely have been treated with RAI, while in 230 (49%) treatment with RAI therapy should have been considered (see Supplemental Table 3 (26)). The 10-year DSS in the control group was 99.8%. Combining the groups in which RAI therapy should not or would not routinely have been given according to the 2015 ATA Guidelines resulted in 246 patients (i.e. 4 + 54 + 188) in which four (1.6%) distant metastatic disease would have been initially missed if no RAI therapy would have been given. The group in which RAI therapy should be considered consisted of 236 patients (i.e. 6 + 70 + 160) in which six (2.5%) distant metastases would have been missed if one had decided to not treat them with RAI. No in-depth investigation of possible risk factors to identify distant metastatic disease before initial therapy was possible in these patients; characteristics of the four Low Risk patients are presented in Table 3.

Table 2. Indication for RAI therapy (2015 ATA Guidelines) in the post-RAI group in which distant metastases would have been missed if RAI therapy was omitted.

RAI indication	ATA Low Risk (n=4)[a]	ATA Intermediate Risk (n=6)[a]	ATA High Risk (n=39)[a]
No	-	-	-
Not routine	4 (8%)	-	-
Consider	-	6 (12%)	-
Yes	-	-	39 (80%)

[a] Values are numbers (percentages).

Table 3. Characteristics of the ATA Low Risk patients in which distant metastatic would have been initially missed.

Gender, Age	Year of Diagnosis	Disease Type	TNM (8th)	Location of Distant Metastases	s-Tg (ng/ml) during first RAI therapy	RAI therapy	Initial DRS	Time until Excellent Response
Female, 48 years	2014	PTC; Follicular variant	pT1bN0M1	Sternal lesion on SPECT/CT	<0.9 (Tg-abs: (15.8 U/mL)	Once; 143 mCi	Excellent	17 months
Female, 46 years	2006	PTC	pT2N0M1	Uptake in Lungs on SPECT/CT	22.3 (Tg-abs: 22.3 U/mL)	Twice; 230 mCi	Structural Incomplete	121 months
Female, 32 years	2014	FTC; minimally invasive	pT2N0M1	Uptake in Lungs on SPECT/CT	2.9	Twice; 193 mCi	Excellent	10 months
Female, 52 years	2013	PTC	pT2N0M1	Uptake in Lungs on SPECT/CT	8.6	Twice; 192 mCi	Indeterminate	38 months

PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; RAI, radioactive iodine; mCi, milliCurie; DRS, Dynamic Risk Stratification; s-Tg, stimulated thyroglobulin; Tg-abs, thyroglobulin antibodies.

Combining the pre- and post-RAI groups, while purposefully not accounting for the knowledge of the presence of distant metastases that was known upfront, resulted in five patients with Low Risk that would not have been treated with RAI therapy according to the 2015 ATA Guidelines. This resulted in 247 patients (i.e. 5 + 54 + 188) in which in five (2.0%) distant metastatic disease would have been missed if no RAI therapy would have been given. The number of Intermediate Risk patients remained the same. Therefore, there was no influence on the percentage of potentially missed distant metastases in the group in which RAI therapy should be considered.

Response to therapy and Survival of patients with distant metastases

At the first DRS after initial therapy (median 10 months), the majority of the patients with distant metastases continued to have structural disease (87%), while an excellent response was seen in only 5 patients (6%). The other patients had either biochemical incomplete (1%) or an indeterminate (6%) response. These percentages were similar for the pre- and post-RAI groups separately (see Table 4). None of the patients with an initial excellent response died from thyroid cancer during follow-up, while the 10-year DSS of patients with an initial structural incomplete response was as low as 54%.

During follow-up, only 14 (17%) patients achieved NED after a median of 45 months. During the rest of follow-up (median 43 months), none of these patients experienced a recurrence. NED occurred significantly more often in the post-RAI group (p=0.044), but using a Cox proportional hazards model accounting for time, this significant difference between both groups disappeared (p=0.106). None of the patients that achieved NED died during remaining follow-up.

As none of the patients experienced a recurrence, at the end of follow-up, 14 patients (17%) had an excellent response. Next to this, 55 patients (67%) still had structural disease (see Table 5). The other patients had either biochemical incom-

Table 4. Response to therapy after first therapy.

	Total population (n=83)[a]	Pre-RAI group (n=33)[a]	Post-RAI group (n=50)[a]	p-value[b]
Excellent	5 (6%)	-	5 (10%)	0.998
Indeterminate	5 (6%)	2 (6%)	3 (6%)	0.991
Biochemical Incomplete	1 (1%)	-	1 (2%)	0.998
Structural Incomplete	72 (87%)	31 (94%)	41 (82%)	0.134
Persistent Disease	73 (88%)	31 (94%)	42 (84%)	0.190

[a] Values are numbers (percentages).
[b] p-value comparing pre- and post-RAI groups.

Table 5. Response to therapy at end of follow-up.

	Total population (n=82)[a]	Pre-RAI group (n=33)[a]	Post-RAI group (n=49)[a]	p-value[b]
Excellent	14 (17%)	2 (6%)	12 (25%)	**0.044**
Indeterminate	12 (15%)	2 (6%)	10 (20%)	0.089
Biochemical Incomplete	1 (1%)	1 (3%)	-	0.998
Structural Incomplete	55 (67%)	28 (85%)	27 (55%)	**0.007**
Local	24 (43%)	11 (39%)	13 (48%)	
Distant	53 (96%)	28 (100%)	25 (93%)	
Both	22 (40%)	11 (39%)	11 (41%)	
Persistent Disease	56 (68%)	29 (88%)	27 (55%)	**0.003**

[a] Values are numbers (percentages).
[b] p-value comparing pre- and post-RAI groups.

plete (1%) or indeterminate (15%) response. In the majority of patients, structural disease was still present as distant metastases (96%), but 40% also had local disease in the neck region in addition to the distant metastases. The patients in which the distant metastases were identified on the post-therapy scan, exhibited an excellent response more often (25% vs. 6%; p=0.044), and less frequently had evidence of structural disease (55% vs. 85%; p=0.007).

With respect to survival, 10-year OS was 48.5% for the whole group, while 5-year and 10-year DSS were 79.8% and 57.0%, respectively (see Figure 1). The pre-RAI group had a significantly lower 10-year DSS (39.3% vs. 68.3% respectively; p=0.042). However, when adjusting for age and sex, the difference between both groups lost statistical significance (p=0.187; see also Table 6).

Of the four patients with distant metastases who would not have been treated with RAI therapy, and of the six in whom RAI therapy would have been considered, according to the 2015 ATA Guidelines (see also Table 3), at the first DRS after initial therapy, three had an excellent response, while the others had either indeterminate (n=1) or structural incomplete response (n=6). Six patients achieved NED, and median time to NED was 28 months. During the rest of follow-up (median 29 months), none of these patients experienced a recurrence nor died. The other four patients had either indeterminate (n=1) or structural incomplete response (n=3) at end of follow-up.

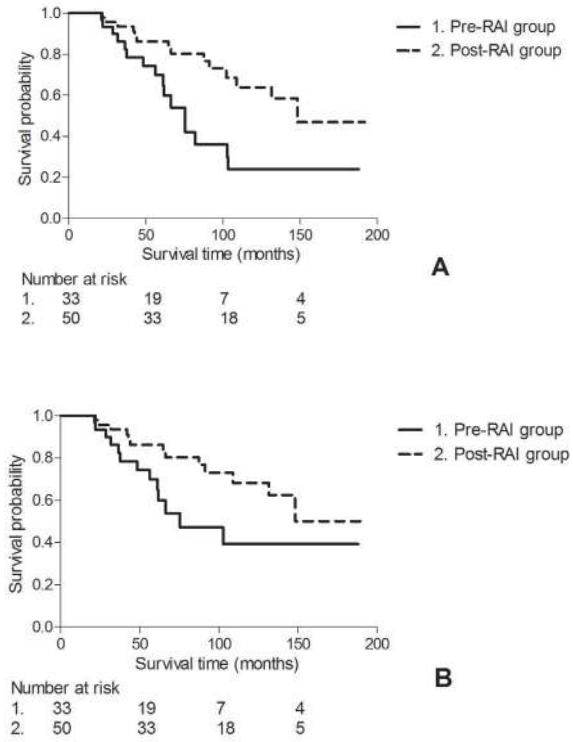

Figure 1. Kaplan-Meier curves for (A) OS and (B) DSS.

Table 6. Effect of metastases detection time on Survival.

	Hazard Ratio (95% CI)	p-value[c]
OS		
Model I [a]	0.38 (0.18 – 0.79)	**0.009**
Model II [b]	0.47 (0.22 – 1.00)	0.050
DSS		
Model I [a]	0.45 (0.21 – 0.99)	**0.047**
Model II [b]	0.58 (0.26 – 1.30)	0.187

[a] Model I; unadjusted.
[b] Model II; adjusted for age and sex.
[c] p-value comparing pre- and post-RAI groups (pre-RAI group as reference group).
OS, Overall Survival; DSS, Disease Specific Survival; CI, confidence interval.

Risk factors

An elevated postoperative stimulated-Tg just before RAI therapy, presence of initial lymph node metastases, older age, and larger tumor size increased the risk of not having an excellent response at end of follow-up in a univariate analysis, while an elevated postoperative stimulated-Tg, older age, larger tumor size, and having FTC increased the risk of having persistent disease at end of follow-up (see Supplemental Table 4a (26)). Presence of RAI refractory disease, older age, and larger tumor size resulted in an increased all-cause and thyroid cancer specific mortality (see Supplemental Table 5a (26)). An elevated postoperative stimulated-Tg, initial presence of lymph node metastases and older age resulted in a lower chance of developing NED during follow-up (see Supplemental Table 6 (26)).

After adjusting for age and sex, an elevated postoperative stimulated-Tg, initial presence of lymph node metastases, and older age increased the risk of not having an excellent response at final follow-up, while these same factors except for an elevated postoperative stimulated-Tg increased the risk of having persistent disease (see Supplemental Table 4b (26)). The presence of RAI refractory disease and older age still increased all-cause and thyroid cancer specific mortality (see Supplemental Table 5b (26)). An elevated postoperative stimulated-Tg, initial presence of lymph node metastases and older age still resulted in a lower chance of developing NED during follow-up (see Supplemental Table 6 (26)).

DISCUSSION

This study shows that 1.6% of the patients who do not have a routine indication for RAI therapy according the 2015 ATA Guidelines, were found to have distant metastases that would have been missed initially if no RAI therapy was given. This percentage was 2.5% in the patient group in whom RAI therapy should have been considered. Further, in patients with initial distant metastases, two-third still had structural disease at end of follow-up, while almost 20% achieved an excellent response. None of the patients with an excellent response experienced a recurrence during the period of follow-up.

The recommendation in the 2015 ATA Guidelines not to give RAI therapy in ATA Low Risk patients is based on systematic reviews which did not find a significant benefit of RAI therapy on cancer-related death (3,27,28). Next to this, others argued that the occurrence of undetected metastatic disease in these patients is low, and rising Tg levels during follow-up would warrant the need for further investigations (27). In addition, it has been reported that RAI therapy in patients with hyperthyroidism might lead to an increased mortality risk from secondary solid, but not hematologic

cancers (29), but a recent meta-analysis in thyroid cancer patients did not provide a clear answer on the possible increased risk of secondary malignancies due to RAI therapy (30). In the current retrospective study we observed that 1.6% of the 246 patients without an indication for RAI therapy according to the 2015 ATA Guidelines had distant metastases. Those metastases would initially have been missed if no RAI therapy was given. Although one might argue that due to the characteristics of these patients (Tg-levels, presence of antibodies), closer follow-up was warranted which would have led to detection of the metastases, but it is unclear if this would have affected prognosis. Due to the small number of identified Low Risk patients with distant metastatic disease, we were unable to search for factors that could have identified those patients with distant metastatic disease before initial therapy. Albano et al. found a slightly higher number as 3.6% of their Low Risk patients with distant metastases that would have been missed using the 2015 ATA Guidelines (13). However, they also included patients in whom metastatic disease was diagnosed during follow-up. This group is probably a different subset of patients, which would probably have been investigated for possible presence of distant metastases during follow-up. In contrast, Agate et al. found a lower number of approximately 1% in Low Risk patients with distant metastases that would have been missed (14). However, these results might not be totally comparable as they used the 2009 ATA Guidelines Low Risk definition. Further, Avram et al. investigated the impact of the first whole-body scan on staging, and demonstrated distant metastases in five out of 116 (4.3%) patients with pT1 tumors (31). However, because no information about the ATA Risk category is available from this study, the possible indications for RAI therapy could not be determined from their study. Furthermore, no separate numbers regarding pT2 tumors were given.

The 2015 ATA Guidelines recommend to consider RAI therapy in ATA Intermediate Risk patients; these recommendations are based on literature investigating the effect of RAI therapy in patients having one or more of the different Intermediate Risk criteria; as data are conflicting, the recommendation is given to consider RAI therapy in these patients (3). As our Dutch guideline recommends to always treat patients with RAI therapy after a total thyroidectomy, our study population is suited to evaluate the proportion of possible undetected distant metastases when omitting RAI therapy in ATA Intermediate Risk patients. We showed that 2.5% of the patients had distant metastases which would have been missed if no RAI therapy was given. Albano et al. showed that 4.9% would have been missed (13), while this was 1.4% in the study of Agate et al. (14). Differences between these two and our study were mentioned in the previous paragraph.

Evaluating the 2015 ATA Risk Stratification System in patients with distant metastases, two-third of our patients still had structural disease at final follow-up,

whereas 17% had no evidence of disease anymore. This suggests that the initial risk stratification of patients with distant metastases as ATA High Risk is valid. Hirsch et al. found an excellent response in 25% of their patients at end of follow-up, but to define an excellent response they used a stimulated Tg <2ng/mL, and not like both us and the 2015 ATA Guidelines, <1ng/mL (15). Earlier research in ATA High Risk patients, thus including patients with distant metastatic disease, showed lower percentages of patients with persistent structural disease and higher numbers of patients with no evidence of disease at final follow-up (32,33). This difference is probably due to the fact that we only studied patients treated at a tertiary referral center with distant metastases. However, metastatic disease did not influence response to therapy in an earlier study (25). Another factor might be age, as we showed that older age increases the risk of having persistent disease and not having an excellent response; our population is older than the populations of two earlier studies (32,33). Data on other factors, such as elevated postoperative stimulated-Tg was unfortunately unavailable for these two studies.

In patients that achieved an excellent response, no recurrences occurred (median time from NED to end of follow-up was 43 months). Similar results were found by Hirsh et al. (15). Chopra et al. found a recurrence rate of 21% in patients with lung metastases (34). However, their definition of an excellent response was different (stimulated Tg <10ng/mL), and therefore their group also included patients having an indeterminate response according to the 2015 ATA Guidelines. Further, earlier studies in ATA High Risk patients found recurrences rates of 14-30% (9,25,32,33,35). One might argue that a successful therapy for distant metastatic disease is also able to destroy other thyroid cancer tissue (e.g. due to gross ETE) resulting into a lower recurrence in these patients. Therefore, the DRS of the ATA Risk Stratification System performs well regarding the prediction recurrent disease after NED.

The 5- and 10-year DSS rates were respectively 80% and 57% in our population. This is similar to earlier studies (20,22,33). On the other hand, Lee et al. (16) and Goffredo et al. (36) showed 10-year DSS rates of respectively 27% and 44%, while Nixon et al. showed a 5-year DSS of 68% (18). Differences might be due to the fact that we studied only patients with distant metastases detected before or during initial therapy, while others also included patients who developed distant metastases later during follow-up (16), or patients that did not receive thyroid surgery (36). RAI refractory disease and older age resulted in an increased all-cause and thyroid cancer specific mortality. These factors were also reported in earlier studies identifying risk factors for decreased survival in patients with distant metastases (15-17,20,21,36).

One of the main strengths of this study is the substantial number of patients having distant metastases of well differentiated thyroid carcinoma discovered before or during initial therapy with a well-documented follow-up. This enabled us

to evaluate the indications for RAI therapy of the 2015 ATA Guidelines, but also to investigate disease outcome and prognostic factors. Further, unlike us, many other studies evaluated well differentiated and poorly differentiated thyroid cancer as one group despite their different behavior (15,16,33). A possible limitation of the current study is that patients were recruited from a single tertiary university hospital, which might attract patients with more aggressive disease because of the availability of advanced treatments. Finally, because of the retrospective character of the study, our dataset was incomplete in 4% of the (potential) risk factors values. As only two patients had insufficient follow-up information. It is therefore highly unlikely that such a small proportion would have altered the overall results.

CONCLUSIONS

In conclusion, this study shows that in DTC patients without an indication for RAI therapy or in whom RAI therapy should be considered respectively 1.6% and 2.5% have distant metastases that initially would be missed if no RAI therapy is given. Further research should therefore focus on factors predicting in which patients RAI therapy could be omitted safely without the risk of missing distant metastatic disease. Secondly, the 2015 ATA Guidelines are an excellent predictor of both persistent disease and recurrence in patients with initial metastatic disease, since at end of follow-up still two-third of the patients had structural disease, and none of the patients with an excellent response during follow-up experienced a recurrence later-on.

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Supplemental Table 1. Characteristics of the different risk factors.

	Total population (n=83)[a]	Pre-RAI group (n=33)[a]	Post-RAI group (n=50)[a]	p-value[b]
Gross ETE	22 (27%)	9 (27%)	13 (26%)	0.919
Incomplete tumor resection	40 (48%)	17 (52%)	23 (46%)	0.648
Postoperative Tg suggestive distant metastases (≥30ng/mL)	58 (70%)	28 (85%)	30 (60%)	0.151
Pathologic N1	41 (49%)	15 (46%)	26 (52%)	0.560
Pathologic N1 with lymph node ≥ 3cm	14 (17%)	7 (21%)	7 (14%)	0.467
Multifocal disease	31 (37%)	8 (24%)	23 (46%)	**0.042**
Angio-invasion	34 (41%)	15 (46%)	19 (38%)	0.300
RAI refractory Disease	34 (41%)	17 (52%)	17 (34%)	0.129

[a] Values are numbers (percentages).
[b] p-value comparing pre- and post-RAI groups.
ETE, extrathyroidal extension; Tg, thyroglobulin.

Supplemental Table 2. Characteristics of the control group.

	Control group (n=472)[a]	Metastases group (n=83)[a]
Age at diagnosis (years)	44.7 ± 14.3	56.3 ± 20.0
Women	340 (72%)	57 (69%)
Histopathological subtype		
Papillary	403 (85%)	53 (64%)
Follicular	69 (15%)	30 (36%)
Hürthle Cell	20 (4%)	7 (8%)
AJCC/TNM Staging system (8th)		
I	425 (90%)	-
II	47 (10%)	36 (43%)
III	-	-
IV	-	47 (57%)
ATA Risk Stratification System (2015)		
Low	312 (65%)	-
Intermediate	166 (35%)	-
High	-	83 (100%)
Tumor Size (cm)	2.0 (1.1 – 3.0)	3.5 (2.0 – 5.2)
Metastatic disease	0 (0%)	83 (100%)
Pulmonary	-	64 (77%)
Bone	-	24 (29%)
Pulmonary and Bone	-	12 (15%)
Surgery (TT or HT)	472 (100%)	83 (100%)

Supplemental Table 2. Characteristics of the control group. (*continued*)

		Control group (n=472)[a]	Metastases group (n=83)[a]
	HT	1 (0%)	1 (1%)
	TT	471 (100%)	82 (99%)
Neck dissection		108 (23%)	40 (48%)
	Central	29 (6%)	6 (7%)
	Lateral	11 (2%)	5 (6%)
	Both	68 (14%)	29 (35%)
RAI treatment		472 (100%)	83 (100%)
	Once	385 (82%)	19 (23%)
	Twice	59 (13%)	21 (25%)
	≥ 3	28 (6%)	43 (52%)
	Cumulative dose (mCi)	50 (50 – 150)	387 (193 – 599)
Other treatments			
	EBRT	1 (0%)	24 (29%)
	TKI	1 (0%)	11 (13%)
Follow-up (months)		106 (47 – 143)	62 (34 – 103)
Death		17 (4%)	30 (36%)
	Thyroid cancer	1 (0%)	26 (31%)

[a] Values are means (± standard deviation), medians (25-75-IQR), or numbers (percentages).
ATA, American Thyroid Association; TT, total thyroidectomy; HT, hemi-thyroidectomy; RAI, radioactive iodine; cm, centimeter; mCi, milliCurie; TKI, Tyrosine Kinase Inhibitor; EBRT, External Beam Radiation Therapy.

Supplemental Table 3. Summary of ATA Low and Intermediate Risk control group regarding indication for RAI therapy.

RAI indication	ATA Low Risk (n=312)[a]	ATA Intermediate Risk (n=160)[a]
No	54 (11%)	-
Not routine	188 (40%)	-
Consider	70 (15%)	160 (34%)
Yes	-	-

[a] Values are numbers (percentages).

Supplemental Table 4a. Effect of risk factors on response to therapy at end of follow-up.

	Excellent		Structural Disease		Persistent disease	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Gross ETE	-	-	2.14 (0.69 – 6.62)	0.118	1.98 (0.64 – 6.16)	0.236
Incomplete tumor resection	0.73 (0.23 – 2.33)	0.592	0.96 (0.38 – 2.46)	0.937	1.08 (0.42 – 2.80)	0.869
Postoperative Tg suggestive distant metastases	0.12 (0.03 – 0.48)	**0.003**	6.14 (1.60 – 23.54)	**0.008**	6.77 (1.75 – 26.12)	**0.006**
Pathologic N1	0.23 (0.06 – 0.89)	**0.034**	1.62 (0.64 – 4.12)	0.309	1.85 (0.72 – 4.77)	0.205
Pathologic N1 with lymph node ≥ 3cm	-	-	2.07 (0.52 – 8.22)	0.302	3.39 (0.69 – 16.52)	0.132
Age (years)	0.94 (0.91 – 0.98)	**0.001**	1.09 (1.05 – 1.13)	**<0.001**	1.08 (1.05 – 1.12)	**<0.001**
Sex	0.31 (0.06– 1.48)	0.141	1.16 (0.43 – 3.14)	0.777	1.07 (0.39 – 2.91)	0.901
Tumor Size (cm)	0.68 (0.48 – 0.97)	**0.032**	1.32 (1.03 – 1.69)	**0.026**	1.34 (1.04 – 1.72)	**0.025**
DTC subtype (PTC reference)	0.41 (0.11 – 1.62)	0.206	3.67 (1.21 – 11.09)	**0.021**	3.39 (1.12 – 10.26)	**0.031**
Multifocal disease	0.60 (0.17 – 2.12)	0.427	0.66 (0.26 – 1.71)	0.394	0.60 (0.23 – 1.56)	0.296
Angio-invasion	0.27 (0.07 – 1.07)	0.063	2.21 (0.81 – 6.03)	0.122	2.00 (0.73 – 5.48)	0.178
Pulmonary metastases	0.71 (0.19 – 2.58)	0.600	1.25 (0.43 – 3.67)	0.679	1.35 (0.46 – 3.96)	0.584
Bone metastases	1.43 (0.43 – 4.83)	0.562	1.70 (0.59 – 4.95)	0.329	1.58 (0.54 – 4.61)	0.403
Pulmonary and Bone metastases	0.97 (0.19 – 4.99)	0.968	2.78 (0.56 – 13.69)	0.209	2.61 (0.53 – 12.87)	0.239
RAI refractory disease	-	-	-	-	-	-

[a] univariate analysis.

[b] p-value for presence of risk factor.

OR, odds ratio; CI, confidence interval; ETE, extrathyroidal extension; Tg, thyroglobulin; N1, lymph node metastases; PTC, papillary thyroid cancer; DTC, differentiated thyroid cancer; RAI, radioactive iodine.

Supplemental Table 4b. Effect of risk factors on response to therapy at end of Follow-up.

	Excellent		Structural Disease		Persistent disease	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Gross ETE	-	-	0.83 (0.17 – 4.03)	0.817	0.85 (0.19 – 3.81)	0.827
Incomplete tumor resection	0.90 (0.24 – 3.48)	0.884	0.47 (0.13 – 1.79)	0.269	0.68 (0.20 – 2.31)	0.536
Postoperative Tg suggestive distant metastases	0.16 (0.03 – 0.78)	**0.024**	3.66 (0.77 – 17.45)	0.103	4.42 (0.96 – 20.32)	0.056
Pathologic N1	0.09 (0.02 – 0.56)	**0.010**	3.94 (0.99 – 15.69)	0.052	4.54 (1.16 – 17.72)	**0.030**
Pathologic N1 with lymph node ≥ 3cm	-	-	5.08 (0.75 – 34.54)	0.096	10.65 (1.34 – 84.65)	**0.025**
Age (years)	0.94 (0.91 – 0.97)	**0.001**	1.09 (1.05 – 1.14)	**<0.001**	1.08 (1.05 – 1.12)	**<0.001**
Sex	0.22 (0.04– 1.19)	0.079	1.71 (0.46 – 6.37)	0.422	1.41 (0.40 – 4.93)	0.591
Tumor Size (cm)	0.63 (0.39 – 1.03)	0.065	1.24 (0.90 – 1.70)	0.190	1.27 (0.92 – 1.73)	0.142
DTC subtype (PTC reference)	1.33 (0.25 – 7.20)	0.741	1.11 (0.27 – 4.49)	0.889	1.11 (0.29 – 4.34)	0.878
Multifocal disease	0.28 (0.06 – 1.37)	0.116	1.54 (0.41 – 5.72)	0.523	1.21 (0.35 – 4.19)	0.767
Angio-invasion	0.47 (0.10 – 2.28)	0.347	0.92 (0.24 – 3.50)	0.899	0.90 (0.25 – 3.25)	0.867
Pulmonary metastases	0.58 (0.13 – 2.53)	0.469	1.43 (0.36 – 5.66)	0.612	1.61 (0.43 – 6.06)	0.485
Bone metastases	5.52 (0.99 – 30.48)	0.050	0.57 (0.14 – 2.34)	0.434	0.56 (0.14 – 2.20)	0.404
Pulmonary and Bone metastases	2.40 (0.32 – 17.76)	0.392	1.06 (0.14 – 7.98)	0.952	1.03 (0.15 – 7.07)	0.976
RAI refractory disease	-	-	-	-	-	-

[a] multivariate analysis adjusted for age and sex.
[b] p-value for presence of risk factor.
OR, odds ratio; CI, confidence interval; ETE, extrathyroidal extension; Tg, thyroglobulin; N1, lymph node metastases; PTC, papillary thyroid cancer; DTC, differentiated thyroid cancer; RAI, radioactive iodine.

Supplemental Table 5a. Effect of risk factors on survival.

	Overall Survival		Disease Specific Survival	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Gross ETE	1.65 (0.77 – 3.52)	0.196	1.52 (0.67 – 3.48)	0.319
Incomplete tumor resection	1.43 (0.67 – 3.09)	0.358	1.38 (0.61 – 3.11)	0.437
Postoperative Tg suggestive distant metastases	4.95 (0.67 – 36.78)	0.118	4.42 (0.59 – 32.99)	0.147
Pathologic N1	1.16 (0.56 – 2.40)	0.683	1.03 (0.48 – 2.25)	0.932
Pathologic N1 with lymph node ≥ 3cm	0.48 (0.16 – 1.46)	0.198	0.59 (0.19 – 1.82)	0.355
Age (years)	1.04 (1.02 – 1.07)	**0.001**	1.04 (1.02 – 1.07)	**0.001**
Sex	1.08 (0.74 – 1.58)	0.682	1.12 (0.75 – 1.68)	0.583
Tumor Size (cm)	1.17 (1.03 – 1.33)	**0.020**	1.17 (1.03 – 1.34)	**0.020**
DTC subtype (PTC reference)	1.97 (0.96 – 4.06)	0.066	1.94 (0.89 – 4.23)	0.095
Multifocal disease	0.75 (0.33 – 1.67)	0.477	0.98 (0.42 – 2.27)	0.961
Angio-invasion	1.83 (0.83 – 4.05)	0.136	1.81 (0.79 – 4.15)	0.160
Pulmonary metastases	1.13 (0.46 – 2.76)	0.797	1.58 (0.54 – 4.61)	0.399
Bone metastases	0.91 (0.42 – 2.00)	0.817	0.96 (0.42 – 2.22)	0.923
Pulmonary and Bone metastases	0.74 (0.26 – 2.12)	0.573	0.90 (0.31 – 2.63)	0.846
RAI refractory disease	6.35 (2.71 – 14.88)	**<0.001**	7.76 (2.91 – 20.67)	**<0.001**

[a] univariate analysis.
[b] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extrathyroidal extension; Tg, thyroglobulin; N1, lymph node metastases; PTC, papillary thyroid cancer; DTC, differentiated thyroid cancer

Supplemental Table 5b. Effect of risk factors on survival.

	Overall Survival		Disease Specific Survival	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Gross ETE	0.99 (0.44 – 2.22)	0.979	0.88 (0.37 – 2.11)	0.777
Incomplete tumor resection	1.40 (0.63 – 3.11)	0.406	1.35 (0.58 – 3.14)	0.482
Postoperative Tg suggestive distant metastases	3.40 (0.45 – 25.75)	0.236	3.08 (0.40 – 23.44)	0.278
Pathologic N1	1.80 (0.84 – 3.88)	0.131	1.52 (0.67 – 3.45)	0.313
Pathologic N1 with lymph node ≥ 3cm	0.66 (0.21 – 2.08)	0.477	0.80 (0.25 – 2.60)	0.713
Age (years)	1.04 (1.02 – 1.07)	**0.001**	1.05 (1.02 – 1.07)	**0.001**
Sex	1.09 (0.74 – 1.60)	0.661	1.14 (0.76 – 1.72)	0.516
Tumor Size (cm)	1.06 (0.91 – 1.23)	0.442	1.07 (0.92 – 1.24)	0.384
DTC subtype (PTC reference)	1.02 (0.47 – 2.25)	0.956	1.00 (0.43 – 2.32)	0.993
Multifocal disease	1.22 (0.51 – 2.91)	0.661	1.82 (0.72 – 4.62)	0.209
Angio-invasion	1.04 (0.45 – 2.41)	0.919	1.05 (0.44 – 2.49)	0.918
Pulmonary metastases	1.85 (0.73 – 4.68)	0.195	2.56 (0.86 – 7.64)	0.092
Bone metastases	0.52 (0.23 – 1.16)	0.110	0.56 (0.24 – 1.32)	0.184

Supplemental Table 5b. Effect of risk factors on survival. (*continued*)

	Overall Survival		Disease Specific Survival	
	HR (95% CI)[a]	p-value[c]	HR (95% CI)[b]	p-value[c]
Pulmonary and Bone metastases	0.53 (0.18 – 1.52)	0.234	0.64 (0.22 – 1.88)	0.416
RAI refractory disease	4.30 (1.71 – 10.77)	**0.002**	5.04 (1.78 – 14.27)	**0.002**

[a] multivariate analysis adjusted for age and sex
[b] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extrathyroidal extension; Tg, thyroglobulin; N1, lymph node metastases; PTC, papillary thyroid cancer; DTC, differentiated thyroid cancer

Supplemental Table 6. Effect of risk factors on developing excellent response.

	Excellent		Excellent	
	HR (95% CI)[a]	p-value[c]	HR (95% CI)[b]	p-value[c]
Gross ETE	0.03 (0.00 – 3.05)	0.134	-	-
Incomplete tumor resection	0.80 (0.27 – 2.33)	0.697	0.68 (0.23 – 2.01)	0.486
Postoperative Tg suggestive distant metastases	0.15 (0.05 – 0.48)	**0.001**	0.23 (0.07 – 0.72)	**0.012**
Pathologic N1	0.27 (0.08 – 0.99)	**0.048**	0.13 (0.03 – 0.56)	**0.006**
Pathologic N1 with lymph node ≥ 3cm	0.03 (0.00 – 5.55)	0.182	-	-
Age (years)	0.96 (0.94 – 0.99)	**0.004**	0.96 (0.93 – 0.99)	**0.002**
Sex	0.61 (0.29 – 1.29)	0.195	0.56 (0.26 – 1.18)	0.127
Tumor Size (cm)	0.72 (0.52 – 1.00)	0.051	0.70 (0.47 – 1.05)	0.081
DTC subtype (PTC reference)	0.45 (0.12 – 1.60)	0.214	1.31 (0.26 – 6.54)	0.746
Multifocal disease	0.60 (0.19 – 1.92)	0.388	0.48 (0.14 – 1.61)	0.232
Angio-invasion	0.37 (0.10 – 1.32)	0.124	0.60 (0.15 – 2.29)	0.450
Pulmonary metastases	0.79 (0.25 – 2.53)	0.695	0.49 (0.14 – 1.74)	0.273
Bone metastases	1.15 (0.39 – 3.44)	0.801	3.27 (0.84 – 12.76)	0.089
Pulmonary and Bone metastases	0.80 (0.18 – 3.58)	0.770	1.50 (0.29 – 7.63)	0.626
RAI refractory disease	0.02 (0.00 – 1.74)	0.086	-	-

[a] univariate analysis.
[b] multivariate analysis adjusted for age and sex
[c] p-value for presence of risk factor.
HR, hazard ratio; CI, confidence interval; ETE, extrathyroidal extension; Tg, thyroglobulin; N1, lymph node metastases; PTC, papillary thyroid cancer; DTC, differentiated thyroid cancer

Chapter 6

The Influence of Age on Disease Outcome in 2015 ATA High Risk Differentiated Thyroid Cancer Patients

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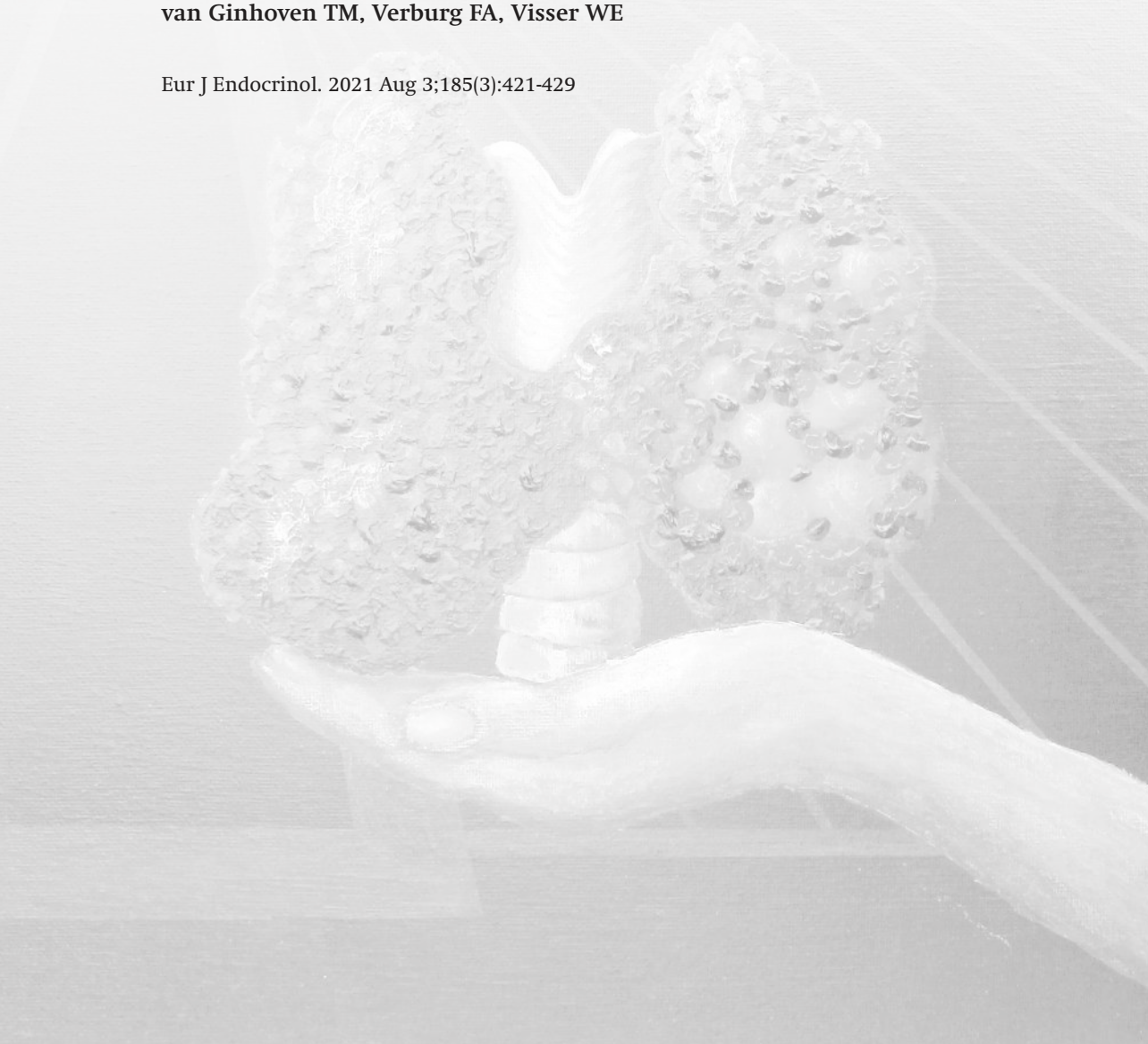

ABSTRACT

Objective

Recent research suggests that the addition of age improves the 2015 American Thyroid Association (ATA) Risk Stratification System for differentiated thyroid cancer (DTC). The aim of our study was to investigate the influence of age on disease outcome in ATA High Risk patients with a focus on differences between patients with papillary (PTC) and follicular thyroid cancer (FTC).

Methods

We retrospectively studied adult patients with High Risk DTC from a Dutch university hospital. Logistic regression and Cox proportional hazards models were used to estimate the effects of age (at diagnosis) and several age cutoffs (per five years increment between 20 and 80 years) on (i) response to therapy, (ii) developing no evidence of disease (NED), (iii) recurrence, and (iv) disease specific mortality (DSM).

Results

We included 236 ATA High Risk patients (32% FTC) with a median follow-up of 6 years. Age, either continuously or dichotomously, had a significant influence on having an excellent response after initial therapy, developing NED, recurrence, and DSM for PTC and FTC. For FTC, an age cutoff of 65 or 70 years showed the best statistical model performance, while this was 50 or 60 years for PTC.

Conclusions

In a population of patients with High Risk DTC, older age has a significant negative influence on disease outcomes. Slightly different optimal age cutoffs were identified for the different outcomes, and these cutoffs differed between PTC and FTC. Therefore, the ATA Risk Stratification System may further improve should age be incorporated as an additional risk factor.

INTRODUCTION

The American Thyroid Association (ATA) Risk Stratification system is designed to predict response to therapy and recurring disease in patients with differentiated thyroid cancer (DTC) (1). Nowadays, it is widely used and several studies have shown its usefulness in predicting response to therapy and recurrence (2-8) and even disease specific survival (DSS) (7-10). In contrast to the joint Union International Contre le Cancer and American Joint Committee on Cancer (UICC/AJCC) Tumor, Node, Metastasis (TNM) staging system (11-13), age was not incorporated into the classification of patients into different prognostic groups. Recently, three studies investigated the influence of age on recurrence and disease outcome in patients with DTC (9, 14, 15), including those with ATA High Risk DTC. Subsequently, it was shown that the 2015 ATA Risk Stratification System can be improved through the addition of age as a factor in the risk classification, especially for ATA High Risk patients (14). Unfortunately, these three studies either comprised relatively small proportions of ATA High Risk patients, only investigated 45 and 55 years as age cutoff, or had low numbers of patients with follicular thyroid cancer (FTC). It is well-established that FTC has a different clinical manifestation than papillary thyroid cancer (PTC) as lymph node metastasis are uncommon, patients are generally older and more often have distant metastasis at initial presentation (16).

The aim of the present study was to investigate the influence of age on recurrence and disease outcome in ATA High Risk patients, and whether the 2015 ATA Risk Stratification System could be improved by adding an age cutoff. The secondary aim of our study was to examine whether differences regarding age and the optimal age cutoff exist between patients with PTC and FTC.

MATERIALS AND METHODS

Study population and Clinical Outcomes

We retrospectively included all patients, aged 18 years or above, who were diagnosed and/or treated for either PTC or FTC (including Hürthle Cell carcinoma (HCC)) between January 2002 and December 2015 at the Erasmus Medical Center, Rotterdam, The Netherlands. Thereafter, using the 2015 ATA Risk Stratification System, we retrospectively identified those patients fulfilling the ATA High Risk criteria (1), i.e. macroscopic invasion of the tumor into the perithyroidal soft tissues (gross extrathyroidal extension (ETE)), incomplete tumor resection, distant metastases or postoperative serum thyroglobulin level (Tg) suggestive for distant metastatic disease (Tg >30 µg/L), any metastatic lymph node larger than 3 cm in size, or FTC with

extensive vascular invasion. The same database was earlier used to evaluate the Risk Stratification System of the 2015 ATA Guidelines for High Risk patients (8). From patient records, we obtained demographic, disease, treatment, response to therapy, recurrence, and mortality characteristics.

Response to therapy was defined according to the four categories defined in the 2015 ATA Guidelines, and was continually assessed during follow-up (i.e. dynamic risk stratification (DRS)) (1). These four response to therapy categories were: excellent response (also called no evidence of disease (NED)), biochemical incomplete response, structural incomplete response, and indeterminate response. Persistent disease was defined as either structural or biochemical incomplete response. Response to therapy was recorded for the first time at 6 to 18 months after the first therapy based upon patients' records; thereafter during and at end of follow-up. A recurrence was defined as new biochemical, structural, or functional disease (e.g. radioiodine scan) after longer than twelve months of NED. Time to last follow-up, survival status, and date and cause of death were recorded. Survival was defined as the time of initial diagnosis to either last date of follow-up, death, or end of study (December 2017), whichever occurred first. Cause of death was obtained from hospital or general practitioner records. The study protocol was approved by the Institutional Review Board of the Erasmus Medical Center.

Age was defined as age at initial diagnosis throughout the whole manuscript. Patients were stratified in either the younger or older group based on different age cutoffs. For this purpose, we investigated age cutoffs at five-year increments from 20 up to and including 80 years. Besides, age was also investigated as a continuous entity. Analyses were performed separately for both PTC and FTC, as well as combined for the overall DTC patient population.

Statistical Analysis

For continuous variables, means and standard deviations (SD), or medians with interquartile ranges (IQR) were calculated. For categorical variables, absolute numbers with percentages were recorded. Differences in characteristics between PTC and FTC were assessed using the Student's t-test or $\chi 2$-test.

