TOMMI HAKALA

# Differentiated Thyroid Cancer

## Diagnostics, prognostic factors and
long-term outcome

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long-term outcome

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# ABSTRACT

Thyroid cancer is the most common endocrine malignancy. Papillary (PTC) and follicular (FTC) thyroid cancers are referred to as well-differentiated thyroid cancers (DTCs). The incidence of thyroid cancer has increased significantly in recent decades. The goal in the treatment of thyroid cancer is to minimise disease-related mortality with surgery and post-operative thyroid remnant ablation with radioiodine (RAI).

The study population consisted of patients treated for thyroid cancer in Pirkanmaa Hospital District in the south of Finland between 1981 and 2002. Altogether 553 patients with thyroid cancer were analysed for trends during past decades for tumour related parameters, treatments, the incidence of thyroid cancer and mortality (**I**). Twenty-seven patients with histologically verified recurrent DTC were compared with matched controls with non-recurrent DTC (n=24) and thyroid adenomas (n=24) to estimate the role of angiogenesis and lymphangiogenesisin the DTC recurrences (**II**). The DTC patients in the first study (n=493) were used in conjunction with the Oulu University Hospital patient cohort (n=427), a total of 920 patients, to evaluate the risks for second primary malignancies (SPMs) in thyroid cancer patients (**III**). The incidence of SPMs in thyroid cancer patients was compared to controls (n=4,542) matched for age, gender and place of residence. A prospective study evaluating the usefulness of core needle biopsy (CNB) in thyroid nodule diagnostics was performed using CNB samples of 52 consecutive patients (**IV**).

The incidence of thyroid cancer rose during the observation period, from 4.5/100,000/year between 1981 and 1991 to 6.0/100,000/year between 1992 and 2002 (Rate ratio [RR] 1.33, confidence interval 1.11-1.60) (**I**). During the respective time periods in DTC patients the change in incidence was accompanied by a rise in the share of PTCs, from 81% to 89% (p=0.02) (**I**). Furthermore, the median size of tumours decreased from 25 mm in 1981–1991 to 15 mm in 1992–2002 (p<0.001) (**I**). No significant differences were observed in 10-year disease-specific survival (92% vs. 94%, p=0.43) (**I**).

Angiogenesis and lymphangiogenesis were studied for recurrent DTCs and compared to DTCs with no recurrence and thyroid adenomas. Recurrent DTCs

had lower microvascular density (MVD) than thyroid adenomas (327 vessels [v]/mm$^2$ vs. 484 v/mm$^2$ respectively, p=0.017) (**II**). Peritumoural lymphatic vessel density (LVD) was higher in recurrent DTCs than in non-recurrent DTCs (101 v/mm$^2$ vs. 56.1 v/mm$^2$, p=0.015) (**II**). The highest peritumoural LVD was observed in recurrent PTCs (102 v/mm$^2$), whereas peritumoural LVD of non-recurrent PTCs was lower (56.0 v/mm$^2$, p=0.044) (**II**).

SPMs were studied in 910 patients and in controls matched for age, gender, and place of residence. Overall SPM incidence was not significantly higher in the patients than the controls (RR 1.12, p=0.269) (**III**). Higher incidence of SPMs was observed in patients diagnosed or treated when younger than 40 years (RR 1.73, p=0.037) and patients diagnosed after 1996 (RR 1.51, p=0.029) (**III**). Higher incidence of sarcomas and soft tissue cancers (RR 4.37, p=0.004) and haematologic and lymphatic malignancies (RR 1.87, p=0.035) was observed in patients than controls (**III**).

CNBs were studied in 52 consecutive patients with malignant or malignant suspicious thyroid tumours and compared to fine-needle aspiration biopsy (FNA). CNB had a definite sensitivity for malignancy of 61% (CI 41%-78%), which was markedly higher than the definitive sensitivity of FNA 22% (CI 10%-42%). Specificity was 97% for CNB (CI 83%-99%) and 100% for FNA (CI 88%-100%) (**IV**). CNB was not beneficial in the diagnosis of follicular thyroid lesions (**IV**).

In summary, the patients with thyroid cancer had a favourable long-term outcome. The incidence of thyroid cancer and the proportion of PTC were rising. The recurrent PTCs had high peritumoural lymphatic vasculature, which correlates with the type of metastatic spread route of PTC. SPMs are more frequent in young DTC patients, and this observation should be taken into account in the treatment and follow-up of DTC patients. CNB may be considered as an auxiliary diagnostic procedure in FNA with suspicion of malignancy.

# TIIVISTELMÄ (ABSTRACT IN FINNISH)

Kilpirauhassyöpä on yleisin umpieritysrauhasten syöpä. Kilpirauhassyövän papillaarista ja follikulaarista alatyyppiä nimitetään yhdessä hyvin erilaistuneeksi kilpirauhassyöväksi. Kilpirauhassyövän ilmaantuvuus on kasvanut merkittävästi viime vuosikymmenten aikana. Kilpirauhassyövän hoito sisältää leikkauksen ja leikkauksen jälkeisen radiojodihoidon; hoidon tavoitteena on minimoida tautiin liittyvä sairastuvuus ja kuolleisuus.

Tutkimuksen aineiston muodostivat Pirkanmaan sairaanhoitopiirin alueella vuosina 1981–2002 todetut 553 kilpirauhassyöpätapausta, joista 77 % oli papillaarista ja 13 % follikulaarista tyyppiä. Viime vuosikymmenten aikana tapahtuneita muutoksia kilpirauhassyövän esiintyvyydessä, kuolleisuudessa, annetuissa hoidoissa ja kasvainten ominaisuuksissa havainnoitiin retrospektiivisessä tutkimuksessa (**I**). Leikkauksella hoidettu hyvin erilaistuneen kilpirauhassyövän uusima todettiin 27 potilaalla, ja näiden potilaiden kasvainten verisuonitusta ja imusuonitusta verrattiin uusiutumattomiin kasvaimiin (n=24) ja hyvänlaatuisiin kilpirauhasen adenoomiin (n=24). (**II**). Tutkimuksen **I** hyvin erilaistunutta kilpirauhassyöpää sairastavat potilaat (n=493) ja Oulun yliopistollisen sairaalan vastaavan tutkimuksen potilaat (n=427) analysoitiin yhdessä kilpirauhassyöpäpotilaiden uusien primaarimaligniteettien esiintyvyyden selvittämiseksi (**III**). Karkeaneulanäytteen käyttökelpoisuutta kilpirauhassyövän leikkausta edeltävässä diagnostiikassa arvioitiin prospektiivisessa tutkimuksessa, jonka aineiston muodostivat 52 Tampereen yliopistollisessa sairaalassa kilpirauhaskasvaimen vuoksi leikattua potilasta (**IV**).

Kilpirauhassyövän ilmaantuvuus kasvoi tutkimusaikana: ikävakioimaton ilmaantuvuus oli 4,5/100000/v vuosina 1981–1991 ja 6,0/100000/v vuosina 1992–2002 (riskisuhde 1,33, luottamusväli 1,11–1,60) (**I**). Hyvin erilaistunutta kilpirauhasyöpää sairastavien joukossa papillaaristen kilpirauhassyöpien suhteellinen osuus kasvoi 81 prosentista 89 prosenttiin (p=0,02) ja kasvaimen keskimääräinen koko laski 25 millimetristä 15 millimetriin (p<0,01). Ajanjaksojen välillä ei havaittu merkittävää eroa siinä, kuinka suuri osa potilaista ei ollut kuollut kilpirauhassyöpään 10 vuoden päästä diagnoosista (92 % vuosina 1981–1991 ja 94 % vuosina 1992–2002, p=0,43).

Uusiutuneet kilpirauhassyövät sisälsivät vähemmän kasvaimensisäisiä verisuonia (327 verisuonta/mm$^2$) kuin hyvänlaatuiset adenoomat (484 verisuonta/mm$^2$, p=0,017) (**II**). Kasvaimen lähiympäristössä sijaitsevien imusuonten määrä oli suurempi uusiutunutta kilpirauhassyöpää sairastavilla potilailla (101 imusuonta/mm$^2$) kuin uusiutumatonta syöpää sairastavilla (56,1 imusuonta/mm$^2$, p=0,015) (**II**). Uusiutuneessa papillaarisessa kilpirauhassyövässä todettiin suurempi määrä kasvaimen lähiympäristön imusuonia (102 imusuonta/mm$^2$) kuin uusiutumattomassa papillaarisessa kilpirauhassyövässä (56,0 imusuonta/mm$^2$, p=0,044) (**II**).

Uusien syöpien kokonaisilmaantuvuudessa ei ollut merkittävää eroa potilaiden (n=910) ja verrokkien (n=4542) välillä (riskisuhde 1,12, p=0,269) (**III**). Korkeampi uusien syöpien ilmaantuvuusaste havaittiin nuorilla, alle 40-vuotiailla potilailla (riskisuhde 1,73, p=0,037) ja potilailla, joiden hoito ja diagnoosi oli tehty vuoden 1995 jälkeen (riskisuhde 1,51, p=0,029) (**III**). Sarkoomien ja pehmytkudossyöpien (riskisuhde 4,37, p=0,004) sekä hematologisten ja lymfaattisten syöpien (riskisuhde 1,87, p=0,035) ilmaantuvuus oli kuitenkin suurempi kilpirauhassyöpäpotilailla kuin verrokeilla (**III**).