DSS was analyzed using the Kaplan-Meier method, and compared across different age cutoffs using the log-rank test. For DTC, and PTC and FTC separately, univariate and multivariate logistic regression (outcome: odds ratio (OR)) or Cox proportional hazards models (outcome: hazard ratio (HR)) were used to examine the effect of age as a continuous entity, and the effect of the previously described age cutoffs on either initial response to therapy (excellent response), developing NED, recurrence, or Disease Specific Mortality (DSM). In the multivariate analyses, the effect of age and the age cutoffs was adjusted for the ATA High Risk criteria (gross ETE, incomplete

tumor resection, distant metastases or postoperative serum Tg suggestive for distant metastatic disease, any metastatic lymph node larger than 3 cm in size, and in case of FTC also for FTC with extensive vascular invasion) to assess whether age is an independent predictor of disease outcome.

To assess the statistical model performance of the 2015 ATA Risk Stratification System with different age cutoffs, we used the concordance index (Harrell's C-index) (17, 18) for the Cox proportional hazards models, the Area under the Curve (AUC) for the logistic regression models, and for both models also the Akaike information criterion (AIC) (19), and the Bayesian information criterion (BIC) (20). Both the C-index and AUC measure the discriminative power of a model and is a measure of goodness-of-fit. It ranges from 0.5 to 1.0, with 0.5 meaning the model predicts no better than random chance, and 1.0 being the perfect prediction model. Furthermore, the AIC and BIC measure the relative quality of a statistical model, and they provide the relative information lost when a statistical model is used to represent the true model. The model with the highest C-index/AUC and lowest AIC and BIC is considered to be the best model for predicting outcomes. Therefore, using these three criteria, we aimed to find the age cutoff that optimizes the statistical performance. The same analyses as for DTC were performed for both PTC and FTC separately.

P-values below 0.05 were considered significant. All analyses were performed using either SPSS Statistics for Windows (version 25.0) or the open source statistical software R (version 3.4.1) with package survC1 for estimating the C-index (21).

RESULTS

Population characteristics

A total of 236 patients fulfilled the inclusion criteria and had sufficient follow-up information. Table 1 lists the characteristics of the study population. Mean age was 56.3 years, and 148 (63%) were women. PTC was present in 160 (68%) patients, and the remaining 76 patients (32%) had FTC, including 29 patients (38%) with Hürthle Cell carcinoma. Median follow-up time was 72 months, and during follow-up 70 patients (30%) died, of which 49 (70%) due to thyroid cancer. Patients with FTC were significantly older (64.1 years vs. 52.6 years; $p<0.001$). Consequently, there were fewer patients with FTC than with PTC in the younger group for each age cutoff (see Supplemental Table 1).

Table 1. Characteristics of the study population.

	DTC (n=236)[a]	PTC (n=160)[a]	FTC (n=76)[a]	p-value[b]
Age at diagnosis (years)	56.3 ± 17.6	52.6 ± 17.8	64.1 ± 14.7	**<0.001**
Women	148 (63%)	102 (64%)	46 (61%)	0.632
AJCC/TNM Staging system (8th edition)				**0.001**
I	84 (36%)	63 (39%)	21 (28%)	
II	79 (34%)	56 (35%)	23 (30%)	
III	24 (10%)	19 (12%)	5 (7%)	
IV	49 (21%)	22 (14%)	27 (36%)	
Hürthle Cell	29 (12%)	-	29 (38%)	-
Tumor Size (cm)	3.4 (2.0 – 5.0)	3.0 (1.6 – 4.2)	5.0 (3.1 – 7.9)	**<0.001**
Metastatic disease	78 (33%)	47 (29%)	31 (41%)	0.082
Pulmonary	58 (25%)	39 (24%)	19 (25%)	
Bone	26 (11%)	10 (6%)	16 (21%)	
Surgery (TT or HT)	236 (100%)	160 (100%)	76 (100%)	-
HT	3 (1%)	2 (1%)	1 (1%)	0.996
TT	233 (99%)	158 (99%)	75 (99%)	
Neck dissection	105 (45%)	88 (55%)	17 (22%)	**<0.001**
RAI treatment	227 (96%)	156 (98%)	71 (93%)	0.126
Once	68 (29%)	38 (24%)	30 (40%)	
Twice	76 (32%)	57 (36%)	19 (25%)	**0.017**
≥ 3	82 (35%)	61 (38%)	21 (28%)	
Cumulative dose (mCi)	295 (150 – 450)	298 (150 – 450)	195 (142 – 400)	**0.019**
Other treatments				
Radiotherapy	41 (17%)	23 (14%)	18 (24%)	0.078
TKI	19 (8%)	11 (7%)	8 (11%)	0.335
Follow-up (months)	72 (44 – 120)	75 (44 – 128)	66 (42 – 103)	0.329
Dead	70 (30%)	39 (24%)	31 (41%)	**0.010**
Thyroid cancer	49 (21%)	28 (18%)	21 (28%)	0.073

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; TT, total thyroidectomy; HT, hemi-thyroidectomy; RAI, radioactive iodine; cm, centimeter; mCi, milliCurie; TKI, Tyrosine Kinase Inhibitor.

Response to therapy and Survival

Seven patients (3%) died within 6 months after initial therapy, precluding assessment of initial response to therapy in these patients. Therefore, these patients were excluded from the response to therapy analyses, leaving 229 patients for the remaining analyses. The youngest patient who died from PTC was 47.5 years at

Table 2. Response to therapy.

	DTC (n=229)[a, b]	PTC (n=157)[a]	FTC (n=72)[a]	p-value[c]
After initial therapy				
Excellent	38 (17%)	27 (17%)	11 (15%)	0.717
Indeterminate	59 (26%)	44 (28%)	15 (21%)	0.250
Biochemical Incomplete	15 (7%)	11 (7%)	4 (6%)	0.681
Structural Incomplete	117 (51%)	75 (48%)	42 (58%)	0.139
Persistent Disease	132 (58%)	86 (55%)	46 (64%)	0.196
Developing NED	79 (35%)	58 (37%)	21 (29%)	0.252
Recurrence	11 (14%)	10 (17%)	1 (5%)	0.137
At end of follow-up				
Excellent	69 (29%)	49 (31%)	20 (26%)	0.497
Indeterminate	38 (16%)	30 (19%)	8 (11%)	0.113
Biochemical Incomplete	9 (4%)	9 (6%)	-	0.997
Structural Incomplete	120 (51%)	72 (45%)	48 (63%)	**0.010**
Local	69 (57%)	48 (67%)	21 (44%)	**0.014**
Distant	89 (74%)	49 (68%)	40 (83%)	0.065
Both	38 (32%)	25 (35%)	13 (27%)	0.379
Persistent Disease	129 (55%)	81 (51%)	48 (63%)	0.072

[a] Values are numbers (percentages).
[b] Seven patients were excluded due to death precluding initial response to therapy assessment.
[c] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; NED, no evidence of disease.

diagnosis, while this was 41.1 years for FTC. After initial therapy, the majority of the remaining 229 patients continued to have structural disease (51%), while an excellent response was seen in only 38 patients (17%). These percentages were similar for PTC and FTC separately (see Table 2). During follow-up, 79 patients (35%) achieved NED after a median of 22 months. In 11 out of 79 patients (14%) who achieved NED, a recurrence occurred during follow-up after a median of 47 months. Both these percentages were similar for PTC and FTC, and also no significant differences were seen taking time into account. The numbers for the different age cutoffs are shown in Supplemental Tables 2 to 5.

Influence of Age

For DTC, we observed that age has a significant influence on having an excellent response after initial therapy (OR 0.98, 95% CI 0.96 – 0.99 per year increase; p=0.039), developing NED (HR 0.98, 95% CI 0.97 – 0.99 per year increase; p<0.001), and DSM (HR 1.06, 95% CI 1.04 – 1.08 per year increase; p<0.001) and a non-significant trend was seen for recurrence (HR 1.03, 95% CI 0.99 – 1.07 per year increase; p=0.132).

Therefore, older patients have a lower chance of having an excellent response, and higher risk of dying due to thyroid cancer. After adjustment for the ATA High Risk criteria, age remained significant for having NED during follow-up (HR 0.95, 95% CI 0.91 – 0.99 per year increase; p=0.042). The different age cutoffs more or less also follow this pattern (see Supplemental Tables 6 to 9). For PTC, we also observed that older age results in a significant lower chance on developing NED (OR 0.98, 95% CI 0.97 – 0.99 per year increase; p=0.009) and having a higher risk of dying due to thyroid cancer (HR 1.08, 95% CI 1.04 – 1.11 per year increase; p<0.001), but no significant influence of age on having an excellent response after initial therapy and on recurrence was found. After adjustment for the ATA High Risk criteria, age had a significant influence on having an excellent response after initial therapy (OR 0.96, 95% CI 0.92 – 0.99 per year increase; p=0.044), developing NED (HR 0.97, 95% CI 0.95 – 0.99 per year increase; p=0.018), and DSM (HR 1.07, 95% CI 1.02 – 1.11 per year increase; p=0.002). The different age cutoffs more or less also follow this pattern (see Supplemental Tables 6 to 9), and it is important to mention that, in a univariate analysis, several age cutoffs (50, 55 and 60 years) showed a significant influence on recurrence; older age resulted into a higher recurrence risk. For FTC, we observed that age has a significant influence on developing NED (HR 0.96, 95% CI 0.94 – 0.99 per year increase; p=0.005), but not on having an excellent response after initial therapy, recurrence and DSM. Therefore, older patients had a significant lower chance on developing NED. After adjustment for the ATA High Risk criteria, age remained to have a significant influence on developing NED (HR 0.95, 95% CI 0.91 – 0.99 per year increase; p=0.042). The different age cutoffs more or less also follow this pattern (see Supplemental Tables 6 to 9).

Statistical Model Performance

Regarding having an excellent response after initial therapy (see Figure 1 and Supplemental Figure 1), the optimal statistical performance (highest AUC, and lowest AIC and BIC) was identified for an age cutoff of 65 years for DTC. This also holds for FTC, but for PTC, an age cutoff of 50 years seemed to be optimal. For developing NED, the optimal statistical performance for DTC and PTC was identified for an age cutoff of 60 years, while this was 65 years for FTC (see Figure 1 and Supplemental Figure 2). For recurrence (see Figure 1 and Supplemental Figure 3) the optimal statistical performance for both DTC and PTC was observed with an age cutoff of 50 years. As there was only 1 patient with FTC that had a recurrence, no separate statistics were performed. Finally, regarding DSM (see Figure 1 and Supplemental Figure 4) for DTC, the highest C-index was found with an age cutoff of 55 years, while the lowest AIC and BIC was found for 45 years. For PTC, these were respectively 50 years and 45 years, while for FTC the optimal statistical performance was identified for 70 years

of age. The odds and hazard ratios for the optimal statistical performing age cutoffs are shown in Table 3, while the corresponding Kaplan-Meier curves are shown in Figures 2 to 4.

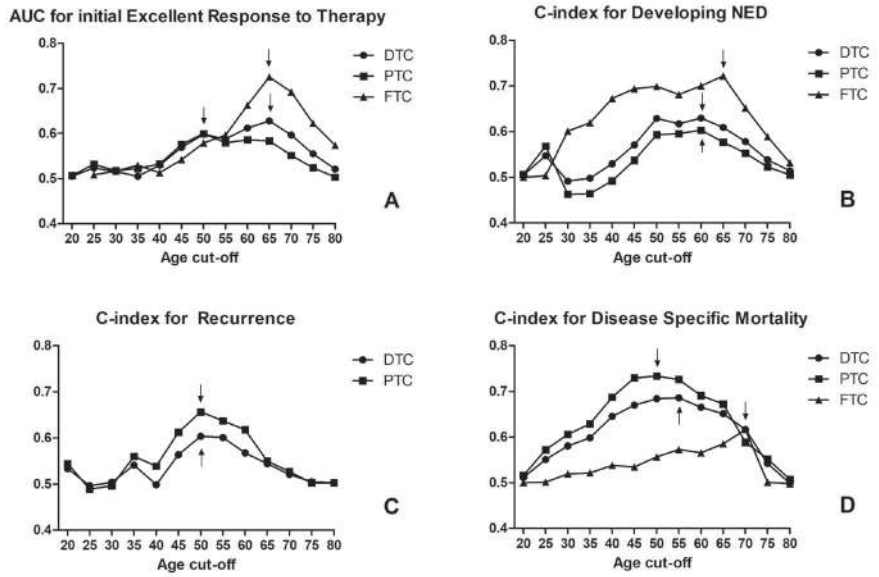

Figure 1. Statistical Model Performance of (A) AUC for initial Excellent Response to Therapy, and C-index for (B) Developing No Evidence of Disease, (C) Recurrence, and (D) Disease Specific Mortality.

Table 3. Influence of age on disease outcome for the age cutoffs with the best statistical performance.

	DTC			PTC			FTC		
	n[a]	OR or HR (95% CI)[b]	p-value[c]	n[a]	OR or HR (95% CI)[b]	p-value[c]	n[a]	OR or HR (95% CI)[b]	p-value[c]
Initial Excellent Response[d]									
50 years cutoff	20/18			17/10	0.45 (0.19 – 1.05)	0.063	3/8		
65 years cutoff	33/5	0.24 (0.09 – 0.64)	**0.004**	23/4			10/1	0.09 (0.01 – 0.70)	**0.022**
Developing NED[e]									
60 years cutoff	60/19	0.33 (0.19 – 0.55)	**<0.001**	47/11	0.35 (0.18 – 0.67)	**0.002**	13/8		
65 years cutoff	69/10			51/7			18/3	0.17 (0.05 – 0.56)	**0.004**
Recurrence[e]									
50 years cutoff	3/8	3.68 (0.97 – 13.91)	0.055	3/7	5.46 (1.39 – 21.52)	**0.015**	0/1		
Disease Specific Mortality[e]									
50 years cutoff	4/45			3/25	2.74 (2.74 – 30.39)	**<0.001**	1/20		
55 years cutoff	8/41	5.34 (2.49 – 11.47)	**<0.001**	5/23			3/18		
70 years cutoff	26/23			17/11			9/12	2.88 (1.19 – 6.99)	**0.019**

[a] numbers below / above cutoff.
[b] univariate analysis.
[c] p-value for influence of age (cutoff).
[d] odds ratio.
[e] hazard ratio.
OR, odds ratio; HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer; NED, no evidence of disease.

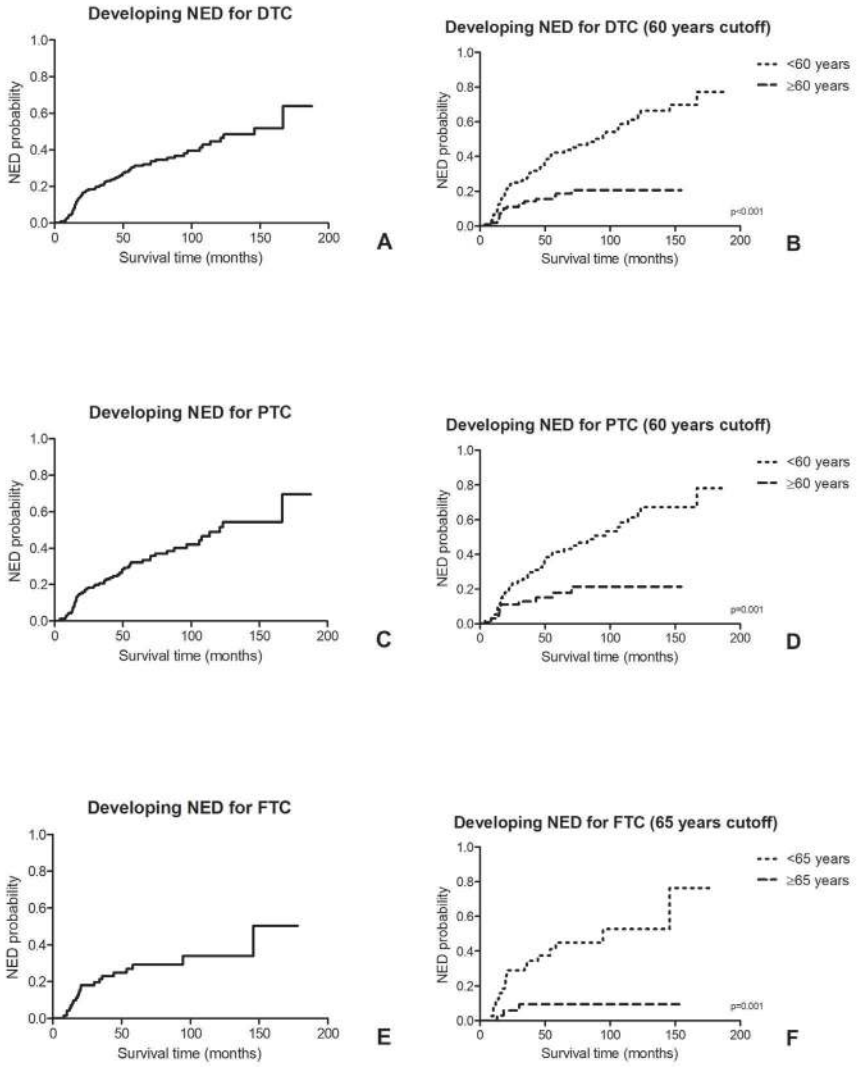

Figure 2. Kaplan-Meier curves for Developing No Evidence of Disease (NED) in (A, B) DTC, (C, D) PTC, and (E, F) FTC for either without an age cutoff or for the best statistical performing age cutoffs.

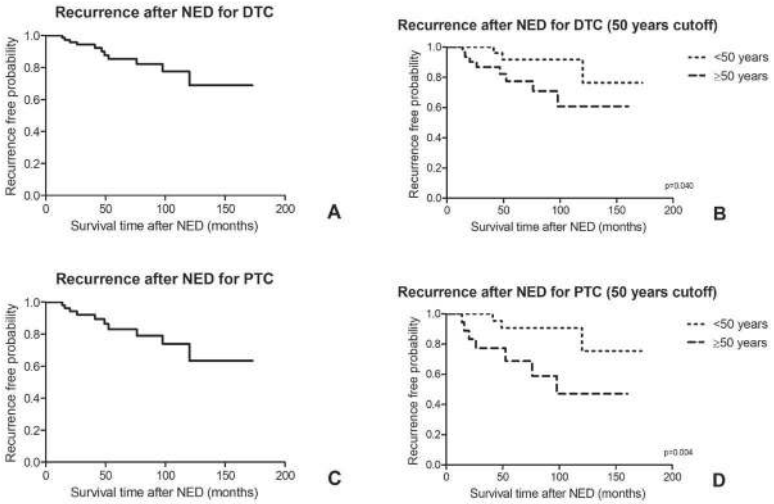

Figure 3. Kaplan-Meier curves for Recurrence in (A, B) DTC or (C, D) PTC for either without an age cutoff or for the best statistical performing age cutoffs.

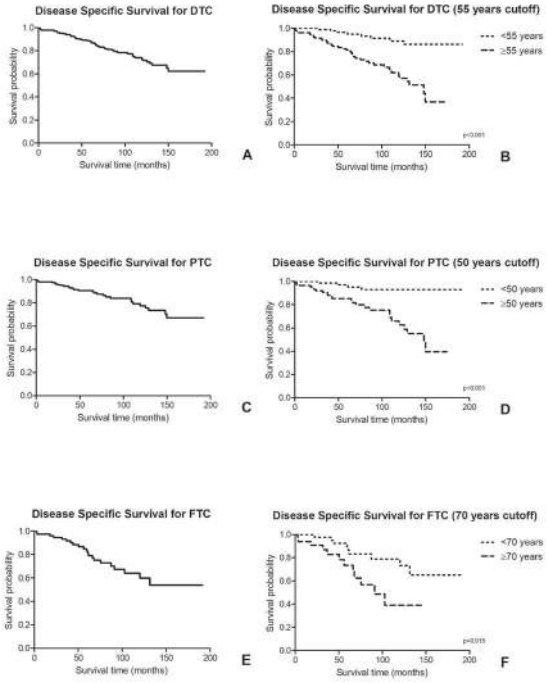

Figure 4. Kaplan-Meier curves for Disease Specific Survival in (A, B) DTC, (C, D) PTC, and (E, F) FTC for either without an age cutoff or for the best statistical performing age cutoffs.

DISCUSSION

This study shows that, in a population of patients with High Risk DTC, older age, either continuously or dichotomously, has a significant negative influence on disease outcome. Slightly different optimal age cutoffs were identified for the different outcomes, and these cutoffs differed between PTC and FTC.

We observed a significant influence of age on having an excellent response after initial therapy, developing NED, recurrence, and DSM for either PTC or FTC. Kim et al. showed no influence of age on recurrence using a 55 years age cutoff in patients with PTC (15); the recurrence rate in their patients was with 16.5% in line with ours (13.9%). On the other hand, Trimboli et al. showed a significant influence of age on relapse using an age cutoff of 55 years of age in patients with High Risk DTC (14). Difference between the latter study and ours is that, although they had fewer High Risk patients (n=87), relapse rates were higher which is probably caused by the fact that they used disease free survival instead of recurrence. On the other hand, in our study 11 (13.9%) out of 79 patients that achieved NED during follow-up experienced a recurrence, and therefore numbers might be too low to observe a significant influence of age as a continuous variable on recurrence. Shah et al. showed that age is a major determinant of response to therapy as there were significant more patients with an age below 55 years that achieved an excellent response at end of follow-up when comparing them to older patients (9). Besides, they also showed that age is a key predictor of Disease Specific Survival (DSS) / DSM which corresponds with our results. In the current study, containing patients with High Risk DTC, including those with distant metastases, no patients younger than 40 years died from DTC. This implies that in the UICC/AJCC TNM Staging System, patients younger than 40 years of age at diagnosis having distant metastases might be better classified as Stage I rather than Stage II. Further research is needed to confirm this proposal.

For DTC, the best statistical performance was observed for an age cutoff of 65 years (excellent response after initial therapy), 60 years (developing NED), 55 years (DSM) or 50 years (recurrence). In patients with FTC results were more consistent, as we showed that an age cutoff of 65 years (excellent response after initial therapy, developing NED) or 70 years (DSM) statistically outperformed the other age cutoffs. For PTC, an age cutoff of 50 years (excellent response after initial therapy, recurrence and DSM) or 60 years (developing NED) had the best statistical performance. These observations are partly in line with our earlier study regarding the 8th edition of the UICC/AJCC TNM Staging System also showing different age cutoffs for PTC and FTC (22). The observed optimal age cutoff for the 8th edition UICC/AJCC TNM Staging System for patients with FTC regarding DSM in that study (40 years) differs from the optimal age cutoff in FTC patients in the current study (70 years). Differ-

ences between these studies are 1) the study population, which comprise only ATA High Risk patients in the current study, 2) the way age is incorporated in the 8th UICC/AJCC TNM Stage System as this includes different tiers, and 3) the relative low number of younger patients with FTC, therewith reducing the statistical power in the lower age cutoffs. To the best of our knowledge, the present study is the first one to investigate the optimal age cutoff specifically for patients with FTC. Compared to those with PTC, patients with FTC in our population were older and had more advanced disease in terms of both local disease and distant metastases, which is in accordance with literature (16).

We showed that age remained significant, when adjusted for the original ATA High Risk factors, for having an excellent response after initial therapy for PTC, developing NED for PTC and FTC, and DSM for PTC. We recently showed, using the same population, that the presence of distant metastases, and an elevated postoperative Tg are also independent predictors of either having an excellent response after initial therapy or developing NED in High Risk DTC patients (8). Combining this implies that age, either continuously or dichotomously, is an independent predictor of excellent response to therapy, developing NED and DSM in High Risk PTC or FTC patients, and therefore should be considered to be included as a risk factor in the ATA Risk Stratification System. Based on our results, different age cutoffs for PTC and FTC are probably needed. One might suggest to use 65 years for FTC, and not 70 years, which was the optimal age cutoff for DSM, as the Risk Stratification System is not designed to predict DSS/DSM. For PTC, the optimal age cutoff was either 50 years or 60 years, and therefore on might argue to use the average of the two which is 55 years of age. Recently, Trimboli et al. showed that ATA High Risk patients could be reclassified in two subgroups based on an age cutoff of 55 years with older patients having the highest relapse risk, while such an age cutoff could not be identified for Low and Intermediate Risk patients (14); their population predominantly contained patients with PTC (91%). Therefore, further research is still needed, which besides ATA High Risk also includes patients with ATA Low and Intermediate Risk to determine in which way age can be incorporated into the ATA Risk Stratification System to further improve its predictive function regarding response to therapy and recurrence in both PTC and FTC patients. For example, single or multiple age cutoffs, or, like Trimboli et al. (14), define new risk categories for High Risk patients younger or older than a certain age cutoff. Therewith, clinical management can be better optimized for these older High Risk patients.

One of the main strengths of the current study is the relatively high number of FTC patients which enabled us to be, to our knowledge, the first to investigate the influence of age in patients with FTC, and consequently, observe differences between PTC and FTC patients. There is substantial evidence suggesting that Hürthle

Cell carcinoma (HCC) is not a subtype of 'regular' FTC (23, 24). However, in our previous study using the same dataset, we did not find any differences regarding disease outcome between HCC en 'regular' FTC (8), and therefore in the current study we did not analyze HCC and 'regular' FTC separately. A possible limitation of the study is that patients were recruited from a single tertiary university hospital, which might attract patients with more aggressive disease, especially FTC, because of the availability of advanced treatments. Another limitation might be the inability to perform a multivariate analysis for recurrence because of the low number of events. Elaborating on this, the number of events in young patients, especially in those with FTC, was relatively low, which lead to less robust results (no estimates or large confidence intervals) for these groups. Further, 16 patients had insufficient information to determine their ATA Risk category, and 19 patients had insufficient follow-up information. It is therefore highly unlikely that such a small proportion would have altered the overall results. Further, it is possible that an (unknown) proportion of patients would at present classified otherwise, e.g. Non-invasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP). Also, different pathologists were involved during this 15-year study period. Unfortunately, the retrospective nature of our study precludes any ascertainment in this respect. Finally, we used three statistical measures (C-index, AIC, and BIC) to be able to define the age cutoff that optimizes statistical performance. These three measures in the various analyses occasionally showed only minor discrepancies, allowing us to weigh the purely statistical analyses with pragmatic clinical considerations to balance the various results.

CONCLUSIONS

The present study shows that in a population of patients with High Risk DTC, harboring a large set of FTC patients, older age, either continuously or dichotomously, has a significant negative influence on disease outcome and therefore should be considered to be included as a risk factor in the ATA Risk Stratification System. Slightly different optimal age cutoffs were identified for the different outcomes, and these cutoffs differed between PTC and FTC. Therefore, our study implies that for an optimal estimate of disease outcome, PTC and FTC should be treated as separate entities. Next to this, further research is needed to determine in which way age can be incorporated as a risk factor in the ATA Risk Stratification System to further improve its predictive function.

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Supplemental Table 1. Number of patients per age cutoff.

	DTC (n=236)[a]	PTC (n=160)[a]	FTC (n=76)[a]	p-value[b]
20 years cutoff	4 (1.7%)	4 (2.5%)	0 (0.0%)	0.164
25 years cutoff	15 (6.4%)	14 (8.8%)	1 (1.3%)	**0.029**
30 years cutoff	24 (10.2%)	22 (13.8%)	2 (2.6%)	**0.008**
35 years cutoff	32 (13.6%)	29 (18.1%)	3 (3.9%)	**0.003**
40 years cutoff	49 (20.8%)	44 (27.5%)	5 (6.6%)	**<0.001**
45 years cutoff	64 (27.1%)	56 (35.0%)	8 (10.5%)	**<0.001**
50 years cutoff	83 (35.2%)	73 (45.6%)	10 (13.2%)	**<0.001**
55 years cutoff	105 (44.5%)	84 (52.5%)	21 (27.6%)	**<0.001**
60 years cutoff	120 (50.8%)	94 (58.8%)	26 (34.2%)	**<0.001**
65 years cutoff	150 (63.6%)	112 (70.0%)	38 (50.0%)	**0.003**
70 years cutoff	174 (73.7%)	132 (82.5%)	42 (55.3%)	**<0.001**
75 years cutoff	204 (86.4%)	146 (93.1%)	58 (76.3%)	**0.002**
80 years cutoff	220 (93.2%)	155 (96.9%)	65 (85.5%)	**0.001**

[a] Values are number (percentages) of patients below the age cutoff.
[b] p-value comparing PTC and FTC.
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 2. Response to therapy numbers per age cutoff.

	DTC (n=38)[a]	PTC (n=27)[a]	FTC (n=11)[a]
No age cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
20 years cutoff	1 (2.6%)	1 (3.7%)	0 (0.0%)
25 years cutoff	1 (2.6%)	1 (3.7%)	0 (0.0%)
30 years cutoff	3 (7.9%)	3 (11.1%)	0 (0.0%)
35 years cutoff	5 (13.2%)	4 (14.8%)	1 (9.1%)
40 years cutoff	10 (26.3%)	9 (33.3%)	1 (9.1%)
45 years cutoff	15 (39.5%)	13 (48.1%)	2 (18.2%)
50 years cutoff	20 (52.6%)	17 (63.0%)	3 (27.3%)
55 years cutoff	23 (60.5%)	18 (66.7%)	5 (45.5%)
60 years cutoff	27 (71.1%)	20 (74.1%)	7 (63.6%)
65 years cutoff	33 (86.8%)	23 (85.2%)	10 (90.9%)
70 years cutoff	35 (92.1%)	25 (92.6%)	10 (90.9%)
75 years cutoff	37 (97.4%)	26 (96.3%)	11 (100.0%)
80 years cutoff	37 (97.4%)	26 (96.3%)	11 (100.0%)

[a] Values are numbers below age cutoff (percentages).
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 3. Developing No Evidence of Disease numbers per age cutoff.

	DTC (n=79)[a]	PTC (n=58)[a]	FTC (n=21)[a]
No age cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
20 years cutoff	2 (2.5%)	2 (3.4%)	0 (0.0%)
25 years cutoff	5 (6.3%)	5 (8.6%)	0 (0.0%)
30 years cutoff	11 (13.9%)	10 (17.2%)	1 (4.8%)
35 years cutoff	15 (19.0%)	13 (22.4%)	2 (9.5%)
40 years cutoff	26 (32.9%)	22 (37.9%)	4 (19.0%)
45 years cutoff	36 (45.6%)	30 (51.7%)	6 (28.6%)
50 years cutoff	44 (55.7%)	37 (63.8%)	7 (33.3%)
55 years cutoff	53 (67.1%)	42 (72.4%)	11 (52.4%)
60 years cutoff	60 (75.9%)	47 (81.0%)	13 (61.9%)
65 years cutoff	69 (87.3%)	51 (87.9%)	18 (85.7%)
70 years cutoff	74 (93.7%)	56 (96.6%)	18 (85.7%)
75 years cutoff	76 (96.2%)	57 (98.3%)	19 (90.5%)
80 years cutoff	77 (97.5%)	57 (98.3%)	20 (95.2%)

[a] Values are numbers below age cutoff (percentages).
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 4. Recurrence numbers per age cutoff.

	DTC (n=11)[a]	PTC (n=10)[a]	FTC (n=1)[a]
No age cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
20 years cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
25 years cutoff	1 (9.1%)	1 (10.0%)	0 (0.0%)
30 years cutoff	2 (18.2%)	2 (20.0%)	0 (0.0%)
35 years cutoff	2 (18.2%)	2 (20.0%)	0 (0.0%)
40 years cutoff	3 (27.3%)	3 (30.0%)	0 (0.0%)
45 years cutoff	3 (27.3%)	3 (30.0%)	0 (0.0%)
50 years cutoff	3 (27.3%)	3 (30.0%)	0 (0.0%)
55 years cutoff	4 (36.4%)	4 (40.0%)	0 (0.0%)
60 years cutoff	6 (54.5%)	5 (50.0%)	1 (100.0%)
65 years cutoff	8 (72.7%)	7 (70.0%)	1 (100.0%)
70 years cutoff	10 (90.9%)	9 (90.0%)	1 (100.0%)
75 years cutoff	11 (100.0%)	10 (100.0%)	1 (100.0%)
80 years cutoff	11 (100.0%)	10 (100.0%)	1 (100.0%)

[a] Values are numbers below age cutoff (percentages).
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 5. Disease Specific Mortality numbers per age cutoff.

	DTC (n=49)[a]	PTC (n=28)[a]	FTC (n=21)[a]
No age cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
20 years cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
25 years cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
30 years cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
35 years cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
40 years cutoff	0 (0.0%)	0 (0.0%)	0 (0.0%)
45 years cutoff	1 (2.0%)	0 (0.0%)	1 (4.8%)
50 years cutoff	4 (8.2%)	3 (10.7%)	1 (4.8%)
55 years cutoff	8 (16.3%)	5 (17.9%)	3 (14.3%)
60 years cutoff	13 (26.5%)	8 (28.6%)	5 (23.8%)
65 years cutoff	19 (38.8%)	11 (39.3%)	8 (38.1%)
70 years cutoff	26 (53.1%)	17 (60.7%)	9 (42.9%)
75 years cutoff	39 (79.6%)	22 (78.6%)	17 (81.0%)
80 years cutoff	47 (95.9%)	28 (100.0%)	19 (90.5%)

[a] Values are numbers below age cutoff (percentages).
DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 6a. Influence of Age on Initial Excellent Response to therapy.

	DTC		PTC		FTC	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI) [a]	p-value[b]
Continuously	0.98 (0.96 – 0.99)	**0.039**	0.99 (0.96 – 1.00)	0.205	0.96 (0.92 – 1.0)	0.054
20 years cutoff	0.59 (0.06 – 5.83)	0.652	0.61 (0.06 – 6.14)	0.678	-	-
25 years cutoff	2.93 (0.37 – 22.95)	0.307	2.89 (0.36 – 23.08)	0.317	-	-
30 years cutoff	1.44 (0.41 – 5.10)	0.571	1.37 (0.38 – 5.00)	0.634	-	-
35 years cutoff	1.09 (0.39 – 3.03)	0.874	1.37 (0.43 – 4.32)	0.592	0.34 (0.03 – 4.10)	0.395
40 years cutoff	0.72 (0.32 – 1.60)	0.420	0.74 (0.30 – 1.79)	0.501	0.70 (0.07 – 6.94)	0.762
45 years cutoff	0.53 (0.26 – 1.10)	0.086	0.52 (0.23 – 1.23)	0.141	0.49 (0.09 – 2.82)	0.425
50 years cutoff	0.44 (0.22 – 0.90)	**0.024**	0.45 (0.19 – 1.05)	0.063	0.35 (0.07 – 1.62)	0.177
55 years cutoff	0.49 (0.24 – 0.99)	**0.050**	0.52 (0.22 – 1.23)	0.136	0.43 (0.11 – 1.59)	0.205
60 years cutoff	0.39 (0.18 – 0.82)	**0.014**	0.46 (0.18 – 1.17)	0.103	0.26 (0.07 – 0.99)	**0.048**
65 years cutoff	0.24 (0.09 – 0.64)	**0.004**	0.38 (0.12 – 1.16)	0.089	0.09 (0.01 – 0.70)	**0.022**
70 years cutoff	0.23 (0.07 – 0.78)	**0.018**	0.37 (0.08 – 1.68)	0.199	0.11 (0.01 – 0.92)	**0.041**
75 years cutoff	0.17 (0.02 – 1.30)	0.089	0.42 (0.05 – 3.37)	0.411	-	-
80 years cutoff	0.37 (0.05 – 2.92)	0.345	1.21 (0.13 – 11.29)	0.866	-	-

[a] univariate analysis; age below age cutoff as reference value.
[b] p-value of influence of age (cutoff).
OR, odds ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 6b. Influence of Age on Initial Excellent Response to therapy.

	DTC		PTC		FTC	
	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]	OR (95% CI)[a]	p-value[b]
Continuously	0.93 (0.85 – 1.01)	0.091	0.96 (0.92 – 0.99)	**0.044**	0.93 (0.85 – 1.01)	0.091
20 years cutoff	-	-	0.249 (0.02 – 3.80)	0.313	-	-
25 years cutoff	-	-	1.29 (0.13 – 12.66)	0.825	-	-
30 years cutoff	-	-	0.82 (0.14 – 4.84)	0.825	-	-
35 years cutoff	0.01 (0.00 – 1.76)	0.083	0.82 (0.18 – 3.84)	0.800	0.01 (0.00 – 1.76)	0.083
40 years cutoff	0.28 (0.01 – 6.30)	0.425	0.34 (0.10 – 1.21)	0.095	0.28 (0.01 – 6.30)	0.425
45 years cutoff	0.18 (0.01 – 2.19)	0.177	0.34 (0.10 – 1.17)	0.089	0.18 (0.01 – 2.19)	0.177
50 years cutoff	0.23 (0.02 – 2.37)	0.215	0.25 (0.07 – 0.92)	**0.037**	0.23 (0.02 – 2.37)	0.215
55 years cutoff	0.07 (0.01 – 1.03)	0.053	0.40 (0.12 – 1.42)	0.158	0.07 (0.01 – 1.03)	0.053
60 years cutoff	0.09 (0.01 – 1.01)	0.051	0.33 (0.08 – 1.32)	0.118	0.09 (0.01 – 1.01)	0.051
65 years cutoff	0.21 (0.02 – 2.41)	0.209	0.09 (0.01 – 0.84)	**0.035**	0.21 (0.02 – 2.41)	0.209
70 years cutoff	0.22 (0.02 – 2.55)	0.228	-	-	0.22 (0.02 – 2.55)	0.228
75 years cutoff	-	-	-	-	-	-
80 years cutoff	-	-	-	-	-	-

[a] multivariate analysis adjusted for ATA High Risk factors; age below age cutoff as reference value.
[b] p-value of influence of age (cutoff).
OR, odds ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 7a. Influence of Age on Developing No Evidence of Disease.

	DTC		PTC		FTC	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Continuously	0.98 (0.97 – 0.99)	**<0.001**	0.98 (0.97 – 0.99)	**0.009**	0.96 (0.94 – 0.99)	**0.005**
20 years cutoff	0.49 (0.12 – 2.01)	0.324	0.51 (0.12 – 2.10)	0.350	-	-
25 years cutoff	1.36 (0.54 – 3.43)	0.509	1.46 (0.57 – 3.75)	0.434	-	-
30 years cutoff	0.86 (0.45 – 1.64)	0.647	0.93 (0.46 – 1.85)	0.827	0.84 (0.11 – 6.71)	0.871
35 years cutoff	0.75 (0.42 – 1.32)	0.317	0.87 (0.47 – 1.63)	0.661	0.39 (0.09 – 1.79)	0.227
40 years cutoff	0.56 (0.35 – 0.90)	**0.017**	0.64 (0.38 – 1.10)	0.104	0.34 (0.11 – 1.03)	0.057
45 years cutoff	0.43 (0.27 – 0.67)	**<0.001**	0.47 (0.28 – 0.80)	**0.005**	0.30 (0.12 – 0.78)	**0.013**
50 years cutoff	0.42 (0.27 – 0.66)	**<0.001**	0.46 (0.27 – 0.79)	**0.004**	0.33 (0.13 – 0.81)	**0.016**
55 years cutoff	0.39 (0.25 – 0.63)	**<0.001**	0.42 (0.24 – 0.76)	**0.004**	0.35 (0.15 – 0.84)	**0.018**
60 years cutoff	0.33 (0.19 – 0.55)	**<0.001**	0.35 (0.18 – 0.67)	**0.002**	0.30 (0.13 – 0.73)	**0.008**
65 years cutoff	0.27 (0.14 – 0.51)	**<0.001**	0.35 (0.16 – 0.77)	**0.009**	0.17 (0.05 – 0.56)	**0.004**
70 years cutoff	0.22 (0.09 – 0.54)	**0.001**	0.20 (0.05 – 0.83)	**0.027**	0.23 (0.07 – 0.77)	**0.018**
75 years cutoff	0.30 (0.09 – 0.95)	**0.040**	0.24 (0.03 – 1.74)	0.159	0.37 (0.09 – 1.58)	0.178
80 years cutoff	0.42 (0.10 – 1.71)	0.225	0.59 (0.08 – 4.27)	0.601	0.35 (0.05 – 2.63)	0.309

[a] univariate analysis; age below age cutoff as reference value.

[b] p-value of influence of age (cutoff).

HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 7b. Influence of Age on Developing No Evidence of Disease.

	DTC		PTC		FTC	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Continuously	0.95 (0.91 – 0.99)	**0.042**	0.97 (0.95 – 0.99)	**0.018**	0.95 (0.91 – 0.99)	**0.042**
20 years cutoff	-	-	0.31 (0.07 – 1.43)	0.133	-	-
25 years cutoff	-	-	0.78 (0.26 – 2.37)	0.666	-	-
30 years cutoff	-	-	0.68 (0.26 – 1.66)	0.378	-	-
35 years cutoff	0.03 (0.00 – 0.55)	**0.018**	0.69 (0.30 – 1.61)	0.391	0.03 (0.00 – 0.55)	**0.018**
40 years cutoff	0.19 (0.04 – 0.83)	**0.027**	0.42 (0.21 – 0.85)	**0.016**	0.19 (0.04 – 0.83)	**0.027**
45 years cutoff	0.16 (0.04 – 0.62)	**0.008**	0.47 (0.23 – 0.96)	**0.037**	0.16 (0.04 – 0.62)	**0.008**
50 years cutoff	0.42 (0.11 – 1.62)	0.210	0.47 (0.22 – 0.99)	**0.049**	0.42 (0.11 – 1.62)	0.210
55 years cutoff	0.22 (0.05 – 0.88)	**0.033**	0.53 (0.24 – 1.19)	0.126	0.22 (0.05 – 0.88)	**0.033**
60 years cutoff	0.33 (0.09 – 1.25)	0.102	0.46 (0.18 – 1.16)	0.099	0.33 (0.09 – 1.25)	**0.102**
65 years cutoff	0.24 (0.05 – 1.29)	0.096	0.30 (0.09 – 1.01)	0.051	0.24 (0.05 – 1.29)	0.096
70 years cutoff	0.29 (0.06 – 1.42)	0.127	-	-	0.29 (0.06 – 1.42)	0.127
75 years cutoff	0.25 (0.03 – 2.00)	0.192	-	-	0.25 (0.03 – 2.00)	0.192
80 years cutoff	0.52 (0.06 – 4.25)	0.542	-	-	0.52 (0.06 – 4.25)	0.542

[a] multivariate analysis adjusted for ATA High Risk factors; age below age cutoff as reference value.

[b] p-value of influence of age (cutoff).

HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 8. Influence of Age on Recurrence.

	DTC		PTC		FTC	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Continuously	1.03 (0.99 – 1.07)	0.132	1.04 (0.99 – 1.08)	0.059	1.11 (0.73 – 1.69)	0.635
20 years cutoff	22.13 (0.00 –)	0.614	22.89 (0.00 –)	0.578	-	-
25 years cutoff	0.77 (0.10 – 6.08)	0.808	0.96 (0.12 – 7.60)	0.966	-	-
30 years cutoff	0.80 (0.17 – 3.71)	0.774	0.93 (0.20 – 4.43)	0.931	-	-
35 years cutoff	1.31 (0.28 – 6.09)	0.727	1.57 (0.33 – 7.42)	0.572	-	-
40 years cutoff	1.62 (0.43 – 6.12)	0.478	2.14 (0.55 – 8.33)	0.272	-	-
45 years cutoff	2.75 (0.73 – 10.40)	0.135	3.82 (0.98 – 14.88)	0.053	-	-
50 years cutoff	3.68 (0.97 – 13.91)	0.055	5.46 (1.39 – 21.52)	**0.015**	-	-
55 years cutoff	3.20 (0.94 – 10.97)	0.064	4.14 (1.15 – 14.83)	**0.029**	-	-
60 years cutoff	2.64 (0.80 – 8.67)	0.110	4.69 (1.35 – 16.29)	**0.015**	-	-
65 years cutoff	3.73 (0.98 – 14.15)	0.053	3.53 (0.91 – 13.68)	0.069	-	-
70 years cutoff	5.83 (0.61 – 56.09)	0.127	9.45 (0.97 – 91.86)	0.053	-	-
75 years cutoff	0.05 (0.00 -)	0.778	0.05 (0.00 -)	0.849	-	-
80 years cutoff	0.05 (0.00 -)	0.818	0.05 (0.00 -)	0.849	-	-

[a] univariate analysis; age below age cutoff as reference value.
[b] p-value of influence of age (cutoff).
HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

Supplemental Table 9a. Influence of Age on Disease Specific Mortality.

	DTC		PTC		FTC	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Continuously	1.06 (1.04 – 1.09)	**<0.001**	1.08 (1.04 – 1.11)	**<0.001**	1.04 (0.99 – 1.08)	0.054
20 years cutoff	20.78 (0.00 –)	0.496	21.06 (0.00 –)	0.540	-	-
25 years cutoff	23.52 (0.29 –)	0.160	25.18 (0.18 –)	0.202	-	-
30 years cutoff	26.09 (0.74 – 919.91)	0.073	28.38 (0.45 – 1802.13)	0.114	-	-
35 years cutoff	27.78 (1.13 – 686.02)	**0.042**	31.06 (0.72 – 1349.76)	0.074	-	-
40 years cutoff	33.44 (2.20 – 508.27)	**0.011**	40.38 (1.50 – 1083.88)	**0.028**	-	-
45 years cutoff	23.91 (3.29 – 173.69)	**0.002**	51.30 (2.27 – 1160.20)	**0.013**	2.50 (0.33 – 18.61)	0.373
50 years cutoff	7.87 (2.82 – 21.97)	**<0.001**	2.74 (2.74 – 30.39)	**<0.001**	3.64 (0.49 – 27.13)	0.208
55 years cutoff	5.34 (2.49 – 11.47)	**<0.001**	7.07 (2.66 – 18.82)	**<0.001**	2.38 (0.70 – 8.10)	0.164
60 years cutoff	3.91 (2.06 – 7.42)	**<0.001**	5.29 (2.30 – 12.15)	**<0.001**	1.75 (0.64 – 4.78)	0.276
65 years cutoff	4.31 (2.40 – 7.75)	**<0.001**	5.91 (2.71 – 12.92)	**<0.001**	2.36 (0.97 – 5.71)	0.057
70 years cutoff	4.96 (2.75 – 8.96)	**<0.001**	6.54 (2.93 – 14.62)	**<0.001**	2.88 (1.19 – 6.99)	**0.019**
75 years cutoff	2.85 (1.41 – 5.77)	**0.004**	6.06 (2.38 – 15.45)	**<0.001**	1.05 (0.35 – 3.14)	0.926
80 years cutoff	0.97 (0.23 – 4.01)	0.961	0.05 (0.00 -)	0.612	0.97 (0.22 – 4.22)	0.970

[a] univariate analysis; age below age cutoff as reference value.

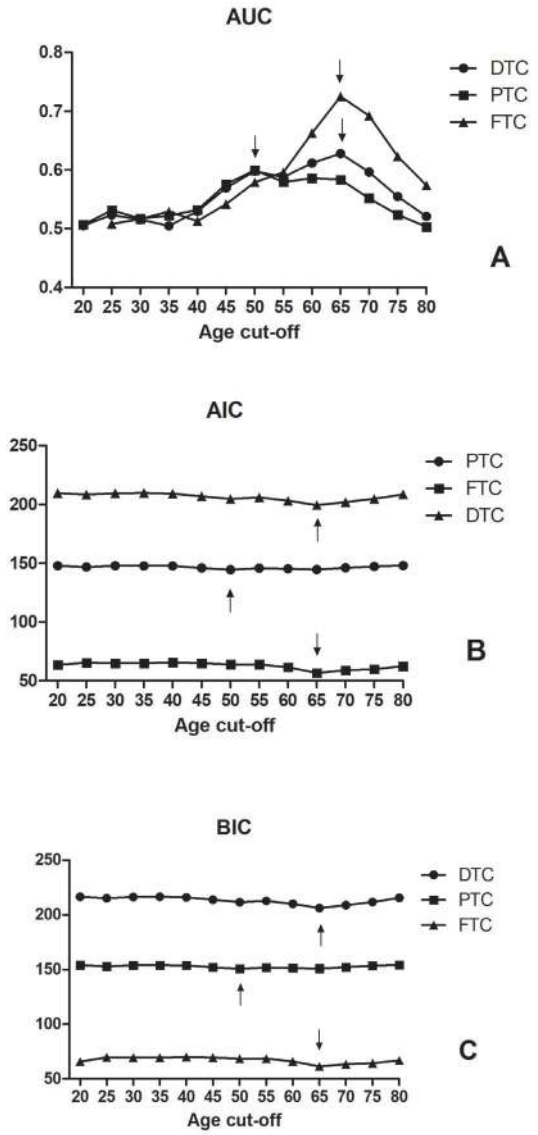
[b] p-value of influence of age (cutoff).

HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.

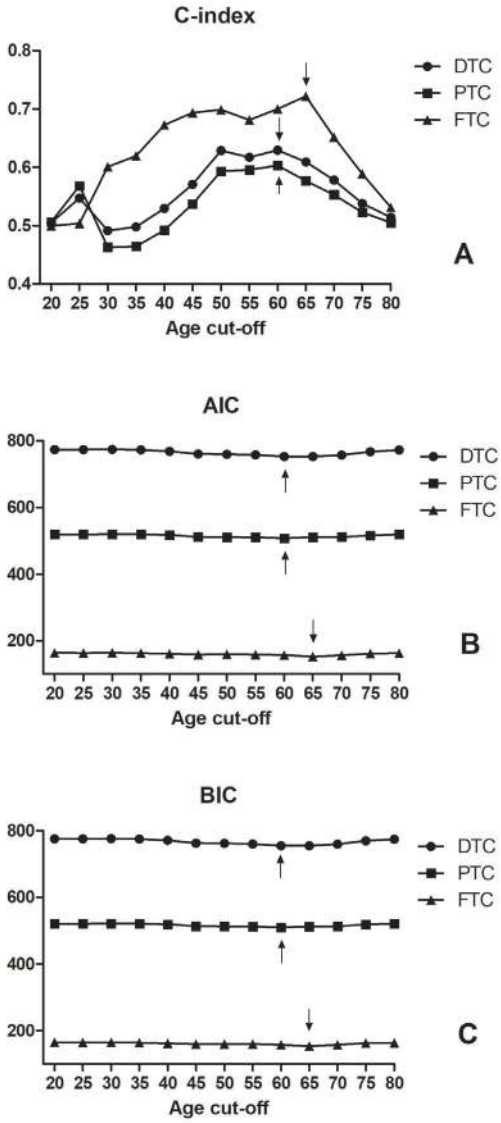
Supplemental Table 9b. Influence of Age on Disease Specific Mortality.

	DTC		PTC		FTC	
	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]	HR (95% CI)[a]	p-value[b]
Continuously	1.00 (0.96 – 1.06)	0.845	1.07 (1.02 – 1.11)	**0.002**	1.00 (0.96 – 1.06)	0.845
20 years cutoff	-	-	-	-	-	-
25 years cutoff	-	-	-	-	-	-
30 years cutoff	-	-	-	-	-	-
35 years cutoff	-	-	-	-	-	-
40 years cutoff	-	-	-	-	-	-
45 years cutoff	1.52 (0.17 – 13.26)	0.705	-	-	1.52 (0.17 – 13.26)	0.705
50 years cutoff	1.86 (0.21 – 16.67)	0.579	10.43 (1.95 – 55.92)	**0.006**	1.86 (0.21 – 16.67)	0.579
55 years cutoff	1.28 (0.24 – 6.97)	0.776	5.28 (1.40 – 19.84)	**0.014**	1.28 (0.24 – 6.97)	0.776
60 years cutoff	0.57 (0.14 – 2.36)	0.437	5.10 (1.46 – 17.79)	**0.011**	0.57 (0.14 – 2.36)	0.437
65 years cutoff	0.61 (0.14 – 2.72)	0.515	3.55 (1.12 – 10.49)	**0.022**	0.61 (0.14 –2.72)	0.515
70 years cutoff	1.43 (0.38 – 5.37)	0.595	2.31 (0.80 – 6.73)	0.124	1.43 (0.38 – 5.37)	0.595
75 years cutoff	0.72 (0.15 – 3.53)	0.684	3.29 (0.90 – 12.01)	0.071	0.72 (0.15 – 3.53)	0.684
80 years cutoff	0.59 (0.07 – 4.92)	0.630	-	-	0.59 (0.07 – 4.92)	0.630

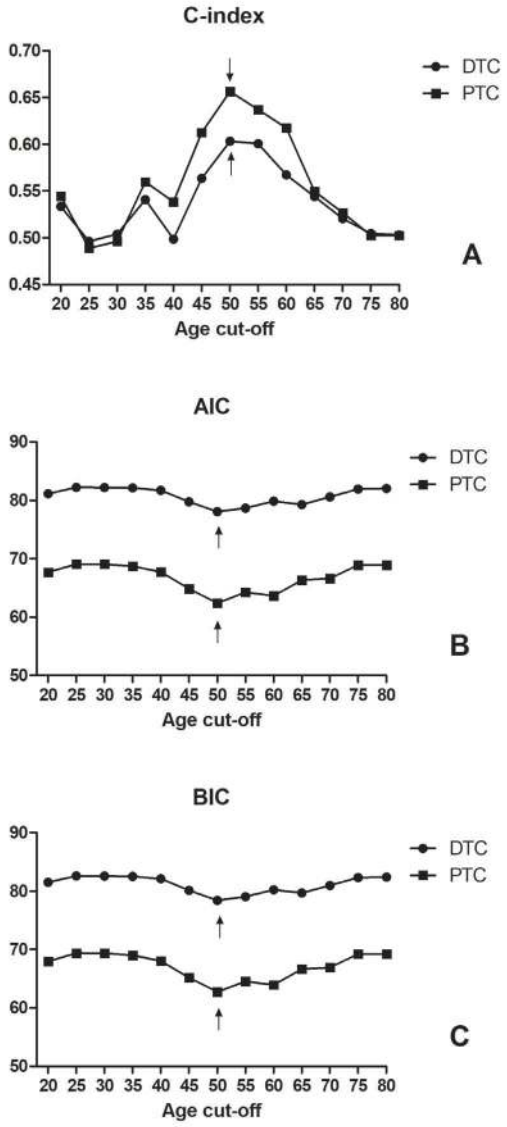
[a] multivariate analysis adjusted for ATA High Risk factors; age below age cutoff as reference value.
[b] p-value of influence of age (cutoff).
HR, hazard ratio; CI, confidence interval; DTC, differentiated thyroid cancer; PTC, papillary thyroid cancer; FTC, follicular thyroid cancer.



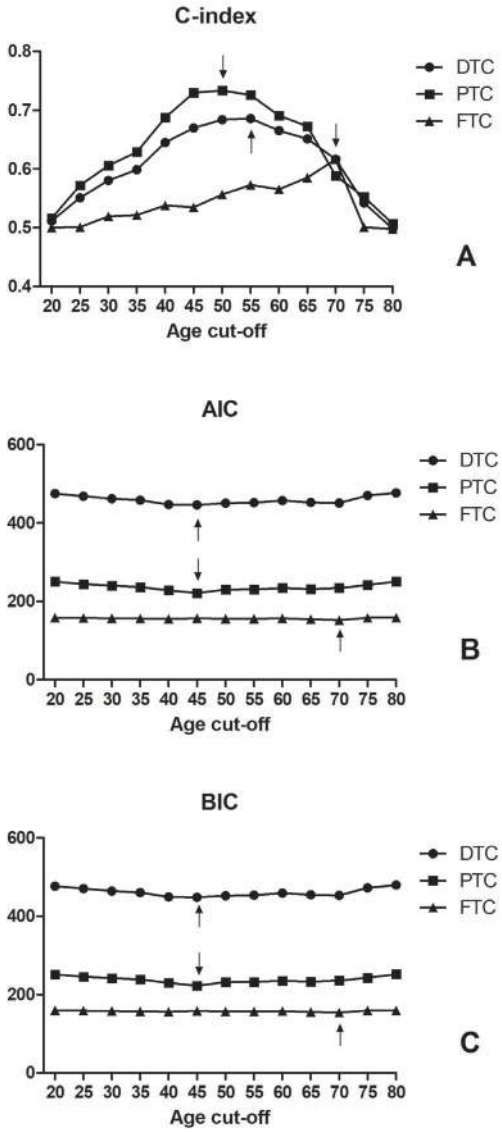
Supplemental Figure 1. Statistical Model Performance for (A) C-index, (B) AIC, and (C) BIC for Initial Excellent Response to therapy.



Supplemental Figure 2. Statistical Model Performance for (A) AUC, (B) AIC, and (C) BIC for Developing No Evidence of Disease.



Supplemental Figure 3. Statistical Model Performance for (A) C-index, (B) AIC, and (C) BIC for Recurrence.



Supplemental Figure 4. Statistical Model Performance for (A) C-index, (B) AIC, and (C) BIC for Disease Specific Mortality.

Part II

Long-term impact of treatment

Chapter 7

Longitudinal Analysis of Quality of Life in Patients treated for Differentiated Thyroid Cancer

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ABSTRACT

Objective

Earlier cross-sectional studies showed that patients with differentiated thyroid cancer (DTC) have a significant reduction of Quality of Life (QoL) compared to controls. However, recent longitudinal studies showed mixed results, and had relative short follow-up or lacked knowledge about QoL before initial surgery. Therefore, we initiated a longitudinal study to assess changes of QoL in patients undergoing treatment for DTC.

Methods

We prospectively included patients, aged 18 to 80 years, who were treated for DTC at a Dutch university hospital. Using questionnaires, QoL was assessed before surgery, just before radioiodine (RAI) therapy, and regularly during follow-up. Repeated measurement analysis was used to assess changes of QoL over time, and we explored the influence of different characteristics on QoL.

Results

Longitudinal QoL assessments were available in 185 patients (mean age 47 years; 71% women). All patients were treated according to the Dutch guidelines with total thyroidectomy followed by RAI (83% after thyroid hormone withdrawal). Median time between baseline and final questionnaire was 31 months, and patients completed a median of three questionnaires. QoL at baseline was lower than in the general population, developed non-linear over time, was lowest around RAI therapy, and recovered over time. Females, younger patients, and patients with persistent hypoparathyroidism had lower QoL scores.

Conclusions

In a population of DTC patients, QoL before initial therapy is already lower than in the general population. Thereafter, QoL develops non-linear over time in general, with lowest QoL around RAI therapy while two to three years later it approximates baseline values.

INTRODUCTION

The worldwide incidence of differentiated thyroid cancer (DTC) has been steadily increasing over the last two decades (1, 2). As patients with DTC have a relative good prognosis in general (3-5), treating these patients is a trade-off between gaining survival on one hand, and preserving quality of life (QoL) on the other hand. Therefore, a less aggressive therapeutic approach is suggested for selected populations (6, 7).

There are numerous questionnaires to measure QoL in patients with thyroid cancer, and the most commonly used to assess general QoL is the Short-Form-36 (SF-36) (8, 9). Besides, thyroid-specific QoL can be assessed using the Thyroid-specific patient reported outcome measure (ThyPRO) questionnaire which was earlier validated and subsequently used in patients with thyroid diseases (10-12).

Several cross-sectional studies showed a decreased QoL in different domains in thyroid cancer survivors compared to the general population (12-17). Additionally, their QoL is at the same level as in patients with other cancers with worse prognosis, and is even worse than in breast cancer survivors (18). Main drawback of the aforementioned cross-sectional studies is that no conclusions can be drawn about QoL evolution over time. Therefore, more recently several longitudinal studies were conducted (16, 17, 19-21). Their results were mixed as some showed increasing QoL (16, 20), and others showed decreasing QoL over time (17, 19). However, these longitudinal studies had one or more limitations such as relative short follow-up with a maximum of two years (19-21), or lack of knowledge about QoL before initial surgery (16, 20). The aim of our study, lacking the abovementioned limitations, was therefore to investigate long-term longitudinal changes of quality of life in patients undergoing treatment for differentiated thyroid cancer.

MATERIALS AND METHODS

Study population

We aimed to include all patients, aged 18 to 80 years, who were treated for DTC at the Erasmus Medical Center, Rotterdam, The Netherlands. Inclusion period was from January 2013 until December 2017, and patients were followed for at least one year. All included patients had tumors ≥ 1cm and were treated in line with the current Dutch guideline with a total thyroidectomy followed by RAI therapy (22). Further, included patients understood Dutch language and did not have any other active malignancy or an active inflammatory disease.

We recorded demographic, disease, and treatment characteristics. Demographical variables included age at diagnosis and gender. Disease characteristics included dis-

ease type, AJCC/TNM-stage (8th edition), and ATA Risk Stratification category (2015). Data regarding treatment consisted of extent of surgery, method of RAI preparation, number of RAI therapies, and cumulative RAI dose. RAI therapy was given either after 3-4 weeks of thyroid hormone withdrawal, or after two subsequent injections with recombinant human TSH (rhTSH). Further, incidence of recurrent nerve paralysis and hypoparathyroidism was recorded.

At baseline, i.e. before surgery, just before RAI therapy, six to nine months later, and every one to two years thereafter, patients were asked to fill in three different QoL questionnaires (see next section for details). Additionally, if a patient received more than one RAI therapy, just before a new therapy, and six to nine months thereafter additional questionnaires were handed-out; thereafter, the regular schedule was continued.

The study was approved by the Institutional Review Board of the Erasmus Medical Center, and consent has been obtained from each patient after full explanation of the purpose and procedures.

Quality of Life questionnaires

The ThyPRO is a thyroid-specific QoL questionnaire which consists of 85 questions summarized in 13 scales (10). Scores vary from 0 to 100 with higher scores indicating more thyroid-related complaints. We used the Composite, Tiredness, Cognitive problems, Anxiety, Depressivity, Social Impairment, and Daylife Impairment scores (23).

The Multidimensional fatigue index-20 (MFI-20) is a 20-item self-report instrument designed to assess fatigue (24). Scores vary from 0 to 20 with higher scores indicating more fatigue. We used the General, Physical and Mental Fatigue scores.

The RAND-36 item health survey (RAND-36) is the validated Dutch version of the SF-36 (8, 9, 25). It is a generic questionnaire aimed at overall health-related QoL during the previous 30 days. It consists of nine subscales and two summary scales. Scores vary from 0 to 100 with higher scores associated with better QoL. We used the General Health, General Mental Health, Vitality, Physical functioning and Social functioning scores.

Statistical analysis

For continuous variables we calculated means and standard deviations (SD), or in case of a non-normal distribution, medians with interquartile ranges (IQR). For categorical variables, absolute numbers with percentages were recorded. In order to assess the changes of QoL over time while accounting for the correlation between the repeated measurements of each patient, we used marginal models; the appropriate covariance matrix that best fitted the data was selected. If needed, appropriate

transformation of the QoL scores was applied to obtain a normal distribution. Further, using a marginal model allows QoL questionnaires to be collected without predefined time points and therewith strengthens the model creation and analysis. Additionally, this model enabled us to determine QoL at each moment in time. Our main analysis assessed changes in QoL over time. Further, additional analyses were performed to explore the effect of different factors on QoL over time; these factors were age (<50 years vs. ≥50 years), sex, presence of permanent hypoparathyroidism, presence of recurrent nerve paralysis, ATA Risk Stratification category (Low vs. Intermediate/High), or number of RAI therapies (Single vs. Multiple). In general, p-values below 0.05 were considered significant. The age cut-off of 50 years was based on the age distribution of the population. The possibility of effect modification was studied using a two-step strategy. Firstly, a screening p-value cut-off for interaction of <0.10 was used, because the statistical power to identify an interaction term is less than for normal covariates. Secondly, to make sure that we do not identify irrelevant modification, we subsequently performed stratified analyses to replicate and quantify the effect modification so that we can interpret whether the differences are of (clinical) relevance. All analyses were performed using either SPSS Statistics for Windows (version 24.0) or R statistical software (version 3.4.1).

RESULTS

Population characteristics

During the study period, a total of 238 patients were eligible for the study of which 18 patients were not included because they either did not want to participate (n=9) or were missed at inclusion (n=9). Further, 35 patients completed less than two questionnaires and were subsequently removed from the analyses (see Supplemental Table 1 for their characteristics). Therefore, the analyses presented here were performed in the remaining 185 patients.

Table 1 lists the characteristics of the study population. Mean age was 47.0 years, and 131 (71%) were women. Papillary thyroid carcinoma was present in 162 (88%) patients, and the remaining 23 patients (12%) had follicular thyroid carcinoma. Patients were almost equally distributed over the three ATA Risk categories. Total thyroidectomy was performed in all patients, and neck dissection in 70 (38%) of them (central in 23 (12%), lateral in one (1%), and both in 46 (25%)). All patients underwent RAI therapy, of which 142 (77%) received one therapy, while the others received two or more therapies. The majority (83%) of the patients received their first RAI therapy after thyroid hormone withdrawal. Thirty-one patients (17%) developed permanent hypoparathyroidism, while 17 patients (9%) had a recurrent nerve paralysis at end

Table 1. Characteristics of the study population.

	Total Population (n=185)[a]
Age at diagnosis (years)	47.0 ± 15.7
Women	131 (71%)
Disease Type	
Papillary Thyroid Cancer	162 (88%)
Follicular Thyroid Cancer	23 (12%)
ATA Risk Stratification System (2015)	
Low Risk	72 (39%)
Intermediate Risk	55 (30%)
High Risk	58 (31%)
AJCC/TNM Staging system (8th edition)	
Stage I	138 (75%)
Stage II	35 (19%)
Stage III	9 (5%)
Stage IV	3 (2%)
Surgery (TT)	185 (100%)
Neck dissection	70 (38%)
Central	23 (12%)
Lateral	1 (1%)
Both	46 (25%)
RAI treatment	185 (100%)
Once	142 (77%)
Twice	33 (18%)
≥ 3	10 (5%)
Withdrawal at first therapy	154 (83%)
first dose (mCi)	143 (48 – 147)
Admission duration first therapy (days)	3 (2 – 3)
Cumulative dose (mCi)	145 (49 – 151)
Hypoparathyroidism	
Transient	37 (20%)
Permanent	31 (17%)
Recurrent nerve paralysis	17 (9%)
Number of questionnaires per patient	3 (2 – 4)
Follow-up (months)	31 (17 – 49)

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
TT, total thyroidectomy; RAI, radioactive iodine; mCi, milliCurie.

of follow-up. Additionally, we found a significant correlation between permanent hypoparathyroidism and neck dissection (p-value <0.001); it is known that a neck dissection is a risk factor for permanent hypoparathyroidism (6). Median time between surgery and completing the final questionnaire was 31 months, and during follow-up, patients completed a median of three questionnaires.

Quality of Life

QoL at baseline and during follow-up for the different QoL scores is listed in Table 2. Further, we also compared our baseline QoL with reference values obtained from literature (13, 25, 26). QoL at baseline in patients was lower than in the general population for all scores, except for the ThyPRO Cognitive Problems score.

Table 2. Quality of Life during Follow-up.

	Reference populations (13, 24, 25)[a]	Baseline[b]	Around RAI therapy[b]	± 24 months[b]	± 48 months[b]
MFI-20					
General fatigue	8.1 ± 3.4	12.5 ± 0.4	14.7 ± 0.4 [c]	13.2 ± 0.4	13.1 ± 0.4
Physical fatigue	6.7 ± 2.6	11.3 ± 0.4	13.4 ± 0.4 [c]	11.5 ± 0.4	11.3 ± 0.4
Mental fatigue	6.9 ± 3.3	10.9 ± 0.4	12.3 ± 0.4 [c]	11.6 ± 0.4 [c]	11.3 ± 0.4
RAND-36					
Vitality	67.4 ± 19.9	56.6 ± 1.9	44.6 ± 1.8 [c]	53.9 ± 1.8	54.3 ± 1.8
General Mental Health	76.8 ± 18.4	66.7 ± 1.3	67.0 ± 1.3	68.2 ± 1.3	69.3 ± 1.5 [c]
General Health Perception	72.7 ± 22.7	57.9 ± 1.4	57.8 ± 1.4	57.4 ± 1.4	56.9 ± 1.7
Physical functioning	81.9 ± 23.2	76.3 ± 1.0	68.3 ± 1.0 [c]	73.9 ± 1.0	75.5 ± 1.0
Social functioning	86.9 ± 20.5	66.9 ± 2.8	57.0 ± 2.4 [c]	70.1 ± 2.7	70.5 ± 2.8
ThyPRO					
Composite	*na*	28.3 ± 1.7	34.2 ± 1.8 [c]	26.6 ± 1.6	25.8 ± 1.6
Tiredness	35 ± 21	40.6 ± 2.1	52.8 ± 2.0 [c]	41.6 ± 2.0	42.0 ± 2.1
Cognitive Problems	14 ± 17	9.4 ± 1.1	19.4 ± 1.1 [c]	14.3 ± 1.1 [c]	14.4 ± 1.1 [c]
Anxiety	13 ± 16	26.7 ± 1.1	15.9 ± 1.1 [c]	13.6 ± 1.1 [c]	12.9 ± 1.1 [c]
Depressivity	21 ± 18	23.9 ± 1.1	22.0 ± 1.1	18.1 ± 1.1 [c]	18.0 ± 1.1 [c]
Social Impairment	*na*	6.2 ± 1.1	6.0 ± 1.1	5.3 ± 1.1	4.7 ± 1.1 [c]
Impaired Daylife	*na*	10.5 ± 1.2	26.7 ± 1.1 [c]	8.2 ± 1.1	7.5 ± 1.1 [c]

[a] Values are means (± standard deviation) obtained from reference populations (13, 24, 25).
[b] Values are means (± standard error).
[c] Significantly different compared to baseline.
RAI, radioactive iodine; na, not available.

The ThyPRO Composite, Tiredness, Cognitive Problems and Impaired Daylife scores showed a significant non-linear development over time (see Figure 1). QoL was the lowest immediately after RAI therapy, and subsequently increased again towards baseline levels in the following years. Patients had significant more Cognitive Problems at end of follow-up compared to baseline, although the end of follow-up score was similar to the value of the general population. Further, Anxiety was high at baseline, and thereafter decreased with a short temporally increase around one year, and it was significantly lower after 24 and 48 months (p<0.001 compared to

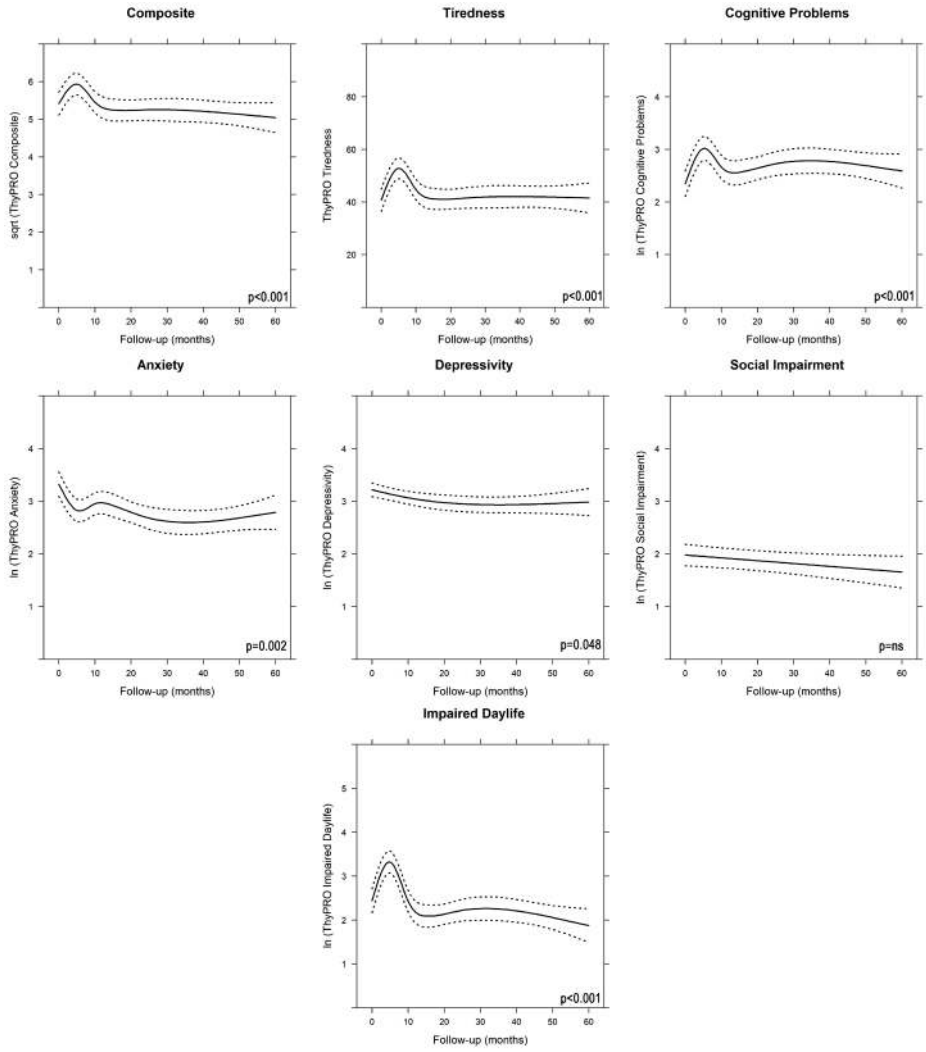

Figure 1. Quality of Life over time for the different ThyPRO scores.

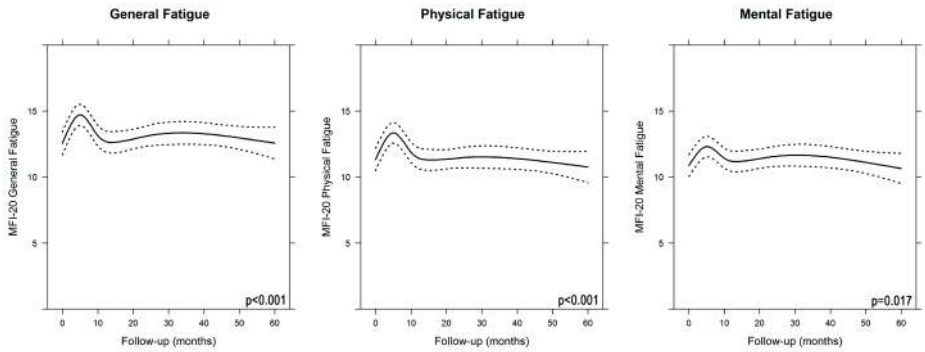

Figure 2. Quality of Life over time for the different MFI-20 scores.

baseline). Depressivity and Social Impairment complaints decreased almost linear over time.

All three MFI-20 scores also showed a significant non-linear development over time (see Figure 2), with the lowest QoL short after RAI therapy. QoL four years later was not significantly different compared to baseline level.

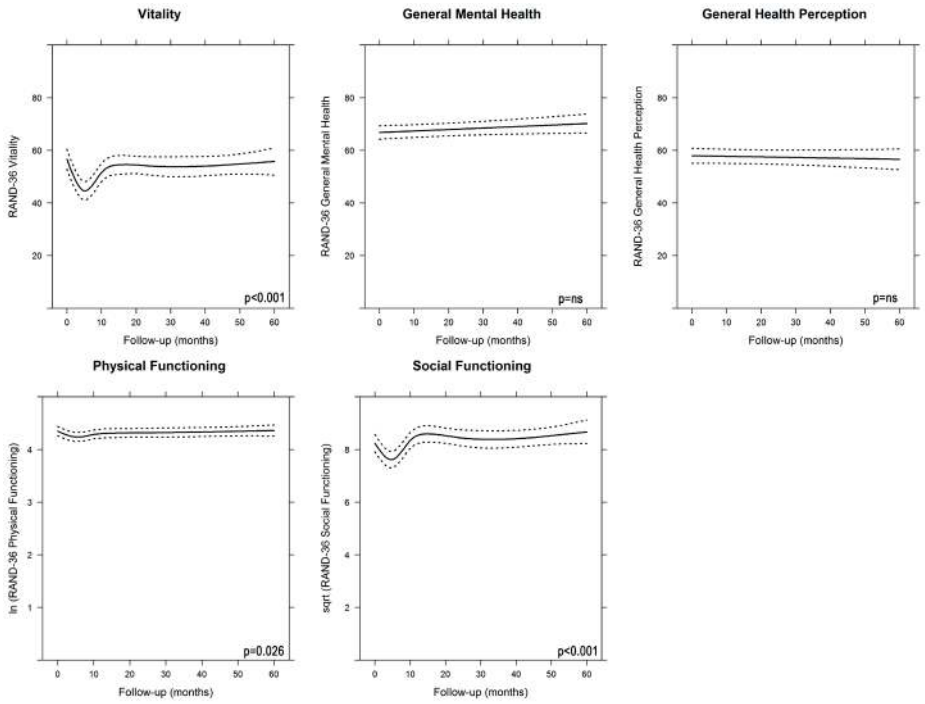

Figure 3. Quality of Life over time for the different RAND-36 scores.

Table 3. Univariate analyses of factors influencing Quality of Life over Time.

	Age[a, b]	Gender[a, c]	Number of RAI therapies[a, d]	ATA Risk Category[a, e]	Persistent Hypo-parathyroidism[a, f]	Recurrent Nerve Paralysis[a, f]	Neck Dissection[a, f]
MFI-20							
General fatigue	**0.002**	**<0.0001**	Interaction	0.585	0.638	0.288	0.162
Physical fatigue	0.121	**<0.0001**	0.469	0.502	0.155	0.791	0.484
Mental fatigue	**0.012**	**0.020**	Interaction	Interaction	0.110	0.077	Interaction
RAND-36							
Vitality	**0.025**	**<0.001**	0.705	0.505	0.257	0.379	0.169
General Mental Health	**0.019**	**0.011**	0.119	0.743	0.318	0.758	0.601
General Health Perception	Interaction	**0.006**	0.846	0.443	**0.048**	0.145	0.370
Physical functioning	0.439	Interaction	0.359	0.508	**0.003**	0.147	0.813
Social functioning	**0.038**	**0.006**	0.494	0.609	**0.020**	0.653	0.929
ThyPRO							
Composite	**0.015**	**0.001**	0.778	0.932	**0.012**	0.229	0.959
Tiredness	0.075	**<0.001**	Interaction	Interaction	**0.034**	0.971	0.227
Cognitive Problems	0.096	Interaction	0.756	0.707	0.258	0.551	0.751
Anxiety	0.174	0.100	0.696	0.827	0.791	**0.030**	0.910
Depressivity	**0.024**	**0.006**	0.176	0.594	0.220	**0.021**	0.945
Social Impairment	**<0.001**	**0.005**	0.210	0.960	**0.040**	0.539	0.762
Impaired Daylife	0.115	Interaction	Interaction	0.395	**<0.001**	0.964	0.287

[a] p-values for influence on Quality of Life over time.
[b] <50 years vs. ≥50 years (<50 years as reference group).
[c] women vs. men (women as reference group)
[d] Single vs. Multiple RAI therapies (Single as reference group).
[e] ATA Low vs. High Risk (Low as reference group).
[f] yes vs. no (no as reference group).

The RAND-36 Vitality, and both the Physical and Social functioning scores also showed a significant non-linear development over time (see Figure 3). Again, QoL is the lowest short after RAI therapy, and subsequently increased in such a way that QoL after two to three years is not significantly different compared to baseline levels. General Mental Health increased linearly over time, and it was significantly better after 48 months than at baseline (p=0.039). General Health Perception showed a slight decrease over time, but after 48 months it was not significantly lower than at baseline.

Influencing factors

Age, gender, persistent hypoparathyroidism and recurrent nerve paralysis affect several QoL scores in a way that older male patients without persistent hypoparathyroidism or without recurrent nerve paralysis had the best QoL (see Table 3 and Supplemental Table 2). Additionally, the number of RAI therapies significantly altered QoL development over time for the MFI-20 Mental and General fatigue, and for the ThyPRO Tiredness and Impaired Daylife scores, while gender significantly influenced this for the RAND-36 Physical function, and the ThyPRO Cognitive Problems and Impaired Daylife scores (see Supplemental Figures 1 to 3). Age, Initial ATA Risk category, and neck dissection significantly influenced QoL development over time either in one or two QoL scores (see Supplemental Figures 1 to 3).

DISCUSSION

This study shows that in patients with DTC who were treated with total thyroidectomy followed by RAI therapy, QoL in general develops non-linear over time. The lowest QoL was observed around RAI therapy, while afterwards it took two to three years to return to baseline QoL levels. This is even despite the fact that QoL before initial therapy was already lower than that of the general population.

We showed a significant non-linear development of QoL over time in the majority of the measured QoL scales. QoL was the lowest around RAI therapy, and afterwards it took two to three years to return to baseline. This pattern of lowest QoL around RAI therapy, and thereafter increasing QoL was also shown by Gamper et al. (16). However, their study did not have data on QoL before surgery. Like in our study, at end of follow-up, QoL was still lower than in the general population. Lubitz et al. showed a temporary decrease of QoL after initial therapy, but after six months QoL was comparable to baseline (21); it must be noted that only half of their population received RAI therapy, and the majority of these patients received RAI therapy after preparation with rhTSH. Gou et al. (17) and Ryu et al. (19) showed a decreased QoL

after initial therapy which thereafter increased again, but was still below baseline at end of follow-up. Ryu et al. had a follow-up of just 12 months so nothing was known about QoL evolution thereafter (19), while Gou et al. followed during 24 months thyroid cancer patients who did not received RAI therapy (17). Therefore, the decreased QoL after initial therapy in their study may have been due to postsurgical complaints and thereafter possibly due to fear of disease/recurrence. This study indicates that our finding of a decreased QoL around RAI therapy might to some extent also be partly explained by postsurgical complaints. Further, Ryu et al. showed that QoL after 12 months is better in patients who received a total thyroidectomy without RAI therapy, but nothing is known what happens thereafter (19). In combination with our results this suggests that less aggressive treatment might be favorable for QoL in the long term. We observed a different development over time with respect to the ThyPRO Anxiety score; it was the highest before initial therapy, and thereafter significantly decreased with a short temporally increase around approximately one year. This pattern is probably due to fear of the disease itself, and later-on of recurrence; this same pattern was also shown in another study (20).

We observed that QoL before initial therapy for all scores was lower in patients than reference values of the general population obtained from literature, except the ThyPRO Cognitive Problems scale (13, 25, 26). This might be due to the fact that patients already were aware of their thyroid cancer diagnosis when completing baseline questionnaires. Knowledge of QoL before initial treatment is important to differentiate between attribution to thyroid cancer and/or other diseases (e.g. cardiovascular) in patients with a decreased QoL years after diagnosis of the thyroid cancer. Unfortunately, it is impossible to have QoL scores of thyroid cancer patients before (suspicion of) diagnosis, so using reference values of the general population is second best. Lubitz et al. (21) and Ryu et al. (19) showed baseline QoL scores that were comparable with the general population, while Gou et al. (17) also showed a lower QoL at baseline. The persistent decreased QoL in our population two to three years after initial therapy, compared to the general population, might explain why earlier cross-sectional studies also found a lower QoL in thyroid cancer survivors compared to the general population (12-17). In addition, earlier studies in patients with benign thyroid disease showed slightly lowered QoL scores in most domains before surgery, while results after surgery were mixed varying from normalization of QoL towards no significant change (26-28). Therefore, it is possible that the decreased baseline QoL score in thyroid cancer patients might also be partly explained by fear of the upcoming therapy.

In a univariate analysis, younger age, female sex, and persistent hypoparathyroidism resulted in lower QoL. These results are in line with earlier studies (29, 30). Persistent hypoparathyroidism resulting in lower QoL might also suggests that

less aggressive treatment, if possible, might be favorable for QoL in the long term. We also observed that the QoL over time trajectories for patients receiving one or multiple RAI therapies were not significantly different, except for two MFI-20 and two ThyPRO scores. A difference between the two groups was expected, but due to the fact that the multiple RAI group is relatively small (23%) and patients received additional therapies at different time-points, their variations in QoL were probably compensated by the rest of the population having a 'normal' QoL at these time-points.

Nine percent of our patients had a recurrent nerve paralysis at end of follow-up. According to the 2015 ATA Guideline, vocal cord paresis or paralysis can be present in up to 8% presurgically, and up to 30% of the patients postsurgically (6). Next to this, 17% of our patients obtained permanent hypoparathyroidism (30% in the neck dissection, and 9% in the group without neck dissection). Earlier studies showed percentages of permanent hypoparathyroidism up to 10% (6, 31-33). However, thyroid cancer surgery and/or central neck dissection are known risk factors for developing hypoparathyroidism (6), and as 38% of patients in our tertiary referral center received a neck dissection already at initial therapy (2/3 with both central and lateral), this might be the reason for the higher percentage found in our study.