Karkeaneulanäytteitä tutkittiin 52 kilpirauhaskasvaimesta ja näytteitä verrattiin ohutneulanäytteiden tuloksiin. Karkeaneulanäyte osoitti kasvaimen suuremmalla herkkyydellä (sensitiivisyydellä) pahanlaatuiseksi (61 %, luottamusväli 41 %–78 %) kuin ohutneulanäyte (21%, luottamusväli 10 %–42 %). Karkeaneulanäytteen tarkkuus (spesifisyys) oli 97 % (luottamusväli 83 %–99 %) ja ohutneulanäytteen 100 % (luottamusväli 88 %–100 %) (**IV**). Karkeaneulanäyte ei ollut hyödyllinen follikulaaristen kilpirauhaskasvainten diagnostiikassa (**IV**).

Yhteenvetona voidaan todeta, että kilpirauhassyöpäpotilaiden pitkän aikavälin ennuste oli suotuisa. Hyvin erilaistuneen kilpirauhassyövän, erityisesti papillaarisen syövän, ilmaantuvuus oli nousussa. Uusiutuneessa papillaarisessa kilpirauhassyövässä kasvaimen ympäristössä oli runsaasti imusuonitusta, mikä sopii hyvin siihen, että papillaarinen kilpirauhassyöpä leviää tyypillisesti kaulan imusolmukkeisiin. Uudet primäärisyövät ovat yleisempiä nuorilla, hyvin erilaistunutta kilpirauhassyöpää sairastavilla potilailla. Tämä havainto tulisi ottaa huomioon kilpirauhassyöpäpotilaiden hoitoa ja jatkoseurantaa suunniteltaessa. Karkeaneulanäytettä voidaan harkita lisätoimenpiteenä, jos kilpirauhaskasvaimesta otettu ohutneulanäyte osoittautuu ei-diagnostiseksi.

# Contents

# 1 ABBREVIATIONS

ATA American Thyroid Association

ATC anaplastic thyroid cancer

AUS/FLUS atypia of undetermined significance or follicular lesion of undetermined significance

CNB core needle biopsy

CNS central nervous system

DTC well-differentiated thyroid cancer

FDG-PET fluorodeoxyglucose positron emission tomography

FNA fine-needle aspiration biopsy

FTC follicular thyroid cancer

GBq gigabecquerel (radioactivity of $10^9$ disintegrations per second)

LN lymph node

LT4 levothyroxine

LVD lymphatic vascular density

mCi millicurie (radioactivity of 3.7 x $10^7$ disintegrations per second)

MTC medullary thyroid carcinoma

MVD microvascular density

OUH Oulu University Hospital

PTC Papillary thyroid cancer

RAI radioiodine ($^{131}$I isotope)

rhTSH recombinant human thyroid-stimulating hormone

SPM second primary malignancy

TAUH Tampere University Hospital

Tg thyroglobulin

TKI tyrosine kinase inhibitor

TSH thyrotropin (thyroid-stimulating hormone)

US ultrasonography

VEGF vascular endothelial growth factor

# 2   LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following four original publications, which are referred to in the text by their Roman numerals **I-IV**.

**I**      Hakala T, Kellokumpu-Lehtinen P, Kholova I, Holli K, Huhtala H, Sand J (2012): Rising incidence of small size papillary thyroid cancers with no change in disease-specific survival in Finnish thyroid cancer patients. Scandinavian Journal of Surgery 101: 301-306.

**II**    Hakala T, Sand J, Kellokumpu-Lehtinen P, Huhtala H, Leinonen R, Kholová I (2014): Recurrent thyroid cancers have more peritumoural lymphatic vasculature. European Journal of Clinical Investigation 44: 825-832.

**III**  Hakala T, Sand J, Jukkola A, Huhtala H, Metso S, Kellokumpu-Lehtinen P (2014): Increased risk of certain second primary malignancies in patients treated for well-differentiated thyroid cancer. Submitted.

**IV**   Hakala T, Kholová I, Sand J, Saaristo R, Kellokumpu-Lehtinen P (2013): A core needle biopsy provides more malignancy-specific results than fine-needle aspiration biopsy in thyroid nodules suspicious for malignancy. Journal of Clinical Pathology 66: 1046-1050.

# 3   INTRODUCTION

Thyroid cancer is the most common endocrine malignancy and accounts for 1-2% of all cancer cases. The incidence of thyroid cancer has increased worldwide in the past few decades, especially among younger population (Elisei, Molinaro et al. 2010). Among people less than 40 years old, thyroid cancer is the fourth most common cancer in female patients and the seventh most common cancer in males (Engholm, Ferlay et al. 2010). Most thyroid cancer patients (70-80%) are female.

Thyroid malignancies are categorized according to their histological properties into papillary thyroid cancers (PTC), follicular thyroid cancers (FTC), anaplastic thyroid cancers (ATC) and medullary thyroid cancers (MTC) (Busnardo, De Vido 2000, Kinder 2003, Pasieka 2003, Moo-Young, Traugott et al. 2009). PTCs and FTCs, both arising from thyroid follicular epithelial cells, are collectively referred to as well-differentiated thyroid cancers (DTCs) (Kinder 2003).

Recent studies have reported a steady increase in incidence of DTC (Welch, Black 2010, Colonna, Grosclaude et al. 2002). The proportion of PTC in particular has been rising and changes in tumour subtypes have occurred (Davies, Welch 2006, Reynolds, Weir et al. 2005, Colonna, Guizard et al. 2007). Less than 10% of PTCs are known to be of familial origin (Fiore, Fuziwara et al. 2009, Guan, Ji et al. 2009, Nikiforova, Kimura et al. 2003).

Thyroid cancer is treated with surgery, followed by radioiodine (RAI) ablation and suppression of thyrotropin (TSH) using levothyroxine (LT4). In most cases total or near total thyroidectomy is performed, accompanied by lymph node (LN) dissection if needed (Shen, Ogawa et al. 2010, Popadich, Levin et al. 2011). The sensitivity of tumours to RAI treatment varies, and some primary tumours are less sensitive to RAI or progress to be insensitive to RAI treatment (Mihailovic, Stefanovic et al. 2009, Kim, Lee et al. 2013).

Despite the generally indolent behaviour of DTC, some patients have recurrent or persistent disease and 8-10% of these patients will eventually die of it (Gilliland, Hunt et al. 1997). The five-year overall survival rate in Finland of all thyroid cancers is 92% for women and 89% for men (Engholm, Ferlay et al. 2010).

The invasion of tumour cells to adjacent structures, especially blood and lymphatic vessels is a marker of a more aggressive tumour. Local LN metastases

are more likely to develop if lymphatic vessel invasion is present at the primary tumour. Likewise, angioinvasion is a risk factor for blood-borne metastasis (Adams, Alitalo 2007, Holopainen, Bry et al. 2011). Angiogenesis of the primary tumour is a predictive marker for LN metastasis and unfavourable prognosis (Bono, Wasenius et al. 2004, Tanaka, Ishiguro et al. 2010).

RAI ablation exposes patients to ionizing radiation and may cause adverse effects. Acute and sub-acute adverse effects include irritation of the mucous membranes of the gastrointestinal tract, salivary glands and respiratory tract (Van Nostrand 2009). Long-term effects include radiation-induced risk of second primary malignancies (SPMs) (Sandeep, Strachan et al. 2006, Sawka, Thabane et al. 2009).

The most commonly used diagnostic procedure is fine-needle aspiration biopsy (FNA), which is performed under ultrasonography (US) guidance. Non-diagnostic and indeterminate FNA samples are frequent and have a significant impact on the treatment of thyroid cancer patients (Yassa, Cibas et al. 2007, Yang, Schnadig et al. 2007, Nayar, Ivanovic 2009). Core needle biopsy (CNB) has been the subject of a few studies, but no conclusive evidence exists as to whether CNB is a useful tool in thyroid nodule diagnostics (Screaton, Berman et al. 2003, Renshaw, Pinnar 2007, Bandyopadhyay, Pansare et al. 2007).

The purpose of this study was to evaluate the long-term prognosis of thyroid cancer patients in Pirkanmaa Hospital District. In addition, our aim was to find prognostic markers, evaluate the risk of SPMs in DTC patients and investigate whether CNB is useful in the diagnostic workup of thyroid nodules.

# 4    REVIEW OF THE LITERATURE

## 4.1    Definitions

The thyroid gland is an endocrine organ located at the caudal part of the neck on the sides and in front of the trachea. The thyroid gland derives its name from the Greek word 'thyreos', meaning shield. The normal weight of the thyroid gland is between 15 and 25 g in adults. Thyroid anatomy consists of lateral lobes, which are connected medially by the isthmus (Fancy, Gallagher III et al. 2010). The lobes are approximately 4 cm in length, 1 to 2 cm in thickness and 2 cm in width. The isthmus is located at the level of second to fourth tracheal ring and measures about 2 to 6 mm in thickness (Hoyes, Kershaw 1985).

The thyroid gland originates from two embryonal structures, the medially located thyroid anlage, which is the source of thyroid follicular cells, and the lateral ultimobrachial bodies from the fourth pharyngeal pouch, which are the source of the parafollicular C-cells (De Felice, Di Lauro 2004). The thyroid anlage and the lateral ultimobrachial bodies descend caudally and fuse together to form the thyroid gland caudally and ventrally from the cricoid cartilage (Boyd 1950). By 10 weeks after conception foetal thyroid follicular cells begin to produce levothyroxine (LT4) (Epstein, Burrow et al. 1994). LT4 is transformed into the active form of the thyroid hormone, triiodothyronine, by deiodinase enzymes.