The main strength of this study is the relative large number of DTC patients that, starting before initial therapy, were followed longitudinally. Furthermore, our follow-up of more than 2.5 years (in 25% of our patients longer than four years) is relatively longer than earlier longitudinal studies with QoL assessment before initial therapy. Besides, using marginal models for repeated measurement analysis it is possible to create a model for QoL development over time, which enabled us to determine QoL at each moment in time. Additionally, these models also enabled us to correct for missing QoL scores. A possible limitation of the study is that patients were recruited from a single tertiary university hospital, which attracts patients with more aggressive disease possibly leading into worsened QoL already at baseline. Next to this, as mentioned earlier, QoL scores before initial treatment are influenced by the fact that patients were already aware of their cancer diagnosis, which probably resulted into lower scores. However, it is impossible to know their QoL scores before (suspicion of) diagnosis. Also, it might be that QoL itself can contribute to the patient's tendency completing questionnaires. However, 84% of the included patients completed two or more questionnaires which is a decent percentage. Although being one of the largest prospective studies, we were unable to build a statistical model including all different studied factors to investigate a full model including all possible interactions between these studied factors. This is caused by the needed of non-linear terms in combination with the current sample size.

CONCLUSIONS

In conclusion, this study shows that in a population of DTC patients treated with total thyroidectomy and RAI therapy, QoL before initial therapy is already lower than in the general population. Thereafter, QoL develops in general non-linear over time with the lowest QoL around RAI therapy, and two to three years after initial therapy it is approximately the same as at baseline. Therefore, clinicians should be aware that it takes years to regain baseline QoL level after initial treatment with total thyroidectomy followed by RAI therapy. Herewith our results seem to support less aggressive treatment, but further longitudinal research, with follow-up of more than 5 years, also including patients who did not receive RAI therapy for DTC, is needed to know more about the QoL in the long term and the impact of less aggressive treatment on it.

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Supplemental Table 1. Characteristics of the included study population.

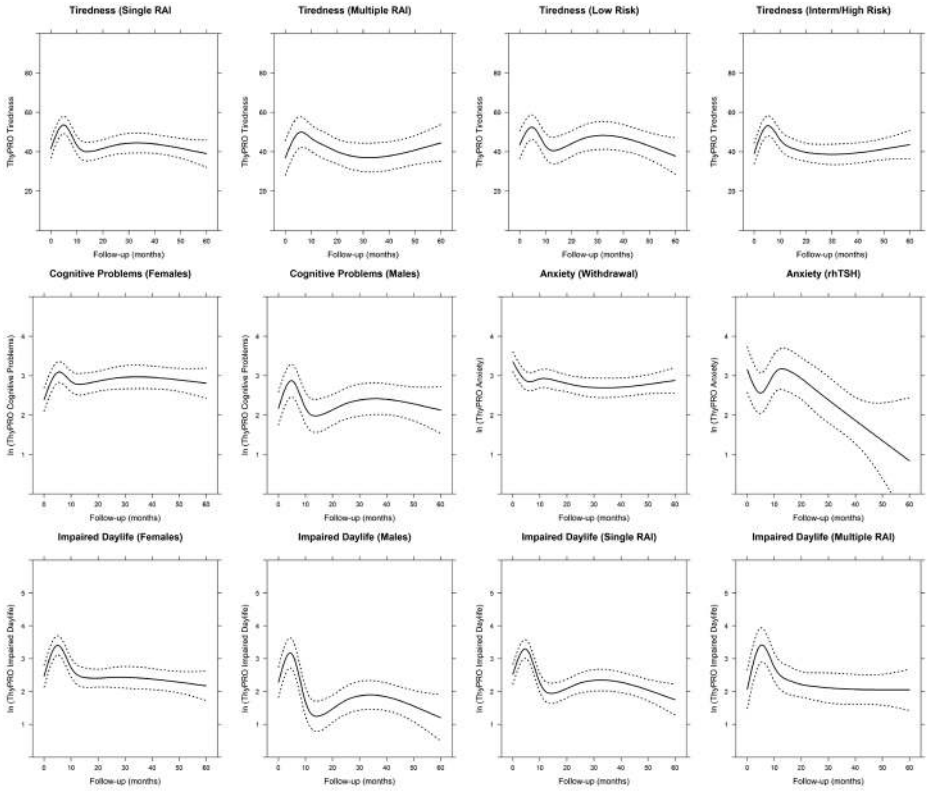
	Insufficient questionnaires (n=35)[a]	Sufficient questionnaires (n=185)[a]
Age at diagnosis (years)	45.7 ± 15.0	47.0 ± 15.7
Women	26 (74%)	131 (71%)
Disease Type		
Papillary Thyroid Cancer	27 (77%)	162 (88%)
Follicular Thyroid Cancer	8 (23%)	23 (12%)
ATA Risk Stratification System (2015)		
Low Risk	12 (34%)	72 (39%)
Intermediate Risk	7 (20%)	55 (30%)
High Risk	16 (45%)	58 (31%)
AJCC/TNM Staging system (8th edition)		
Stage I	28 (80%)	138 (75%)
Stage II	4 (11%)	35 (19%)
Stage III	-	9 (5%)
Stage IV	3 (8%)	3 (2%)
Surgery (TT)	35 (100%)	185 (100%)
Neck dissection	14 (40%)	70 (38%)
Central	2 (6%)	23 (12%)
Lateral	-	1 (1%)
Both	12 (34%)	46 (25%)
RAI treatment	35 (100%)	185 (100%)
Once	21 (60%)	142 (77%)
Twice	10 (29%)	33 (18%)
≥ 3	4 (11%)	10 (5%)
Withdrawal at first therapy	30 (85%)	154 (83%)
first dose (mCi)	144 (49 – 147)	143 (48 – 147)
Admission duration first therapy (days)	3 (2 – 3)	3 (2 – 3)
Cumulative dose (mCi)	146 (50 – 293)	145 (49 – 151)
Hypoparathyroidism		
Transient	1 (3%)	37 (20%)
Permanent	3 (9%)	31 (17%)
Recurrent nerve paralysis	1 (3%)	17 (9%)
Number of questionnaires per patient	1 (0 – 1)	3 (2 – 4)
Follow-up (months)	0 (0 – 11)	31 (17 – 49)

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
TT, total thyroidectomy; RAI, radioactive iodine; mCi, milliCurie.

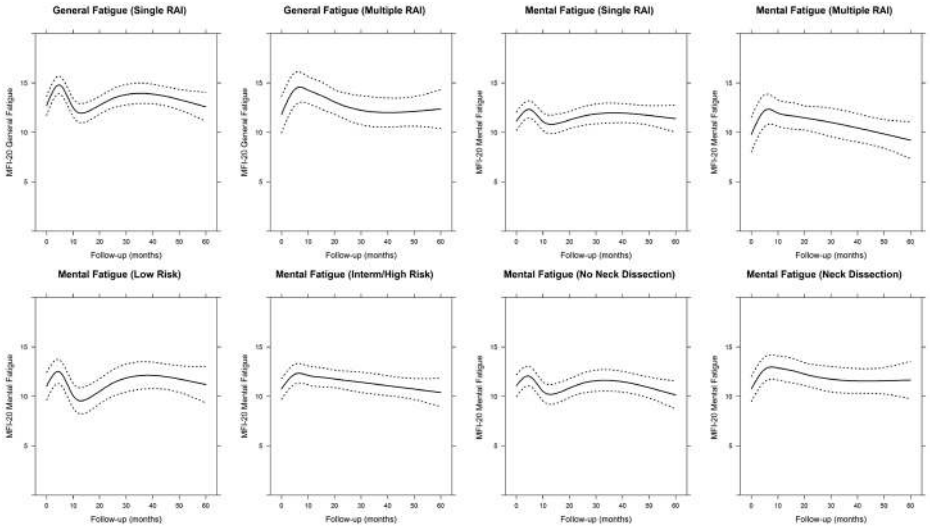
Supplemental Table 2. Estimated values of factors significantly influencing Quality of Life over time.

	Age[a, b]	Gender[a, c]	Persistent Hypo-parathyroidism[a, d]	Recurrent Nerve Paralysis[a, d]
MFI-20				
General fatigue	-1.79 ± 0.58	-2.67 ± 0.61		
Physical fatigue		-2.72 ± 0.59		
Mental fatigue	-1.50 ± 0.59	-1.51 ± 0.65		
RAND-36				
Vitality	5.95 ± 2.66	10.94 ± 2.82		
General Mental Health	5.70 ± 2.42	6.77 ± 2.63		
General Health Perception		7.96 ± 2.90	-3.55 ± 1.79	
Physical functioning[e]			-0.12 ± 0.04	
Social functioning[f]	0.50 ± 0.24	0.71 ± 0.26	-0.42 ± 0.18	
ThyPRO				
Composite[f]	-0.57 ± 0.24	-0.88 ± 0.25	0.39 ± 0.16	
Tiredness		-13.05 ± 3.31	4.85 ± 2.28	
Cognitive Problems[e]				
Anxiety[e]				0.48 ± 0.22
Depressivity[e]	-0.26 ± 0.12	-0.35 ± 0.13		0.36 ± 0.16
Social Impairment[e]	-0.68 ± 0.19	-0.58 ± 0.21	0.29 ± 0.14	
Impaired Daylife[e]			0.61 ± 0.15	

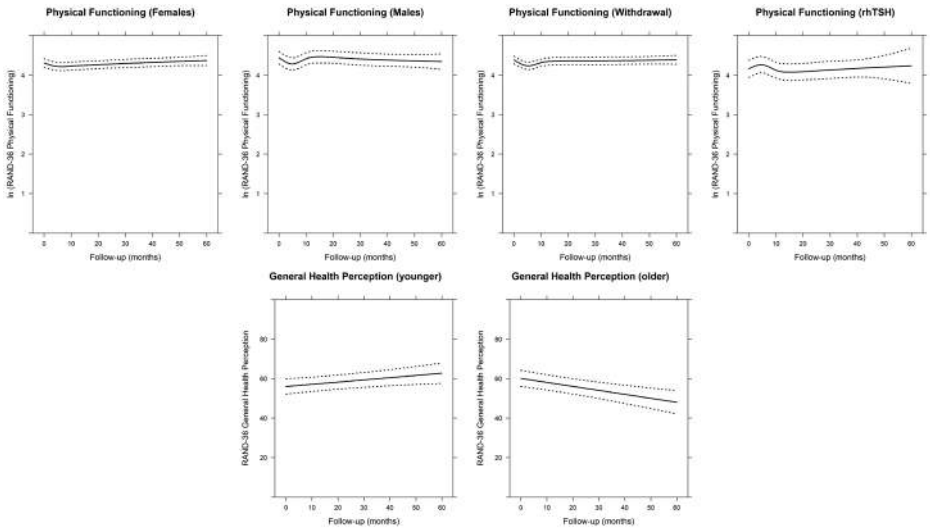
[a] estimated values ± standard errors.
[b] <50 years vs. ≥50 years (<50 years as reference group).
[c] women vs. men (women as reference group).
[d] yes vs. no (no as reference group).
[e] natural logarithmic transformed.
[f] square root transformed.



Supplemental Figure 1. Quality of Life (ThyPRO scores) over time in case of significant interaction.



Supplemental Figure 2. Quality of Life (MFI-20 scores) over time in case of significant interaction.



Supplemental Figure 3. Quality of Life (RAND-36 scores) over time in case of significant interaction.

Chapter 8

Longitudinal Analysis of the Effect of Radioiodine Therapy on Ovarian Reserve in Females with Differentiated Thyroid Cancer

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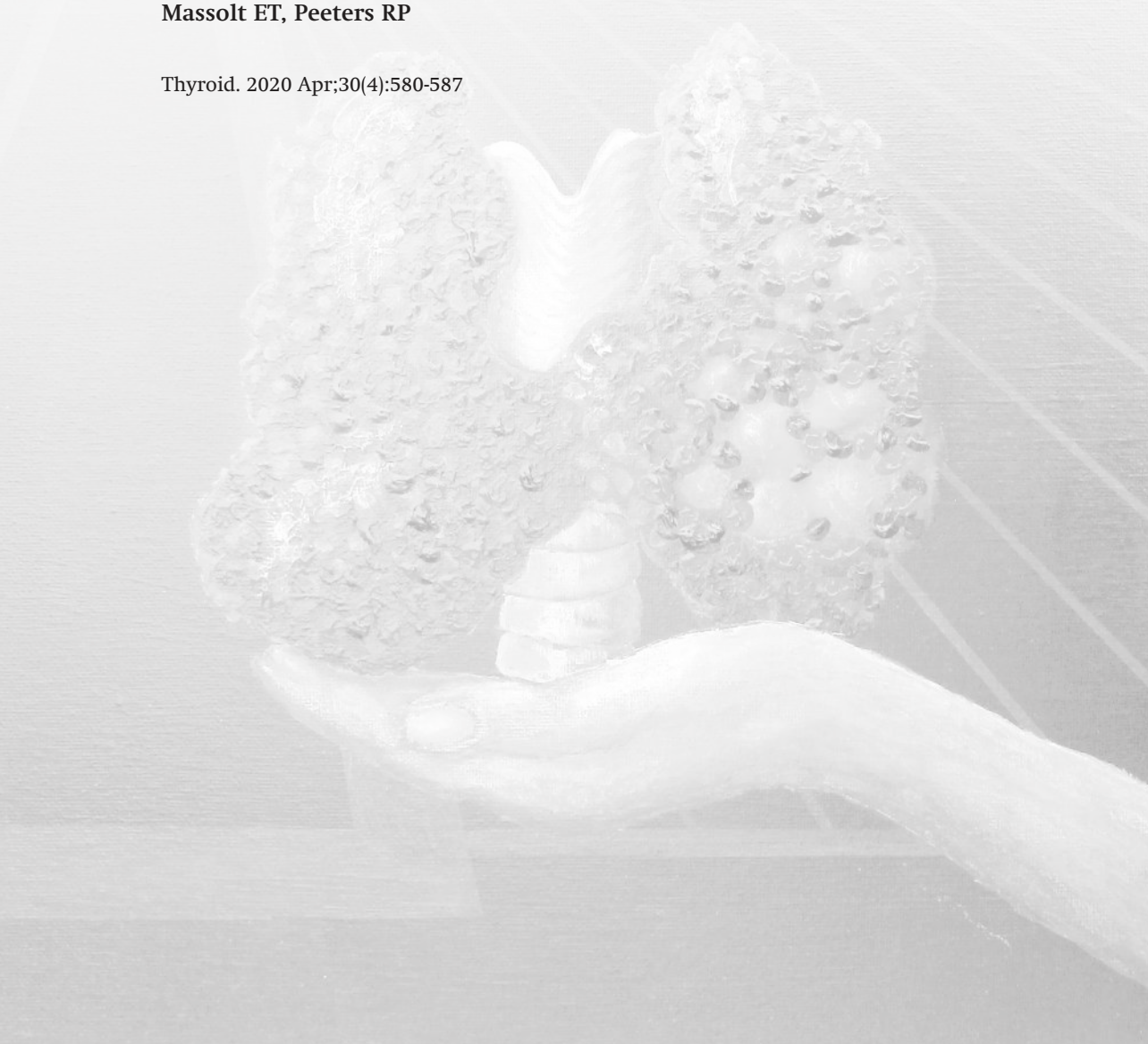

ABSTRACT

Background

Although international guidelines have become more conservative on the use of radioactive iodine (RAI) therapy, it is still one of the cornerstones of the treatment of patients with advanced differentiated thyroid cancer (DTC). As a large proportion of females diagnosed with DTC is in their reproductive years, knowledge about the effect of RAI on their gonadal and reproductive function is important. Earlier studies evaluating Anti-Müllerian hormone (AMH) as a representative of ovarian reserve were either cross-sectional, had relative low numbers, had no patients with multiple RAI therapies, or had a relative short follow-up. The primary aim of our study was therefore to prospectively evaluate the effect of RAI on AMH in women undergoing treatment for DTC.

Methods

We included females, aged 16 years until menopause, who were scheduled to undergo their first RAI treatment for DTC at our hospital. Serum AMH was measured before initial therapy and regularly thereafter. Repeated measurement analysis was used to assess the changes of AMH concentrations over time, and how it is influenced by age and cumulative RAI dose.

Results

Longitudinal AMH assessments were available in 65 patients (mean age 32 years, median of five measurements during median follow-up of 34 months). AMH concentrations changed non-linear over time, decreased until 12 months in the Single RAI group (-55%), and stabilized thereafter. In the Multiple RAI group, after stabilization, a further decrease occurred (-85% after 48 months). Age in both RAI groups significantly influenced AMH change over time with younger patients (<35 years of age) showing a less steep decrease.

Conclusions

In a population of female DTC patients treated with total thyroidectomy and a single RAI therapy, AMH concentrations significantly dropped during the first year after initial therapy, and thereafter remained stable. In patients receiving multiple RAI therapies, a further decrease was seen. Age at baseline significantly influenced AMH change over time. Herewith our results support a less aggressive treatment with RAI in low risk patients as is advocated in the current ATA guidelines, especially in females over 35 years of age with the desire to have a child.

INTRODUCTION

The worldwide incidence of differentiated thyroid cancer (DTC) has been steadily increasing over the last two decades (1, 2). As mortality has remained stable, a less aggressive therapy seems more appropriate (1-3). For this reason, current ATA guidelines recommend less extensive surgery in low risk tumors, and more restricted use of radioiodine (RAI) therapy (3), but controversies remain (4). Nevertheless, RAI is still one of the cornerstones of the treatment of patients with DTC, particularly in more advanced stages (3, 5). DTC occurs more frequent in females than it does in males, and a large proportion of these female patients is in their reproductive years (2). Disease specific survival in these young females is generally very good (6, 7), leading to important questions about the influence of RAI therapy on their reproductive function.

Earlier studies showed a transient change of the menstrual cycle in 12-31%, and a temporary increase of Follicle Stimulation Hormone (FSH) during the first year after RAI therapy (8-10). However, no increased infertility rates or adverse obstetric outcomes were seen in patients after RAI therapy (8, 9). Further, four recent studies evaluated Anti-Müllerian hormone (AMH) as a representative of ovarian reserve in patients with DTC receiving RAI therapy (11-14). AMH is relatively insensitive to inter- and intra-cycle variability and oral contraceptives use, is known to gradually decline with age, and is undetectable at menopause (15-18). Therefore, AMH seems to be a good marker for ovarian reserve. Cross-sectionally, results were mixed as one study showed that AMH concentrations were significantly lower in patients who received RAI than in controls (13), but another study showed no significant differences (14). Earlier longitudinal studies showed a significant decrease of AMH concentrations when comparing concentrations just before RAI therapy with concentrations 12 months later (11, 12). So, these earlier studies investigated the effects of RAI on AMH concentrations were either cross-sectional, had relative low numbers, had no patients with multiple RAI therapies, or had a relative short follow-up. The primary aim of our study was therefore to investigate the long-term effects of RAI therapy on AMH concentrations in female patients undergoing treatment for differentiated thyroid cancer.

MATERIALS AND METHODS

Study population

We aimed to include all females, aged 16 years until menopause, who were scheduled to undergo treatment for DTC at the Erasmus Medical Center, Rotterdam, The

Netherlands. Inclusion period was from January 2013 until December 2017, and patients were followed for at least one year. All included patients had tumors ≥ 1cm and received a total thyroidectomy followed by RAI therapy according to the current Dutch guidelines (19). At baseline, i.e. before surgery, just before RAI therapy, six to nine months later, and regularly thereafter, serum AMH measurements were performed. Additionally, if a patient received more than one RAI therapy, just before a new therapy, and six to nine months thereafter additional AMH measurements were performed. As AMH concentrations typically do not fluctuate, the blood samples were not taken at a specific point during the menstrual cycle. Serum AMH concentrations were measured using the AMH Gen II enzyme-linked immunosorbent assay (Beckman Coulter, Brea, CA), and expressed in µg/l.

Next to AMH measurements, we recorded demographic, disease, and treatment characteristics. Demographical variables included age at diagnosis and Body Mass Index (BMI). Disease characteristics included disease type, AJCC/TNM-stage (8th edition), and ATA Risk Stratification category (2015). Data regarding treatment consisted of number of RAI therapies, method of TSH stimulation before first RAI therapy (either 3-4 weeks of thyroid hormone withdrawal, or two subsequent injections with recombinant human TSH (rhTSH)), and cumulative dose of RAI. Further, childbirth rates were recorded during follow-up, and at end of follow-up, using a questionnaire, patients were asked about the influence of thyroid cancer diagnosis and treatment on their desire to have a child. The study was approved by the Institutional Review Board of the Erasmus Medical Center.

Statistical analysis

For continuous variables, means and standard deviations (SD), or medians with interquartile ranges (IQR) were calculated. For categorical variables, absolute numbers with percentages were recorded. In order to assess the changes of AMH concentrations over time while accounting for the correlation between the repeated measurements of each patient, we used marginal models; the appropriate covariance matrix that best fitted the data was selected. To obtain a normal distribution for AMH concentrations, a (natural) log transformation was applied. Further, using a marginal model allows AMH measurements to be collected without predefined time points and therewith strengthens analysis. Analyses were performed separately for patients receiving one or multiple RAI therapies (Single RAI and Multiple RAI group respectively). Our main goal was to assess changes in AMH concentrations over time. Further, additional analyses were performed to explore the effect of age (<35 years vs. ≥35 years), RAI preparation method (withdrawal vs. rhTSH), and cumulative RAI dose on AMH changes over time. In general, p-values below 0.05 were considered significant. The possibility of effect modification was studied using a two-step strat-

egy. Firstly, a screening p-value cut-off for interaction of <0.10 was used, because the statistical power to identify an interaction term is less than for normal covariates. Secondly, to make sure that we did not identify irrelevant modification, we subsequently performed stratified analyses to replicate and quantify the effect modification so that we can interpret whether the differences are of (clinical) relevance. All analyses were performed using either SPSS Statistics for Windows (version 24.0) or R statistical software (version 3.4.1).

RESULTS

Population characteristics

During the inclusion period, a total of 85 female patients were eligible for the study of which 12 were not included because they either refused to participate (n=5), or were missed at inclusion (n=7). Next to this, eight patients had less than two AMH measurements and were subsequently excluded. Therefore, the analyses presented here were performed in the remaining 65 patients.

Table 1 lists the characteristics of the study population. Mean age was 32.0 years, and 59 (91%) had papillary thyroid carcinoma. Almost all patients (94%) had Stage I disease. All patients received a total thyroidectomy followed by RAI therapy in correspondence with the current Dutch guidelines. Forty-seven patients received one RAI therapy (median dose 50 mCi; 25-75IQR: 30-146 mCi), and the other 18 patients received two or three therapies (median dose 291 mCi; 25-75IQR: 284-293 mCi).

AMH

For the total population, median follow-up from first RAI therapy to final AMH measurement was 34 months, and during this time a median of five measurements (25-75 IQR: 3-5) were taken. Median follow-up was six months longer for the Multiple RAI than for the Single RAI group.

Using repeated measurement analysis, AMH concentrations showed a significant non-linear change over time in both RAI groups (see Figure 1 and Table 2). For the Single RAI group, after initial therapy AMH, concentrations significantly dropped with the lowest values after 12 months (-55%; $p<0.0001$). Thereafter, AMH concentrations remained stable. For the Multiple RAI group, after initial therapy, AMH concentrations significantly dropped (-74%; $p<0.001$), and after a short stabilization phase again started to decrease. AMH concentrations after 48 months were significantly lower in the Multiple RAI than in the Single RAI group ($p=0.005$).

Age significantly influenced AMH change over time in both RAI groups (see Figure 2 and 3). In patients below 35 years of age, AMH concentrations showed a less

Table 1. Characteristics of the study population.

	Total Population (n=65)[a]	Single RAI group (n=47)[a]	Multiple RAI group (n=18)[a]	p-value[b]
Age at diagnosis (years)	32.0 ± 8.4	32.9 ± 7.8	29.7 ± 9.7	0.230
< 35 years	44 (68%)	32 (68%)	12 (67%)	0.913
≥ 35 years	21 (32%)	15 (32%)	6 (33%)	
BMI (kg/m2)	25.5 ± 6.6	26.2 ± 7.0	23.4 ± 4.9	0.110
Children at Baseline	35 (54%)	28 (60%)	7 (39%)	0.092
Disease Type				
Papillary Thyroid Cancer	59 (91%)	42 (89%)	17 (94%)	0.526
Follicular Thyroid Cancer	6 (9%)	5 (11%)	1 (6%)	
ATA Risk Stratification System (2015)				
Low	33 (51%)	32 (68%)	1 (6%)	
Intermediate	14 (22%)	19 (9%)	5 (28%)	**<0.001**
High	18 (28%)	6 (13%)	12 (67%)	
AJCC/TNM Staging system (8th edition)				
I	61 (94%)	47 (100%)	14 (78%)	
II	4 (6%)	-	4 (22%)	**0.001**
III	-	-	-	
IV	-	-	-	
Surgery (TT)	65 (100%)	47 (100%)	18 (100%)	-
RAI treatment				
Once	47 (72%)	47 (100%)	-	
Twice	15 (23%)	-	15 (83%)	**<0.001**
≥ 3	3 (5%)	-	3 (17%)	
Withdrawal at first therapy	51 (79%)	34 (72%)	17 (94%)	0.052
Cumulative dose (mCi)	144 (48 – 226)	50 (30 – 146)	291 (284 – 293)	**<0.001**
Number of AMH measurements per patient	5 (3 – 5)	5 (3 – 5)	5 (3 – 6)	0.543
Follow-up (months)	34 (19 – 48)	31 (16 – 47)	37 (29 – 51)	0.134

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
[b] p-value comparing Single and Multiple RAI group.
AMH, Anti-Müllerian hormone; ATA, American Thyroid Association; TT, total thyroidectomy; RAI, radioactive iodine; mCi, milliCurie.

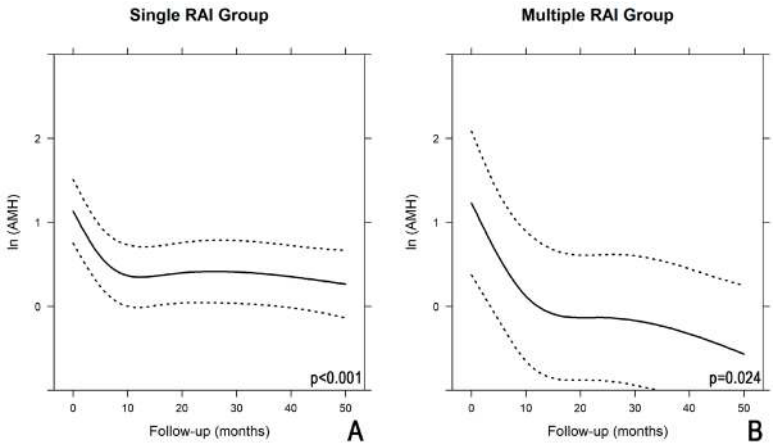

Figure 1. AMH concentrations over time for (A) Single RAI and (B) Multiple RAI group.

Table 2. AMH concentrations during follow-up.

	AMH concentrations[a] (Total Population)	AMH concentrations[a] (Single RAI group)	AMH concentrations[a] (Multiple RAI group)
Baseline	3.34 ± 1.20	3.05 ± 1.21	3.37 ± 1.54
± 12 months after RAI	1.37 ± 1.19[b]	1.38 ± 1.20[b]	0.86 ± 1.48[b]
± 24 months after RAI	1.22 ± 1.19[b]	1.46 ± 1.21[b]	0.82 ± 1.47[b]
± 36 months after RAI	1.07 ± 1.21[b]	1.42 ± 1.21[b]	0.74 ± 1.49[b]
± 48 months after RAI	0.95 ± 1.23[b]	1.25 ± 1.23[b]	0.52 ± 1.52[b]

[a] Values are means (± standard error) in µg/l obtained from created model.
[b] Significant change from baseline ($p<0.05$).
RAI, radioactive iodine.

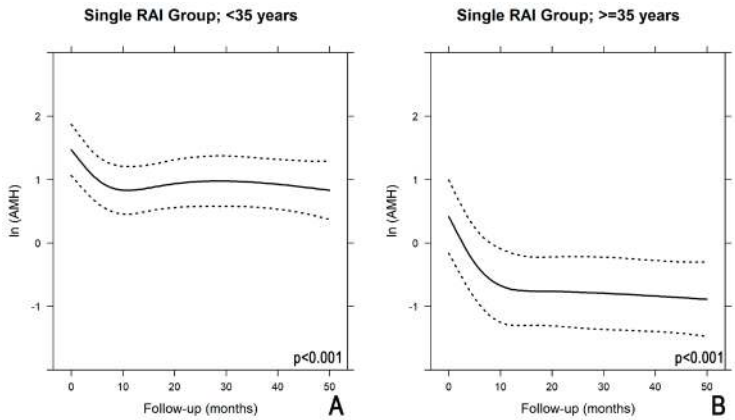

Figure 2. AMH concentrations over time for females (A) <35 years of age and (B) ≥ 35 years of age in the Single RAI group.

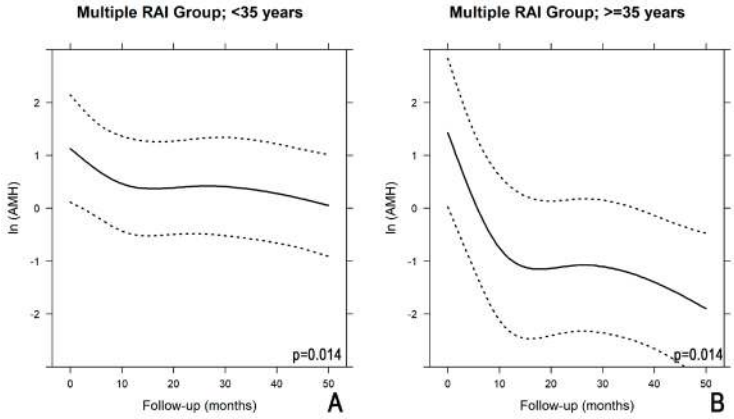

Figure 3. AMH concentrations over time for females (A) <35 years of age and (B) ≥ 35 years of age in the Multiple RAI group.

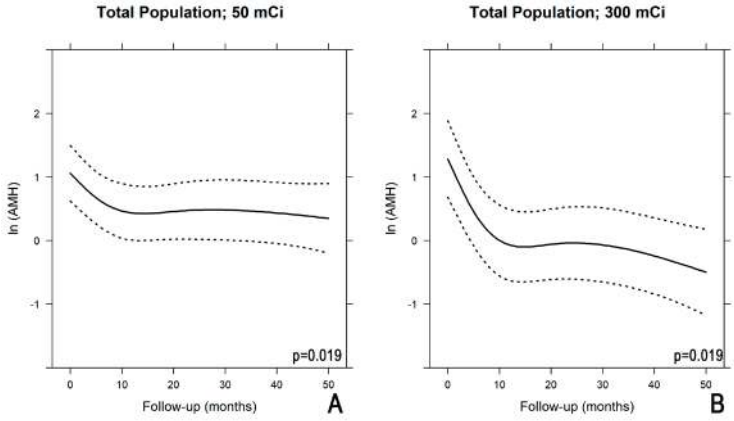

Figure 4. AMH concentrations over time for females (A) 50 mCi and (B) 300 mCi in the total population.

steep decrease during the first year compared to patients older than 35 years (For Single RAI group: -46% vs. -71%). Cumulative RAI dose did not significantly influence AMH concentrations in both RAI group separately. Additionally, we also did not find a difference in the Single RAI group between females receiving <50 mCi and those receiving ≥50 mCi (p=0.370). However, after combining both RAI groups, we did see a significant effect of cumulative RAI dose on AMH change over time (p=0.019; see also Figure 4). Further, we did not find a significant influence of RAI preparation method on AMH concentrations in the Single RAI group. As only one patient in the Multiple RAI group received her initial RAI therapy after rhTSH, we were unable to analyze the influence of RAI preparation method on AMH in the Multiple RAI group.

Desire to have a child and Childbirth

Median time between first RAI therapy and end-of-follow-up was 43 months. During follow-up, seven children (11%) were born (see also Table 3); all children were born in patients below 35 years of age at diagnosis. Median time from initial RAI therapy to birth was 34 months. During follow-up, eight patients became menopausal; seven (88%) were above 35 years of age at diagnosis.

Fifty-two patients (80%) completed the questionnaire about the influence of the diagnosis and treatment of thyroid cancer on their desire to have a child. Of these patients, 16 (40%) in the Single RAI, and four (33%) in Multiple RAI group stated that their desire to have a child was indeed influenced by the diagnoses or treatment. In the majority of these patients this was either not wanting a child anymore (40%), or the medicalization of an upcoming pregnancy (30%), while the rest had other reasons or did not specify them. There were more women in whom their desire to have a child was influenced in the below 35 than in the above 35 years of age group (47% vs. 19% respectively; p=0.051).

Table 3. Outcomes related to childbearing.

	Total Population (n=65)[a]	Single RAI group (n=47)[a]	Multiple RAI group (n=18)[a]
Influence on desire to have child	20/52 (38%)[b]	16/40 (40%)[c]	4/12 (33%)[d]
Childbirth	7 (11%)	6 (13%)	1 (6%)
Time to birth (months)	34 (15 – 48)	25 (15 – 50)	44

[a] Values are medians (25-75-IQR), or numbers (percentages).
[b] Information missing in 13 subjects.
[c] Information missing in 7 subjects.
[d] Information missing in 6 subjects.
RAI, radioactive iodine.

DISCUSSION

This study shows that in women with DTC, who were treated with total thyroidectomy followed by a single RAI therapy, AMH concentrations significantly dropped by 55% in the first year after initial therapy, and thereafter were more-or-less stable. In patients receiving multiple RAI therapies, after a short stabilization, a further decrease was seen (-85% after 48 month). Next to this, age significantly influenced AMH change over time, with a more pronounced decrease in AMH concentrations in women above 35 years of age. Further, DTC diagnosis and treatment influences the desire to have a child in almost 40% of the patients.

We showed a significant non-linear change over time for both RAI groups. For the Single RAI group, AMH concentrations significantly dropped by 55% during the

first 12 months, and thereafter stabilized. In two earlier longitudinal studies with follow-up of 12 months, also a significant decrease of AMH concentrations were seen after initial RAI therapy (11, 12); in these studies, AMH concentrations after 12 months were approximately 30% and 58% lower than at baseline. Nadir in these studies was seen after three months. Thereafter a slight recovery occurred, followed by stabilization. We did not have measurements that early after RAI therapy, and we were therefore unable to compare our results with these findings. However, one might argue that in these studies stabilization of AMH concentrations was already seen at 12 months, and, based on our results, one could speculate that thereafter AMH concentrations would remain stable. Therewith, AMH concentrations later-on were lower than at baseline which is in line with the cross-sectional findings of Acibucu et al. showing significant decreased AMH concentrations compared to healthy controls (13). In the Multiple RAI group, we also showed a significant drop of AMH concentrations in the first year (-74%). This drop is followed by short stabilization, and thereafter a further decrease is seen. This latter decrease is most likely due additional RAI therapy, but the decrease is less severe than the initial drop. This pattern might be caused by the fact that the patients received their therapies at different points in time (median time between first and second therapy was 9 months) and therewith the expected step-wise decrease was not found, or oocytes of less quality were already damaged by the initial RAI therapy and those with better quality prevail. As we were the first to study women receiving multiple RAI therapies, we cannot compare our results with other studies, and further research is needed to confirm our findings.

We observed a significant influence of age on AMH change over time in both RAI groups; females younger than 35 years of age showed a less steep decrease than those older than 35 years. In correspondence with our results, two other longitudinal studies also showed a less steep decrease after RAI therapy in younger females (11, 12). The steeper decrease of AMH concentrations in females above 35 years compared to those younger than 35 years (-71% vs. -46% in the Single RAI group) indicates that they might be more prone to become menopausal after receiving RAI therapy; taking into account that AMH concentrations are related to menopause (20, 21). This steeper decline might be caused by their older oocytes of less quality which are therefore more easily damaged by RAI therapy. Additionally, in the Single RAI group, baseline AMH concentrations were also already lower in females above 35 years compared to those younger than 35 years. Further, although causality cannot be proven due to the lack of a control group, 88% of the patients that became menopausal after RAI therapy were above 35-years of age at initial diagnosis. Therefore, these results suggests that clinicians should thoroughly balance the indication for RAI therapy in females over 35 years of age with the desire to have a child.

We did not find an effect of cumulative RAI dose on AMH change over time in both RAI groups separately, which is in correspondence with earlier studies which did not show an effect of cumulative RAI dose on AMH (11, 12, 14). However, one might argue that there is indeed an effect of cumulative RAI dose in our study because 1) AMH concentrations after 48 months were significantly lower in the Multiple RAI than in the Single RAI group, and 2) combining both RAI groups we indeed found a significant effect of cumulative RAI dose on AMH change over time suggesting that the cumulative RAI dose distribution in each RAI group separately is probably too small to find an effect.

DTC diagnosis and treatment influenced the desire to have a child in almost 40% of our female patients. This high percentage reflects the struggles of patients after being diagnosed and treated for thyroid cancer, especially at a young age. Therefore, one might argue that this is one of the reasons why younger females showed lower quality of life in earlier studies (22, 23). Carefully counseling these patients is therefore very important.

The main strength of this study, compared to previous studies, is the relative large number of women with DTC who were studied prospectively. Furthermore, the availability of follow-up AMH measurements with a median of almost three years, positions our study, as far as we know, the longest longitudinal study available. Besides, the use of marginal models for repeated measurement analysis allowed us to create a model for AMH change over time, thereby enabling the calculation of AMH at each moment in time. Additionally, these models also enabled us to correct for missing AMH measurements. A possible limitation of the study might be the lack of a control group, and therefore, one might argue that the discovered decline over time in our study is the biological decrease. However, both the stabilization one year after RAI therapy, and the AMH decline in our study, which is far beyond the expected age-related decline (16), suggests causality. Further, it is important to note that AMH concentrations do not reflect the quality of the oocytes and therewith diminished AMH concentrations do not necessarily mean reduced fertility (24), but undetectable concentrations are related to menopause (15). Although being the largest and longest prospective study available, the statistical power in the Multiple RAI group might have been hampered due to the availability of only 18 patients. However, an indication of the effect of multiple RAI therapies on AMH concentrations can still be extracted from our results.

CONCLUSIONS

In conclusion, this study shows that in a population of female DTC patients treated with a single RAI therapy, AMH concentrations significantly dropped the first year after initial therapy by 55%. Thereafter, AMH concentrations remained stable but no recovery was seen. In patients receiving multiple RAI therapies, AMH concentrations decreased further after a short plateau phase. In addition, patients older than 35 years showed a much stronger decrease than those younger than 35 years. Finally, DTC diagnosis and treatment influenced the desire to have a child in almost 40% of the female patients. Therefore, clinicians should be aware of both the psychological and biological impact of DTC diagnosis and treatment on female patients in their reproductive years. Our results support a less aggressive treatment with RAI in low risk patients as is advocated in the current ATA guidelines, especially in females over 35 years of age with the desire to have a child.

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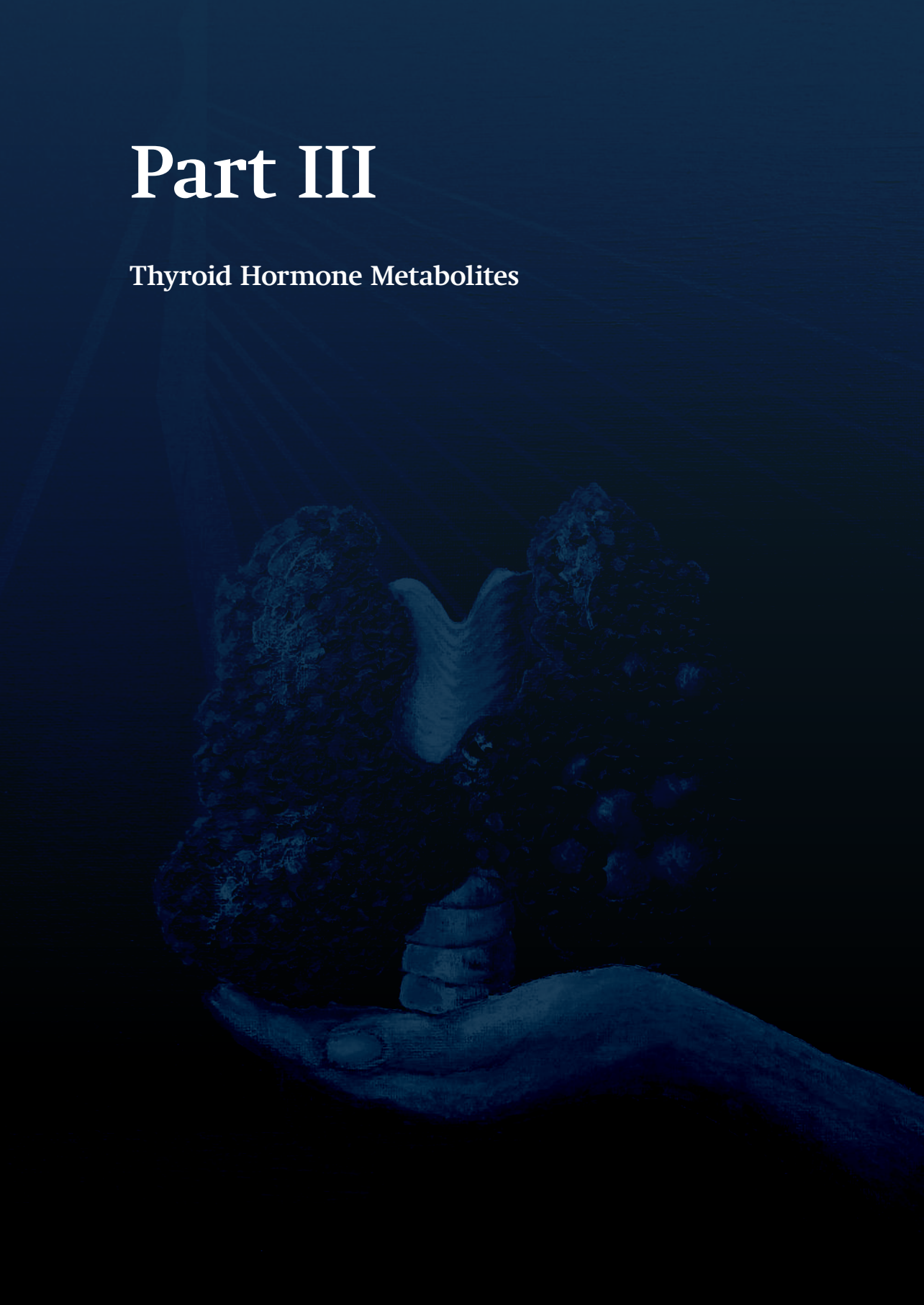
Part III

Thyroid Hormone Metabolites



Chapter 9

Change in Thyroid Hormone Metabolite concentrations across Different Thyroid States

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ABSTRACT

Background

In contrast to the thyroid hormones (TH) T3 and T4, current literature on thyroid hormone metabolite concentrations in the hypothyroid and hyperthyroid state is inconclusive. It is unknown how thyroidectomy affects thyroid hormone metabolite concentrations, and if LT4 replacement therapy after thyroidectomy restores thyroid hormone metabolite concentrations in those without a thyroid gland. The treatment of patients with differentiated thyroid cancer (DTC) covers the euthyroid, hypothyroid and (subclinical) hyperthyroid states and therefore provides a unique model to answer this. Here, we prospectively studied nine TH and its metabolites (THM) across different thyroid states in a cohort of patients treated for DTC. Also, three potentially important determinants for THM concentrations were studied.

Methods

We prospectively included patients aged 18 to 80 years who were scheduled for DTC treatment at the Erasmus MC. Peripheral blood samples were obtained before surgery (euthyroid, endogenous thyroid hormone production), after surgery just before RAI therapy (hypothyroid), and six months later on LT4 therapy ((subclinically) hyperthyroid, exogenous T4 supplementation). Nine THM were quantified in serum with an established liquid chromatography-tandem mass-spectrometry method. Repeated measurement analysis was used to compare the three different thyroid states with each other for each THM, while linear regression was used to determine the association between THM concentrations and age, sex and kidney function.

Results

In total 77 patients (mean age 49 years; 65% women) were eligible for the study. 3,5-diiodothyronine and 3,3',5-triiodothyroacetic acid were below the lower limit of detection. Compared to the euthyroid state, all THM were significantly decreased in the hypothyroid state, and significantly increased in the (subclinically) hyperthyroid state with T3 concentrations remaining within the reference interval. Higher age was associated with higher 3-monoiodothyronine concentrations ($p<0.001$). Women had higher L-thyronine concentrations than men ($p=0.003$). A better kidney function was associated with lower 3-monoiodothyronine concentrations ($p<0.001$).

Conclusions

All THM decrease after a thyroidectomy and increase under TSH suppressive LT4-therapy suggesting that formation of thyroid hormone metabolites is dependent on peripheral extrathyroidal metabolism of T4. This is also reflected by T3 concentra-

tions that remained within the reference interval in patients receiving TSH suppressive LT4-therapy as T3 has some thyroidal origin.

INTRODUCTION

The thyroid hormones (TH) thyroxine (T4) and 3,3',5-triiodothyronine (T3) are essential for a proper function of virtually every tissue in the human body. The thyroid gland predominantly secretes the prohormone T4. T4 is metabolized by deiodination into the active metabolite T3. The major biological activity of TH is mediated by binding of T3 to nuclear thyroid hormone receptors. T4 and T3 can be further metabolized to form other thyroid hormone metabolites (1). Potential roles for thyroid hormone metabolites have been described in processes such as energy metabolism, lipid metabolism, bone remodeling and cardiac hypertrophy (2-6).

Hypothyroidism is a very common endocrine disorder which can be treated with levothyroxine (LT4) (7). Despite LT4 treatment, 10-15% of the patients show significant physical and psychological impairment compared to matched controls or the general population (8-13). Altered thyroid hormone metabolite concentrations could possibly contribute to these residual complaints. Although it has been reported that patients on T4 supplementation generally have lower concentrations of serum T3 than euthyroid controls, it is currently unknown if T4 supplementation can adequately restore concentrations of other thyroid hormone metabolites (14, 15).

Current literature on thyroid hormone metabolite concentrations in the euthyroid, hypothyroid and hyperthyroid state are inconclusive for iodothyronines such as 3-monoiodothyronine (3-T1), 3,5-diiodothyronine (3,5-T2) and 3,3'-diiodothyronine (3,3'-T2), and for iodothyroacetic acids such as 3,3',5-triiodothyroacetic acid (triac, TA3) and 3,3',5,5'-tetraiodothyroacetic acid (tetrac, TA4) (Table 1) (16-35). These inconsistencies between studies are likely due to the fact that most studies have small sample sizes (N<30), and thyroid hormone metabolites were measured with (radio) immunoassays. Low selectivity is an important limitation of (radio)immunoassays. Compared to (radio)immunoassays, liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods are more reliable due to increased selectivity as selection is based on chromatographic behavior, mass and fragmentation pattern. Further, thyroid hormone metabolite concentrations at different thyroid states were not compared within the same subject in these earlier studies. In addition, while age, sex and kidney function are potentially important determinants for concentrations of TH and its metabolites (THM), only very limited clinical information was reported (32, 36-40). Until now, it therefore remains unclear if all THM are affected to the

Table 1. Literature on Thyroid Hormone Metabolites in euthyroid, hypothyroid and hyperthyroid state.

	Analytical method	Euthyroid		Hypothyroid		Hyperthyroid		
		Concentration (pmol/L)	n	Concentration (pmol/L)	n	Concentration (pmol/L)	n	Ref
3-T1	RIA	72.5 +/- 42.5	93	52.5 +/- 37.5[a]	24	155 +/- 97.5	30	(16)
3,5-T2	IA	290 +/- 10	99	430 +/- 40[a]	31	310 +/- 20[a] 480 +/-30	24 100	(17)
	IA					860	143	(18)
	RIA	16.2 +/- 6.4	62	0.75 +/- 1.6	8	57 +/- 57.1	9	(19)
	RIA	138.7 +/- 66.5	30	180.5 +/- 95[a]	16	161.5 +/- 68.4[a]	16	(20)
	RIA	81.7 +/- 3.8	70	26.6 +/- 5.7	10	349.6 +/- 43.7	14	(21)
	RIA	105 +/- 51	52	NS[a]		232 +/- 187	17	(22)
	RIA	7.6 +/- 3.42	20	8.17 +/- 1.14[a]	20	33.06 +/- 5.32	17	(23)
	RIA	74.1 +/- 7.6	17	72.2 +/- 11.4[a]	10	76 +/- 9.5[a]	17	(24)
3,3'-T2	RIA	46.6 +/- 20	62	14.9 +/- 9.2	12	85.4 +/- 43.0	9	(25)
	RIA	70.3 +/- 5.7 85.5 +/- 11.4	17 18	55.1 +/- 11.4	10	201.4 +/- 22.8	17	(24)
	RIA	36.1 +/- 15.2	34	9.5 +/- 7.6	13	148.2 +/- 79.8	27	(26)
	RIA	136.8		< 57		209 – 1216		(27)
	RIA	114 +/- 19	13	51.3 +/- 20.9	17	171 +/- 87.4	25	(28)
	RIA	81.7 +/- 38	31	< 24.7	9	224.2 +/- 51.3	17	(29)
	RIA	323 +/- 19	18	< 114 – 418	11	551 +/- 38	5	(30)
	RIA	144.4 +/- 45.6	44	114 +/- 41.8	8	383.8 +/- 142.5	19	(31)
	LC-MS/MS	12.73 – 43.7	260	35.9 (25.8 – 44.8)		35.0 (23.8 – 42.4)	26	(32)
TA3	RIA	< 55 (LLoD)	14	< 55 (LLoD)	10	< 55 (LLoD)	10	(33)
	RIA	139.2	11	118.4	3	155.2	10	(34)
TA4	LC-MS/MS	104.5 +/- 64.7 140.1 +/- 159.8	58 23			232.2 +/- 226.3 249.1 +/- 121.2	88 33	(35)

[a] non-significant compared to the euthyroid subjects.

LLoD, lower limit of detection; THM, Thyroid hormone metabolites.

same extent in hypothyroid and hyperthyroid states, or if compensatory metabolic mechanisms are in place. In addition, it is unclear if LT4 therapy is able to restore serum concentrations of all these THM after thyroidectomy.

Historically, patients with differentiated thyroid cancer (DTC) are treated with total thyroidectomy followed by radioiodine (RAI) therapy, and subsequently with LT4 in a TSH-suppressive dose (41). Patients are usually euthyroid before surgery, hypothyroid after 3-4 weeks of thyroid hormone withdrawal (THW) just before RAI therapy, and (subclinically) hyperthyroid during TSH-suppressive therapy. These patients therefore provide a unique model to study how thyroidectomy without and later with LT4 replacement therapy affects THM concentrations.

Recently, we developed a LC-MS/MS panel for seven iodothyronines and two iodothyroacetic acids in human serum, and subsequently established reference intervals in healthy adults (38). Here, we use this extensive THM panel to prospectively analyze how thyroidectomy without and with T4 supplementation affects THM concentrations in patients treated for DTC.

MATERIALS AND METHODS

Study population

Patients aged 18 to 80 years who were scheduled for a total thyroidectomy for DTC followed by RAI therapy between April 2013 and September 2018 at the Erasmus MC University Medical Center, Rotterdam, The Netherlands, were deemed eligible for this study. All included patients had tumor diameters larger than 1 cm and were treated in line with the current Dutch guideline with a total thyroidectomy followed by RAI therapy (42). Exclusion criteria were inadequate understanding of the Dutch language, another active malignancy, known thyroid disease, an active inflammatory disease, or using any drugs known to influence thyroid hormone metabolism.

We recorded demographic, physical, disease, and treatment characteristics. Demographical variables included age at diagnosis and sex. Physical characteristics included height, weight, and BMI. Disease characteristics included disease type and AJCC/TNM stage (8th edition). Data regarding treatment consisted of extent of surgery, RAI therapy dose, and (weight-corrected) LT4 dose. The study was approved by the Institutional Review Board of the Erasmus Medical Center (MEC-2012-561).

Peripheral blood samples were obtained from all participants at three visits, i.e. before surgery when patients were euthyroid (V=0), just before RAI therapy when patients were hypothyroid due to 3 to 4 weeks of THW (V=1), and six to nine months thereafter when patients were (subclinically) hyperthyroid during TSH-suppressive therapy by LT4 (V=2) (Figure 1). DTC-patients receiving RAI therapy after recom-

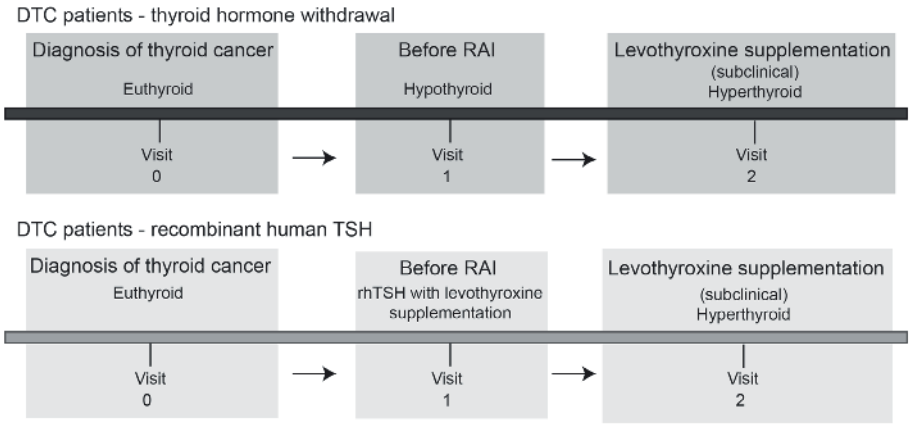

Figure 1. Differentiated thyroid cancer model.

DTC, differentiated thyroid cancer; RAI, radioactive iodine; rhTSH, recombinant human TSH.

binant human TSH (rhTSH) stimulation, instead of THW, were included to assess the influence of THW on thyroid hormone metabolism; these patients treated with rhTSH were (subclinically) hyperthyroid and not hypothyroid at V=1 (Figure 1).

Laboratory measurements

Peripheral blood sample measurements were processed on the same day. We measured TSH and free-T4 (FT4) as markers of thyroid function. Serum TSH was measured by a sandwich immunoassay (Immulite 2000 XPi; Siemens, Los Angeles, CA, USA), and serum FT4 was measured by a competitive immunoassay (Vitros ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Rochester, MI, USA). Of note, the FT4 immunoassay was calibrated to an in-house established equilibrium dialysis method, and as such a 23% correction above the manufacturer's instruction was needed. The magnitude of correction was comparable to the correction needed to agree with the reference measurement procedure (43). Creatinine was measured with an enzymatic method in heparin plasma (Cobas 8000 modular analyzer series; Roche, Indianapolis, IN, USA). Kidney function was estimated by calculation of estimated glomerular filtration rate (eGFR) using the CKD-epi equation (44).

We determined nine THM in serum samples stored at -80°C using our previously published method (38). Briefly, an internal standard and an anti-oxidant mixture was added to 500 µL of serum. Samples were precipitated with acetonitrile, followed by a solid phase extraction and evaporation to dryness under a stream of nitrogen at 50°C. After reconstitution, samples were injected onto a UPLC column (Waters Acquity UPLC BEH C18 1.7 µm, 130 Å, 1.0 x 100 mm) separating THM with a gradient of MilliQ + 0.1% formic acid as mobile phase A and acetonitrile + 0.1% formic acid

as mobile phase B. After liquid chromatography, compounds were detected with targeted mass spectrometry (Sciex QTRAP 6500+). Data were analyzed with analyst 1.7 software and multiQuant 3.0.3 software packages.

Statistical analysis

For continuous variables, we calculated means and standard deviations (SD), or in case of a non-normal distribution, medians with 25th – 75th interquartile ranges (IQR). For categorical variables, absolute numbers with percentages were recorded.

To assess differences in THM concentrations across the three different thyroid states, we used repeated measurement analysis to compare the three different thyroid states with each other for each THM; we used marginal models, and the appropriate covariance matrix that best fitted the data was selected to account for the correlation between the repeated measurements of each patient. In these analyses, we adjusted for age and sex. If needed, appropriate (natural) logarithmic transformation of the THM concentrations was applied to obtain normal distributions. We used linear regression to compare THM concentrations between the THW and rhTSH patients at V=2 adjusted for age, sex and weight-corrected LT4 dose. Correlations between the different THM were assessed at V=0 using the non-parametric Spearman correlation test as THM concentrations were non-Gaussian distributed. Linear regression was performed (at V=0) to determine the association between THM concentrations and age, sex or kidney function; all these analyses were performed unadjusted. To account for multiple testing in the different analyses, we used Bonferroni correction, and therefore p-values below 0.005 were considered statistically significant.

All analyses were performed using either SPSS Statistics for Windows (version 25.0) or R statistical software, including the 'nephro' package to calculate the eGFR with the CKD-Epi equation (version 3.4.1)(45).

RESULTS

Population characteristics

During the inclusion period, 119 patients were eligible for the study of which 42 patients were not included because they were not willing to participate (n=7), were missed to be included (n=11), or did not have a blood sample at each thyroid state (n=24). The remaining 77 patients were included.

Descriptive characteristics of the participants are shown in Table 2. Mean age of the participants was 49.0 years (range 19 to 80 years), BMI at baseline was 27.1 kg/m^2, 65% were women, 88% had papillary thyroid carcinoma, eGFR was 90.8 ml/

Table 2. Characteristics of the study population.

	Total Population (n=77)[a]	THW (n=68)[a]	rhTSH (n=9)[a]
Age at diagnosis (years)	49.0 ± 16.5	48.9 ± 16.3	49.2 ± 19.2
Women	50 (65%)	44 (65%)	6 (67%)
BMI at baseline (kg/m2)	27.1 ± 5.6	26.9 ± 5.6	28.2 ± 5.5
Papillary Thyroid Cancer	68 (88%)	62 (90%)	7 (78%)
AJCC/TNM Staging system (8th edition)			
Stage I	48 (62%)	41 (60%)	7 (78%)
Stage II	21 (27%)	21 (31%)	-
Stage III	6 (8%)	5 (7%)	1 (11%)
Stage IV	2 (3%)	1 (2%)	1 (11%)
Surgery (TT)	77 (100%)	68 (100%)	9 (100%)
Neck dissection	40 (52%)	39 (57%)	1 (1%)
RAI treatment	77 (100%)	68 (100%)	9 (100%)
After Withdrawal	68 (88%)	68 (100%)	-
Cumulative dose (mCi)	145 (50 – 148)	145 (133 – 148)	30 (29 – 66)
Time until V=2 (months)	6 (4 – 7)	6 (4 – 8)	4 (3 – 6)
eGFR (ml/min/1.73 m^2)	90.8 ± 22.1	-	-

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
TT, total thyroidectomy; RAI, radioactive iodine; mCi, milliCurie; THW, thyroid hormone withdrawal; rhTSH, recombinant human thyroid stimulating hormone.

min/1.73 m^2, and 88% received RAI therapy after THW and 12% after rhTSH. Median time between RAI therapy and V=2 was six months.

TSH, FT4 and THM

At V=0, all patients were euthyroid as confirmed by TSH and FT4 concentrations within the reference interval (Figure 2A). At V=1, patients undergoing THW were hypothyroid with a median TSH of 71.8 mU/L and a median FT4 of 2 pmol/L (Figure 2A). At V=1, patients treated with rhTSH had a median FT4 of 23.1 pmol/L (Figure 2A). At V=2, patients who underwent THW and patients treated with rhTSH were both (subclinically) hyperthyroid as confirmed by a median TSH of respectively 0.026 mU/L and 0.069, and a median FT4 of respectively 27.6 pmol/L and 22.8 pmol/L (Figure 2A).

The majority of the measurements of 3,5-T2 (>90%) and TA3 (100%) were below the lower limit of detection, and could therefore not be used in further analyses.

In patients undergoing THW, all THM were significantly decreased after thyroidectomy (hypothyroid state (V=1) compared to euthyroid state (V=0)), and all THM significantly increased after LT4 supplementation (hyperthyroid state (V=2) compared to hypothyroid state (V=1)) (Figure 2B and Table 3). When comparing the

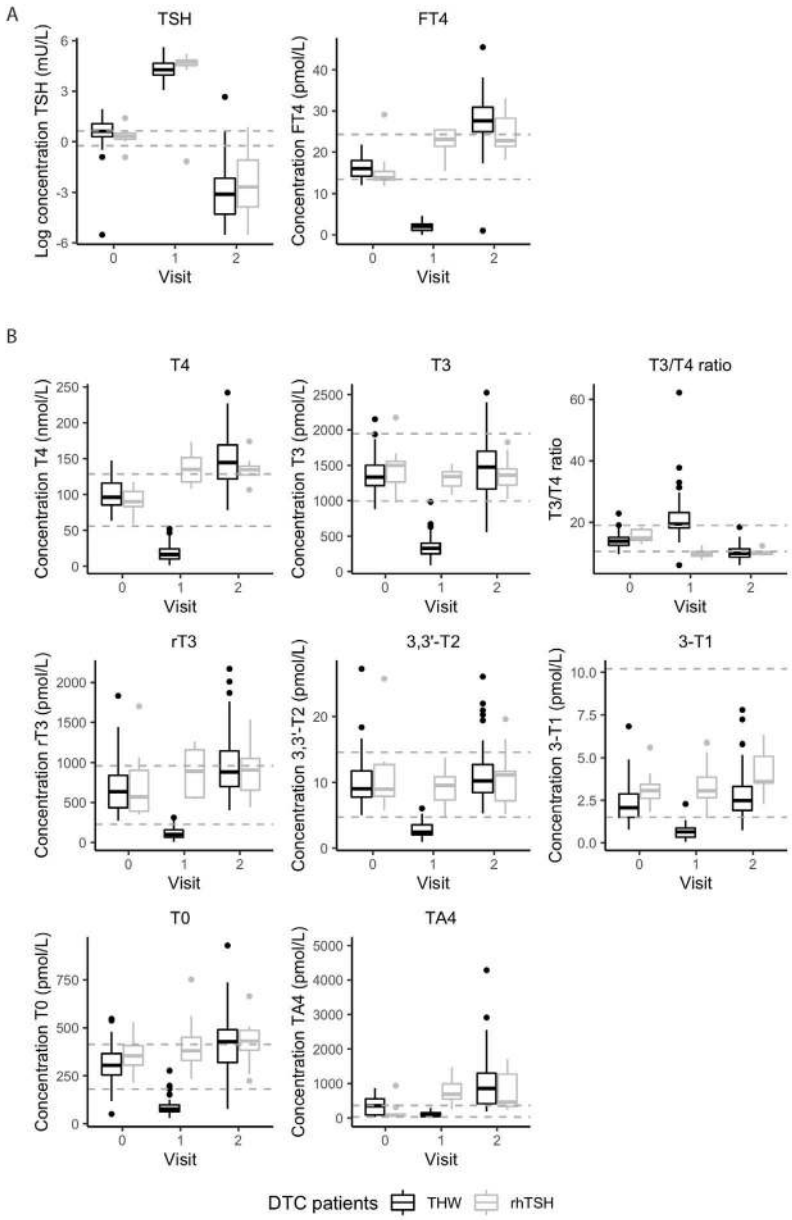

Figure 2. (A) TSH, FT4, and (B) Thyroid hormone metabolites at diagnosis of differentiated thyroid cancer (visit 0), after thyroidectomy with thyroid hormone withdrawal or recombinant human TSH (visit 1) and on LT4 replacement therapy (visit 2).

Box and Whiskers plots show the distribution in the thyroid hormone withdrawal (THW) group (black) and the recombinant human TSH (rhTSH) group (grey). The line located in the middle of the box represents the median, the top of the box represents the 75th percentile and the bottom of the box represents the 25th percentile. The ends of the whiskers are the 25th or 75th percentile ± 1.5x interquartile range. Solid circles represent outliers. Grey dashed lines represents the reference interval.

Table 3. Comparison of the three different thyroid states with each other and between patients treated with THW or rhTSH for each Thyroid Hormone Metabolite, TSH and FT4.

	V=0 vs. V=1		V=1 vs. V=2		V=0 vs. V=2		THW vs. rhTSH	
	β ± s.e.[a]	p-value[b]	β ± s.e.[a]	p-value[b]	β ± s.e.[a]	p-value[b]	β ± s.e.[c]	p-value[d]
TSH (mU/L)	3.78 ± 0.12	<0.0001	-7.77 ± 0.27	<0.0001	-4.00 ± 0.29	<0.0001	1.13 ± 0.72	0.122
FT4 (pmol/L)	-3.68 ± 0.39	<0.0001	4.22 ± 0.40	<0.0001	0.54 ± 0.02	<0.0001	-0.12 ± 0.07	0.067
T0 (pmol/L)	-1.29 ± 0.06	<0.0001	1.59 ± 0.06	<0.0001	0.30 ± 0.03	<0.0001	0.06 ± 0.13	0.645
3-T1 (pmol/L)	-1.33 ± 0.07	<0.0001	1.52 ± 0.07	<0.0001	0.20 ± 0.04	<0.0001	0.42 ± 0.14	0.0047
3,3'-T2 (pmol/L)	-1.32 ± 0.05	<0.0001	1.43 ± 0.05	<0.0001	0.11 ± 0.04	0.0046	-0.01 ± 0.12	0.965
T3 (pmol/L)	-1.46 ± 0.05	<0.0001	1.50 ± 0.05	<0.0001	0.04 ± 0.03	0.1382	-0.03 ± 0.08	0.741
rT3 (pmol/L)	-1.88 ± 0.07	<0.0001	2.25 ± 0.08	<0.0001	0.37 ± 0.04	<0.0001	-0.08 ± 0.14	0.551
T4 (mmol/L)	-1.85 ± 0.07	<0.0001	2.23 ± 0.07	<0.0001	0.38 ± 0.02	<0.0001	-0.03 ± 0.08	0.683
TA4 (pmol/L)	-1.10 ±0.10	<0.0001	2.39 ± 0.10	<0.0001	1.30 ± 0.12	<0.0001	-0.34 ± 0.32	0.290

[a] values are the differences between both thyroid states expressed in natural log transformed units.
[b] p-Values (Age and Sex adjusted) for comparing both thyroid states in THW patients.
[c] values are the differences between THW and rhTSH expressed in natural log transformed units.
[d] p-Values (Age, Sex and weight-corrected LT4 dose adjusted) for comparing rhTSH and TWH patients at V=2.
p-values below 0.005 are considered significant.
THW, thyroid hormone withdrawal; rhTSH, recombinant human thyroid stimulating hormone; s.e., standard error.

hyperthyroid state with the euthyroid state, all THM were significantly increased except for T3 (p=0.138) (Table 3).

In patients treated with rhTSH, all THM remained normal or increased when comparing V=1 to V=0 (Figure 2B). At V=2, adjusted for age, sex, and weight-corrected LT4 dose, none of the THM, except for 3-T1 (p=0.0047), were significantly different between rhTSH patients and THW patients.

Serum levels of T3 and T4 are dependent on deiodinating enzymes (deiodinases), thyroid function and the binding capacity of binding proteins. Therefore, ratios between these THM are thought to better reflect peripheral deiodination. Therefore, we calculated the T3/T4 ratios in each thyroid state. In patients undergoing THW, the T3/T4 ratio significantly increased in the hypothyroid state and significantly decreased after TSH suppressive LT4 therapy compared to the euthyroid state. (Figure 2B). In patients treated with rhTSH, the T3/T4 ratio was similarly decreased at V=1 and at V=2 compared to the euthyroid state (Figure 2B).

TSH did not correlate with THM and FT4 concentrations in the euthyroid state (Supplemental Table 1). In the euthyroid state, FT4 was positively correlated with all THM, except for 3-T1 and TA4 (Supplemental Table 1). Every THM was correlated to its precursor except for 3-T1 with 3,3'-T2 and TA4 with T4 (Supplemental Table 1).

Association of THM concentrations with age, gender and kidney function

At V=0, higher age was significantly associated with higher 3-T1 concentrations ($p<0.001$) and lower T4 concentrations (p=0.002). Besides, a higher eGFR was significantly associated with lower 3-T1 concentrations ($p<0.001$), and higher T4 concentrations (p=0.003) (Supplemental Figure 1 and Supplemental Table 2). Of the other THM, women had significantly higher L-thyronine (T0) concentrations than men (p=0.003) (Supplemental Figure 1 and Supplemental Table 2).

DISCUSSION

To our knowledge, this is the first prospective study investigating the course of different THM concentrations and thyroid hormone metabolism across different thyroid states in the same patient. At the individual level, all THM decreased after THW and all THM increased with TSH suppressive LT4 therapy, with T3 concentrations remaining within the reference interval. All measured THM, except for T3, follow the same trend as T4, suggesting that all measured thyroid hormone metabolites are produced by peripheral metabolism of T4 and have no or negligible thyroidal origin. This is also reflected by T3 which has thyroidal origin (approximately 20%)

and remained within the reference interval in the absence of a thyroid gland and treatment with TSH suppressive LT4 therapy.

In our study, patients treated for DTC by total thyroidectomy followed by RAI therapy are used as a model to study the course of different THM concentrations across different thyroid states. This DTC model is a known concept (40, 46-48), and has several advantages: 1) Three thyroid states can be studied in the same patient in a prospective manner, 2) Hypothyroidism without residual TH production by the thyroid gland is ensured by total thyroidectomy without LT4 supplementation, and 3) In absence of the thyroid gland, (subclinical) hyperthyroidism is caused by exogenous oral LT4 therapy, and therewith all thyroid hormone metabolites are directly or indirectly derived from LT4.

In the hypothyroid state compared to the euthyroid state, all THM decreased significantly in patients undergoing THW, while the T3/T4 ratio increased. This is in correspondence with most literature on 3-T1, 3,3'-T2 and T3/T4 ratios (16, 24-31, 49). The decrease of all THM after thyroidectomy and subsequent rise after LT4 therapy in our study emphasizes the dependence of the formation of these thyroid hormone metabolites on T4. The increase of the T3/T4 ratio in patients undergoing THW is in line with reports showing that in a hypothyroid state, the body's response is to ensure sufficient T3 concentrations via transporters, deiodinases, and thyroid hormone receptors (50). In the hypothyroid state, T3 concentrations remain at approximately 400 pmol/L, while these patients do not have a thyroid gland and do not receive exogenous oral LT4 therapy. The T3 concentration in the circulation at this hypothyroid state is most likely explained by T3 production via peripheral metabolism of residual T4 produced before the thyroidectomy. However, it might be that a small residual of the thyroid gland, which still produces a small amount of T3, was left after surgery.

In the (subclinically) hyperthyroid state compared to the euthyroid state, all THM increased and the T3/T4 ratio decreased after TSH suppressive LT4 therapy in patients that underwent THW and in patients that were treated with rhTSH. Increased 3,3'-T2, T3, T4 and T3/T4 ratios are in correspondence with earlier reports (32, 49). This suggests that exogenous LT4 restores thyroid hormone metabolite concentrations via peripheral metabolism in extrathyroidal tissues, as all patients underwent a total thyroidectomy. All thyroid hormone metabolites follow the same trend as T4. T3 concentrations in (subclinically) hyperthyroid thyroidectomized patients on LT4 therapy are similar to euthyroid concentrations. This is likely explained by the lack of thyroidal T3 production since T3 can be formed via two different routes: 1) T3 production by the thyroid gland (approximately 20% of serum T3 is derived from thyroidal T3 secretion in the euthyroid state) and 2) Peripheral metabolism of T4 via deiodination. In our study, T3 formation at V=2 is completely dependent on

peripheral metabolism, as patients usually do not have any remaining thyroid tissue after surgery and RAI therapy. Peripheral metabolism likely explains our finding that T3 concentrations were within the reference interval while FT4 levels were elevated, which is a known phenomenon (14, 15).

To our knowledge, this is the first study investigating the correlation of TSH or FT4 with T0, 3-T1 and 3,3'-T2, which was previously impeded by the lack of selective analytical methods to quantify thyroid hormone metabolites. Unexpectedly, TSH was not correlated with FT4 or any of the other THM in the euthyroid state. The previously reported log-linear relationship between TSH and FT4 in a large population-based study was weak (51), suggesting that our study might be underpowered to identify such a relationship. The euthyroid state itself is another possible explanation for the absence of the correlation between TSH and FT4 as this relationship is mainly observed in diseased states. The absence of a correlation between TSH and TA4 was in accordance with previous literature in healthy individuals and patients with Graves' disease (35).

Associations between serum creatinine levels and T3 and T4 have been reported in the literature, also across different thyroid states (36, 37, 40). The relationship between TH levels and kidney function is well-known, but the causality remains a topic of interest in both health and disease states (52). In the euthyroid state, our data are in agreement with these studies for T4, but not for T3. This difference might be ascribed to our smaller sample size, the lower selectivity of (radio) immunoassays and/or only looking at a single thyroid state instead of comparing different thyroid states. Especially in kidney dysfunction, potential interferences of TH binding proteins such as thyroxine-binding globulin, transthyretin and albumin become more relevant. We discovered that women had significantly higher T0 concentrations than men, but we do not have a good explanation for this sex difference. Therefore, further research is needed to understand this result. We were also the first to discover the significant association between age and 3-T1 and T4. Earlier longitudinal studies showed mixed results for FT4 and T3 changes with increasing age (53). Therefore, further studies on this topic are needed. Further research into the relation between THM and other factors such as body weight, smoking or quality of life is also of interest.

The current study has some limitations. First, we would have preferred to measure all THM with the current THM panel, but unfortunately lower concentrations of 3,5-T2 and TA3 could not be measured suggesting our method might not be sensitive enough. The strength of our innovative method is that we measure a panel of metabolites, but this approach also restricts the possibilities for optimizing the sample preparation, the liquid chromatography and the mass spectrometry settings. Other thyroid hormone metabolites with only a positive charge such as

3-iodothyronamine are also of interest, but the selectivity of our sample preparation for negatively charged ions limits this possibility. Second, our study might be underpowered especially for the group of patients treated with rhTSH. Third, kidney function measurements were unfortunately not available in the hypothyroid and (subclinically) hyperthyroid state impeding further thorough investigations.

CONCLUSIONS

All THM decrease after a thyroidectomy and increase under TSH suppressive LT4 therapy, indicating that formation of thyroid hormone metabolites is dependent on peripheral extrathyroidal metabolism of T4, with the exception of T3 that has some thyroidal origin. Further research into the relation between THM and quality of life is needed to assess whether THM concentrations play a role in the persistent complaints in patients with hypothyroidism undergoing LT4 replacement therapy.

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Supplemental Table 1. Correlations between Thyroid Hormone Metabolites in THW patients in the euthyroid state.

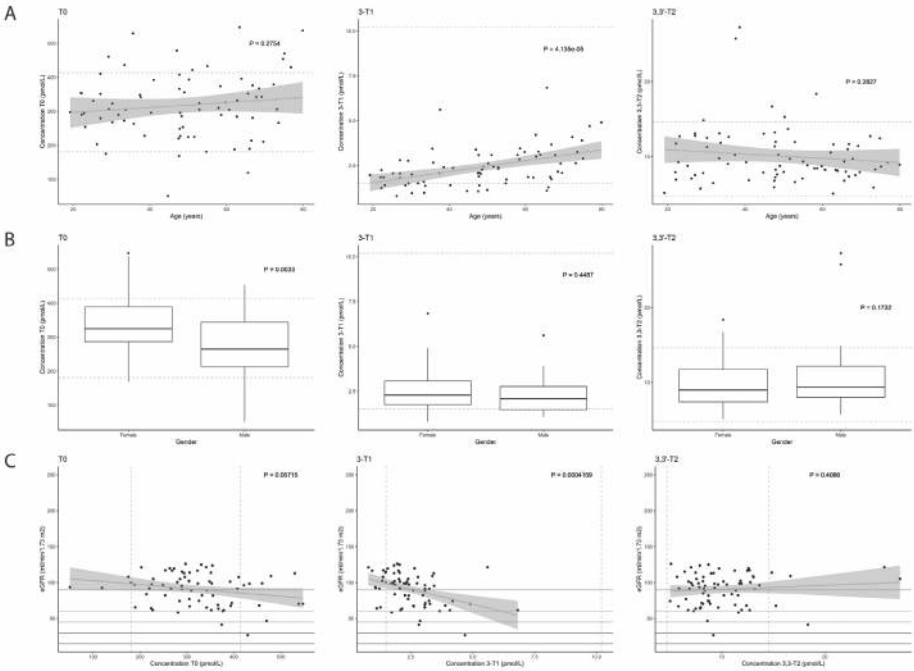
	TSH	FT4	T0	3-T1	3,3-T2	T3	rT3	T4	TA4
TSH	-								
FT4	-0.053 (0.680)	-							
T0	0.169 (0.200)	**0.402 (0.002)**	-						
3-T1	0.006 (0.965)	0.201 (0.126)	**0.586 (<0.001)**	-					
3,3'-T2	0.071 (0.585)	**0.367 (0.003)**	0.274 (0.027)	0.109 (0.386)					
T3	0.112 (0.384)	**0.405 (0.001)**	0.125 (0.319)	-0.062 (0.624)	**0.566 (<0.001)**	-			
rT3	0.106 (0.413)	**0.517 (<0.001)**	0.220 (0.078)	0.015 (0.906)	**0.778 (<0.001)**	**0.708 (<0.001)**	-		
T4	0.009 (0.942)	**0.437 (<0.001)**	0.107 (0.369)	-0.089 (0.479)	0.309 (0.010)	**0.712 (<0.001)**	**0.502 (<0.001)**	-	
TA4	-0.117 (0.363)	-0.004 (0.976)	-0.043 (0.736)	-0.100 (0.426)	-0.160 (0.194)	0.085 (0.490)	0.026 (0.833)	0.211 (0.084)	-

Values are Spearman Correlation Coefficients with (corresponding p-Value).
Significant p-values (p<0.005) in bold.

Supplemental Table 2. Linear regression Thyroid Hormone Metabolites with age, gender and kidney function.

THM	Age Slope (β); se (P-value[a,b])	Gender Slope (β); se (P-value[a,c])	eGFR Slope (β); se (P-value[a,d])
T0 (pmol/L)	0.728; 0.663 (0.275)	-68; 22.4 **0.0033**	-0.054; 0.028 (0.057)
3-T1 (pmol/L)	0.0302; 0.00692 **($4.1*10^{-5}$)**	-0.21; 0.276 0.449	-8.3; 2.24 **(0.00042)**
3,3'-T2 (pmol/L)	-0.0288; 0.0266 (0.283)	1.25; 0.91 0.173	0.56; 0.67 (0.41)
T3 (pmol/L)	-5.17; 1.8 (0.00527)	-56.4; 64.8 0.387	0.0232; 0.0094 (0.0161)
rT3 (pmol/L)	-3.07; 2.19 (0.166)	-35.9; 76.3 0.64	0.004; 0.008 (0.629)
T4 (nmol/L)	-0.286; 0.0902 **(0.00224)**	-6.96; 3.2 0.033	0.561; 0.184 **(0.003)**
TA4 (pmol/L)	-1.97; 1.83 (0.285)	-68.1; 62.8 0.282	0.00654; 0.00979 (0.507)

[a] p-value < 0.005 is considered significant
[b] per year increase.
[c] female as reference.
[d] per ml/min/1.73m2 increase.
Significant p-values (p<0.005) in bold.
eGFR, estimated glomerular filtration rate.



Supplemental Figure 1. Linear regression of T0, 3-T1, 3,3'-T2 with (A) age, (B) gender, and (C) kidney function at the euthyroid state (V=0).

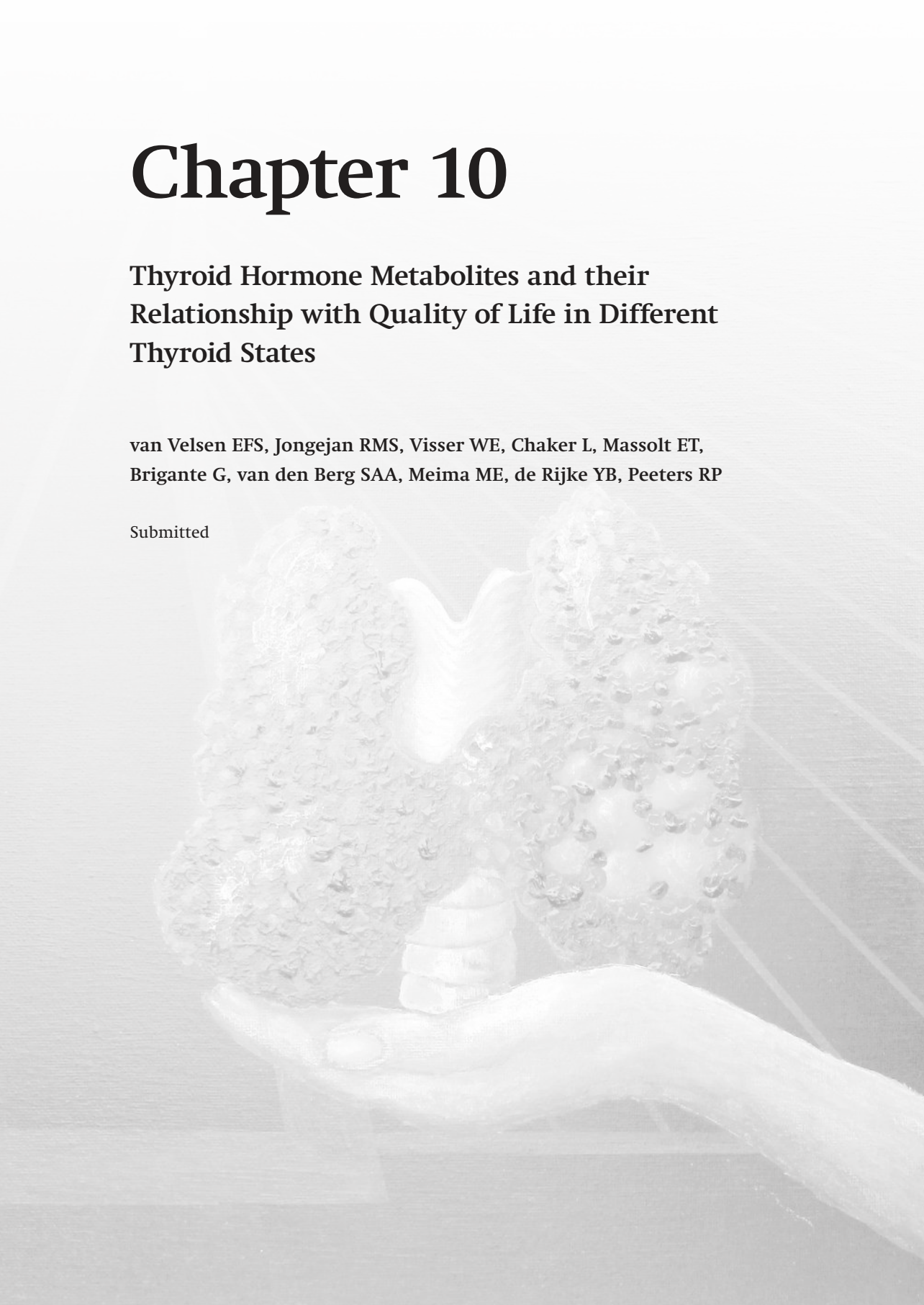
T0, L-thyronine; 3-T1, 3-monoiodothyronine; 3,3'-T2, 3,3'-diiodothyronine; eGFR, estimated glomerular filtration rate.

Chapter 10

Thyroid Hormone Metabolites and their Relationship with Quality of Life in Different Thyroid States

van Velsen EFS, Jongejan RMS, Visser WE, Chaker L, Massolt ET, Brigante G, van den Berg SAA, Meima ME, de Rijke YB, Peeters RP

Submitted



ABSTRACT

Background

Up to 15% of the hypothyroid patients have persistent complaints, despite achieving euthyroidism with levothyroxine (LT4) treatment. The cause for this decreased Quality of Life (QoL) remains unknown, but changes in concentrations of thyroid hormone metabolites (THM) have been proposed as a possible mechanism. Therefore, the aim of our study was to explore a possible relationship between THM and QoL using patients with differentiated thyroid cancer (DTC).

Methods

We prospectively included DTC patients, aged 18 to 80 years, subjecting them to different thyroid states during clinical management of their disease. Peripheral blood samples and QoL questionnaires were obtained at three visits, i.e. before surgery (euthyroid), before radioiodine therapy (hypothyroid), and six months later on TSH suppressive therapy using LT4 replacement therapy ((subclinical) hyperthyroid). Seven THM, and T3 and T4 [THM] were measured in serum with a recently developed LC-MS/MS method. To investigate the relationship between THM and QoL, we used linear regression at each of the three visits (cross-sectional analysis), and repeated measurement analysis to investigate changes between each of the three visits (longitudinal analysis).

Results

In 63 patients (mean age 49 years; 64% women), THM measurements and QoL scores were available. Cross-sectionally, there was no association between THM and QoL. Longitudinally, higher concentrations of T0, 3-T1, 3,3'-T2, T3, rT3 and TA4 were associated with significantly less complaints in six different QoL domains compared to those with lower concentrations of these metabolites. However, no consistent patterns in the relationships between THM and QoL domains were seen.

Conclusions

We showed that 3-T1, 3,3'-T2, T3, rT3, T4 and TA4 are significantly associated with changes in QoL between euthyroid, hypothyroid and (subclinical) hyperthyroid state for specific QoL scores, but no consistent relationship was found for either THM or QoL scores. Also, no cross-sectional associations were observed at either hypo, (subclinical) hyper on LT4 replacement therapy, or euthyroid state. Further research in patients with thyroid disease is needed to confirm our findings of the absence of an association between THM and QoL.