The prevalence of thyroid nodules in general population is high, 20-50%, but only 5% of the nodules prove to be malignant. PTCs and FTCs arise from the thyroid follicular cells (Kinder 2003). PTC is the most common thyroid malignancy and accounts for 70-80% of all thyroid cancer cases, while the share of FTC is 10-20%. The share of aggressive and fatal ATCs is 5-10% and that of C-cell derived MTCs is 2-10% (Busnardo, De Vido 2000, Kinder 2003, Pasieka 2003, Moo-Young, Traugott et al. 2009). Poorly differentiated DTC represents intermediate disease in the progression from DTC to ATC (Patel, Shaha 2014).

## 4.2  Aetiology

### 4.2.1  Ionizing radiation

Radiation exposure is a well-established risk factor for thyroid cancer. External radiotherapy administered to the neck-region increases the incidence of thyroid cancer later in life. Radiation is measured in Becquerels, which expresses the number of nuclear decays per second. Administered radioiodine activity of 3.7 gigabecquerel (GBq) is common in treatment of thyroid cancer, and this dose is equivalent to 100 millicuries (mCi), which is an old unit of radioactivity. Gray (Gy) is a unit of adsorbed radiation, and indicates the adsorbed radiation energy in joules per kilogram. Radiation exposure increases thyroid cancer risk most markedly in childhood (Ron, Lubin et al. 1995).

After the Chernobyl nuclear disaster in 1986, the incidence of childhood and adolescent thyroid cancer rose in regions with the highest contamination of radioiodine in Belarus, Ukraine and Russia. The thyroid cancer risk was elevated after an exposure dose of 0.2 Gy, and the risk increased linearly to the dose of 1.5-2.0 Gy. The estimated odds ratio after a dose of 1 Gy was estimated to be 5.5-8.4 when compared to unexposed children (Cardis, Kesminiene et al. 2005). In Belarussian children, the minimal latent period after exposure was short, only four years after the accident, and the maximum increase in incidence was reached after seven years. Children less than 15 years of age were most susceptible to radiation-induced carcinogenesis. The majority of radiation-induced thyroid cancers were PTCs, with a solid or solid-follicular microscopic appearance. Chromosomal rearrangements, such as *RET/PTC*, *AKAP8-BRAF* and *TRK*, were more common than point mutations in thyroid tumours after the Chernobyl nuclear disaster (Nikiforov 2006).

### 4.2.2  Supply of iodine

The overall incidence of DTC is generally not considered to be dependent on the iodine intake of the population but the supply of iodine has an effect on the histological subtypes of thyroid cancer. In iodine-deficient areas the incidence of FTC is relatively high (Dal Maso, Bosetti et al. 2009). The incidence of PTC is higher in iodine-rich areas (Feldt-Rasmussen 2001).

### 4.2.3 Hormonal factors

Thyroid disorders occur more often in females than in males. The difference between genders in the incidence of thyroid disorders is highest at puberty and declines thereafter. Oestrogen and progesterone receptors are expressed in the normal thyroid tissue and in the benign thyroid lesions. The immunoreactivity of oestrogen and progesterone receptors is lost in most PTCs, although the oestrogen gene transcript messenger-RNA is present in most PTCs (Bonacci, Pinchera et al. 1996, Egawa, Miyoshi et al. 2001). In FTC, low level of estrogen receptor beta is a sign of unfavourable prognosis (Heikkilä, Hagström et al. 2013). Obesity has been associated with increased risk of thyroid cancer, possibly through hormonal factors (Kitahara, Platz et al. 2011, Pazaitou-Panayiotou, Polyzos et al. 2013).

### 4.2.4 Thyroid stimulating hormone

TSH is the most important regulator of the growth and function of thyroid gland. Most DTCs will retain sensitivity to TSH regulation. Higher TSH levels have been observed in patients with PTC than in patients with benign thyroid nodular disease (Boelaert, Horacek et al. 2006). Serum TSH levels correlate with the risk of PTC; higher TSH levels are associated with higher risk of PTC (Fiore, Rago et al. 2009). In addition to the correlation with TSH, the risk of PTC has a weak correlation with thyroglobulin antibodies (Fiore, Rago et al. 2011). Thyroid autoimmune disease, Hashimoto's thyroiditis, causes thyroid damage by an autoimmune process and leads to subclinical or clinical hypothyroidism and a rise in serum TSH levels. Hashimoto's thyroiditis is associated with an increase in PTC frequency if TSH levels are elevated or in the upper half of normal range. On the other hand, if serum TSH levels are suppressed to the lower half of normal range with LT4-treatment, patients with the Hashimoto's thyroiditis do not have significantly increased incidence of PTC (Fiore, Rago et al. 2011). During pregnancy, human chorionic gonadotropin mimics the effects of TSH and the risk of thyroid cancer increases with the number of pregnancies (Xhaard, Rubino et al. 2014).

### 4.2.5 Geographic and ethnic factors

Geographic location and ethnic group affect the incidence of thyroid cancer (Dal Maso, Bosetti et al. 2009). In the United States of America a high incidence of

thyroid cancer has been observed in certain ethnic groups, most noticeably in Filipino patients (Haselkorn, Bernstein et al. 2000). A high incidence of thyroid cancer has been observed in volcanic areas, *e.g.* in Sicily (Pellegriti, De Vathaire et al. 2009).

In Finland the incidence of thyroid cancer varies from region to region. The incidence of thyroid cancer in the catchment area of Oulu University Hospital is the highest in Finland. Between 2007 and 2011, the annual age-adjusted incidence of thyroid cancer per 100,000 persons was 13.0 for females and 3.6 for males in the Oulu region and 8.6 for females and 2.9 for males in the Tampere region further south. Nationally, the figures were 8.0 for females and 2.6 for males (Engholm, Ferlay et al. 2010).

## 4.2.6    Hereditary thyroid cancer

In DTC the majority of cancer cases are sporadic and familial tumours account for less than 10% of all non-medullary thyroid cancers cases (Kebebew 2008). First-degree relatives of thyroid cancer patients have a three to 11-fold risk of DTC (Hemminki, Eng et al. 2005).

In some genetic disorders, the incidence of thyroid cancer is increased. Familial cancer syndromes with increased incidence of DTC but without predominance of DTC include Cowden's syndrome (*PTEN* mutation), familial adenomatous polyposis (*APC* mutation), McCune-Albright syndrome (*GNAS1* mutation), Carney's complex (*PRKAR1a* mutation) and Werner's syndrome (*WRN* mutation) (Nosé 2011).

Familial cancer syndromes with a predominance of DTC remain more obscure, but a few potential chromosomal locations have been identified; familial multinodular goitre (locus 14q31), thyroid cancer with oxyphilia (19p13.2), PTC with papillary renal neoplasia (1q21), a follicular variant of PTC (2q21), PTC (8p23 1-p22), PTC (1q21 & 6q22) (Nosé 2011).

In MTC, 20-25% of all cancer cases are associated with known hereditary syndromes involving *RET* gene. Multiple endocrine neoplasia syndrome type IIA is associated with bilateral MTC, pheochromocytoma and hyperparathyroidism. Multiple endocrine neoplasia syndrome type IIB is associated with MTC, pheochromocytoma, mucosal ganglioneuromas and marfanoid habitus. Familial medullary carcinoma without associated endocrinopathies has also been reported (Nosé 2011, Kloos, Eng et al. 2009).

## 4.2.7    Genetic alterations in well-differentiated thyroid cancer

PTCs commonly have activating mutations of genes that code for proteins associated with mitogen-activated protein kinase pathway. *BRAF*, *RET* or *RAS* proto-oncogenes are found in 70% of PTCs and seldom overlap in the same tumour (Adeniran, Zhu et al. 2006). *BRAF* mutations are common and only found in PTCs and ATCs arising from PTCs (Nikiforova, Kimura et al. 2003). *RET* receptor tyrosine kinase gene may be activated by rearrangements known as *RET/PTC* proto-oncogene (Nikiforov 2002). *RET/PTC* can be found in 5% to 30% of sporadic adult PTCs and in 45 to 60% of PTCs occurring in children and adolescents (Fenton, Lukes et al. 2000).

## 4.3    Epidemiology

Thyroid cancer is the most common endocrine malignancy, and accounts for 1-2% of all malignant tumours. Increased incidence of thyroid cancer has been reported worldwide in recent decades. Recent studies have confirmed rising incidence of thyroid cancer in Europe, in the United States of America, in Canada and in the Philippines (Colonna, Grosclaude et al. 2002, Colonna, Guizard et al. 2007, Reynolds, Weir et al. 2005, Leenhardt, Bernier et al. 2004, Galanti, Hansson et al. 1997, Davies, Welch 2006, Burke, Hay et al. 2005, Haselkorn, Bernstein et al. 2000, Mangano 1996, Mulla, Margo 2000, Kent, Hall et al. 2007, Kus, Shah et al. 2010)

In Finland, the age-adjusted incidence of thyroid cancer has increased from 3.5 to 8.3 per 100,000 in women and from 0.9 to 3.4 per 100,000 in men between 1970 and 2011 (Engholm, Ferlay et al. 2010). Among the Nordic countries, the incidence of thyroid cancer is highest in Finland (Kilfoy, Zheng et al. 2009).

In France the incidence of thyroid cancer rose annually by 8% in men and by 9% in women between 1983 and 2000 (Colonna, Guizard et al. 2007). In women, the incidence of PTC increased from 2.65 per 100,000 in 1983-1988 to 7.5 in 1995-2000. The incidence of FTC was stable in women and declining in men. The increase in incidence of small size PTCs was most notable, and the incidence of large (>40mm) PTCs remained stable.