INTRODUCTION

Hypothyroidism is a very common endocrine disorder (1) and treatment is readily available. Although biochemical euthyroidism can be achieved by replacement therapy with levothyroxine (LT4), a substantial part of the patients (± 10-15%) show significant impairment of physical and psychological well-being compared to matched controls or the general population (2-7). Several possible explanations for these persistent symptoms exist. First, thyroid autoimmunity in itself, apart from its effects on thyroid function, could cause persisting symptoms (8-10). However, thyroid cancer survivors that have underwent thyroidectomy also continue to have a decreased quality of life (QoL) in different domains despite being on replacement therapy (11-17). Second, several studies have shown LT4 replacement therapy to be inadequate in restoring physiological T4 and T3 concentrations in serum and tissue (18, 19). Patients on LT4 replacement therapy require higher concentrations of free-T4 in order to maintain a normal T3 (20), and as a consequence, the T3/T4 ratio in these patients is significantly altered. Third, next to T3, thyroid hormone metabolite (THM) concentrations may be altered in hypothyroid patients on LT4 replacement therapy as well (13). Altered serum concentrations of reverse-T3 (rT3), 3,3'-diiodothyronine (3,3'-T2), 3,5-T2, 3-monoiodothyronine (3-T1), L-thyronine (T0), 3,3',5,5'-tetraiodothyroacetic acid (TA4), and TA3 have been reported in hypo- and hyperthyroidism (21). Further, one study found higher 3,5-T2 concentrations in thyroid cancer patients after thyroidectomy compared to healthy controls (22). Besides, effects for 3,5-diiodo-L-thyronine (3,5-T2) (23) and 3, 5, 3'-triiodothyroacetic acid (TA3) on metabolism have been reported (24).

Earlier studies investigating the relationship between thyroid function and QoL were inconclusive and predominantly performed in patients with autoimmune thyroid disease (2-4, 25). In thyroid cancer survivors, regularly measured thyroid hormones (TH) like T4, T3, and THM like rT3, but also 3,5-T2, were not associated with QoL (13, 15). To our knowledge, the relationship between other THM and QoL has not been studied previously. One of the factors limiting such a study is that until recently, only (radio)immunoassays were available for 3-T1, 3,5-T2, 3,3'-T2, TA3 and TA4, which showed large variations in measured concentrations (26). Possible causes for these large variations are the use of nonselective antibodies and nonstandardized methods (27). We recently developed a LC-MS/MS method that can adequately quantify a panel of seven THM and T3 and T4 [THM] in human serum and established reference intervals in healthy adults (26). Subsequently, we established THM concentrations in the hypo, hyper, and euthyroid state of the patients who were treated for differentiated thyroid cancer (DTC) (21).

Historically, patients with DTC have been treated with total thyroidectomy followed by radioiodine (RAI) therapy, and subsequently TSH-suppressive therapy using LT4 replacement (28-30). During treatment, patients are usually euthyroid before surgery, hypothyroid after three to four weeks of thyroid hormone withdrawal, and (subclinical) hyperthyroid during TSH-suppressive therapy using LT4. Although current treatment protocols for DTC have become less aggressive (i.e. hemithyroidectomy instead of total thyroidectomy, and additional RAI therapy in only a subgroup of patients), patients undergoing the full treatment protocol are a unique model to study THM and QoL during altered thyroid states as individual patients have these three different thyroid states (21, 22, 31, 32). Therefore, the aim of our study was to explore a possible relationship between THM and QoL in patients treated for DTC.

MATERIALS AND METHODS

Study population

In correspondence with our earlier study regarding measurements of THM across hypo, hyper, and euthyroid state (21), we included patients aged 18 to 80 years who were scheduled for their first treatment for DTC at the Erasmus Medical Center, Rotterdam, The Netherlands between April 2013 and September 2018. All included patients had tumors above one cm and were treated in line with the current Dutch guideline with a total thyroidectomy followed by RAI therapy (33). Exclusion criteria were inadequate understanding of the Dutch language, receiving RAI therapy after recombinant human TSH (rhTSH) stimulation, another active malignancy, known thyroid disease, an active inflammatory disease, or use of drugs known to influence thyroid hormone metabolism.

We recorded demographic, disease, and treatment characteristics. Further, presence of recurrent nerve paralysis and hypoparathyroidism was recorded.

Peripheral blood samples were obtained from all participants at three different visits, i.e. before surgery when patients were euthyroid (V=0), just before RAI therapy when patients were hypothyroid due to three to four weeks of thyroid hormone withdrawal (V=1), and six months thereafter when patients were (subclinical) hyperthyroid on TSH-suppressive therapy using LT4 replacement (V=2). At every visit, all participants also filled in three different QoL questionnaires, namely the ThyPRO, the Multidimensional fatigue index-20 (MFI-20) and the RAND-36; see section on Quality of Life questionnaires for details.

The study was approved by the Institutional Review Board of the Erasmus Medical Center (MEC-2012-561).

Thyroid function and Thyroid Hormone Metabolites

In fresh serum samples, TSH was measured by an immunometric assay (Immulite 2000 XPi; Siemens, Los Angeles, CA, USA) and free-T4 was measured by a chemoluminescence assay (Vitros ECI Immunodiagnostic System; Ortho-Clinical Diagnostics, Rochester, MI, USA).

An in-house LC-MS/MS method was used to determine T0, 3-T1, 3,5-T2, 3,3'-T2, T3, rT3, T4, TA3 and TA4 in serum (26). Briefly, serum, internal standards and antioxidants were mixed, followed by protein precipitation with acetonitrile and solid phase extraction. After solid phase extraction, samples were evaporated to dryness under a stream of nitrogen at 50°C and reconstituted in 10% acetonitrile with 0.1% ammonia solution. Samples were injected onto a column (Waters Acquity UPLC BEH C18 1.7 µm, 130 Å, 1.0 x 100 mm) and THM were separated with a gradient of mobile phase A (MilliQ with 0.1% formic acid) and mobile phase B (Acetonitrile with 0.1% formic acid) followed by detection with mass spectrometry (Sciex Qtrap 6500+). Analyst software (version 1.7) and multiQuant (version 3.0.3) software packages were used for data analysis.

Quality of Life questionnaires

The ThyPRO, the MFI-20 and the RAND-36 questionnaires were used to assess QoL.

The ThyPRO is a thyroid-specific QoL questionnaire, which consists of 85 questions summarized in 13 scales (34). Scores vary from 0 to 100 with higher scores indicating more thyroid-related complaints. We used the Composite, Tiredness, Cognitive problems, Anxiety, Depressivity, Social Impairment, and Daylife Impairment scores (35).

The MFI-20 is a 20-item self-report instrument designed to assess fatigue (36). Scores vary from 0 to 20 with higher scores indicating more fatigue. We used the General, Physical and Mental Fatigue scores.

The RAND-36 item health survey (RAND-36) is the validated Dutch version of the Short Form (36) Health Survey (SF-36) (37-39), which is designed to assess overall health-related QoL during the previous 30 days. It consists of nine subscales and two summary scales. Scores vary from 0 to 100 with higher scores associated with better QoL. We used the General Health, General Mental Health, Vitality, Physical functioning and Social functioning scores.

Statistical analysis

To assess the association between THM and QoL cross-sectionally, we used linear regression at each of the three thyroid states separately. Next, we used marginal models for continuous data to explore the (possible) association of THM with differences in QoL between the thyroid states, while accounting for the correlation

between the repeated measurements of each patient, and for the fact that also THM change over time; the appropriate covariance matrix that best fitted the data was selected. We compared euthyroid with hypothyroid, euthyroid with hyperthyroid, and hypothyroid with hyperthyroid. For the assessment of a possible interaction of THM with the visits, i.e. investigating whether the (possible) association between THM and QoL is different for higher or lower THM concentrations, p-values below 0.05 were considered significant.

If needed for cross-sectional or longitudinal analyses, appropriate transformations ((natural) logarithmic, square root) of the QoL scores were applied to obtain normal distributions. For both cross-sectional and longitudinal analyses, to correct for the large number of statistical tests we used the Benjamini-Hochberg false discovery rate (FDR) controlling procedure (40). All analyses were performed unadjusted, and also adjusted for age and sex, using either SPSS Statistics for Windows (version 25.0) or R statistical software (version 3.4.1) (41).

RESULTS

Population characteristics

A total of 104 patients were eligible for the study of which 41 patients were not included because they either did not want to participate (n=7), were missed at inclusion (n=10), or completed less than two questionnaires or had less than two blood samples (n=24). Therefore, the data analyses presented here were performed in the remaining 63 patients.

Table 1 lists the descriptive characteristics of the study population. Mean age was 49.4 years and, 40 patients (64%) were women. At V=2, 15 patients (24%) had (temporary) hypoparathyroidism, and 7 patients (11%) had recurrent nerve paralysis. Median time between RAI therapy and V=2 was six months (25-75 IQR: 4 – 7 months).

Thyroid function and Thyroid Hormone Metabolites

In accordance with our previous study (21), TSH and free-T4 concentrations confirmed that patients were euthyroid at V=0, hypothyroid at V=1, and (subclinical) hyperthyroid at V=2. Interestingly, all THM follow the same trend as free-T4 at V=2, except for T3, 3-3'-T2 and 3-T1, which remained within the reference interval at V=2 (Table 2). 3,5-T2 and TA3 could not be used for QoL analysis as the majority of the measurements were below the lower limit of the measurement interval (LLMI). Further, THM concentrations were missing for 5, 7 and 2 patients at respectively V=0, V=1 and V=2.

Table 1. Characteristics of the study population.

		Total Population (n=63)[a]
Age at diagnosis (years)		49.4 ± 16.7
Women		40 (64%)
BMI a baseline (kg/m^2)		27.1 ± 5.5
Papillary Thyroid Cancer		56 (89%)
AJCC/TNM Staging system (8th edition)		
	Stage I	38 (60%)
	Stage II	19 (30%)
	Stage III	5 (8%)
	Stage IV	1 (2%)
Total thyroidectomy		63 (100%)
Neck dissection		35 (56%)
RAI treatment after Withdrawal		63 (100%)
	Cumulative dose (mCi)	145 (130 – 148)
Hypoparathyroidism at V=2		15 (24%)
Recurrent nerve paralysis at V=2		7 (11%)

[a] Values are means (± standard deviation), medians (25-75 IQR) or numbers (percentages).
RAI, radioactive iodine; mCi, milliCurie.

Table 2. Thyroid Function tests and Thyroid Hormone (Metabolites) across different thyroid states.

	V=0[a]	V=1[a]	V=2[a]	Reference intervals[b]
TSH (mU/l)	1.85 (1.34 – 2.89)	72.3 (53.1 – 110.0)	0.02 (0.01 – 0.11)	0.4 – 4.3
free-T4 (pmol/L)	16.2 (14.3 – 18.0)	2.1 (0.9 – 2.6)	27.6 (25.1 – 31.0)	11 – 25
T0 (pmol/L)	304 (254 – 372)	77 (63 – 102)	429 (321 – 497)	181 - 413
3-T1 (pmol/L)	2.08 (1.60 – 2.96)	0.68 (0.34 – 0.91)	2.56 (1.98 – 3.34)	1.5 – 10.2
3,3'-T2 (pmol/L)	8.79 (7.49 – 11.72)	2.42 (1.99 – 3.57)	10.13 (8.39 – 13.00)	4.7 – 14.6
T3 (pmol/L)	1329 (1189 – 1516)	329 (227 – 398)	1463 (1144 – 1702)	995 - 1948
rT3 (pmol/L)	623 (429 – 833)	99 (61 – 152)	870 (699 – 1107)	226 - 961
T4 (nmol/L)	96.0 (84.7 – 116.4)	17.0 (9.8 – 24.2)	145.0 (123.4 – 169.1)	56 – 128
TA4 (pmol/L)	370 (85 – 565)	95 (39 – 150)	862 (406 – 1343)	30 - 363

[a] Values are medians (25-75-IQR).
[b] Values are 2.5th - 97.5th percentiles.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state.

Quality of Life

Table 3 lists the QoL scores at each visit for the three different questionnaires. In the majority of the QoL scores, lowest QoL was observed at V=1, i.e. after thyroid hormone withdrawal. QoL at V=2 was slightly lower than QoL at V=0. Exceptions

Table 3. Quality of Life across different thyroid states.

	V=0[a]	V=1[a]	V=2[a]	Reference populations (15,37,44)[b]
MFI-20[c]				
General fatigue	13 (7 – 17)	16 (12 – 19)	14 (9 -17)	8.1 ± 3.4
Physical fatigue	9 (6 – 14)	14 (10 – 18)	13 (7 – 16)	6.7 ± 2.6
Mental fatigue	11 (6 – 15)	12 (9 – 16)	12 (8 – 16)	6.9 ± 3.3
RAND-36[d]				
Vitality	56 (44 – 75)	44 (31 – 63)	50 (38 – 69)	67.4 ± 19.9
General Mental Health	70 (55 – 80)	75 (60 – 85)	70 (53 – 85)	76.8 ± 18.4
General Health Perception	60 (50 – 75)	60 (45 – 70)	55 (40 – 70)	72.7 ± 22.7
Physical functioning	95 (75 – 100)	70 (55 – 85)	85 (68 – 95)	81.9 ± 23.2
Social functioning	75 (63 – 100)	63 (38 – 75)	75 (50 -100)	86.9 ± 20.5
ThyPRO[e]				
Composite	26 (11 – 39)	36 (20 – 47)	30 (15 – 47)	*na*
Tiredness	32 (21 – 52)	54 (39 – 64)	43 (21 – 64)	35 ± 21
Cognitive Problems	13 (0 – 31)	25 (8 – 46)	25 (8 – 50)	14 ± 17
Anxiety	29 (21 – 48)	25 (8 – 38)	25 (13 – 46)	13 ± 16
Depressivity	21 (11 – 36)	21 (14 – 39)	29 (7 – 43)	21 ± 18
Social Impairment	6 (0 – 16)	13 (0 – 19)	13 (0 – 25)	*na*
Impaired Daylife	8 (0 – 36)	46 (20 – 60)	21 (0 – 50)	*na*

[a] Values are medians (25-75-IQR).
[b] Values are means (± standard deviation) obtained from reference populations (15,37,44).
[c] For MFI-20, higher scores indicating more fatigue.
[d] For RAND-36, higher scores indicating with better Quality of Life.
[e] For ThyPRO, higher scores indicating more thyroid-related complaints.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state; na, not available.

were the RAND-36 General Mental Health (best QoL at V=1) and General Health Perception (QoL at V=0 and V=1 around same level), and the ThyPRO Cognitive Problems (QoL at V=1 and V=2 around same level), Anxiety (best QoL at V=1 and V=2), Depressivity (worst QoL at V=2) and Social Impairment scores (QoL at V=1 and V=2 around same level). It must be noted that questionnaires were missing in 8 (V=0, V=1) and 10 (V=2) patients.

Thyroid Hormone Metabolites and Quality of Life

With the cross-sectional analysis, the association between THM and QoL in each thyroid state was investigated separately. There were no significant associations between THM and QoL, either unadjusted or adjusted (Supplemental Table 3).

With the longitudinal analysis, the association of THM with QoL, comparing the different thyroid states within individuals with each other, was investigated (see

Supplemental Table 4 to 6). Using V=0 and V=1, after adjustments, 3,3'-T2 and rT3 for the MFI-20 Mental Fatigue score, T0 and 3-T1 for the RAND-36 Physical Function score, and TA4 for the ThyPRO Impaired Daylife score showed a significant interaction with QoL differences between the visits, indicating that changes in QoL between V=0 and V=1 differ for patients with higher or lower concentrations of these THM (see Figure 1 to 3). Higher rT3 and 3-3'-T2 concentrations at V=1 were significantly associated with less Mental Fatigue complaints compared to lower concentrations, while at V=0 there was a small difference between lower and higher concentrations of these THM. This same pattern was seen for T0 and 3-T1 for the RAND-36 Physical Function score, and for TA4 for the ThyPRO Impaired Daylife score.

Using V=1 and V=2, after adjustments, T0 for the RAND-36 Physical Function, and TA4 for the ThyPRO Impaired Daylife score showed significant interactions with QoL differences between the visits (see Figure 2 and 3). Higher TA4 concentrations at V=1 were associated with less complaints, while at V=2 there was hardly any difference in QoL between higher and lower TA4 concentrations. Higher T0 concentrations at V=1 were significantly associated with less RAND-36 Physical Function complaints compared to lower concentrations, while at V=2 there was a smaller difference between lower and higher concentrations.

Using V=0 and V=2, after adjusting, rT3 for the RAND-36 Vitality score, T3 for the ThyPRO Social Impairment score, and TA4 for the ThyPRO Tiredness score showed a significant interaction with QoL differences between the visits, indicating that differences in QoL between V=0 and V=2 differ for patients with higher or lower concentrations of these THM (see Figure 2 and 3). Lower rT3 concentrations at V=0 were associated with a higher RAND-36 Vitality score compared to those with higher rT3 concentrations, while at V=2, this differences was less pronounced. Higher T3

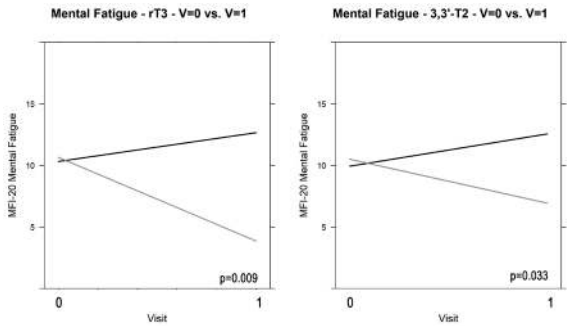


Figure 1. Longitudinal Associations between Thyroid Hormone Metabolites and MFI-20 changes between thyroid states.

Lines show significant association for lower THM (Black) and for higher THM (Grey) in case of interaction. For MFI-20, higher scores indicating more fatigue. p-values < 0.05 are considered significant in case of interaction.

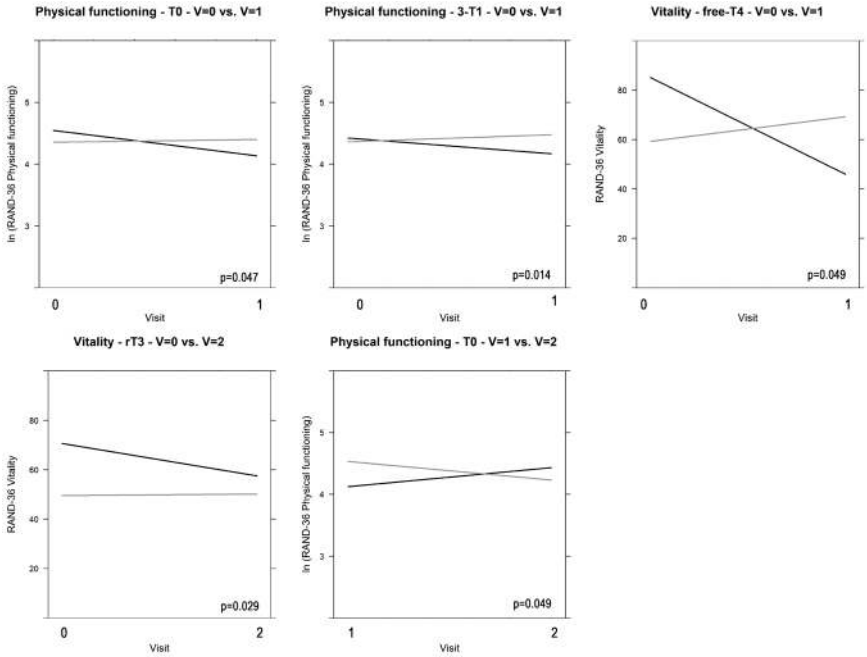


Figure 2. Longitudinal Associations between Thyroid Hormone Metabolites and RAND-36 changes between thyroid states.

Lines show significant association for lower THM (Black) and for higher THM (Grey) in case of interaction. For RAND-36, higher scores indicating better quality of life. p-values < 0.05 are considered significant in case of interaction.

concentrations were associated with a lower ThyPRO Social Impairment score at V=0, but a higher score at V=2 compared to lower concentrations. Higher TA4 concentrations at V=0 were associated with a higher ThyPRO Tiredness score compared to those with lower scores, while at V=2 there was hardly any difference anymore.

DISCUSSION

To our knowledge, this is the first study that prospectively investigated a possible relationship between a full spectrum of THM and QoL. This study shows that T0, 3-T1, 3,3'-T2, T3, rT3 and TA4 are significantly associated with QoL differences between euthyroid, hypothyroid and (subclinical) hyperthyroid state in specific QoL domains during treatment for DTC. In general, those with higher concentrations of these THM have less complaints, but no consistent patterns were found between THM concentrations and QoL domains. Cross-sectionally, THM were not associated with QoL at either hypo, (subclinical) hyper, or euthyroid state.

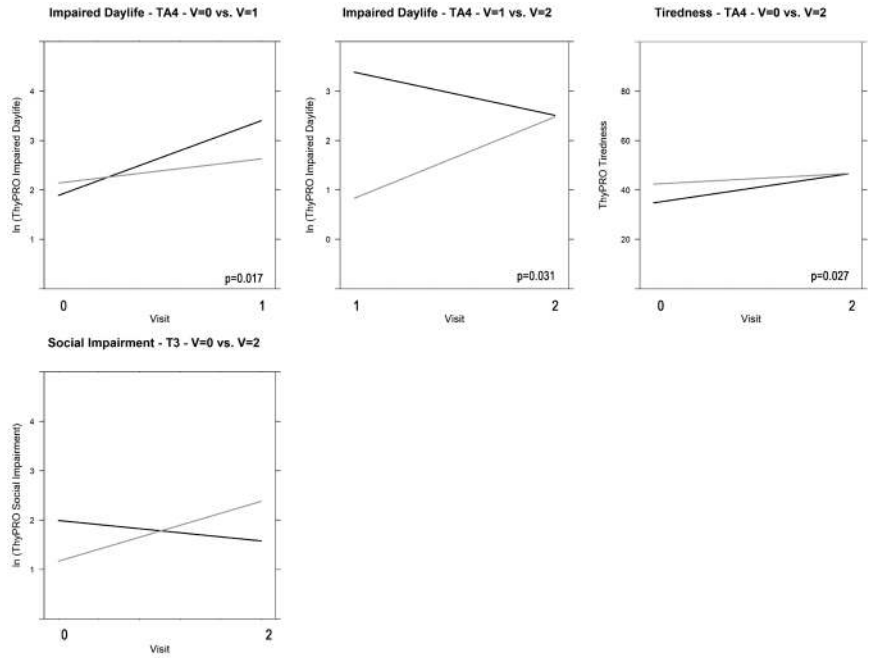


Figure 3. Longitudinal Associations between Thyroid Hormone Metabolites and ThyPRO changes between thyroid states.

Lines show significant association for lower THM (Black) and for higher THM (Grey) in case of interaction. For ThyPRO, higher scores indicating more thyroid-related complaints. p-values < 0.05 are considered in significant case of interaction.

Cross-sectionally, none of the THM were associated with QoL in either of the three thyroid states. In previous cross-sectional studies in DTC-patients on LT4 replacement therapy, 3,5-T2, T3, rT3 and T4 were also not associated with QoL (13, 15). In hypothyroid patients on LT4 replacement therapy, T3, rT3 and free-T3 were not associated with psychological well-being (3, 25). One study suggested that residual complaints were related to low free-T3 concentrations (42), but no standardized questionnaires were used. In our study, 3,5-T2 concentration were below LLMI and free-T3 was not measured, and therefore we cannot relate to some of these previous studies. At the hypothyroid state (V=1), thyroid function and QoL were not associated despite decreased QoL (for the majority of the scores) and decreased THM concentrations. An explanation may be that a certain decrease in THM concentrations results into such a low QoL that individual differences in THM hardly have any influence on QoL anymore. Further, with respect to all three thyroid states, the impact of cancer diagnosis, surgery or RAI therapy on QoL can be an explanation for not finding any associations as this might create QoL differences unrelated to THM. Finally, the lack of spread of THM concentrations in either thyroid state might be another reason

no associations were discovered. However, one might also argue that there is no significant association between QoL and THM.

Longitudinally, TA4 (three QoL scores), rT3 and T0 (two scores), 3-T1, 3,3'-T2, and T3 were significantly associated with QoL differences between euthyroid, hypothyroid and (subclinical) hyperthyroid state for six QoL scores (RAND-36 Physical Functioning (three times), MFI-20 Mental Fatigue (twice), ThyPRO Impaired Daylife (twice), RAND-36 Vitality, ThyPRO Social Impairment and Tiredness). In general, those with higher concentrations of these THM have less complaints, but no consistent patterns were found between specific THM and QoL domains. To our knowledge, this is the first study establishing that QoL differs between different thyroid states in patients treated for DTC for those with higher compared to lower concentrations of the above mentioned THM. However, higher concentrations of T3 were associated with more ThyPRO Social Impairment complaints in those on LT4 replacement therapy when comparing euthyroid state with (subclinical) hyperthyroid state. No other significant associations were found comparing these two states that suggests that a shortage of (specific) THM may be the explanation of persisting symptoms in patients with hypothyroidism on LT4 replacement therapy. Therefore, we could not identify a specific THM that may explain the persistent symptoms in patients with hypothyroidism on LT4 replacement therapy. However, as previously mentioned, results might be influenced by the impact of cancer diagnosis, surgery or RAI therapy on QoL as this might create QoL differences unrelated to THM, or our study population was too small to detect differences. Therefore, larger studies in different populations are needed to confirm our results.

Longitudinal QoL studies have been performed in thyroid cancer patients, and QoL in these patients was lower compared to the general population before surgery and remained low during follow-up (16, 43, 44). In the current study, QoL at V=2 ((subclinical) hyperthyroid state) was also lower compared to baseline in the majority of the QoL scores. The period of thyroid hormone withdrawal or thyroid cancer itself may play a role in these lower QoL scores, but also the fact that patients were on LT4 replacement therapy leading to residual complaints may be. However, we did not discover specific THM that were consequently associated with these lower QoL scores, making the latter explanation less likely. Additionally, for the euthyroid state, the majority of the QoL scores were lower than that of the general population (15, 37, 45), and here we also did not observe an association between THM and QoL. A possible explanation for the decreased QoL at baseline is the awareness of patients of their thyroid cancer diagnosis resulting into anxiousness and stress. Therefore, one might argue that treated DTC patients are not an optimal model to study the relationship between THM and QoL, as QoL is also influenced by DTC diagnosis and treatment. On the other hand, advantages of our model are 1) three thyroid states

can be studied in the same patient in a prospective manner, and 2) hypothyroidism is caused by total thyroidectomy and (subclinical) hyperthyroidism by LT4 replacement therapy, and not by autoimmune thyroid disease, therewith eliminating this possible confounder/modifier.

Main strength of the current study is that it is the first study, to our knowledge, to look at the relationship of an extensive panel of THM from two metabolic pathways with QoL comparing different thyroid states. As described earlier, due to the different thyroid hormone states that the patients went through, we were able to investigate this relationship both cross-sectionally and longitudinally. Limitations of the study include that since 3,5-T2 was below the lower limit of quantification and TA3 was not detected, we were not able to analyze the effect of these THM on QoL in the current study. A previous study did not observe an association between 3,5-T2 and QoL (13), while for TA3 no earlier studies on associations with QoL are known. Previous literature on 3,5-T2 is inconclusive and we should remain vigilant in interpreting this data as (radio)immunoassays are predominantly used to quantify 3,5-T2 (27). Second, we may not have observed any cross-sectional, and no consistent longitudinal associations between THM and QoL because our sample size may not be large enough. Finally, THM were measured in serum, which might not reflect tissue specific concentrations as previous studies showed in rats that this was not the case for T3 and T4 (46, 47). However, serum measurements are currently the best available method to determine thyroid state in patients.

CONCLUSIONS

In conclusion, this study shows that T0, 3-T1, 3,3'-T2, T3, rT3 and TA4 are significantly associated with QoL differences between different thyroid states for specific QoL scores in patients treated for DTC, but no consistent patterns in the relationship between THM concentrations and QoL domains were observed. Cross-sectionally, the measured THM were not associated with QoL at either hypo, (subclinical) hyper or euthyroid state. Therefore, we could not identify a specific THM that may explain the persistent symptoms in patients with hypothyroidism on LT4 replacement therapy. Further research in larger and different populations are needed to confirm our findings.

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Supplemental Table 1. Cross-sectional Associations between Thyroid Hormone Metabolites and MFI-20.

	T0[a]	3-T[a]	3,3'-T2[a]	T3[a]
General fatigue[b]				
V=0	-0.0001 ± 0.0004 (0.710)	-0.04 ± 0.04 (0.322)	0.009 ± 0.008 (0.296)	-0.00002 ± 0.0001 (0.846)
V=1	-0.0001 ±0.0005 (0.869)	-0.02 ± 0.06 (0.791)	-0.012 ± 0.018 (0.519)	-0.00003 ± 0.0001 (0.838)
V=2	0.0001 ±0.0003 (0.698)	-0.02 ± 0.02 (0.642)	0.009 ± 0.007 (0.213)	0.00016 ± 0.0001 (0.068)
Physical fatigue[b]				
V=0	-0.0002 ± 0.0003 (0.546)	-0.05 ± 0.04 (0.196)	0.003 ± 0.008 (0.727)	-0.00003 ± 0.0001 (0.783)
V=1	0.0000 ± 0.0004 (0.954)	-0.04 ± 0.05 (0.472)	-0.012 ± 0.016 (0.462)	-0.00013 ± 0.0001 (0.254)
V=2	0.0002 ± 0.0003 (0.494)	0.000 ± 0.03 (0.995)	0.006 ± 0.007 (0.393)	0.00007 ± 0.00009 (0.455)
Mental fatigue[b]				
V=0	-0.0005 ± 0.0004 (0.154)	-0.04 ± 0.04 (0.360)	0.002 ± 0.009 (0.822)	-0.00008 ± 0.0001 (0.499)
V=1	-0.0004 ± 0.0006 (0.562)	0.04 ± 0.08 (0.623)	-0.027 ± 0.023 (0.246)	-0.00009 ± 0.0002 (0.573)
V=2	-0.0003 ± 0.0002 (0.170)	-0.02 ± 0.02 (0.371)	0.005 ± 0.007 (0.451)	0.00008 ± 0.00008 (0.348)

[a] Values are β ± standard error (p-values) per unit increase in THM; adjusted for age and sex.
[b] All MFI-20 scores are log transformed.
p-values < 0.0005 are considered significant.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state.

rT3[a]	T4[a]	TA4[a]	free-T4[a]
0.00009 ±0.0001 (0.350)	0.001 ± 0.001 (0.451)	-0.0001 ± 0.0001 (0.612)	0.014 ± 0.012 (0.234)
0.00001 ± 0.0003 (0.968)	-0.0002 ± 0.002 (0.901)	-0.0004 ± 0.0002 (0.153)	0.004 ± 0.015 (0.788)
0.00007 ± 0.0001 (0.368)	0.001 ± 0.001 (0.094)	-0.0000 ± 0.0000 (0.212)	0.007 ± 0.006 (0.204)
0.00006 ± 0.0001 (0.548)	0.0003 ± 0.001 (0.838)	-0.0000 ± 0.0001 (0.943)	0.013 ± 0.012 (0.266)
-0.00013 ± 0.0002 (0.596)	-0.002 ± 0.002 (0.192)	-0.0004 ± 0.0002 (0.088)	-0.006 ± 0.013 (0.649)
-0.00001 ± 0.00008 (0.897)	0.001 ± 0.001 (0.224)	-0.0000 ± 0.0000 (0.316)	0.003 ± 0.006 (0.632)
-0.00005 ± 0.0001 (0.604)	0.001 ± 0.002 (0.645)	0.0001 ± 0.0001 (0.457)	-0.004 ± 0.013 (0.766)
-0.00042 ± 0.0004 (0.240)	-0.000 ± 0.003 (0.896)	-0.0002 ± 0.0003 (0.629)	0.011 ± 0.019 (0.558)
0.00008 ± 0.0001 (0.285)	0.0005 ± 0.001 (0.571)	-0.0000 ± 0.0000 (0.233)	-0.0001 ± 0.006 (0.986)

Supplemental Table 2. Cross-sectional Associations between Thyroid Hormone Metabolites and RAND-36.

	T0[a]	3-T1[a]	3,3'-T2[a]	T3[a]
Vitality				
V=0	0.020 ± 0.037 (0.601)	-0.33 ± 4.29 (0.939)	-1.82 ± 0.84 (0.035)	-0.013 ± 0.012 (0.287)
V=1	-0.031 ± 0.072 (0.666)	13.47 ± 8.06 (0.102)	-0.06 ± 2.67 (0.983)	0.012 ± 0.018 (0.488)
V=2	-0.030 ± 0.033 (0.376)	2.15 ± 3.06 (0.486)	-1.63 ± 0.86 (0.065)	-0.024 ± 0.010 (0.025)
General Mental Health				
V=0	0.020 ± 0.030 (0.510)	0.75 ± 3.51 (0.832)	-0.79 ± 0.71 (0.276)	-0.008 ± 0.010 (0.389)
V=1	-0.015 ± 0.069 (0.823)	14.43 ± 7.48 (0.060)	1.99 ± 2.51 (0.431)	0.004 ± 0.017 (0.819)
V=2	0.054 ± 0.028 (0.062)	5.10 ± 2.59 (0.054)	-0.61 ±0.77 (0.434)	-0.008 ± 0.009 (0.336)
General Health Perception				
V=0	-0.027 ± 0.033 (0.419)	-1.21 ± 3.91 (0.758)	-0.76 ± 0.78 (0.337)	-0.008 ± 0.011 (0.461)
V=1	-0.081 ± 0.066 (0.223)	7.76 ± 7.69 (0.318)	-1.53 ± 2.49 (0.542)	-0.011 ± 0.017 (0.509)
V=2	-0.012 ± 0.029 (0.681)	2.03 ± 2.70 (0.455)	-0.87 ± 0.75 (0.254)	-0.007 ± 0.009 (0.428)
Physical functioning[b]				
V=0	-0.0004 ± 0.0002 (0.049)	-0.03 ± 0.02 (0.279)	-0.01 ±0.01 (0.097)	0.000 ± 0.000 (0.930)
V=1	0.0009 ± 0.0006 (0.121)	0.13 ± 0.05 (0.025)	0.02 ± 0.02 (0.369)	0.000 ± 0.000 (0.093)
V=2	-0.0002 ± 0.0002 (0.275)	0.01 ± 0.02 (0.500)	-0.00 ± 0.01 (0.501)	-0.000 ± 0.000 (0.576)
Social functioning[b]				
V=0	-0.001 ± 0.001 (0.287)	-0.11 ± 0.10 (0.269)	-0.02 ± 0.02 (0.298)	0.000 ± 0.000 (0.541)
V=1	-0.002 ± 0.003 (0.602)	0.11 ± 0.35 (0.744)	-0.04 ± 0.11 (0.728)	-0.000 ± 0.001 (0.528)
V=2	-0.0002 ± 0.001 (0.859)	-0.06 ± 0.09 (0.497)	0.001 ± 0.03 (0.969)	0.000 ± 0.000 (0.571)

[a] Values are β ± standard error (p-values) per unit increase in THM; adjusted for age and sex.
[b] These RAND-36 scores are log transformed.
p-values < 0.0005 are considered significant.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state.

rT3[a]	T4[a]	TA4[a]	free-T4[a]
-0.020 ± 0.010 (0.044)	-0.099 ± 0.155 (0.527)	0.001 ± 0.012 (0.924)	-2.238 ± 1.254 (0.081)
0.009 ± 0.040 (0.818)	0.248 ± 0.278 (0.377)	0.030 ± 0.038 (0.428)	1.869 ± 2.135 (0.081)
-0.016 ± 0.010 (0.091)	-0.157 ± 0.104 (0.135)	0.002 ± 0.001 (0.064)	-0.594 ± 0.709 (0.406)
-0.004 ± 0.008 (0.623)	-0.223 ± 0.124 (0.078)	-0.012 ± 0.009 (0.194)	-1.083 ± 1.063 (0.314)
0.009 ± 0.038 (0.807)	0.039 ± 0.265 (0.884)	-0.003 ± 0.036 (0.935)	0.561 ± 2.035 (0.784)
-0.009 ± 0.008 (0.309)	-0.077 ± 0.091 (0.401)	0.002 ±0.001 (0.143)	-0.107 ± 0.627 (0.865)
-0.013 ± 0.009 (0.144)	-0.074 ± 0.143 (0.610)	-0.000 ±0.010 (0.975)	-1.053 ± 1.168 (0.372)
-0.050 ± 0.037 (0.181)	-0.173 ± 0.263 (0.515)	-0.001 ± 0.035 (0.977)	-0.767 ± 2.025 (0.706)
-0.010 ± 0.008 (0.216)	-0.151 ± 0.088 (0.092)	0.000 ± 0.001 (0.902)	-0.429 ± 0.614 (0.488)
-0.000 ± 0.000 (0.149)	0.000 ± 0.001 (0.740)	0.000 ± 0.000 (0.655)	-0.022 ± 0.001 (0.0006)
0.000 ± 0.000 (0.508)	0.004 ±0.002 (0.104)	0.000 ± 0.000 (0.227)	0.028 ± 0.018 (0.123)
-0.000 ± 0.000 (0.978)	-0.001 ± 0.001 (0.374)	0.000 ± 0.000 (0.226)	-0.006 ± 0.005 (0.208)
-0.000 ± 0.000 (0.343)	0.003 ±0.004 (0.431)	-0.000 ± 0.000 (0.841)	-0.018 ± 0.032 (0.571)
-0.002 ± 0.002 (0.178)	-0.004 ± 0.011 (0.703)	-0.001 ± 0.001 (0.570)	-0.085 ± 0.087 (0.334)
0.000 ± 0.000 (0.621)	0.003 ± 0.003 (0.294)	0.000 ± 0.000 (0.816)	-0.015 ± 0.022 (0.506)

Supplemental Table 3. Cross-sectional Associations between Thyroid Hormone Metabolites and ThyPRO.

	T0[a]	3-T1[a]	3,3'-T2[a]	T3[a]
Composite[c]				
V=0	-0.002 ± 0.003 (0.447)	-0.074 ± 0.321 (0.818)	0.068 ± 0.065 (0.301)	0.000 ± 0.001 (0.723)
V=1	-0.001 ± 0.006 (0.821)	-0.636 ± 0.664 (0.342)	-0.052 ± 0.211 (0.807)	-0.000 ± 0.001 (0.964)
V=2	-0.003 ± 0.003 (0.287)	-0.179 ± 0.237 (0.453)	0.071 ± 0.070 (0.311)	0.001 ± 0.001 (0.135)
Tiredness				
V=0	-0.016 ± 0.037 (0.664)	0.438 ± 4.244 (0.918)	0.887 ± 0.869 (0.312)	0003 ± 0.012 (0.803)
V=1	0.026 ± 0.066 (0.699)	-5.405 ± 7.637 (0.482)	1.449 ± 2.462 (0.559)	0.011 ± 0.017 (0.528)
V=2	0.018 ± 0.032 (0.586)	1.610 ± 2.937 (0.586)	1.375 ± 0.861 (0.116)	0.013 ± 0.010 (0.208)
Cognitive Problems[b]				
V=0	-0.002 ± 0.003 (0.516)	-0.005 ± 0.310 (0.988)	0.076 ± 0.063 (0.232)	-0.000 ± 0.001 (0.828)
V=1	-0.001 ± 0.004 (0.700)	-0.116 ± 0.437 (0.792)	-0.148 ± 0.135 (0.279)	-0.000 ± 0.001 (0.939)
V=2	-0.003 ± 0.002 (0.092)	-0.349 ± 0.160 (0.034)	0.004 ± 0.049 (0.938)	0.001 ±0.001 (0.125)
Anxiety[b]				
V=0	-0.004 ± 0.001 (0.022)	-0.028 ± 0.180 (0.879)	0.021 ± 0.037 (0.566)	0.000 ± 0.000 (0.932)
V=1	0.000 ± 0.004 (0.917)	-0.618 ± 0.421 (0.148)	0.084 ± 0.142 (0.556)	0.001 ± 0.001 (0.551)
V=2	-0.002 ± 0.002 (0.131)	-0.068 ± 0.146 (0.642)	0.035 ± 0.042 (0.410)	0.001 ± 0.000 (0.134)
Depressivity[b]				
V=0	-0.001 ± 0.001 (0.381)	-0.029 ± 0.106 (0.784)	-0.004 ± 0.022 (0.858)	-0.000 ± 0.000 (0.685)
V=1	-0.001 ± 0.003 (0.658)	-0.003 ± 0.324 (0.992)	-0.083 ± 0.100 (0.410)	-0.000 ± 0.001 (0.084)
V=2	0.000 ± 0.001 (0.730)	-0.044 ± 0.101 (0.667)	0.063 ± 0.028 (0.030)	0.001 ± 0.000 (0.019)
Social Impairment[b]				
V=0	-0.001 ± 0.001 (0.848)	-0.166 ± 0.304 (0.586)	-0.028 ± 0.064 (0.659)	-0.001 ± 0.001 (0.317)
V=1	-0.001 ± 0.005 (0.774)	-0.354 ± 0.573 (0.539)	-0.130 ± 0.181 (0.475)	-0.001 ± 0.001 (0.357)
V=2	-0.001 ± 0.002 (0.783)	-0.008 ± 0.189 (0.967)	0.079 ± 0.055 (0.157)	0.001 ± 0.001 (0.086)

rT3[a]	T4[a]	TA4[a]	free-T4[a]
0.001 ± 0.001 (0.476)	0.017 ± 0.011 (0.146)	0.001 ± 0.001 (0.421)	0.102 ± 0.095 (0.287)
-0.000 ± 0.003 (0.990)	0.006 ± 0.022 (0.789)	-0.001 ± 0.003 (0.671)	0.0064 ± 0.170 (0.707)
0.001 ± 0.001 (0.251)	0.016 ± 0.008 (0.061)	-0.000 ± 0.000 (0.370)	0.050 ± 0.058 (0.393)
0.012 ± 0.010 (0.229)	0.073 ± 0.155 (0.639)	0.015 ± 0.011 (0.186)	1.278 ± 1.302 (0.332)
0.032 ± 0.037 (0.391)	0.167 ± 0.260 (0.523)	-0.022 ± 0.035 (0.535)	0.642 ± 1.995 (0.749)
0.012 ± 0.009 (0.194)	0.132 ± 0.105 (0.214)	-0.000 ± 0.001 (0.897)	0.843 ± 0.723 (0.249)
0.001 ± 0.001 (0.365)	0.003 ± 0.011 (0.762)	-0.000 ± 0.001 (0.735)	-0.022 ± 0.092 (0.813)
-0.001 ± 0.002 (0.639)	0.009 ± 0.014 (0.523)	-0.000 ± 0.002 (0.805)	0.102 ± 0.109 (0.357)
0.000 ± 0.001 (0.819)	0.010 ± 0.006 (0.096)	-0.000 ± 0.000 (0.027)	0.013 ± 0.040 (0.751)
0.000 ± 0.000 (0.856)	0.009 ± 0.006 (0.179)	-0.000 ± 0.000 (0.557)	-0.041 ± 0.056 (0.466)
0.002 ± 0.002 (0.389)	0.013 ± 0.015 (0.379)	0.000 ± 0.002 (0.805)	0.072 ± 0.115 (0.535)
0.001 ± 0.000 (0.076)	0.014 ± 0.005 (0.004)	-0.000 ± 0.000 (0.305)	0.048 ± 0.035 (0.180)
-0.000 ± 0.000 (0.547)	-0.001 ± 0.004 (0.826)	0.000 ± 0.000 (0.664)	-0.031 ± 0.033 (0.354)
-0.001 ± 0.002 (0.667)	0.004 ± 0.011 (0.732)	0.000 ± 0.001 (0.914)	0.015 ± 0.081 (0.857)
0.001 ± 0.000 (0.083)	0.004 ± 0.004 (0.246)	-0.0000 ± 0.000 (0.888)	0.000 ± 0.025 (0.986)
-0.001 ± 0.001 (0.240)	0.006 ± 0.011 (0.582)	0.001 ± 0.001 (0.364)	0.017 ± 0.009 (0.855)
-0.004 ± 0.003 (0.164)	-0.010 ± 0.019 (0.614)	-0.001 ± 0.003 (0.588)	-0.093 ± 0.147 (0.527)
0.001 ± 0.001 (0.100)	0.009 ± 0.007 (0.161)	-0.000 ± 0.000 (0.740)	0.046 ± 0.046 (0.342)

Supplemental Table 3. Cross-sectional Associations between Thyroid Hormone Metabolites and ThyPRO. (*continued*)

	T0[a]	3-T1[a]	3,3'-T2[a]	T3[a]
Impaired Daylife[b]				
V=0	-0.003 ± 0.003 (0.332)	-0.240 ± 0.301 (0.428)	-0.001 ± 0.063 (0.989)	-0.001 ± 0.001 (0.529)
V=1	-0.004 ± 0.003 (0.127)	-0.762 ± 0.305 (0.016)	-0.071 ± 0.101 (0.486)	-0.001 ± 0.001 (0.386)
V=2	-0.002 ± 0.002 (0.384)	-0.201 ± 0.185 (0.283)	0.036 ± 0.055 (0.509)	0.001 ± 0.001 (0.284)

[a] Values are β ± standard error (p-values) per unit increase in THM; adjusted for age and sex.
[b] These ThyPRO scores are log transformed.
[c] This ThyPRO scores is square root transformed.
p-values < 0.0005 are considered significant.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state.

Supplemental Table 4. Longitudinal Associations between Thyroid Hormone Metabolites and MFI-20.

	T0[a]	3-T1[a]	3,3'-T2[a]	T3[a]
General fatigue[b]				
V=0 vs. V=1	-0.008 ± 0.006 (0.192)	-1.327 ± 0.639 (0.040)	0.156 ± 0.155 (0.316)	0.0001 ± 0.0020 (0.984)
V=1 vs. V=2	0.004 ± 0.004 (0.351)	-0.241 ± 0.400 (0.548)	0.134 ± 0.124 (0.283)	0.0031 ± 0.0013 (0.017)
V=0 vs. V=2	0.002 ± 0.005 (0.617)	-0.303 ± 0.490 (0.538)	0.152 ± 0.117 (0.198)	0.0005 ± 0.0015 (0.700)
Physical fatigue[b]				
V=0 vs. V=1	-0.007 ± 0.005 (0.189)	-1.359 ± 0.592 (0.024)	0.091 ± 0.143 (0.525)	-0.0008 ± 0.0019 (0.691)
V=1 vs. V=2	0.003 ± 0.004 (0.416)	-0.299 ± 0.426 (0.484)	0.100 ± 0.131 (0.446)	0.0015 ± 0.0014 (0.274)
V=0 vs. V=2	0.002 ± 0.005 (0.729)	-0.375 ± 0.492 (0.447)	0.134 ± 0.120 (0.266)	0.0005 ± 0.0015 (0.732)
Mental fatigue[b]				
V=0 vs. V=1	-0.006 ± 0.006 (0.292)	-0.802 ± 0.619 (0.198)	Interaction	-0.0001 ± 0.0020 (0.954)
V=1 vs. V=2	-0.004 ± 0.004 (0.367)	-0.790 ± 0.407 (0.055)	0.119 ± 0.128 (0.354)	0.0038 ± 0.0013 (0.0038)
V=0 vs. V=2	-0.000 ± 0.004 (0.940)	-0.348 ± 0.461 (0.451)	0.350 ± 0.102 (0.731)	0.0009 ± 0.0013 (0.487)

[a] Values are β ± standard error (p-values) per unit increase in THM; adjusted for age and sex.
[b] All MFI-20 scores are (natural) log transformed.
p-values < 0.0002 are considered significant.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state.

rT3[a]	T4[a]	TA4[a]	free-T4[a]
0.000 ± 0.001 (0.979)	0.014 ± 0.011 (0.208)	0.001 ± 0.001 (0.377)	-0.019 ± 0.093 (0.841)
-0.000 ± 0.002 (0.737)	-0.008 ± 0.011 (0.461)	-0.003 ± 0.001 (0.065)	-0.075 ± 0.081 (0.358)
0.000 ± 0.001 (0.699)	0.011 ± 0.006 (0.091)	-0.000 ± 0.000 (0.299)	-0.006 ± 0.045 (0.894)

rT3[a]	T4[a]	TA4[a]	free-T4[a]
0.003 ± 0.002 (0.080)	0.018 ± 0.028 (0.531)	-0.0004 ± 0.0022 (0.864)	0.13 ± 0.22 (0.548)
0.002 ± 0.001 (0.081)	0.034 ± 0.015 (0.025)	-0.0001 ± 0.0001 (0.208)	0.27 ± 0.10 (0.010)
0.002 ± 0.001 (0.218)	0.024 ± 0.017 (0.171)	-0.0001 ± 0.0001 (0.314)	0.20 ± 0.12 (0.095)
0.002 ± 0.002 (0.148)	-0.006 ± 0.026 (0.805)	0.0015 ± 0.0019 (0.443)	0.20 ± 0.20 (0.332)
0.001 ± 0.001 (0.396)	0.026 ± 0.016 (0.108)	-0.0001 ± 0.0001 (0.462)	0.18 ± 0.11 (0.120)
0.001 ± 0.001 (0.429)	0.017 ± 0.017 (0.338)	-0.0001 ± 0.0001 (0.265)	0.12 ± 0.12 (0.322)
Interaction	0.019 ± 0.027 (0.473)	0.0013 ± 0.0020 (0.501)	0.08 ± 0.20 (0.684)
0.004 ± 0.001 (0.0015)	0.021 ± 0.016 (0.184)	-0.0001 ± 0.0001 (0.434)	0.14 ± 0.11 (0.209)
0.000 ± 0.001 (0.716)	0.005 ± 0.016 (0.775)	-0.0002 ± 0.0001 (0.066)	0.10 ± 0.11 (0.372)

Supplemental Table 5. Longitudinal Associations between Thyroid Hormone Metabolites and RAND-36.

	T0[a]	3-T1[a]	3,3'-T2[a]	T3[a]
Vitality				
V=0 vs. V=1	-0.002 ± 0.025 (0.939)	1.39 ± 2.68 (0.606)	-1.79 ± 0.58 (0.002)	-0.009 ± 0.009 (0.302)
V=1 vs. V=2	-0.036 ± 0.021 (0.088)	1.10 ± 2.25 (0.627)	-0.71 ± 0.64 (0.271)	-0.013 ± 0.007 (0.065)
V=0 vs. v=2	-0.027 ± 0.024 (0.263)	0.77 ± 2.48 (0.757)	-1.31 ± 0.52 (0.013)	-0.014 ± 0.007 (0.055)
General Mental Health				
V=0 vs. V=1	0.010 ± 0.022 (0.665)	2.85 ± 2.40 (0.238)	-0.02 ± 0.55 (0.973)	0.000 ± 0.008 (0.998)
V=1 vs. V=2	0.014 ± 0.021 (0.499)	4.38 ± 2.19 (0.049)	0.28 ± 0.61 (0.652)	-0.005 ± 0.007 (0.436)
V=0 vs. v=2	0.011 ± 0.020 (0.918)	2.21 ± 2.02 (0.276)	-0.47 ± 0.42 (0.269)	-0.009 ± 0.006 (0.143)
General Health Perception				
V=0 vs. V=1	-0.027 ± 0.023 (0.246)	-0.55 ± 2.53 (0.829)	0.32 ± 0.58 (0.580)	0.004 ± 0.008 (0.669)
V=1 vs. V=2	-0.041 ± 0.020 (0.047)	-0.43 ± 2.17 (0.844)	-0.24 ± 0.61 (0.692)	-0.003 ± 0.007 (0.654)
V=0 vs. v=2	0.002 ± 0.020 (0.918)	1.91 ± 2.13 (0.373)	-0.01 ± 0.42 (0.978)	-0.003 ± 0.006 (0.632)
Physical functioning[b]				
V=0 vs. V=1	Interaction	Interaction	-0.01 ± 0.01 (0.123)	0.000 ± 0.000 (0.510)
V=1 vs. V=2	Interaction	-0.00 ± 0.03 (0.870)	-0.00 ± 0.01 (0.944)	0.000 ± 0.000 (0.640)
V=0 vs. v=2	-0.001 ± 0.000 (0.009)	-0.01 ± 0.03 (0.855)	-0.01 ± 0.01 (0.043)	-0.000 ± 0.000 (0.722)
Social functioning[b]				
V=0 vs. V=1	-0.001 ± 0.001 (0.779)	0.01 ± 0.09 (0.929)	-0.02 ± 0.02 (0.284)	0.000 ± 0.000 (0.386)
V=1 vs. V=2	-0.000 ± 0.000 (0.858)	-0.05 ± 0.06 (0.410)	-0.01 ± 0.02 (0.702)	-0.000 ± 0.000 (0.728)
V=0 vs. v=2	-0. 001 ± 0.001 (0.402)	-0.05 ± 0.08 (0.487)	-0.01 ± 0.02 (0.500)	0.000 ± 0.000 (0.351)

[a] Values are β ± standard error (p-values) per unit increase in THM; adjusted for age and sex.
[b] These RAND-36 scores are (natural) log transformed.
p-values < 0.0002 are considered significant.
V=0, euthyroid state; V=1, hypothyroid state;V=2, hyperthyroid state.

rT3[a]	T4[a]	TA4[a]	free-T4[a]
-0.025 ± 0.007 (0.0004)	-0.087 ± 0.118 (0.464)	-0.002 ± 0.009 (0.835)	Interaction
- 0.010 ± 0.007 (0.124)	-0.150 ± 0.079 (0.061)	0.002 ± 0.001 (0.044)	-0.94 ± 0.53 (0.076)
Interaction	-0.112 ± 0.085 (0.190)	0.001 ± 0.001 (0.186)	-0.64 ± 0.55 (0.248)
-0.000 ± 0.007 (0.955)	-0.189 ± 0.104 (0.072)	-0.012 ± 0.007 (0.113)	-0.71 ± 0.80 (0.378)
-0.003 ± 0.007 (0.628)	-0.055 ± 0.076 (0.472)	0.001 ± 0.001 (0.335)	-0.07 ± 0.51 (0.898)
-0.006 ± 0.005 (0.230)	-0.110 ± 0.069 (0.115)	0.001 ± 0.001 (0.275)	-0.44 ± 0.45 (0.331)
-0.004 ± 0.007 (0.611)	-0.018 ± 0.112 (0.871)	-0.008 ± 0.008 (0.305)	-0.33 ± 0.86 (0.706)
-0.007 ± 0.007 (0.302)	-0.156 ± 0.074 (0.038)	0.000 ± 0.001 (0.929)	-0.35 ± 0.51 (0.493)
-0.005 ± 0.005 (0.289)	-0.113 ± 0.070 (0.111)	0.000 ± 0.001 (0.998)	-0.12 ± 0.47 (0.793)
-0.000 ± 0.000 (0.125)	0.002 ± 0.002 (0.332)	0.000 ± 0.000 (0.848)	-0.04 ± 0.01 (0.001)
-0.000 ± 0.000 (0.950)	0.000 ± 0.001 (0.768)	-0.000 ± 0.000 (0.998)	-0.03 ± 0.01 (0.0002)
-0.000 ± 0.000 (0.035)	-0.001 ± 0.001 (0.329)	0.000 ± 0.000 (0.093)	-0.02 ± 0.01 (0.009)
-0.000 ± 0.000 (0.343)	0.002 ± 0.004 (0.616)	-0.000 ± 0.000 (0.209)	-0.02 ± 0.03 (0.489)
-0.000 ± 0.000 (0.370)	0.000 ± 0.002 (0.890)	-0.000 ± 0.000 (0.507)	-0.03 ± 0.01 (0.030)
-0.000 ± 0.000 (0.687)	0.000 ± 0.003 (0.926)	0.000 ± 0.000 (0.748)	-0.01 ± 0.02 (0.646)

Supplemental Table 6. Longitudinal Associations between Thyroid Hormone Metabolites and ThyPRO.

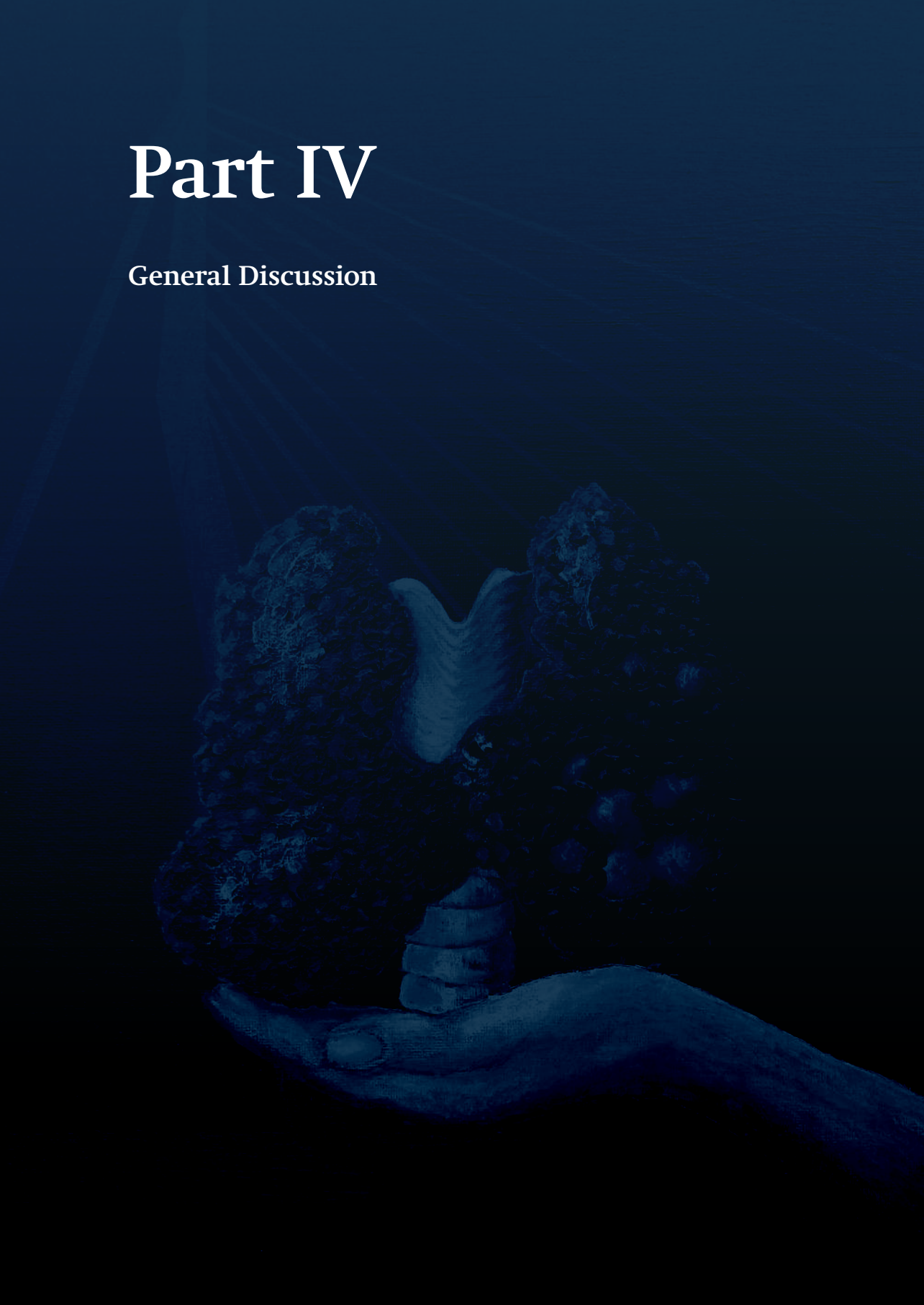
	T0[a]	3-T1[a]	3,3'-T2[a]	T3[a]
Composite[c]				
V=0 vs. V=1	-0.000 ± 0.001 (0.799)	-0.05 ± 0.09 (0.596)	0.015 ± 0.019 (0.451)	-0.000 ± 0.000 (0.779)
V=1 vs. V=2	-0.000 ± 0.001 (0.858)	-0.02 ± 0.08 (0.809)	-0.004 ± 0.023 (0.857)	0.000 ± 0.000 (0.245)
V=0 vs. v=2	0.000 ± 0.001 (0.839)	-0.01 ± 0.08 (0.896)	0.016 ± 0.016 (0.318)	0.000 ± 0.000 (0.338)
Tiredness				
V=0 vs. V=1	-0.039 ± 0.025 (0.111)	-4.19 ± 2.67 (0.119)	0.692 ± 0.641 (0.283)	0.003 ± 0.009 (0.755)
V=1 vs. V=2	0.022 ± 0.023 (0.328)	2.03 ± 2.22 (0.362)	0.656 ± 0.694 (0.346)	0.007 ± 0.007 (0.318)
V=0 vs. v=2	0.020 ± 0.024 (0.403)	1.82 ± 2.44 (0.457)	0.882 ± 0.569 (0.124)	0.005 ± 0.007 (0.534)
Cognitive Problems[b]				
V=0 vs. V=1	-0.001 ± 0.002 (0.655)	0.04 ± 0.21 (0.852)	0.094 ± 0.048 (0.054)	0.001 ± 0.001 (0.334)
V=1 vs. V=2	-0.001 ± 0.001 (0.260)	-0.08 ± 0.12 (0.464)	-0.024 ± 0.036 (0.503)	0.000 ± 0.000 (0.215)
V=0 vs. v=2	-0.001 ± 0.002 (0.477)	-0.18 ± 0.16 (0.273)	0.027 ± 0.038 (0.482)	0.000 ± 0.001 (0.362)
Anxiety[b]				
V=0 vs. V=1	-0.001 ± 0.001 (0.328)	-0.12 ± 0.15 (0.413)	-0.011 ± 0.034 (0.748)	-0.000 ± 0.000 (0.460)
V=1 vs. V=2	-0.001 ± 0.001 (0.604)	-0.01 ± 0.11 (0.955)	-0.020 ± 0.034 (0.564)	0.000 ± 0.000 (0.441)
V=0 vs. v=2	-0.002 ± 0.001 (0.070)	-0.02 ± 0.12 (0.885)	0.009 ± 0.028 (0.761)	0.000 ± 0.000 (0.608)
Depressivity[b]				
V=0 vs. V=1	-0.000 ± 0.001 (0.939)	0.01 ± 0.13 (0.924)	-0.013 ± 0.030 (0.668)	-0.000 ± 0.000 (0.318)
V=1 vs. V=2	0.001 ± 0.001 (0.154)	0.08 ± 0.10 (0.388)	0.023 ± 0.029 (0.423)	0.000 ± 0.000 (0.150)
V=0 vs. v=2	-0.000 ± 0.001 (0.723)	0.01 ± 0.10 (0.946)	0.020 ± 0.023 (0.375)	0.000 ± 0.000 (0.452)
Social Impairment[b]				
V=0 vs. V=1	-0.000 ± 0.002 (0.832)	-0.32 ± 0.20 (0.105)	-0.042 ± 0.048 (0.381)	-0.001 ± 0.001 (0.224)
V=1 vs. V=2	-0.001 ± 0.001 (0.647)	-0.08 ± 0.14 (0.567)	0.033 ± 0.043 (0.449)	0.000 ± 0.000 (0.291)
V=0 vs. v=2	-0.000 ± 0.002 (0.859)	-0.01 ± 0.16 (0.934)	0.026 ± 0.036 (0.472)	Interaction
Impaired Daylife[b]				
V=0 vs. V=1	-0.002 ± -0.002 (0.293)	-0.28 ± 0.22 (0.200)	-0.015 ± 0.054 (0.776)	-0.001 ± 0.001 (0.156)
V=1 vs. V=2	0.000 ± 0.001 (0.919)	-0.04 ± 0.15 (0.812)	0.009 ± 0.048 (0.852)	0.000 ± 0.000 (0.830)
V=0 vs. v=2	-0.000 ± 0.002 (0.892)	-0.15 ± 0.17 (0.367)	0.045 ± 0.040 (0.263)	0.000 ± 0.001 (0.375)

[a] Values are β ± standard error (p-values) per unit increase in THM; adjusted for age and sex.
[b] These ThyPRO scores are (natural) log transformed.
[c] This ThyPRO scores is square root transformed.
p-values < 0.0002 are considered significant.
V=0, euthyroid state; V=1, hypothyroid state; V=2, hyperthyroid state.

rT3[a]	T4[a]	TA4[a]	free-T4[a]
0.000 ± 0.000 (0.504)	0.004 ± 0.004 (0.230)	-0.000 ± 0.000 (0.963)	0.02 ± 0.03 (0.489)
0.000 ± 0.000 (0.269)	0.007 ± 0.003 (0.007)	-0.000 ± 0.000 (0.925)	0.03 ± 0.02 (0.097)
0.000 ± 0.000 (0.158)	0.008 ± 0.003 (0.007)	0.000 ± 0.000 (0.658)	0.02 ± 0.02 (0.225)
0.012 ± 0.001 (0.139)	0.099 ± 0.120 (0.413)	0.014 ± 0.009 (0.114)	0.26 ± 0.95 (0.787)
0.008 ± 0.008 (0.301)	0.109 ± 0.085 (0.209)	-0.000 ± 0.001 (0.957)	0.71 ± 0.60 (0.239)
0.010 ± 0.007 (0.132)	0.103 ± 0.088 (0.244)	Interaction	0.60 ± 0.60 (0.317)
0.001 ± 0.001 (0.030)	0.006 ± 0.009 (0.474)	-0.001 ± 0.001 (0.388)	0.06 ± 0.07 (0.387)
0.000 ± 0.000 (0.404)	0.010 ± 0.004 (0.041)	-0.000 ± 0.000 (0.184)	0.02 ± 0.03 (0.626)
0.000 ± 0.000 (0.326)	0.010 ± 0.006 (0.101)	-0.000 ± 0.000 (0.214)	0.04 ± 0.04 (0.355)
-0.000 ± 0.000 (0.685)	0.010 ± 0.007 (0.125)	-0.000 ± 0.000 (0.851)	0.02 ± 0.05 (0.760)
0.000 ± 0.000 (0.203)	0.010 ± 0.004 (0.018)	0.000 ± 0.000 (0.540)	0.06 ± 0.03 (0.033)
0.000 ± 0.000 (0.188)	0.010 ± 0.004 (0.023)	0.000 ± 0.000 (0.095)	0.05 ± 0.03 (0.061)
-0.000 ± 0.000 (0.480)	0.002 ± 0.005 (0.779)	0.000 ± 0.000 (0.901)	0.01 ± 0.04 (0.753)
0.000 ± 0.000 (0.190)	0.005 ± 0.004 (0.150)	0.000 ± 0.000 (0.343)	0.03 ± 0.02 (0.221)
0.000 ± 0.000 (0.557)	0.005 ± 0.004 (0.190)	0.000 ± 0.000 (0.397)	0.01 ± 0.03 (0.840)
-0.001 ± 0.001 (0.236)	0.001 ± 0.0009 (0.907)	0.001 ± 0.001 (0.130)	0.01 ± 0.07 (0.910)
0.000 ± 0.000 (0.311)	0.006 ± 0.005 (0.274)	0.000 ± 0.000 (0.411)	0.03 ± 0.04 (0.482)
0.000 ± 0.000 (0.343)	0.008 ± 0.006 (0.151)	-0.000 ± 0.000 (0.847)	0.02 ± 0.04 (0.548)
-0.000 ± 0.001 (0.923)	0.001 ± 0.009 (0.939)	Interaction	-0.05 ± 0.07 (0.475)
0.000 ± 0.001 (0.889)	0.006 ± 0.006 (0.280)	Interaction	0.01 ± 0.04 (0.810)
0.001 ± 0.000 (0.264)	0.013 ± 0.006 (0.032)	-0.000 ± 0.000 (0.332)	-0.00 ± 0.04 (0.998)

Part IV

General Discussion



Chapter 11

General Discussion

THYROID CANCER

Epidemiology and Treatment

Thyroid cancer is the most common endocrine malignancy with approximately 700 new cases in The Netherlands in 2018 (1). The worldwide incidence has been steadily increasing over the last two decades (2, 3). Different subtypes of thyroid cancer exist of which well differentiated thyroid cancer (DTC), comprising both papillary (PTC) and follicular thyroid cancer (FTC), to be the most frequent (80-85%) (4).

Initial treatment of DTC is multidisciplinary and is usually multistep wise (4). According to the current 2015 Dutch Thyroid Cancer Guidelines, a total thyroidectomy is recommended if the tumor is larger than 1cm, is multifocal, has extrathyroidal extension, or if there are lymph node or distant metastases (4); otherwise a hemithyroidectomy is sufficient. Postoperatively, the tumor is staged according to the TNM system (5, 6). After a total thyroidectomy, the current 2015 Dutch Guidelines recommend to always perform subsequent radioiodine (RAI) therapy with I-131. To ensure optimal RAI uptake, this takes place under an iodine-deficient diet (during one week) and TSH stimulation. TSH stimulation can be achieved either after 3-4 weeks of thyroid hormone withdrawal, or using two injections of recombinant human TSH (rhTSH). After thyroid surgery, patients are treated with thyroid hormone replacement therapy with supraphysiological doses of LT4 to suppress TSH.

Prognosis and prognostic factors

The survival of patients with DTC is relatively good, with a 10-year disease specific survival (DSS) over 90% (7). Therefore, day-to-day treatment of patients is to a large extent based on the risk of recurrence. However, there are certain patients in which survival is worse and it is important to identify them. Different systems have been proposed to predict the risk of recurrence and survival in patients with DTC to better determine the need for aggressive therapy and optimize follow-up strategies. The AJCC/TNM staging system is designed to predict DSS (5, 8), while the ATA and European Thyroid Association (ETA) risk stratification systems are designed to estimate the risk of disease recurrence (7, 9).

The 8th edition of the AJCC/TNM staging system for DTC has been developed for better prediction of survival in patients with DTC, and was introduced in clinical practice in January 2018 (6). The most important differences with the previous 7th edition are 1) a raised age cut-off from 45 to 55 years, 2) removal of minor extrathyroidal extension from the definition of T3 tumors, and 3) N1 disease does no longer automatically result in staging into stage III or IV in older patients, but into stage II (8). Shortly after introduction, several studies showed superiority of the 8th to the 7th edition in predicting survival (10-14), but these studies comprised only patients

with PTC (12, 13), or had low numbers of patients with FTC and did not distinguish between PTC and FTC (10, 11, 14). Therefore, it is valuable to know if the 8th edition performs equally well for both FTC and PTC.

Therefore in **Chapter 2**, we compared the new 8th edition of the AJCC/TNM classification system to the previous 7th in 792 patients with DTC with respect to differences in staging and survival. Thereafter, we assessed potential differences between PTC and FTC. We showed that using this 8th edition, there is no significant difference between PTC and FTC anymore, regarding survival rates per stage, implicating that TNM stage predicts well for both DTC subtypes. The 8th edition of the TNM staging system is a better predictor of both overall survival (OS) and DSS than the previous 7th edition for both PTC and FTC. Until recently, several other studies compared the performance of the 8th with the 7th edition in patients with DTC (10-23), and like us, the majority showed superiority of the 8th edition with respect to predicting survival. We were the first to compare the 8th and 7th edition in patients with FTC. Subsequently, two studies from Japan and South Korea showed mixed results as one also showed superiority of the 8th edition (16), while the other showed no difference (18). One explanation that the latter study showed no difference is the fact that stage III and IV comprised respectively only two and 11 patients in the 8th edition, and therewith the study probably lacks power to show differences. Therefore, it is plausible that also in patients with FTC, the 8th edition performs better than the 7th edition.

However, although raising the age cutoff from 45 to 55 years was based on three earlier studies (24-26), these studies were performed using the 7th edition's TNM. Consequently this does not necessarily imply that this age cutoff is also the best suited for the 8th edition's TNM. Therefore in **Chapter 3,** we investigate if another age cutoff than 55 years leads to improvement of the prognostic value of the AJCC/TNM 8th edition, and again we also focused on potential differences between patients with PTC and FTC. We showed, using 3074 patients from the Netherlands and Germany, that the overall optimal age cutoff employing the histopathological staging criteria of the 8th edition of the TNM system to predict DSS is 50 years for patients with PTC, but not for those with FTC, as in these patients 40 years was the optimal cutoff. It is well-established that FTC and PTC have different clinical manifestations (22), and our study further emphasizes this. Hence, the present study implies that for an optimal estimate of prognosis, PTC and FTC should be staged as separate entities. However, our results do not necessarily imply that using a dichotomic age classification is optimal; it still remains a dichotomization of what most likely is a sliding scale. Therefore, further research is needed to investigate whether there is a better method to incorporate age into the TNM staging system, e.g. adjusting for relative survival rates (28, 42), combining histopathological staging

criteria with age (continuously or e.g. per decade) in a risk calculator, or considering employing multiple age cutoffs. While doing this, it is important to not only focus on statistical performance, but also on feasibility in clinical practice.

The ATA Risk Stratification System is widely used and several studies have shown its usefulness in predicting disease recurrence (13, 27-32). The majority of these studies either comprised relatively small proportions of ATA High Risk patients (13, 30), evaluated the previous 2009 edition (27-29, 31), only comprised patients with PTC (13, 29, 30), or had low numbers of patients with FTC. Therefore, it is important to know how the 2015 ATA Risk Stratification System performs in High Risk patients, especially with respect to recurrence.

In **Chapter 4**, we therefore evaluated the prognostic value of the 2015 ATA Risk Stratification System in 236 ATA High Risk DTC patients and compared PTC and FTC. We showed that the 2015 ATA Risk Stratification System is not only an excellent predictor of persisting disease, but also of survival as the response to therapy category determined after initial therapy is a strong predictor of survival. During follow-up, 14% of the High Risk patients with an excellent response to therapy experienced a recurrence (median time to recurrence 47 months), which is in contrast with the recurrence rate of 1-4% stated in the 2015 ATA Guidelines (7). Previous studies in High Risk patients also showed much higher rates of 14-30% (27, 29, 30, 32, 33). This indicates that the recurrence rates after excellent response are much higher in High Risk patients than in those with Low or Intermediate Risk, illustrating that it is important to be aware of this substantial high recurrence risk when treating and following up on these High Risk patients. Further research should focus on factors that could predict which patients are at risk for recurrence.

Earlier studies showed that patients with distantly metastasized DTC have a relative poor prognosis (34-37). Risk factors such as age, RAI avidity, tumor size, and follicular type influence this prognosis (34-36, 38-43). However, no studies yet evaluated the 2015 ATA Risk Stratification System in DTC patients with distant metastases with respect to its ability to predict prognosis, recurrence and survival. Therefore in **Chapter 5**, we evaluated the performance of the 2015 ATA Risk Stratification System in 83 patients with distant metastases. We showed that the 2015 ATA Risk Stratification System is an excellent predictor of both persistent disease and recurrence in patients with initial metastatic disease. None of the patients with an excellent response during follow-up experienced a recurrence later-on, which is in contrast with **Chapter 4**, but similar to the results of Hirsh et al. (36). One might argue that a successful therapy for distant metastatic disease is also able to destroy other thyroid cancer tissue (e.g. due to gross extra thyroidal extension) resulting into a lower recurrence rates in these patients.

The current 2015 ATA Guidelines use their own Risk Stratification System to determine the need for RAI therapy after surgery (7). However, this approach has been challenged by several experts in the field (44-46). Because one of the aims of RAI therapy is to treat any remaining unknown cancer tissue, omitting RAI therapy might therewith leave metastases unknown and untreated (45). One study claimed that 4 to 5% of the patients with distant metastatic disease would not have been treated with RAI, and thereby metastases would have been missed (47), while another study showed, in Low and Intermediate Risk patients defined by the 2009 ATA Guidelines, that 1 to 1.4% of the distant metastases would have been missed with omission of RAI therapy (48). In **Chapter 5**, we therefore also investigated the proportion of possible distant metastases that initially would be missed if no RAI therapy is given. We showed that in DTC patients without an indication for RAI therapy or in whom RAI therapy should be considered, 1.6% of the Low Risk and 2.5% of the Intermediate Risk patients have distant metastases that initially would be missed if no RAI therapy is given. Albano et al. found a slightly higher number as 3.6% of their Low Risk and 4.6% in their Intermediate Risk patients having distant metastases that would have been missed using the 2015 ATA Guidelines (47). However, they also included patients in whom metastatic disease was diagnosed during follow-up. In contrast, Agate et al. found a lower number of approximately 1% in Low Risk and 1.4% in Intermediate Risk patients having distant metastases that would have been missed (48). However, these results might not be totally comparable as they used the Low and Intermediate Risk definitions of the 2009 ATA Guidelines. In conclusion, not giving RAI therapy to ATA Low Risk patients might lead to initially missing 1-4% of the patients with distant metastases, while this percentage is 2-5% in those with ATA Intermediate Risk. However, because all studies are retrospective, it is unclear if omitting RAI therapy would have affected prognosis, and studies were too small to determine factors in which patients RAI therapy could be omitted safely. Therefore, further research should focus on determining such factors to be able to predict in which patients RAI therapy could be omitted safely without the risk of missing distant metastatic disease.

Recently it was shown that the 2015 ATA Risk Stratification System can be improved by adding age as a risk classifying factor, especially for ATA High Risk patients (49). However, as this study comprised relatively few patients High Risk DTC, in **Chapter 6** we first assessed the influence of age on disease outcome, and subsequently assessed whether an age cutoff could improve the 2015 ATA Risk Stratification System. We showed that older age, either continuously or dichotomously, have a significant negative influence on excellent response after initial therapy, developing no evidence of disease (NED), recurrence, and disease specific mortality (DSM) for either PTC or FTC. Further, age remained significant, when adjusted for the

original ATA High Risk factors, for having an excellent response after initial therapy for PTC, developing NED for PTC and FTC, and DSM for PTC, implicating that age should be considered to be included as a risk factor in the ATA Risk Stratification System. One might suggest to use an age cutoff of 55 years for PTC and 65 years for FTC in ATA High Risk patients. Trimboli et al. showed that ATA High Risk patients could be reclassified in two subgroups based on an age cutoff of 55 years with older patients having the highest relapse risk, while such an age cutoff could not be identified for Low and Intermediate Risk patients (49); their population predominantly contained patients with PTC (91%). Therefore, further research is still needed, which besides ATA High Risk also includes patients with ATA Low and Intermediate Risk to determine in which way age can be incorporated into the ATA Risk Stratification System to further improve its predictive function regarding response to therapy and recurrence in both PTC and FTC patients. For example, single or multiple age cutoffs, or, like Trimboli et al. (49), define new risk categories for High Risk patients younger or older than a certain age cutoff. Therewith, clinical management can be better optimized for these older High Risk patients.

Long-term impact of treatment

As mentioned and showed earlier, the survival of the majority of the patients with DTC is relatively good. For these patients, it is therefore very important to minimize adverse effects of (initial) therapy and preserve Quality of Life (QoL). It is known from earlier cross-sectional studies that QoL is decreased in different domains in thyroid cancer survivors compared to the general population (50-54). Their QoL is at the same level of patients with other cancers with worse prognosis, and is even worse than QoL of breast cancer survivors (55). Main drawback of these cross-sectional studies is that no conclusions can be drawn about QoL changes over time. Therefore, more recently several longitudinal studies were conducted (50, 56-59), but their results were mixed, and also had one or more limitations such as relative short follow-up with a maximum of two years (57-59), or lack of knowledge of QoL before initial surgery (50, 58). Further, one cross-sectional study showed that younger age, female sex, and lower education are related to lower QoL (60). Unfortunately, none of these factors were investigated longitudinally.

In **Chapter 7**, we therefore investigate long-term longitudinal changes of QoL in 185 patients undergoing treatment for DTC with a total thyroidectomy followed by RAI therapy. We showed that QoL before initial therapy is already lower than in the general population. This might be due to the fact that patients already were aware of their thyroid cancer diagnosis when completing baseline questionnaires. Unfortunately, it is impossible to have QoL scores of thyroid cancer patients before (suspicion of) diagnosis, so using reference values of the general population is

second best. During follow-up, QoL develops in general non-linear over time with the lowest QoL around RAI therapy, and two to three years after initial therapy it is approximately the same as at baseline. This pattern was also shown in earlier studies (50, 59), but these studies were either relative short or lack knowledge of QoL before surgery. Further, one study showed that QoL after 12 months is worse in patients who received a total thyroidectomy with RAI therapy compared to those without RAI therapy, but nothing is known what happens thereafter (59). In addition, younger age, female sex, and persistent hypoparathyroidism resulted in lower QoL, which is in line with earlier studies (60-62). Nowadays, a less aggressive therapeutic approach is suggested for selected populations (6, 7), and therefore less often a total thyroidectomy followed by RAI therapy is performed. Due to the nature of our DTC population, we were only able to include patients receiving a total thyroidectomy followed by RAI therapy. Therefore, our results cannot be compared or extrapolated to patients treated differently. However, our results are still important to create awareness that it takes years to regain baseline QoL level after initial treatment, and younger patients, females, and those with persistent hypoparathyroidism in general have a lower QoL. Further longitudinal research, with follow-up of more than 5 years, also including patients who did not receive RAI therapy for DTC, is needed to know more about QoL (including anxiety/fear for recurrence) in the long term, and the impact of less aggressive treatment on it.

Adverse effects of (initial) therapy are for example hypoparathyroidism, voice alterations, salivary gland dysfunction, severe bone marrow dysfunction, adverse cardiovascular effects, and osteoporosis (7, 63). I-131 has probably also an effect on the gonadal system. In men, abnormalities in testicular function are common several months after one single therapeutic dose, while the risk of persistent gonadal dysfunction is increased after repeated or high RAI dose (64). Therefore, it is advised to cryopreserve semen (4). In women, a transient change of the menstrual cycle in 12-31%, and a temporary increase of Follicle Stimulation Hormone (FSH) during the first year after RAI therapy has been described (65-67). However, no increased infertility rates or adverse obstetric outcomes were seen in patients after RAI therapy (65, 66, 68). Recently, several studies evaluated Anti-Müllerian hormone (AMH) as a representative of ovarian reserve in patients with DTC receiving RAI therapy (68-72). AMH is relatively insensitive to inter- and intra-cycle variability and oral contraceptives use, is known to gradually decline with age, and is undetectable at menopause. Therefore, AMH seems to be a good marker for ovarian reserve (73-76). Two earlier longitudinal studies showed a significant decrease of AMH concentrations when comparing concentrations just before RAI therapy and 12 months later (70, 72), but no studies with follow-up longer than 12 months, or including patients with multiple RAI therapies exist.

In **Chapter 8**, we investigated the long-term effects of RAI therapy on AMH concentrations in 65 female patients undergoing treatment for DTC. In patients who received a single RAI therapy, we showed that AMH concentrations significantly dropped the first year after initial therapy by 55%. Thereafter, AMH concentrations remained stable but no recovery was seen. In two earlier longitudinal studies with follow-up of 12 months, also a significant decrease of AMH concentrations (-30% and -58%) was seen after initial RAI therapy (70, 72). In patients receiving multiple RAI therapies, we also showed a significant drop of AMH concentrations in the first year (-74%). This drop was followed by short stabilization, and thereafter a further decrease is seen. This latter decrease was most likely due to additional RAI therapy, but this decrease was less severe than the initial drop. This pattern might be caused by the fact that the patients received their therapies at different points in time (median time between first and second therapy was 9 months), and therewith the expected step-wise decrease was not found, or oocytes of less quality were already damaged by the first initial RAI therapy and those with better quality prevail. As we were the first to study women receiving multiple RAI therapies, we cannot compare our results with other studies, and further research is needed to confirm our findings. In addition, patients older than 35 years showed a much stronger decrease than those younger than 35 years. In correspondence with our results, two other longitudinal studies also showed a less steep decrease after RAI therapy in younger females (70, 72). At same time of our study, Mittica et al. published a longitudinal study in 43 females with a mean follow-up of 31 months, and they did not find an influence of RAI therapy on AMH concentrations (77); however, they did not perform longitudinal analysis. More recently, Nies et al. did not find effects of RAI therapy on AMH concentration in female survivors of childhood DTC (68). Main difference with our study is the young age at which these survivors were treated, and therefore the initial quality of the oocytes might have been better. A possible limitation of our study might be the lack of a control group, and therefore, one might argue that the discovered decline over time in our study is the biological decrease. However, both the stabilization one year after RAI therapy, and the AMH decline in our study, which is far beyond the expected age-related decline (74), suggests causality. Further, it is important to note that AMH concentrations do not reflect the quality of the oocytes and therewith diminished AMH concentrations do not necessarily mean reduced fertility (78). Finally, DTC diagnosis and treatment influenced the desire to have a child in almost 40% of the female patients. Therefore, it is important to create awareness about both the psychological and biological impact of DTC diagnosis and treatment on female patients in their reproductive years. Our results support a less aggressive treatment with RAI in Low Risk patients as is advocated in the current 2015 ATA Guidelines, especially in females over 35 years of age with the desire to

have a child. Further research should focus on the relationship between the AMH drop and fertility and pregnancy outcomes, and therewith inevitably, larger populations are needed.