In the United States, the incidence of thyroid cancer has increased 2.4-fold in the past three decades, from 3.6 per 100,000 in 1973 to 8.7 per 100,000 in 2002 (Davies, Welch 2006). The increase has mainly been due to the rising incidence of PTC, which has increased from 2.7 to 7.7 per 100 000, a nearly threefold increase.

The incidence of small cancers rose significantly, as 49% of the increase consisted of cancers with ≤1 cm diameter or less. No major changes in the incidence of MTC, ATC or FTC were observed.

Most patients diagnosed with a DTC are female (70-80%), and the mean age at the time of diagnosis is 40-50 years depending on the patient cohort (Kent, Hall et al. 2007, Elisei, Molinaro et al. 2010).

## 4.4 Histology of well-differentiated thyroid cancer (DTC)

### 4.4.1 Histology of papillary thyroid cancer

More than ten microscopic variants of PTC have been documented (Nikiforov, Biddinger et al. 2012). The most common variant is classical type PTC, which may also be referred to as typical PTC or usual type PTC. The majority of PTCs are of the classical type. Diagnosis is based on typical nuclear features, which should be present in tumour cells. Papillary structures are not present in all tumours. Psammona bodies are sometimes present. Pseudoinclusions may be visible in nuclei, as well as nuclear grooves, as a sign for nuclear membrane folding. Due to chromatin margination tumour nuclei may resemble the "Eye of Little Orphan Annie", the character in the comic strip.

Small PTCs sized 10 mm or less are categorized as PTC microcarcinomas. The malignancy potential of these lesions is low and microcarcinomas are a frequent incidental finding in autopsy studies. If sub-millimeter carcinomas are included, the prevalence of PTC microcarcinomas may be as high as 35% in autopsy series (Lee, Lim et al. 2014).

Follicular variant is the most common subtype of PTC after the classical type. It consists of follicles surrounded by cells with the typical nuclear features of PTC. The follicular variant of PTC has a comparable or more favourable prognosis than the classical type, but the typical papillary structures are rare or absent in tumours (Kesmodel, Terhune et al. 2003).

A diffuse sclerosing variant of PTC occurs predominantly in young patients and has a diffuse growth pattern and dense sclerosis, but with typical PTC elements. LN and distant metastases are more common than in the classical type, but the prognosis appears to be as good as in classical PTC (Chow, Chan et al. 2003).

Tall cell, columnar cell and hobnail variants of PTC are rare but more aggressive tumours than classical PTC. The tall cell variant is a PTC subtype with tall cells, at least twice as high as they are long. A tall cell variant of PTC has a high risk of recurring and causing mortality (Leung, Chow et al. 2008).
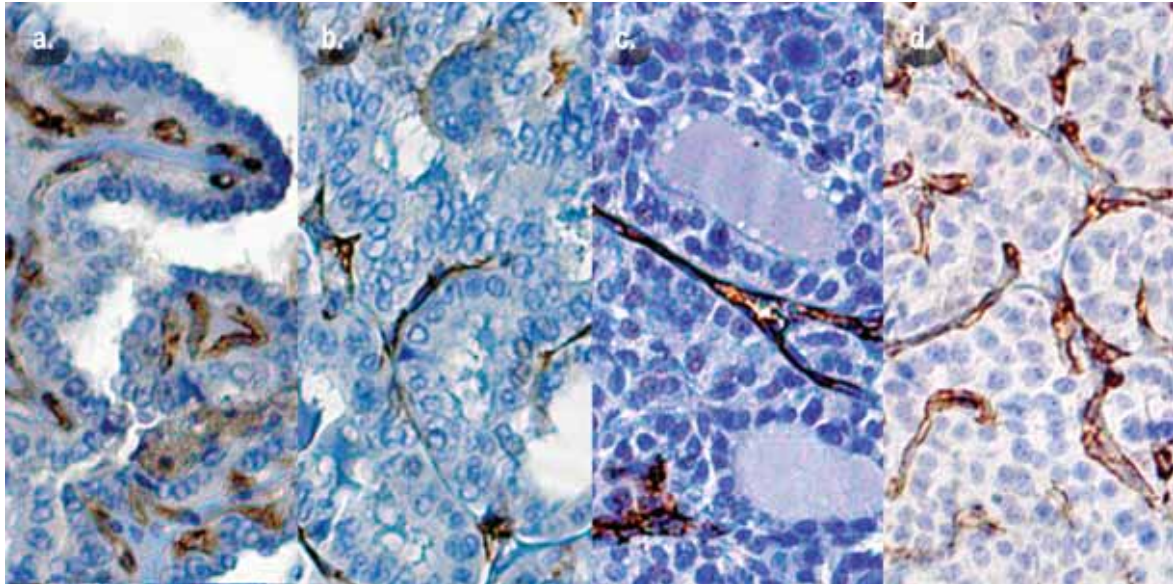


Figure 1. **a.** Papillary thyroid cancer (PTC) classical type expresses typical nuclear features and papillary growth pattern. **b.** PTC follicular variant has follicular growth pattern and typical PTC nuclear features. **c.** Follicular thyroid cancer shows follicular growth pattern. **d.** Thyroid adenoma with microfollicles and trabeculae is displayed for comparison. All cases are presented with vascular marker CD31 immunohistochemistry, magnification ad 200x.

## 4.4.2 Histology of follicular thyroid cancer

FTC may be classified as minimally invasive, if only minor invasion of the tumour capsule or minimal vascular invasion is present. Minimally invasive FTC has a favourable prognosis. Widely invasive FTCs have poorer prognosis, may be vascular invasive and often show distant metastases (Huang, Hsueh et al. 2011).

The oncocytic variant of FTC is also called Hürtle cell carcinoma. It is characterized by the presence of oncocytes, which are large oxyphilic cells with granular eosinophilic cytoplasm and hyperchromatic nuclei. This tumor is currently designated as a histopathologic variant of FTC, although the biological behaviour differs from FTC. Genetic evidence suggests that oncocytic tumors develop via unique molecular mechanisms and therefore represent a distinct type of DTC (Ganly, Ricarte Filho et al. 2013).

## 4.5 Prognostic markers and classifications

### 4.5.1 Clinical and moleculopathological markers

The main clinical prognostic factors are the patient's age and gender. Young age, less than 40 years old, is associated with good prognosis independent of the disease spread (Edge, Compton 2010). Gender affects prognosis in younger patients, especially in patients <55 years the prognosis of PTC is more favourable in females than in males (Jonklaas, Nogueras-Gonzalez et al. 2012). Histology-derived factors include tumour size, histological variant of the tumour, multifocality, possible extrathyroidal spread and nodal status. Multifocal tumours are common, and multifocality has in some studies been associated with poorer prognosis (Mazzaferri, Jhiang 1994). Vascular invasion has been associated with a more aggressive disease course and with more frequent recurrence of the disease (Nishida, Katayama et al. 2002).

*BRAF*-mutation is a new and potentially useful prognostic marker in PTCs. The presence of *BRAF*-mutation has been associated with poorer prognosis (Xing, Alzahrani et al. 2013) and other clinical risk factors in PTC (Knauf, Fagin 2009, Xing, Westra et al. 2005). However, research evidence to the contrary has also been presented (Ito, Yoshida et al. 2009, Fugazzola, Puxeddu et al. 2006).

### 4.5.2 Angiogenesis and lymphangiogenesis

Angiogenesis as a term stands for the development of vasculature. Tumour angiogenesis has been studied extensively in recent decades (Holopainen, Bry et al. 2011, Adams, Alitalo 2007). Studies have indicated that the vascular environment of malignant tumours differs from normal tissue. The vascular environment of malignant tumours is associated with high interstitial fluid pressure, hypoxia and low pH, and therefore the growth environment of malignant tumours is hostile (Carmeliet, Jain 2011). In response the hostile environment triggers changes in tumour cells, which begin to secrete excess amounts of vascular proliferative meditators (vascular endothelial growth factors, VEGFs), to attract more vasculature (De Bock, Mazzone et al. 2011). Various subtypes of VEGFs have

been identified, VEGF-A primarily promotes angiogenesis, and VEGF-C and VEGF-D promote lymphangiogenesis (Ferrara, Gerber et al. 2003, Nieves, D'Amore et al. 2009, De Bock, Mazzone et al. 2011). Serum VEGF levels are elevated in breast cancer patients (Salven, Perhoniemi et al. 1999).

Tumour cell invasion of the vascular structures is considered a marker of a more aggressive than average tumour. Invasion of the blood vessels is a risk factor for metastatic disease. Lymphatic vessel invasion is considered a risk factor for LN metastases (Adams, Alitalo 2007, Holopainen, Bry et al. 2011). Increased lymphangiogenesis in the primary tumour is possibly a risk factor for LN metastasis and poorer prognosis (Bono, Wasenius et al. 2004, Tanaka, Ishiguro et al. 2010).