THYROID HORMONE METABOLITES

Hypothyroidism is a very common endocrine disorder (79). Primary hypothyroidism due to thyroid auto-immunity (Hashimoto's disease) is the most frequent cause. Replacing the deficient hormone is the basis for the treatment of hypothyroidism, and therefore levothyroxine (LT4) is given to restore euthyroidism. Although biochemical euthyroidism can be achieved by LT4, a substantial part of the patients (± 10-15%) show significant impairment of physical and psychological well-being compared to matched controls or the general population (80-85).

Several possible explanations for these persistent symptoms exist. First, thyroid autoimmunity in itself could cause persisting symptoms (86-88). Second, the inability of LT4-treatment to restore physiological T4 and T3 concentrations in serum and tissue (89, 90). Third, next to T3, thyroid hormone metabolites (THM) may be altered in these patients as well (53), and T3 independent effects have been reported for some of these metabolites such as 3,5-T2 (91) and 3,5,3'-triiodothyroacetic acid (TA3) (92). However, it is currently unknown if T4 supplementation can adequately restore concentrations of THM. Next to this, QoL was not associated with T4, T3, rT3, and 3,5-T2 concentrations in thyroid cancer survivors (53, 93). However, the relationship between other THM and QoL has not been studied previously. One of the factors limiting such a study is that until recently, only (radio)immunoassays were available to measure the different THM. Recently, a LC-MS/MS panel for nine thyroid hormone and thyroid hormone metabolites (THM) in human serum was developed, and subsequently reference intervals in healthy adults were established (94).

During treatment for DTC, patients are usually euthyroid before surgery, hypothyroid after three to four weeks of thyroid hormone withdrawal, and (subclinical) hyperthyroid during TSH-suppressive therapy using LT4. Combining these three different thyroid states in individual patients results into a unique model to study THM and QoL during altered thyroid states. Using DTC patients as a model to study differences between altered thyroid states within the same patient is a known concept (93, 95, 96).

In **Chapter 9,** we used an extensive THM panel (94) to prospectively analyze how thyroidectomy without and with T4 supplementation affects THM concentrations in 77 patients treated for DTC. All THM decreased after thyroid hormone withdrawal, and all THM, except for T3, (subclinically) increased during TSH suppressive LT4

therapy. T3 remains within the reference interval with LT4 therapy. As all measured THM, except for T3, follow the same trend as T4, this indicates that the measured THM are produced by peripheral metabolism of T4 and have no or negligible thyroidal origin. This is also reflected by T3, which is synthesized in the thyroid gland for approximately 20%, and does not follow the same trend as T4. With our own research we showed that the newly established LC-MS/MS panel is capable to measure several THM in hypo, hyper en euthyroid state, but further research into the relation between THM and QoL is needed to assess whether THM concentrations play a role in the persistent complaints in patients with hypothyroidism that undergo LT4 therapy.

Therefore, in **Chapter 10** we investigated the possible relationship between THM and QoL in 63 patients treated for DTC. Cross-sectionally, THM were not associated with QoL at either hypo, (subclinical) hyper, or euthyroid state. In previous cross-sectional studies in DTC-patients on LT4-treatment, 3,5-T2, T3, rT3 and T4 were also not associated with QoL (51, 53). In hypothyroid patients on LT4 replacement therapy, T3, rT3 and free-T3 were not associated with psychological well-being (84, 97). A possible explanation may be that a certain decrease in THM concentrations results into such a low QoL that individual differences THM hardly have an influence on QoL anymore. Further, the lack of spread of THM concentrations in either thyroid state might be another reason no associations were found. Longitudinally, TA4 (three QoL scores), rT3 and T0 (two scores), 3-T1, 3,3'-T2, and T3 were significantly associated with QoL differences between euthyroid, hypothyroid and (subclinical) hyperthyroid state for six QoL scores (RAND-36 Physical Functioning (three times), MFI-20 Mental Fatigue (twice), ThyPRO Impaired Daylife (twice), RAND-36 Vitality, ThyPRO Social Impairment and Tiredness). To our knowledge, this is the first study establishing that QoL differs between different thyroid states in patients treated for DTC for those with higher compared to lower concentrations of the above mentioned THM. However, no consistent patterns were found between specific THM and QoL domains.

Longitudinal QoL studies in thyroid cancer patients, including the study presented in **Chapter 7**, showed a persistent decrease in QoL compared to the general population (56, 59). In **Chapter 10**, QoL at the (subclinical) hyperthyroid state was also lower compared to baseline in the majority of the QoL scores. The period of thyroid hormone withdrawal or thyroid cancer itself may play a role in these lower QoL scores, but we did not identify a specific THM that was associated with these lower QoL scores. Additionally, at the euthyroid state, the majority of the QoL scores were lower than those of the general population (51, 98, 99), and again we did not identify a specific THM that is associated to these lower QoL scores. Therefore, the impact of cancer diagnosis, surgery or RAI therapy on QoL can be an explanation for not finding any associations

as this might create QoL differences unrelated to THM. Therefore, one might argue that treated DTC patients are not an optimal model to study the relationship between THM and QoL, but on the other hand, advantages of the current model are 1) three thyroid states can be studied in the same patient in a prospective manner, and 2) hypothyroidism is caused by total thyroidectomy and (subclinical) hyperthyroidism by LT4 therapy and not by autoimmune thyroid disease, therewith eliminating this possible confounder/modifier. In conclusion, we could not identify a specific THM to explain the persistent symptoms in patients with hypothyroidism. This can be either caused by the fact that there is no influence at all, our current model is not optimal, or we lacked statistical power. Consequently, further research in larger and different populations is needed to confirm our findings.

The presence of relatively lower T3 in patients receiving LT4 treatment is a known phenomenon (89, 90), and one of the reasons why T4/T3-combination therapy is advocated for. However, although more than ten randomized controlled trials (RCTs) and meta-analyses were conducted, there is still insufficient evidence for routinely adding LT3 to LT4-treatment outside a formal clinical trial or N-of-1 trial according to 2014 ATA Guidelines (100). Further, the 2012 ETA Guidelines argue to try combination therapy in selected patients (101). Further clinical trials, dealing with the shortcomings of the previous ones, are needed to elucidate if T4/T3-combination therapy is useful (102). The LT4/LT3 combination therapy for hypothyroidism trial (T3-4-Hypo trial) will start soon in hospitals around The Netherlands, and results are expected from 2024 onwards.

CONCLUSIONS AND FURTHER RESEARCH

The studies presented in this thesis aimed at the prognosis and prognostic factors of patients with DTC, the long-term impact of treatment on patients with DTC, and THM in different thyroid states including their possible association with QoL to explore a potential cause of persistent complaints in patients with hypothyroidism.

The two major existing systems that have been designed to predict recurrence and survival in patients with DTC were evaluated. Results show that both systems perform well. For the AJCC/TNM staging system our results imply that for an optimal estimate of prognosis, PTC and FTC should be staged as separate entities. Besides we identified new optimal age cutoffs for both PTC (50 years) and FTC (40 years). Further research should focus whether there is a better method to incorporate age into the TNM staging system. However, while doing this, it is important to not only focus on statistical performance, but also on feasibility for clinical practice.

Regarding the 2015 ATA Guidelines, we showed that 14% of the ATA High Risk patients with an excellent response to therapy experienced a recurrence during follow-up, which is in contrast with the recurrence rate of 1-4% stated in the 2015 ATA Guidelines. This implicates that it is important to be aware of this substantially higher recurrence risk when treating and following up on these ATA High Risk patients. We also showed that, as older age has a significant negative influence on disease outcomes, adding age as a risk factor to the ATA Risk Stratification System could improve the predictive value of this system. Further research should focus on factors that could predict which patients are at risk for recurrence, on other potential factors that may improve the Risk Stratification System, and on which is the best method to incorporate age in this System. Evaluating the 2015 ATA Guidelines, we showed that in DTC patients without an indication for RAI therapy or in whom RAI therapy should be considered, respectively 1.6% and 2.5% have distant metastases that initially would be missed if no RAI therapy is given. Although omitting RAI therapy is not incorporated in the current 2015 Dutch Guidelines, it is important to keep these percentage into mind if the Guidelines might change in the future. Further research should therefore focus on factors predicting in which patients RAI therapy could be omitted safely without the risk of not detecting and not treating distant metastatic disease.

We showed that both diagnosis of and treatment for DTC have a major impact on QoL of the patients, and it takes years to regain baseline QoL level after initial treatment with total thyroidectomy followed by RAI therapy. Further, we showed that younger patients, females, and those with persistent hypoparathyroidism in general have a lower QoL. These results are important to create awareness, but also to create recognition for patients. Further longitudinal research, with follow-up of more than 5 years, also including patients who did not receive RAI therapy for DTC, is needed to know more about the QoL (including anxiety/fear for recurrence) in the long term, and the impact of less aggressive treatment (e.g. in Low Risk patients) on it.

We showed that RAI treatment in female patients with DTC has significant impact on ovarian reserve (measured as AMH). Additionally, patients older than 35 years showed a much stronger decrease than those younger 35 years. Therefore, it is important to create awareness about the impact of DTC treatment on female patients in their reproductive years. Our results support a less aggressive treatment with RAI in Low Risk patients as is advocated for in the current 2015 ATA Guidelines, especially in females over 35 years of age with the desire to have a child. Further research should focus on the relationship between the AMH decrease and fertility and pregnancy outcomes, and therewith inevitably, large(r) populations are needed.

By assessing THM in different thyroid states using a recently established LC-MS/MS panel, our results strongly suggest that all measured THM are produced by

peripheral metabolism of T4 and have no or negligible thyroidal origin. This is also reflected by T3, which is synthesized in the thyroid gland for approximately 20%, and does not follow the same trend as T4. Subsequently, we showed that T0, 3-T1, 3,3'-T2, T3, rT3, and TA4 are significantly associated with QoL differences between different thyroid states in patients treated for DTC, but no consistent patterns were found between THM concentrations or QoL domains. Further, cross-sectionally, the measured THM were not associated with QoL at either hypo, (subclinical) hyper or euthyroid state. Therefore, it seems that THM are not an explanation for persistent symptoms in patients with hypothyroidism, but it might be that our results are hampered by either the sample size, possible cons of the used DTC model, or both. Consequently, further research is needed in larger and different populations to confirm our findings.

In conclusion, in this thesis, the two major existing systems that have been designed to predict recurrence and survival in patients with DTC were evaluated, and results show that both systems perform well. Subsequently, suggestions to improve both the AJCC/TNM staging system and ATA Risk Stratification System were given. Thereafter, it was shown that treatment of patients with DTC has a long-term impact on both QoL and ovarian reserve. Finally, it seems that THM 1) have no or negligible thyroidal origin, but are produced by peripheral metabolism of T4, and 2) are not an explanation for persistent symptoms in patients with hypothyroidism.

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Chapter 12

Summary

ENGLISH SUMMARY

The thyroid is an endocrine gland located in the lower part of the anterior neck, just below the larynx. The main function of the thyroid is to produce thyroid hormones (T3 and T4), hormones which are crucial for the normal development and metabolism regulation of all tissues. Abnormal thyroid hormone concentrations affect the functioning of several other organs possibly resulting in a myriad of clinical symptoms. Hypothyroidism, a very common endocrine disorder, reflects shortness of thyroid hormone at the tissue level. Its most common form is primary hypothyroidism due to thyroid auto-immunity (Hashimoto's disease). Symptoms include tiredness, depressiveness, cold tolerance, constipation, bradycardia, and weight gain.

Development of abnormal density of thyroid cells could cause nodules, which in non-iodine deficient countries can be found in 4-7% of the population. A small proportion of these nodules are malignant which means thyroid cancer. Thyroid cancer is the most common endocrine malignancy with approximately 700 new cases in The Netherlands in 2018. The worldwide incidence has been steadily increasing over the last two decades. Different subtypes of thyroid cancer exist of which well differentiated thyroid cancer (DTC), comprising both papillary (PTC) and follicular thyroid cancer (FTC), to be the most frequent (80-85%). Initial treatment of DTC is multidisciplinary and is usually multistep wise. According to the current 2015 Dutch Thyroid Cancer Guidelines, a total thyroidectomy is recommended if the tumor is larger than 1cm, is multifocal, has extrathyroidal extension, or if there are lymph node or distant metastases; otherwise a hemithyroidectomy is sufficient. Postoperatively, the tumor is staged according to the TNM system. After a total thyroidectomy, the 2015 Dutch guidelines recommend to perform subsequent radioiodine (RAI) therapy with I-131. To ensure optimal RAI uptake, therapy takes place under an iodine-deficient diet (during one week) and TSH stimulation. TSH stimulation can be achieved either after 3-4 weeks of thyroid hormone withdrawal, or using two injections of recombinant human TSH (rhTSH). After thyroid surgery, patients are treated with thyroid hormone replacement therapy with levothyroxine (LT4) in supraphysiological doses to suppress TSH. Rationale behind this is that TSH can stimulate growth of thyroid cancer cells, and therefore lower TSH levels are thought to prevent re-growth of remaining cells.

The survival of patients with DTC is relatively good, with a 10-year disease specific survival (DSS) over 90%. Therefore, day-to-day treatment of patients is to a large extent based on the risk of recurrence. However, there are certain patients in which survival is worse and it is important to identify them. Different systems have been proposed to predict the risk of recurrence and survival in patients with DTC to better determine the need for aggressive therapy and optimize follow-up

strategies. The AJCC/TNM staging system is designed to predict DSS, while the ATA risk stratification system is designed to estimate the risk of recurrence. In **Chapter 2 and 3** we evaluated and refined the most recent version (8th edition) of the AJCC/TNM staging system, while in **Chapter 4, 5 and 6** we assessed the performance and subsequently proposed improvements of the 2015 ATA Guidelines including its Risk Stratification System.

In **Chapter 2** we compared the new 8th edition of the AJCC/TNM classification system to the previous 7th edition in patients with DTC with respect to differences in staging and survival, and thereafter we assessed potential differences between PTC and FTC. We showed that using the 8th edition, there is no significant difference between PTC and FTC anymore regarding survival rates per stage, implicating that AJCC/TNM stage predicts well for both DTC subtypes. The 8th edition of the AJCC/TNM staging system is a better predictor of both overall survival (OS) and DSS than the previous 7th edition for both PTC and FTC. In **Chapter 3,** we investigated if another age cutoff than the currently used 55 years in the 8th edition, leads to improvement of the prognostic value of the AJCC/TNM 8th edition, and again we also focused on potential differences between patients with PTC and FTC. We showed that the overall optimal age cutoff, employing the histopathological staging criteria of the 8th edition of the TNM system, to predict DSS is 50 years for patients with PTC, but not for those with FTC, as in these patients 40 years was the optimal cutoff. Therewith, this implies that that for an optimal estimate of prognosis, PTC and FTC should be staged as separate entities.

In **Chapter 4**, we evaluated the prognostic value of the 2015 ATA Risk Stratification System in a large population of ATA High Risk DTC patients, and compared PTC and FTC. We showed that the 2015 ATA Risk Stratification System is not only an excellent predictor of persisting disease, but also of survival as the response to therapy category determined after initial therapy is a strong predictor of survival. In addition, 14% of the High Risk patients with an excellent response to therapy experienced a recurrence during follow-up (median time to recurrence 47 months), which is in contrast with the 2015 ATA Guidelines stating recurrence rates of 1-4% in DTC patients with an excellent response. In **Chapter 5**, we evaluated the performance of the 2015 ATA Risk Stratification System in patients with distant metastases, and showed that it is an excellent predictor of both persistent disease and recurrence in patients with initial metastatic disease. Also in **Chapter 5**, we investigated the proportion of possible distant metastases that initially would be missed if no RAI therapy is given when following the 2015 ATA Guidelines. We showed that in DTC patients without an indication for RAI therapy or in whom RAI therapy should be considered, 1.6% of the Low Risk and 2.5% of the Intermediate Risk patients have distant metastases that initially would be missed if no RAI therapy is given. In

Chapter 6, we investigated the influence of age on disease outcome in patients with High Risk DTC, and subsequently whether an age cutoff could improve the 2015 ATA Risk Stratification System. We showed that in patients with High Risk DTC, harboring a large set of FTC patients, older age, either continuously or dichotomously, has a significant negative influence on disease outcome, and therefore should be considered to be included as a risk factor in the ATA Risk Stratification System. Slightly different optimal age cutoffs were identified for the different outcomes, and these cutoffs differed between PTC and FTC. Therefore, also this study implies that for an optimal estimate of disease outcome, PTC and FTC should be treated as separate entities.

As the survival of patients with DTC is relatively good, it is therefore very important to minimize adverse effects of (initial) therapy while retaining a good prognosis. Adverse effects of (initial) therapy are for example hypoparathyroidism due to surgical damage of the parathyroid glands, voice alterations due to recurrent nerve damage caused by surgery, salivary gland dysfunction due to I-131, severe bone marrow dysfunction after high doses of I-131, adverse cardiovascular effects or osteoporosis due to TSH-suppressive therapy, and I-131 has probably also an effect on the gonadal system. Next to these, it is known from earlier studies that Quality of Life (QoL) is decreased in different domains in thyroid cancer survivors compared to the general population, and their QoL is at the same level of patients with other cancers with worse prognosis, like breast cancer.

In **Chapter 7**, we investigate long-term longitudinal changes of QoL in patients undergoing treatment for DTC with a total thyroidectomy followed by RAI therapy. We showed that QoL before initial therapy is already lower than in the general population. During follow-up, QoL changes in general non-linear over time with the lowest QoL around RAI therapy. Two to three years after initial therapy QoL is approximately the same as at baseline, which is still lower than in the general population. In addition, younger age, female sex, and persistent hypoparathyroidism resulted in lower QoL.

In **Chapter 8**, we investigated the long-term effects of RAI therapy on female gonadal reserve, represented by serum AMH concentrations, in female patients undergoing treatment for DTC. In patients that received a single RAI therapy we showed that AMH concentrations significantly dropped during the first year after initial therapy by 55%, while in patients receiving multiple RAI therapies this is -74% in the first year. In addition, patients older than 35 years showed a much stronger decrease than those younger than 35 years. Finally, DTC diagnosis and treatment influenced the desire to have a child in almost 40% of the female patients.

Replacing the deficient hormone is the basis for the treatment of hypothyroidism, and therefore LT4 is given to restore euthyroidism. Although biochemical

euthyroidism can be achieved by LT4, a substantial part of the patients (± 10-15%) show significant impairment of physical and psychological well-being compared to matched controls or the general population. Several potential hypotheses for these persistent symptoms exist. First, thyroid autoimmunity in itself could cause persisting symptoms. Second, the inability of LT4 replacement therapy to restore physiological T4 and T3 concentrations in serum and tissue. Third, besides T4 and T3, it might be that thyroid hormone metabolites, which may have physiological functions, are decreased. Of these explanations, literature on the latter is scarce, and therefore in **Chapter 9 and 10** we assessed nine thyroid hormones and thyroid hormone metabolites (THM) during hypo, hyper and euthyroidism using a newly developed and validated Liquid chromatography - mass spectrometry / mass spectrometry (LC-MS/MS) panel. For this, we used patients with DTC as during treatment they experience each of the three thyroid states, i.e. patients are usually euthyroid before surgery, hypothyroid after 3-4 weeks of thyroid hormone withdrawal, and (subclinical) hyperthyroid during TSH-suppressive therapy with LT4.

In **Chapter 9**, we investigate how THM changes across hypo, hyper and euthyroidism. We showed that the newly LC-MS/MS panel is capable to measure several THM in hypo, hyper and euthyroid state. All THM decreased after thyroid hormone withdrawal, and all THM, except for T3, increased during TSH suppressive LT4 therapy. These results strongly suggest that all measured THM are produced by peripheral metabolism of T4 and have no or negligible thyroidal origin. This is also reflected by T3, which is synthesized in the thyroid gland for approximately 20%, and does not follow the same trend as T4.

In **Chapter 10**, we investigate a possible relationship between THM and QoL. We showed that QoL differs between different thyroid states for those with higher compared to lower concentrations of T0, 3-T1, 3,3'-T2, T3, rT3, and TA4, but no consistent patterns in the relationship between THM concentrations or QoL domains were observed. Further, cross-sectionally, none of the measured THM were associated with QoL at either hypo, (subclinical) hyper or euthyroid state. Therefore, it seems that THM are not an explanation for persistent symptoms in patients with hypothyroidism. This can be either caused by the fact that there is no influence, our current model is not optimal, or we lacked statistical power. Consequently, further research is needed in larger and different populations to confirm our findings.

In **Chapter 11,** the results presented in this thesis were shown and discussed, and recommendations for further research were given.

NEDERLANDSE SAMENVATTING

De schildklier is een vlindervormig orgaan dat zich aan de voorzijde van de hals bevindt. De schildklier is een hormoonproducerend orgaan en produceert met name T4 (thyroxine) en T3 (trijodothyronine). T4 is het inactieve hormoon dat door speciale enzymen (dejodases) wordt omgezet in het actieve hormoon T3. Schildklierhormoon is cruciaal voor de normale ontwikkeling en de stofwisseling van alle weefsels in het lichaam. Abnormale schildklierhormoonconcentraties kunnen leiden tot het afwijkend functioneren van verschillende organen waardoor veel verschillende klachten (symptomen) kunnen ontstaan. Een tekort aan schildklierhormoon wordt ook wel hypothyreoïdie genoemd, en de bekendste vorm wordt veroorzaakt door een auto-immuun ziekte van de schildklier (Ziekte van Hashimoto). Symptomen die aanwezig kunnen zijn bij een hypothyroïdie zijn vermoeidheid, somberheid, koude-intolerantie, constipatie, gewichtstoename en een trage hartslag.

In landen waar geen tekort aan jodium bij de bevolking aanwezig is, is bij ongeveer 4-7% van de mensen een knobbel in de schildklier aanwezig. Een klein deel van deze knobbels zijn kwaadaardig wat betekent dat er sprake is van schildklierkanker/carcinoom. Schildklierkanker is de meest voorkomende kanker van de hormoonproducerende organen, en in 2018 werden er in Nederland ongeveer 700 patiënten gediagnosticeerd met schildklierkanker. De laatste decennia is er wereldwijd een toename van het aantal nieuwe patiënten met schildklierkanker. Er zijn verschillende vormen van schildklierkanker, waarbij het (goed) gedifferentieerde schildkliercarcinoom (DTC) het meest voorkomend is (80-85%); deze is nog verder onder te verdelen in papillair schildkliercarcinoom (PTC) en folliculair schildkliercarcinoom (FTC). De behandeling van DTC is multidisciplinair. In de laatste Nederlandse Schildkliercarcinoom Richtlijn uit 2015 wordt een verwijdering van de gehele schildklier geadviseerd (totale thyreoïdectomie) wanneer de tumor groter is dan 1cm, er meerdere tumorhaarden zijn, de tumor buiten de schildklier groeit, of er uitzaaiingen (metastasen) zijn in de lokale lymfeklieren of in andere organen (afstandsmetastasen). In andere gevallen is een verwijdering van de halve schildklier (hemithyreoïdectomie) afdoende. Na de operatie wordt het stadium van het schildkliercarcinoom bepaald met behulp van het zogenaamde TNM systeem. Volgens de Nederlandse Schildkliercarcinoom Richtlijn uit 2015 is er na een totale thyreoïdectomie een indicatie voor vervolgbehandeling met radioactief jodium (RAI; I-131). Om er voor te zorgen dat het radioactieve jodium optimaal wordt opgenomen moeten patiënten gedurende een week een jodiumarm dieet volgen en wordt ervoor gezorgd dat het schildklier stimulerend hormoon (TSH) verhoogd is. Dit laatste kan worden gedaan door patiënten gedurende 3-4 weken geen levothyroxine toe te dienen, of door middel van twee injecties met synthetisch TSH (recombinant human

TSH (rhTSH)). Na een totale thyreoïdectomie worden de patiënten behandeld met levothyroxine in een dosering die hoger is dan bij gewone patiënten zodat het TSH kan worden onderdrukt (TSH-suppressieve therapie). Het idee hierachter is dat TSH de groei van schildklierkankercellen kan stimuleren, en van een lager TSH wordt gedacht dat het voorkomt dat eventuele overbleven schildklierkanker cellen gaan (terug)groeien.

De prognose van patiënten met DTC is relatief goed, waarbij de 10-jaars schildklierkanker specifieke overleving (DSS) meer dan 90% bedraagt. Daarom is de behandeling van patiënten ook voor een groot deel gebaseerd op het risico op een recidief. Daarentegen zijn er ook patiënts wiens prognose slecht is, en het is dan ook van belang om deze patiënten te identificeren. Verschillende 'systemen' zijn ontwikkeld om de overleving en het risico op een recidief beter te kunnen voorspellen, en daarmee te bepalen wie er agressief behandeld en veelvuldig gecontroleerd moet worden, en bij wie dit juist allemaal wat minder nodig is. Het AJCC/TNM Staging System is ontwikkeld om de DSS te voorspellen, terwijl het ATA Risk Stratification System (beschreven in de richtlijn van de Amerikaanse schildklier vereniging (ATA Guidelines)) ontwikkeld is om het risico op het ontstaan van een recidief te voorspellen. In **Hoofdstuk 2 en 3** wordt de meest recente versie (8e editie) van het AJCC/TNM Staging System geëvalueerd en vervolgens een voorstel voor verbetering gedaan, terwijl in **Hoofdstuk 4, 5 en 6** de prestatie van de ATA Guidelines (uit 2015) en haar Risk Stratification System wordt geëvalueerd en ook hier wordt vervolgens een voorstel voor verbetering gedaan.

In **Hoofdstuk 2** wordt in patiënten met DTC de nieuwe 8e editie van het AJCC/TNM Staging System vergeleken met de vorige 7e editie met betrekking tot verschillen in stadiëring en overleving. Daarnaast wordt ook gekeken of er mogelijke verschillen tussen PTC en FTC zijn. De resultaten tonen dat in de 8e editie er geen significant verschil meer is tussen PTC en FTC wat betreft de sterfte per stadium van de ziekte, wat betekent dat de 8e editie evengoed werkt voor patiënten met PTC als voor patiënten met FTC. Daarom wordt de 8e editie van het AJCC/TNM Staging System gezien als een betere voorspeller van zowel de algemene overleving (OS) als DSS dan de 7e editie voor zowel PTC als FTC. In **Hoofdstuk 3** wordt onderzocht of een andere leeftijdsgrens dan 55 jaar, wat de grens is die op dit moment wordt gebruikt in de 8e editie, leidt tot verbetering van de voorspellende waarde van het AJCC/TNM Staging System wat betreft DSS. Dit onderzoek is wederom verricht in patiënten met DTC, waarbij er ook weer gekeken is naar mogelijke verschillen tussen patiënten met PTC en FTC. De resultaten laten zien dat de optimale leeftijdsgrens voor de 8e editie voor patiënten met PTC 50 jaar is, maar voor patiënten met FTC is 40 jaar de optimale leeftijdsgrens. Daarom impliceert deze studie dat voor een optimale voorspelling van de prognose, PTC en FTC moeten worden gezien als aparte entiteiten.

In **Hoofdstuk 4** wordt in ATA High Risk (hoog risico) patiënten met DTC de voorspellende waarde van het ATA Risk Stratification System (uit 2015) onderzocht, en wederom ook patiënten met PTC en FTC met elkaar vergeleken. De resultaten laten zien dat in deze High Risk patiënten het ATA Risk Stratification System een goede voorspeller is van persisterende ziekte, maar ook van overleving. Dit laatste omdat de ziekterespons op de eerste behandeling goed de overlevingskansen voorspelt. Daarnaast werd een recidief gezien in 14% van de ATA High Risk patiënten die na behandeling geen ziekteactiviteit meer hadden. Dit percentage is duidelijk hoger dan de 1-4% die in de recentste ATA Guidelines (2015) wordt genoemd. In **Hoofdstuk 5** wordt het ATA Risk Stratification System (uit 2015) onderzocht in patiënten met afstandsmetastasen. De resultaten tonen aan dit systeem een goede voorspeller is van zowel persisterende ziekte als ook van de kans op een recidief in deze patiënten. In **Hoofdstuk 5** is ook onderzocht in welk percentage van de patiënten mogelijke afstandsmetastasen gemist zouden zijn wanneer een bepaalde patiëntengroep, de ATA Guidelines (2015) volgend, geen RAI behandeling zouden hebben gehad. In 1.6% van de ATA Low Risk (laag risico) en 2.5% van de ATA Intermediate Risk (intermediair risico) patiënten, die volgens deze Amerikaanse richtlijn geen indicatie voor RAI hebben, of bij wie RAI overwogen zou moeten worden, werden afstandsmetastasen gevonden. Deze metastasen zouden zeer waarschijnlijk in eerste instantie gemist zijn wanneer er geen RAI gegeven zou zijn. In **Hoofdstuk 6** is onderzocht wat het effect van leeftijd is op de ziekte uitkomst in ATA High Risk patiënten. Daarnaast is er ook gekeken of een leeftijdsgrens de voorspellende waarde van het ATA Risk Stratification System (uit 2015) zou kunnen verbeteren. De resultaten tonen aan dat een hogere leeftijd, zowel continu als dichotoom, een significant negatieve invloed heeft op de ziekte uitkomst en daarom moet overwogen worden om leeftijd toe te voegen als een risicofactor in het ATA Risk Stratification System. Voor de verschillende ziekte uitkomsten werden net verschillende leeftijdsgrenzen gevonden, en deze grenzen verschillen tussen PTC en FTC. Daarom impliceert ook deze studie dat voor een optimale inschatting van de ziekte uitkomst, PTC en FTC moeten worden gezien als aparte entiteiten.

Omdat de overleving van patiënten met DTC relatief goed is, is het erg belangrijk om de bijwerkingen van de behandeling(en) te minimaliseren, terwijl de prognose daardoor niet slechter mag worden. Bijwerkingen van de behandeling van schildklierkanker zijn bijvoorbeeld onvoldoende werking van de bijschildklieren (hypoparathyreoïdie) door beschadiging tijdens de operatie, stemveranderingen door beschadiging van de nervus recurrens (stembandzenuw) gedurende de operatie, verminderde speekselklier- en beenmergfunctie door RAI, hart-en vaatproblematiek (zoals ritmestoornissen) en botontkalking (osteoporose) door de TSH-suppressieve therapie, en mogelijk heeft RAI ook invloed op de vruchtbaarheid (fertiliteit).

Daarnaast hebben eerdere onderzoeken laten zien dat de kwaliteit van leven is verminderd in patiënten die schildklierkanker hebben overleefd ten opzichte van de gewone bevolking. Hun kwaliteit van leven is op hetzelfde niveau als van patienten met andere kankersoorten, en is zelfs slechter dan dat van patiënten met borstkanker.

In **Hoofdstuk 7** wordt het verloop van de kwaliteit van leven van patiënten met DTC die behandeld zijn met een totale thyreoïdectomie en RAI onderzocht. Resultaten laten zien dat al voor het starten van de behandeling de kwaliteit van leven van de patiënten verlaagd is. Tijdens de follow-up, ontwikkelt de kwaliteit van leven zich niet-lineair waarbij de slechtste kwaliteit van leven wordt gezien ten tijde van de RAI behandeling. Daarna herstelt de kwaliteit zich langzaam waarbij na 2 tot 3 jaar deze weer op de uitgangswaarde van voor de behandeling is. Het is goed om u hierbij te realiseren dat dit nog steeds lager is dan die van de algemene bevolking. Jongeren, vrouwen, en patiënten met een blijvend tekort aan bijschildklierhormoon (als complicatie van de schildklieroperatie) hadden met name een verminderde kwaliteit van leven.

In **Hoofdstuk 8** wordt het lange termijnseffect van RAI op de vrouwelijke fertiliteit onderzocht in patiënten met DTC die behandeld zijn met een totale thyreoïdectomie en RAI. Het Anti-Müllerse hormoon (AMH) wat gemeten wordt in het bloed wordt hierbij gebruikt als maat voor de vrouwelijke fertiliteit. In de groep patiënten die één RAI behandeling ondergingen was er in het eerste jaar na deze behandeling sprake van een significante afname van de AMH concentratie met 55%. In de groep patiënten die meerder RAI behandelingen ondergingen was er sprake van een daling van de AMH concentratie met 74% in het 1^{e} jaar. Bij patiënten ouder dan 35 jaar was er sprake van een sterkere daling dan bij patiënten jonger dan 35 jaar. Ook gaf 40% van de patiënten aan dat de diagnose en behandeling van DTC invloed hadden gehad op hun kinderwens.

De behandeling van patiënten met een hypothyroïdie bestaat uit behandeling met het schildklierhormoon levothyroxine wat bestaat uit T4. Hoewel de schildklierhormoonwaarden in het bloed weer normaal kunnen worden blijft een deel van de patiënten (± 10-15%) zowel fysieke als psychische klachten houden. Hoewel de oorzaak hiervan nog niet goed wordt begrepen, zijn er verschillende mogelijke verklaringen. Ten eerste zou de onderliggende auto-immuunziekte kunnen zorgen voor de blijvende klachten. Ten tweede zou het onvermogen van de behandeling met levothyroxine om normale schildklierhormoonwaarden van T4, en met name ook van T3, in het bloed en weefsels te bewerkstelligen, leiden tot blijvende klachten. En ten derde zou het kunnen dat afgeleiden van schildklierhormoon (schildklierhormoon metabolieten) verlaagd zijn gedurende behandeling met levothyroxine en daardoor leiden tot klachten. Van deze drie verklaringen is er met name weinig

onderzoek gedaan naar de laatste verklaring, en daarom hebben we in **Hoofdstuk 9 en 10** gekeken naar deze schildklierhormoon metabolieten gedurende hypothyreoidie, hyperthyreoïdie en euthyreoïdie (normale hoeveelheid schildklierhormoon). Schildklierhormoon en de metabolieten zijn bepaald met behulp van een recent ontwikkelde en gevalideerde Liquid chromotography - mass spectrometry / mass spectometry (LC-MS/MS) methode. Voor dit onderzoek worden patiënten met DTC gebruikt omdat zij tijdens hun behandeling zowel euthyreoot, hypothyreoot als hyperthyreoot zijn. Dat wil zeggen euthyreoot voorafgaand aan de operatie, hypothyreoot na 3-4 weken onttrekking van schildklierhormoon voorafgaand aan de RAI behandeling, en hyperthyreoot gedurende de TSH-suppressieve therapie met levothyroxine. Het feit dat verschillen tussen eu-, hypo- en hyperthyreoïdie onderzocht kunnen worden in één patiënt, gecombineerd met het feit dat het merendeel van deze patiënten geen auto-immuunziekte heeft, leidt er toe dat DTC patiënten een uniek model zijn om schildklierhormoon metabolieten te onderzoeken.

In **Hoofdstuk 9** is onderzocht op welke manier schildklierhormoon en de metabolieten veranderen gedurende euthyreoïdie, hypothyreoïdie, hyperthyreoïdie. De recent ontwikkelde en gevalideerde LC-MS/MS methode werd hiervoor gebruikt. Zowel schildklierhormoon als alle metabolieten zijn verlaagd tijdens de hypothyreoïdie die de patiënten ontwikkelden na het onttrekken van schildklierhormoon. Dit suggereert dat de vorming van de metabolieten afhankelijk is van schildklierhormoon geproduceerd door de schildklier. Gedurende hyperthyreoïdie veroorzaakt door levothyroxine zijn zowel het schildklierhormoon als alle metabolieten, behalve T3, verhoogd wat suggereert dat de metabolieten worden gevormd in weefsel anders dan de schildklier. Dat het T3 niet verhoogd is kan komen doordat er nu geen T3 meer wordt gevormd door de schildklier, en het vormen van T3 nu uitsluitend kan plaatsvinden in de andere weefsels, wat onvoldoende lijkt te zijn.

In **Hoofdstuk 10** wordt de mogelijke relatie tussen zowel schildklierhormoon als de metabolieten met de kwaliteit van leven onderzocht. Voor meerdere kwaliteit van leven scores wordt gezien dat deze scores verschillen tussen euthyreoïdie, hypothyreodie en hyperthyreoïdie voor patiënten met hogere concentraties ten opzichte van hen met lagere concentraties van schildklierhormoon en verschillende metabolieten (T0, 3-T1, 3,3'-T2, T3, rT3 en TA4). Echter, er werd geen consistent patroon gezien in de relatie tussen één van de schildklierhormonen/metabolieten en de kwaliteit van leven. Daarnaast is tijdens euthyreoïdie, hypothyreoïdie en hyperthyreoïdie apart ook gekeken naar een mogelijke relatie tussen de metabolieten en de kwaliteit van leven, maar hierbij werd geen relatie gevonden. Samenvattend lijken schildklierhormoon metabolieten niet de verklaring te zijn waarom een deel van de patiënten zowel fysieke als psychische klachten houdt. Echter, het is ook mogelijk dat patiënten met DTC niet het optimale model zijn om dit te onderzoeken,

of dat er meer patiënten nodig zijn om de (mogelijke) rol van de schildklierhormoon metabolieten aan te tonen. Daarom is er verder onderzoek nodig om onze resultaten te bevestigen.

In **Hoofdstuk 11** worden tenslotte de resultaten van de diverse onderzoeken gepresenteerd en bediscussieerd, en worden aanbevelingen voor toekomstig onderzoek gedaan.

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PhD period	May 2016 – December 2021
Promotor	Prof. dr. Robin P. Peeters
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Congress visits: oral presentations	**Year**	**ECTS**
European Thyroid Association, Belgrade	2017	1.4
Junior Dutch Endocrine Meeting, Leiden	2017	0.6
Dutch Endocrine Meeting, Noordwijkerhout	2018	0.6
Dutch Endocrine Meeting, Noordwijkerhout	2019	0.6
Dutch Endocrine Meeting, Noordwijkerhout	2020	0.6

Congress visits: poster presentations	**Year**	**ECTS**
Science Days Internal Medicine, Antwerp	2018	0.4
European Congress of Endocrinology, Barcelona	2018	1.4
European Thyroid Association, Newcastle	2018	1.4
ENDO 2019, New Orleans	2019	1.4
Science Days Internal Medicine, Sint-Michielsgestel	2019	0.4
Science Days Internal Medicine, Sint-Michielsgestel	2020	0.4
e-European Congress of Endocrinology	2021	0.9

Congress visits: other	**Year**	**ECTS**
Dutch Thyroid Research Foundation Symposium, Amsterdam	2016	0.3
European Thyroid Association, Copenhagen	2016	1.4
Science Days Internal Medicine, Antwerp	2017	0.4
Dutch Endocrine Meeting, Noordwijkerhout	2017	0.3
Dutch Thyroid Research Foundation Symposium, Amsterdam	2017	0.3
Dutch Thyroid Research Foundation Symposium, Amsterdam	2018	0.3
DTCG Symposium, Rotterdam	2018	0.3
e-Dutch Endocrine Meeting	2021	0.3

Teaching activities	Year	ECTS
Lectures on thyroid (dys)function, first year medical students	2016	0.3
Lecture on thyroid (dys)function, nurse practitioners	2016	0.3
Lectures on thyroid (dys)function, first year medical students	2017	0.3
Lecture on thyroid cancer, radiologists	2018	0.3
Lectures on thyroid (dys)function, first year medical students	2018	0.3
Internal Medicine Research Meeting	2018	0.4
Supervising the research project of Merel Stegenga	2018	1.0
Lecture on thyroid (dys)function, junior med school	2019	0.3
Lecture on adrenal (dys)function, nurse practitioners	2020	0.3
Lecture on thyroid (dys)function, junior med school	2020	0.3
Lectures on thyroid (dys)function, first year medical students	2021	0.3
THANC Foundation Virtual Journal Club	2021	0.3

Research skills	Year	ECTS
Scientific integrity	2016	0.3
BROK® (Basic course Rules and Organization for Clinical researchers)	2016	1.6
CPO-course: Patient Oriented Research	2017	0.3
Survival Analysis Course	2017	0.6
Joint Models for Longitudinal and Survival Data Course	2017	0.7
Repeated Measurements Course	2018	1.7
Biomedical English Writing and Communication	2018	3.0
Local data manager of RIFTOS study	2017-2019	1.0
Peer reviewer for several journals (including Thyroid and JCEM)	2017-2021	4.0

Clinical courses / activities	Year	ECTS
Marburg Summer School of Thyroid Cancer Management	2016	1.2
Course and Workshop Basic and Translational Endocrinology	2016	3.0
Rotterdamse Internistendag 2016	2016	0.3
Challenges in Thyroid Cancer Management Symposium	2016	0.6
Clinical Update on Thyroid and Pregnancy	2017	0.1
Schildklierziekten: een update voor de klinische praktijk	2017	0.1
Regionale Endocrinologie Bespreking Rotterdam	2019	0.1
Schildklierziekte anno nu: een update voor de klinische praktijk	2019	0.3
Internistendagen 2021	2021	0.6
Tumor board meetings	2016-2021	4.0

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CURRICULUM VITAE

About the author

Evert van Velsen was born on August 29th, 1982 in Rotterdam. He completed secondary school at the C.S.G. Johannes Calvijn in 2000, and afterwards studied Computer Sciences at the Utrecht University. He received his Master's degree in August 2015, and his Master's thesis was entitled '*VAMIRE: Lumen Path Definition and Lumen Segmentation of Atherosclerotic Vessels in CT Angiography*'. Thereafter, he started his study of Medicine at the Erasmus University Rotterdam, and received his medical degree in May 2012. During his study, he also obtained a Master's degree in Clinical Epidemiology from the Netherlands Institute of Health Sciences in 2010. He started as a resident (ANIOS) in Internal Medicine at the Albert Schweitzer Hospital of Dordrecht in June 2012, and subsequently started his residency (AIOS) in January 2013 under supervision of Dr. E.F.H. van Bommel. He continued his residency at the Erasmus Medical Center in January 2016. In May 2016 he started as a PhD-student at the Academic Center for Thyroid Diseases at the Erasmus Medical Center under supervision of Prof. dr. R.P. Peeters and Dr. W.E. Visser. The results of his research are presented in this thesis. He presented his work at several national and international meetings, and was one of the winners of the Presidential Poster Competition at ENDO2019 (New Orleans, USA). During his PhD, at the outpatient clinic, Evert treated patients with different (para)thyroid diseases, with a focus on thyroid nodules and thyroid cancer. He continued his residency in September 2019 under supervision of Dr. A.A.M. Zandbergen, and started his fellowship in Endocrinology under supervision of Dr. R.A. Feelders in May 2020. He lives together with Lenneke Hamelink and they have a beautiful daughter Milou.