Angiogenesis-inhibiting advanced therapies have been studied in recent decades and introduced into clinical use in several carcinomas, including thyroid cancer. VEGF humanised monoclonal antibodies act by inhibiting the effects of VEGF and tyrosine kinase inhibitors (TKIs) work by suppressing the response of vascular growth factors on the tyrosine kinase -segment of VEGF receptors. The TKI sorafenib is indicated in metastatic or locally advanced RAI refractory thyroid cancer. Sorafenid improves progression-free survival for five months, but the effect on the survival of DTC patients remains unclear (Brose, Nutting et al. 2014, Wells, Santoro 2014). Sorafenib has an affinity to a number of different tyrosine kinases and therefore sorafenib has an inhibiting effect on multiple kinase-dependent functions in thyroid cancer cells and adjacent vascular endothelial cells (Fallahi, Ferrari et al. 2013, Makita, Iiri 2013). When excessive vascular growth stimulus is inhibited, the tumour blood flow will be altered. This in turn results in depletion of tumour oxygen and nutrient supply. When VEGF stimulus has been reduced with TKIs, resulting in hypoxic conditions, this may trigger tumour cell mesenchymal transformation and metastatic process (Ebos, Lee et al. 2009).

### 4.5.3 TNM and Staging

Multiple staging algorithms have been introduced to estimate the prognosis of thyroid cancer patients. Most staging classifications have been developed and validated with thyroid cancer related death as the outcome. The TNM -classification of the International Union against Cancer (UICC) (Sobin, Gospodarowicz et al. 2010) is the most used and clinically relevant classification worldwide, including Finland. Other significant classification systems include the

Joint Committee on Cancer staging system (AJCC) (Edge, Compton 2010), the Mayo Clinic staging system (MACIS) (Hay, Bergstralh et al. 1993) and the Age Metastasis Extent Size (AMES)-classification (Cady, Rossi 1988). TNM classification and staging for thyroid cancer are presented in Table 1 (Sobin, Gospodarowicz et al. 2010).

Age is a major factor in the TNM staging of thyroid cancer. Disease in patients under 45 years is defined as Stage I if distant metastasis is absent and as Stage II if distant metastasis in present. In patients 45 years or older, staging is based on tumour size (T), nodal metastasis (N) and distant metastasis (M). In the case of lymph node negative patients without distant metastasis, ≤20 mm (T1) tumours are defined as Stage I and 21-40 mm (T2) tumours as Stage II. Stage III is defined as a >40 mm (T3) tumour without nodal metastasis or T1-T3 tumours with central compartment nodal metastasis. Stage IVa is defined as a T1-T3 tumour with lateral compartment nodal metastasis or invasive tumour (T4a) without distant metastasis. Grossly invasive tumour (T4b) without distant metastasis is defined as Stage IVb and with distant metastasis Stage IVc.

**Table 1.** TNM classification and staging in thyroid cancer. Modified from Sobin et al 2009, TNM Classification of Malignant Tumours, pages 53-55. LN=lymph node

| Category | Definition |
|---|---|
| **Primary tumour** | |
| TX | Primary tumours cannot be assessed |
| T0 | No evidence of primary tumours |
| T1a | Tumour ≤10 mm, limited to the thyroid |
| T1b | Tumour 11-20 mm (greatest dimension), limited to the thyroid |
| T2 | Tumour 21-40 mm (greatest dimension), limited to the thyroid |
| T3 | Tumour ≥40 mm (greatest dimension), limited to the thyroid or any tumour with minimal extrathyroidal extension |
| T4a | Tumour of any size extending beyond the thyroid capsule to invade (moderately advanced disease) |
| T4b | Tumours invade prevertebral fascia or encase carotid artery or mediastinal vessels (very advanced disease) |
| **Regional LNs** | |
| NX | Regional LNs cannot be assessed |
| N0 | No regional LNs metastasis |
| N1 | Regional LN metastasis |
| N1a | Metastasis to level VI (pretracheal, paratracheal, and prelanryngeal/Delphian LNs) |
| N1b | Metastasis to unilateral, bilateral, or contralateral cervical or superior mediastinal LNs |
| **Distant metastases** | |
| MX | Distant metastasis cannot be assessed |
| M0 | No distant metastasis |
| M1 | Distant metastasis |
| **Staging** | |
| Stage I | T1 N0 M0 (if patients is <45 years: any T, any N, M0) |
| Stage II | T2 N0 M0 (if patients is <45 years: any T, any M, M1) |
| Stage III | T3 N0 M0 / T1-3 N1a M0 |
| Stage IVA | T4a N0-N1a M0 / T1-3 N1b M0 |
| Stage IVB | T4b any N M0 |
| Stage IVB | Any T any N M1 |

## 4.6  Diagnosis of thyroid cancer

The most common clinical sign of thyroid cancer is a solid, slowly growing and painless nodule, discovered by the patient or at a clinical examination. Although thyroid nodules are fairly common, only 5% of them prove to be malignant. The diagnosis of thyroid cancer is based on US and FNA. FNA is performed under US guidance and is indicated in cases of clinical or US suspicion of a malignancy (Pacini, Castagna et al. 2010).

### 4.6.1  Ultrasonography

US is a recommended imaging procedure in the evaluation of thyroid nodules. US can identify the size and sonographic characteristics of the nodule, possible additional nodules and cervical lymphadenopathy (Frates, Benson et al. 2005). Thyroid US should be performed in the evaluation of suspected thyroid nodule, nodular goitre, or thyroid nodule detected incidentally in another imaging study, *e.g.* computed tomography, magnetic resonance imaging or fluorodeoxyglucose positron emission tomography (FDG-PET) (Marqusee, Benson et al. 2000).

US is useful in the guiding of FNA and in the detection of cervical LNs with suspicion of metastasis. The FNA needle should be introduced to into the thyroid nodule under US guidance. (Hegedüs 2001). FNA is the most important diagnostic procedure in the evaluation of thyroid nodules found in US. Any hypoechoic solid nodule ≥ 10 mm should be biopsied under US guidance unless the nodule is hyper-functioning. According to current guidelines, if the patient has a high-risk history, *e.g.* a history of thyroid cancer in a first-degree relative or exposure to radiation in childhood, then nodules >5 mm should be biopsied. If the risk factors are absent, then nodules <10 mm should be observed and biopsied if a growth pattern emerges or if suspicious patterns arise. In goitre with multiple nodules, the FNA should be performed on the dominant nodules (Pacini, Schlumberger et al. 2006, Cooper, Doherty et al. 2009).

Suspicious characteristics of a thyroid nodule include irregular borders, hypoechogenity, absence of peripheral halo, microcalcifications and taller-than-wide appearance, and any combination of these may contribute to a malignancy-suspicion of the nodule (Smith-Bindman, Lebda et al. 2013). However, in a recent meta-analysis, individual US features were not seen to be accurate predictors of

thyroid cancer (Brito, Gionfriddo et al. 2013). Table 2 presents US appearance and risk stratification of thyroid nodules in the proposed future ATA guidelines.

A novel US technique, real-time elastography, enables the determination of tissue elasticity and has yielded promising results in the investigation of thyroid nodules. Elastography has provided encouraging results, and in some studies has demostrated high sensitivity and specificity in the evaluation of thyroid nodules (Bojunga, Herrmann et al. 2010).

Table 2.  Ultrasonographic (US) appearance and risk stratification of thyroid nodules. Modified from the American Thyroid Association proposed 2015 guidelines. *Suspicious features are: irregular margins, microcalcifications, taller-than-wide shape, rim calcifications or extrathyroidal extension. Suspicious lymph nodes detected in US should also be biopsied. Suspicious lymph node features are: size >1cm, ratio of long axis to short axis <2.0, microcalcifications and peripheral hypervascularity.

| Sonographic pattern | US features | Estimated risk of malignancy | Consider biopsy |
|---|---|---|---|
| High suspicion | Solid hypoechoic nodule or partially cystic nodule *with* one or more suspicious feature* | >70-90% | ≥1cm |
| Intermediate suspicion | Hypoechoic solid nodule with smooth margins without suspicious features* | 10-20% | ≥1cm |
| Low suspicion | Isoechoic or hyperechoic solid nodule, or partially cystic nodule with eccentric solid areas, *without* suspicious features* | 5-10% | ≥1.5cm |
| Very low suspicion | Spongiform or partially cystic nodules *without* suspicious features* | <3% | ≥2cm |
| Benign | Purely cystic nodule (no solid component) | <1% | No biopsy |

## 4.6.2    Fine-needle aspiration biopsy

PTCs have a unique histologic appearance, which can be identified in aspiration biopsy. Capsular invasion of follicular neoplasms is cannot be identified in FNA samples. The expertise of pathologists is very important in the evaluation of thyroid FNAB samples. In recent years the Bethesda system (Cibas, Ali et al. 2009) for reporting cytopathology has been widely adopted (Table 3). The Bethesda system has replaced the former Papanicolau cytology classification in Finland (Arola, Kholova et al. 2010). In surgical specimens removed due to suspicion of

malignancy, the proportion of malignancies could be as low as 14% prior to the introduction of thyroid FNAs. After the adoption of routine FNA, the proportion of malignancies reached over 50% (Cibas, Ali et al. 2009).

The limitations of FNA include inadequate samples and follicular neoplasia (Marqusee, Benson et al. 2000, Frates, Benson et al. 2005). The sensitivity or specificity of FNA is not ideal, and non-adequate samples are frequent (Wang, Friedman et al. 2011, Alexander, Kennedy et al. 2012). Non-diagnostic and indeterminate thyroid nodule FNA samples are common, up to 35% of cases. FNA samples of unknown malignant potential are a problem of everyday clinical work (Yassa, Cibas et al. 2007, Yang, Schnadig et al. 2007, Nayar, Ivanovic 2009). In case of an inadequate sample, the FNA should be repeated (Braga, Cavalcanti et al. 2001).

The FNA biopsy result of the thyroid nodule determines the need for surgical treatment. The preoperative FNA is often indeterminate regarding malignancy. (Raab, Vrbin et al. 2006). Surgery is needed if the FNA shows a suspicion of malignancy, but up to 79% of tumours prove to be benign in final pathology after surgery (Sangalli, Serio et al. 2006). The need for additional preoperative diagnostic methods is obvious, and enhanced diagnostic methods could reduce the number of unnecessary surgical procedures and associated complications. Operations performed due to inconclusive FNA results also cause significant financial costs and affect quality of life (Adler, Sippel et al. 2008).

Table 3.    Categories of Bethesda system and usual management guidelines. Modified from Cibas and Ali 2009.

| Diagnostic category | Risk of Malignacy | Proposed Management |
|---|---|---|
| I. Non-diagnostic or unsatisfactory | 1-4% | Repeat FNA |
| II. Benign | 0-3% | Clinical follow-up |
| III. Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS) | ~5-15% | Repeat FNA |
| IV. Follicular neoplasm or suspicion of follicular Neoplasm | 15-30% | Surgical lobectomy |
| V. Suspicion of malignancy | 60-75% | Total or near-total thyroidectomy or surgical lobectomy |
| VI. Malignant | 97-99% | Total or near-total thyreoidectomy |

## 4.6.3   Core needle biopsy

CNB provides a larger tissue sample that retains its cellular architechture and may enable more accurate histologic diagnosis (Screaton, Berman et al. 2003). CNB is performed under ultrasound guidance by a radiologist, and the technique is comparable to that of FNA. Thyroid FNA and CNB are feasible during the same ultrasound examination. Thyroid CNB has been performed using a 16-21-gauge needle and primarily non-advancing CNB techniques have been used (Renshaw, Pinnar 2007, Screaton, Berman et al. 2003, Karstrup, Balslev et al. 2001).

After CNB, a histologic sample of the tumour is available for immunohistochemial analysis, which may be crucial in selected cases, for example when there is a suspicion of MTC, ATC or parathyroid lesion. FNA cellblocks also enable additional immunohistochemical analysis of tumour cells. (Bhanot, Yang et al. 2007, Absher, Truong et al. 2002). Unfortunately FNA samples are adequate to perform cellblocks in only a minority of cases (VanderLaan, Marqusee et al. 2011, Mills, Poller et al. 2005).

CNB has been studied in thyroid nodule diagnostics and the results of these studies are inconclusive. (Zhang, Ivanovic et al. 2008, Carpi, Nicolini et al. 2000, Bandyopadhyay, Pansare et al. 2007). The role of CNB in thyroid nodule preoperative diagnostics is currently not well established. The present management guidelines do not include recommendations on the use of CNB (Cooper, Doherty et al. 2009, Pacini, Schlumberger et al. 2006). Some studies have advocated the use of CNB in cases of repeated FNAs results with insufficient or atypia of undetermined significance or follicular lesion of undetermined significance (AUS/FLUS) (Na, Kim et al. 2012, Renshaw, Pinnar 2007). No conclusive evidence exists as to whether CNB is beneficial in the diagnostics workup of thyroid nodules with suspicion for malignancy. Due to the limited number of studies available, the complication risks of thyroid nodule CNB have not been thoroughly assessed, but the existing studies estimate the risk to be equal or slightly higher than the complication risks of thyroid FNA. Severe morbidity is rare in both CNB and FNA, and the most common complication in both methods is haematoma formation (Renshaw, Pinnar 2007, Karstrup, Balslev et al. 2001).

### 4.6.4 Other diagnostic procedures

After US and FNA have been performed, other diagnostic methods may be used if necessary. Measurement of serum TSH may help to reveal hyperfunctioning thyroid nodule (Cooper, Doherty et al. 2009). If serum TSH is subnormal, thyroid scintigraphy may be performed. Scintigraphy can reveal the presence of autonomous hyperfunctioning nodules, which seldom harbour malignancy (Pacini, Burroni et al. 2004). Measuring serum calcitonin may assist in diagnosis of MTC (Elisei, Bottici et al. 2004). Computed tomography and magnetic resonance imaging with intravenous contrast is recommended as an adjunct to ultrasound for patients with clinical suspicion for advanced disease including invasive primary tumor, or clinically apparent multiple or bulky lymph node involvement.

Although FDG-PET is not recommended in the primary diagnostic workup of thyroid nodules, a suspicious thyroid lesion may be an incidental finding in FDG-PET scan done for other indications. FDG-PET shows good sensitivity in distinguishing malignancies with high metabolic activity, but specificity is not optimal, as some benign lesions, *e.g.* focal thyroiditis, may also have marked metabolic activity (de Geus-Oei, Pieters et al. 2006).

## 4.7    Treatment

### 4.7.1    Surgical treatment

If thyroid nodule needle biopsy is malignant or malignancy is suspected, surgery is generally recommended. Watchful waiting without immediate surgery may be considered in patients with serious co-morbidities or in patients expected to have a relatively short life span. Patients with very low risk tumours, ≤10mm PTCs without metastases may be treated with watchful waiting as an alternative to immediate surgery. Clinical progression of PTC microcarcinomas is extremely slow in majority of cases, especially in older patients (Sugitani, Toda et al. 2010, Ito, Uruno et al. 2003).

Total or near-total thyroidectomy is recommended if the primary tumour is >4cm in size, if extrathyroidal extension, clinically apparent LN metastasis or distant metastasis is present. Near-total thyroidectomy, meaning leaving a

maximum of 1g of thyroid tissue per side to protect the recurrent laryngeal nerve and parathyroid glands, has equally succesfull treatment results as total thyroidectomy. On the other hand, small quantity thyroid tissue remaining after surgery is an independent prognostic factor (Bilimoria, Bentrem et al. 2007).

The current guidelines recommend total or near-total thyroidectomy in thyroid cancers larger than 10 mm (Cooper, Doherty et al. 2009, Pacini, Schlumberger et al. 2006). However, in tumours 1-4cm in size, the recent data suggest that the initial surgical procedure may be either total thyroidectomy or lobectomy (Nixon, Ganly et al. 2012). Although thyroid lobectomy alone may suffice for low-risk PTCs and FTCs, total thyroidectomy enables RAI ablation and the use of thyroglobulin in clinical follow-up, and a multidisciplinary treatment team may advocate total thyroidectomy on this basis. After total thyroidectomy, RAI scintigraphy may be used to reveal residual disease.

Lobectomy is a sufficient procedure in cases of small ≤ 10 mm unifocal intrathyroidal PTC microcarcinomas, in the absence of LN metastases and prior neck irradiation (Cooper, Doherty et al. 2009). The prognosis of node-negative PTC microcarcinomas is excellent and RAI ablation is not indicated postoperatively. Furthermore, the postoperative complication rate is higher after total thyroidectomy than after lobectomy (Hauch, Al-Qurayshi et al. 2014).

Central neck dissection, the removal of central LNs (region VI) near the trachea, is advocated in patients with metastatic central LNs. In patients with clinically uninvolved central LNs, a prophylactic central neck dissection should be considered if the patient has an advanced primary tumour (>4 cm) or metastatic lateral LNs or if LN status will affect further treatments. Prophylactic central neck dissection may be unnecessary in node-negative T1-T2 tumours and for most follicular cancers. Long-time follow-up studies are not available and the benefit/morbidity-ratio cannot be accurately assessed. The impact of prophylactic central neck dissection on survival and recurrences and is yet to be determined (Brito, Hay et al. 2014). Therapeutic lateral neck compartmental LN dissection should be performed to patients with biopsy-proven lateral cercival lymphadenopathy.

In case of inconclusive FNA cytology, patients may be offered FNA aspirate mutation marker panel testing, which has a negative predictive value of up to 94% (Alexander, Schorr et al. 2013). A negative mutation panel may suggest non-surgical treatment, as the negative predictive value of the mutation marker panel is of the same order as negative FNA biopsy. When surgery is considered in patients with cytologically indeterminate nodules, lobectomy is the recommended initial

surgical approach. Conversion total thyroidectomy may be perfomed if a thyroid nodule suspected of malignancy is malignant in intraoperative evaluation or in frozen section. Intra-operative frozen section may also be used in tumours with preoperative malignancy-suspicious FNA.

Typical complications of thyroid surgery include infection and bleeding, hypoparathyroidism (<1%) and paralysis of the recurrent laryngeal nerve (1-2%) (Bergenfelz, Jansson et al. 2008, Rosato, Avenia et al. 2004). Intra-operative nerve monitoring may be used in the localization of recurrent laryngeal nerve during the surgery, although no conclusive evidence has been presented as to whether the monitoring decreases nerve injuries (Pisanu, Porceddu et al. 2014).

## 4.7.2   Radio-iodine ablation

Surgery may be followed by RAI treatment, which has three main goals: ablation of residual thyroid tissue in order to facilitate thyreoglobulin based follow-up (remnant ablation), eradication of possible microscopic disease left after surgery (adjuvant therapy) and elimination of known macroscopic residual tumour (RAI therapy).

RAI used in the treatment of DTC is $^{131}$I-isotope with a half-life of approximately eight days. RAI is actively transported to the thyroid follicular cells by sodium/iodine-symporter and the radiation dose to the thyroid gland is 1,000 to 10,000 times higher than in the surrounding tissues. Sodium/iodine symporter expression and iodine update are also found is other organs, namely the salivary glands, (lactating) mammary glands, stomach, choroid pleaxus, ciliae body, cutis and placenta. Most of the excess iodine is excreted through the kidneys to the urine. RAI emits beta particles (electrons) and gamma radiation. Beta particles account for 90 % of the radiation, have a penetration depth of 2 mm and are responsible for the treatment effects in thyroid cancer. Gamma-radiation (10%) is used to perform scintigraphy (Maenpaa 2014).

According to ATA recommendations, thyroid remnant ablation after surgery is recommended for patients with intermediate and high-risk disease, which is presented in Table 4 (Cooper, Doherty et al. 2009). The European consensus statement recommends thyroid remnant ablation in tumours with high risk of recurrence, in other words RAI ablation is recommended in tumours with maximum diameter of >40 mm (T3-T4) and when distant metastasis or gross extrathyroidal extension of tumours is present. Furthermore, selective use of

remnant ablation is recommended for DTCs with maximum diameter of 11-40 mm and for tumours with minimal extrathyroidal extension. Table 5 summarises the current recommendations of ATA and the European Thyroid Association. Remnant ablation is not recommended for non-metastatic ≤10 mm DTCs without extrathyroidal extension (Pacini, Schlumberger et al. 2006). The European Society for Medical Oncology (ESMO) guidelines recommends RAI ablation of all DTCs greater than ≥20 mm (Pacini, Castagna et al. 2012).

Table 4. Risk assessment for disease recurrence according to the American Thyroid Association Guidelines (modified from Cooper et al. 2009).

|  | Invasion | Histology | Metastasis | Surgery |
|---|---|---|---|---|
| Low-risk | Intrathyroidal tumour, no invasion | No aggressive histology and no vascular invasion | No local or distant metastasis | Complete |
| Intermediate-risk | Microscopic invasion | Aggressive histology or vascular invasion | Cervical lymph node metastasis (N1) | Complete |
| High-risk | Macroscopic invasion |  | Distant metastasis (M1) | Incomplete tumour resection |

Table 5. Radio-iodine remnant ablation: American (ATA) and European (ETA) Thyroid Association recommendations and risk assessment for disease recurrence according to ETA Guidelines (Cooper, Doherty et al. 2009, Pacini, Schlumberger et al. 2006)

| TNM Classification | ATA guidelines | ETA guidelines | Estimated risk of recurrence (ETA) |
|---|---|---|---|
| T1a | No (also if multifocal) | No (Possibly if multifocal) | Very low (Low if multifocal) |
| T1b | Selective use | Probable indication | Low |
| T2 | Selective Use | Probable indication | Low |
| T3 | Yes (selective use in the presence of minimal extrathyroidal extension) | Yes | High |
| T4 | Yes | Yes | High |
| N1 | Selective use | Yes | High |
| M1 | Yes | Yes | High |

According to current knowledge, RAI ablation decreases the risk of recurrence and the risk of DTC-related death if distant metastasis is present or if the tumour has gross extrathyroidal extension. Patients ≥45 years old with >40 mm tumours benefit from RAI ablation. Otherwise the research provides either conflicting or inadequate data regarding the benefit of RAI ablation for DTC recurrence or cancer related death (Podnos, Smith et al. 2007).

TSH facilitates the uptake of RAI into thyroid follicular cells, and therefore high serum TSH levels are required to achieve optimal RAI ablation effect. A adequate RAI uptake can be achieved when serum TSH levels are above 25-30 $\mu$U/ml. Traditional endogenous TSH stimulation, *i.e.* LT4 withdrawal, produces symptoms of hypothyroidism for 2-4 weeks. The patient's quality of life and working ability may be affected for several weeks before the administration of RAI. Use of exogenous recombinant human TSH (rhTSH) does not require LT4 withdrawal, and therefore has fewer side effects. The efficacy of rhTSH in RAI ablation has been studied, and when compared to LT4 withdrawal, no major differences were observed in the success of ablation (Schlumberger, Catargi et al. 2012, Mallick, Harmer et al. 2012). RhTSH is approved for use in RAI ablation of low-risk DTC patients, using the high or low dose of RAI. For high-risk patients, LT4 withdrawal is the recommended method of TSH stimulation unless there are co-morbidities, which favour the use of rhTSH.

Optimal dose in RAI ablation has been debated throughout the past decade. Recent studies have proven that traditional high doses (3.7 GBq) and low dose (1.1 GBq) yield comparable results in ablation rates. Lower dose requires shorter hospital stay, involves lower extrathyroidal tissue toxicity and less environmental RAI contamination (Mäenpää, Heikkonen et al. 2008, Schlumberger, Catargi et al. 2012). Higher doses (3.7 GBq) are recommended if there is a high risk of recurrence; *i.e.* incomplete surgery, tumour size is large or tumour has extrathyroidal extension (T3-T4), patient has nodal or distant metastasis (Pacini, Schlumberger et al. 2005).

RAI treatment is perfomed usually with hospitalization of a few days after administration of RAI. There are standard guidelines on postrelease precations to minimize radiation exposure to other family members but in future these may be based on the individual kinetics of RAI (Tenhunen, Lehtonen et al. 2013).

RAI therapy produces various acute or subacute adverse effects in organs concentrating iodine and in the gastrointestinal and urinary tract. Contraindications for RAI ablation are pregnancy and breastfeeding. Relative contraindications

include xerostomia, clinically relevant bone marrow depression and pulmonary function restriction in case of lung metastases. When compared to LT4 withdrawal, the use of rhTSH in euthyroid patient leads to faster clearance of RAI, and therefore lowers exposure to radiation (Menzel, Kranert et al. 2003).

In metastatic disease RAI treatment is indicated if metastases are iodine-avid and surgical treatment is unfeasible. Although LT4 withdrawal has been the preferred method of TSH stimulation in high-risk patients and patients with metastatic disease, the efficacy of rhTSH stimulated RAI treatment has been successfully demonstrated in these patients (Klubo-Gwiezdzinska, Burman et al. 2012).

## 4.7.3    Thyroxine therapy

After total thyroidectomy, permanent thyroid hormone substitution is needed. Generally LT4 is administered in doses that suppress endogenous TSH, thus preventing the growth promoting effect of TSH in possible residual disease. TSH suppression in patients with low-risk PTCs is probably not needed, as low-risk patients without TSH suppression (TSH target level ≥0.5 mU/l) are not at increased risk of recurrence (Sugitani, Fujimoto 2010). In advanced disease the benefits of TSH suppression have been demonstrated (Diessl, Holzberger et al. 2012). Due to adverse effects, total TSH suppression (TSH target level <0.1 mU/l) is only recommended for high-risk DTC patients  (Cooper, Doherty et al. 2009, Pacini, Schlumberger et al. 2006, Pacini, Castagna et al. 2012).

## 4.7.4    Treatment options in RAI-refractory metastatic disease

RAI-refractory metastatic disease may be controlled with surgery, especially when the lesions are confined to the neck. In selected inoperable or comorbid patients metastatic disease of the neck region may also be treated with US-guided ethanol injections or interstitial laser photocoagulation (Leenhardt, Erdogan et al. 2013). Bisphosphonates may be used for pain management in bone lesions. External beam radiotherapy may be used to control the unresectable gross residual disease or recurrent cervical metastasis, painful bone metastases or other metastasis in critical locations; *e.g.* bone metastasis and central nervous system (CNS) metastasis (Cooper, Doherty et al. 2009). Intra-arterial embolization may be used for liver metastases. Radiofrequency ablation techniques may be used for lung, bone and

liver metastases. Endotracheal laser ablation has been used in controlling the intratracheal extension of the DTC. Chemotherapy is ineffective in metastatic DTC (Pacini, Castagna et al. 2012).

RAI-refractory and progressive DTC may be treated with TKIs when other treatment options are not available. TKI sorafenib improves progression-free survival in metastatic RAI-refractory DTC and has been approved for clinical use (Brose, Nutting et al. 2014). Phase III clinical trial is in progress with lenvatinib and Phase II trials are ongoing with various other TKIs (Schlumberger, Tahara et al. 2014, Xing, Haugen et al. 2013).

## 4.8 Follow-up and prognosis

A schematic view of the follow-up guidelines for DTC patients is shown in Figure 2. A post-therapy whole body scan is acquired 5-7 days after RAI ablation. LT4-therapy begins after surgery, if rhTSH is to be used in RAI ablation; otherwise LT4-therapy is initiated after RAI ablation. LT4-dose is adjusted to a level with sufficient TSH suppression and controlled after three months. Between six to 12 months after RAI ablation, rhTSH stimulated thyroglobylin (Tg) values are obtained. Neck US is required in cases of elevated Tg. In cases of undetectable stimulated Tg and normal US the risk of recurrence is low and the patient is monitored yearly with unstimulated Tg and neck US. If the US is normal and Tg is at a low detectable level (1-2 μg/l), then TSH-stimulated Tg is re-evaluated after one year. If Tg levels are decreasing, the patient may be monitored yearly using stimulated Tg. RAI treatment is indicated if levels of Tg or Tg-antibodies are increasing or neck US is abnormal. Surgical treatment may be considered in cases of recurrent tumour or biopsy-confirmed LN metastasis in neck US (Cooper, Doherty et al. 2009, Pacini, Schlumberger et al. 2006).

### 4.8.1 Morbidity and mortality

The prognosis of DTC is generally favourable, and long-term survival rates exceed 90% (Ito, Kudo et al. 2012). Up to one third of patients may suffer from recurrence. Most recurrences occur during the first decade after the diagnosis. Most recurrences are local recurrences in the thyroid bed or neck region, distant recurrences account for 20% of cases (Mazzaferri, Jhiang 1994). In the study by

Bilimoria et al. (Bilimoria, Bentrem et al. 2007), the 10-year recurrence rate was 9.4%. Survival and recurrence rates were associated with tumour size.
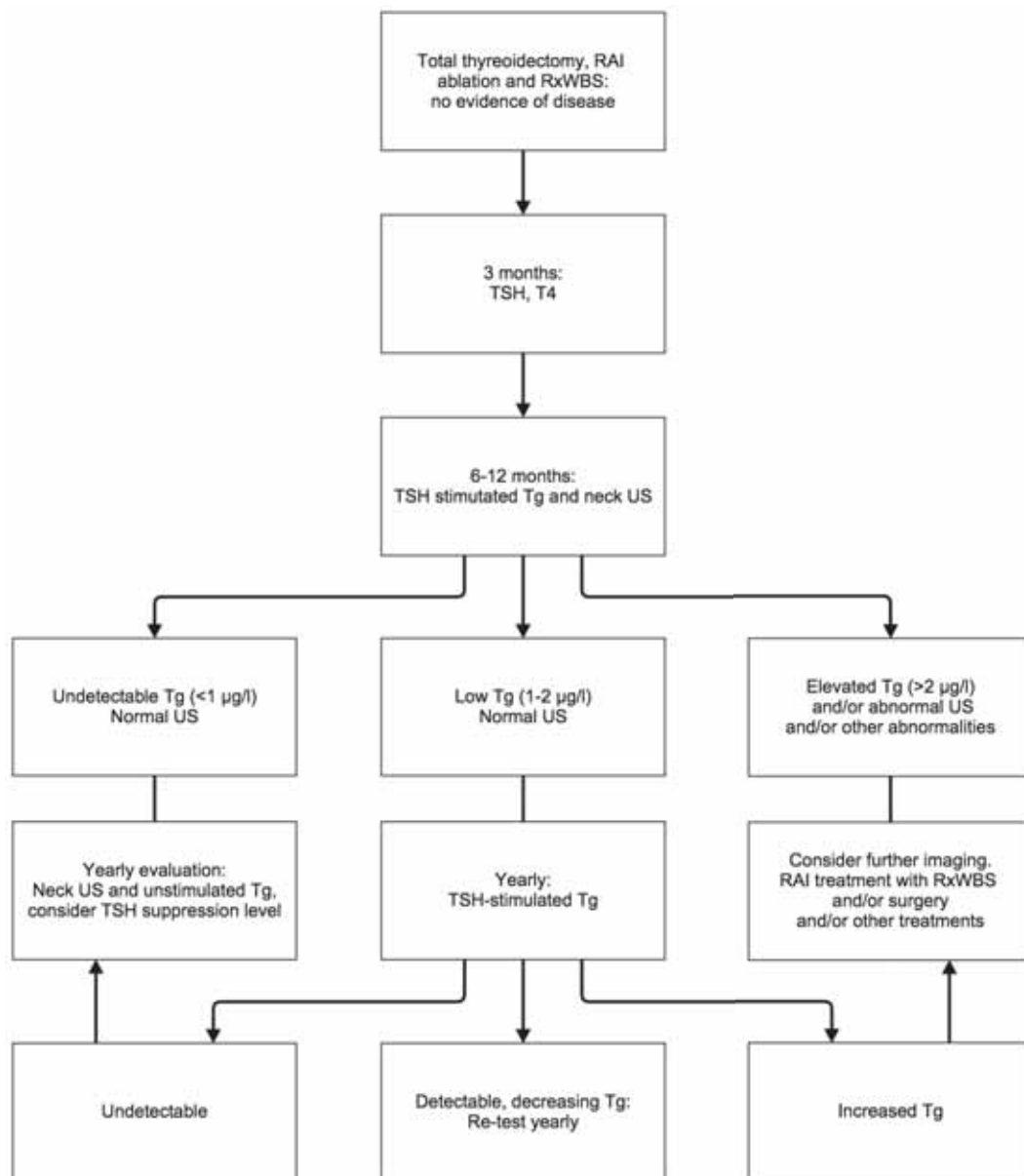


**Figure 2.** Flow chart for the follow-up of DTC patients after initial total thyroidectomy and post-operative radioiodine (RAI) ablation. (Modified from Pacini et al. 2006, Cooper et al. 2009 and Pacini et al. 2012). WBS=post-therapy whole body scan, TSH= thyroid stimulating hormone, T4 = thyroxine, Tg=thyroglobulin, US=ultrasonography.

## 4.8.2 Risk of second primary malignancies (SPMs)

DTC is treated with radioactive iodine, which emits gamma and beta radiation. Radiation is a well-known carcinogenic factor, and younger patients are more sensitive to radiation-induced carcinogenesis (Shuryak, Sachs et al. 2010). Because DTC affects relatively young patients, radiation induced SPMs are a source of concern (Sandeep, Strachan et al. 2006, Sawka, Thabane et al. 2009, Hay, Gonzalez-Losada et al. 2010).

In a meta-analysis the risk of SPM was 18-20% higher in RAI-treated DTC patients than in patients without RAI treatment (Sawka, Thabane et al. 2009). In a recent study, the risk of SPMs in RAI treated DTC-patients was 12% higher than in DTC patients without RAI treatment (de Gonzalez, Curtis et al. 2011). Female RAI-treated DTC patients have been reported to have a 54% higher risk of SPMs than general population (Lang, Wong et al. 2012).

Table 6 summarises recent large cancer register-based studies regarding the risk of SPMs in DTC patients. In a study of 30,278 patients, 9% higher incidence of SPMs was observed in DTC patients than in the general population in United States (Brown, Chen et al. 2008). In Europe a 27% higher risk of SPMs has been reported in DTC patients (Rubino, De Vathaire et al. 2003). In a multination study with 13 population-based cancer registries in Europe, Canada, Australia and Singapore, Sandeep et al. observed a 31% increase in the incidence of SPMs in DTC patients (Sandeep, Strachan et al. 2006). In Taiwan, DTC patients had 33% more SPMs than their counterparts from general population (Lu, Lee et al. 2013).

SPMs associated with DTC include malignancies of the salivary glands, breasts, kidneys and urinary tract, haematopoietic and lymphatic systems, CNS and connective tissue (Rubino, De Vathaire et al. 2003, Brown, Chen et al. 2008, Berthe, Henry-Amar et al. 2004, Ronckers, McCarron et al. 2005, Verkooijen, Smit et al. 2006, Kim, Bi et al. 2013, Iyer, Morris et al. 2011, Lu, Lee et al. 2013).

The above-mentioned studies are cancer registry-based and lack important information on clinical parameters, *e.g.* RAI doses, which are not reliably available in registries. Incidence of SPMs in Finnish DTC patients has not been previously studied.

**Table 6.** Recent registry-based studies with large patient material comparing incidence of second primary malignancies (SPMs) in patients with well-differentiated thyroid cancer (DTC) to incidence of malignancies in general population. RR=risk ratio, CI= confidence interval, SEER = Surveillance, Epidemiology and End Results program, RAI=radioiodine

| Study | Patients, years, countries | SPM RR (CI) | Major findings |
|---|---|---|---|
| Brown et al. 2008 | 30,278 DTC patiens, 1973-2002, USA/SEER | 1.09 (1.05-1.14) | Radiation-treated patients RR 1.20 (1.07-1.33), unirradiated RR 1.05 (1.00-1.10). Greatest risk of SPMs within 5 years of diagnosis and in younger patients. |
| Gonzalez et al. 2011 | 27,775 thyroid cancer patients, 1973-2002, USA/SEER | 1.12 (1.01-1.25) | Relative risk between RAI treated and RAI non-treated patients. Estimated number of radiation-related excess cancers 67. |
| Iyer et al. 2011 | 14,589 RAI-treated DTC patients, 1973-2007, USA/SEER | 1.18 (1.10-1.25) | RAI-treated patients. T1N0 RAI-treated patients RR 1.21 (0.93-1.54). |
| Kim et al. 2013 | 52,013 DTC patients, 1973-2008, USA/SEER | 1.09 (1.06-1.12) | RAI-treated RR 1.25 (1.17-1.33). T1a tumours RR 1.27 (1.17-1.37). 1994-2003 RR 1.21 (1.14-1.28), 2004-2008 RR 1.45 (1.28-1.62). |
| Lu et al. 2013 | 19,068 patients, 1979-2006, Taiwan | 1.33 (1.23-1.44) | Highest risk within 5 years of diagnosis (RR 5.29, CI 4.43-6.26) and in patients <50 years (RR 1.81, CI 1.52-2.14). |
| Rubino et al. 2003 | 6,841 thyroid cancer patients, 1934-1995, Sweden, Italy, France | 1.27 (1.15-1.40) | Equal overall risk between radiation treated and non-treated patients. |
| Sandeep et al. 2006 | 39,002 patients, 1943-2000, EU, Canada, Australia, Singapore | 1.31 (1.26-1.36) | High risk of salivary gland, bone, soft tissue sarcoma and adrenal gland cancers (RR>3). After any first primary cancer, RR 1.54 (CI 1.17-2.00) for thyroid cancer. |

# 5    AIMS OF THE STUDY

The aim of this study was to evaluate the diagnostics, prognostic factors and long-term outcome of patients with thyroid cancer.

The specific aims of the study were:

- To assess retrospectively the outcome of thyroid cancer patients in Pirkanmaa Hospital District between 1981 and 2002

- To identify angiogenesis and lymphangiogenesis related prognostic factors for metastatic spread of DTC using immunohistochemical methods

- To compare the incidence of second primary malignancies in DTC patients with matched controls

- To evaluate the feasibility of core needle biopsy in thyroid cancer diagnostics

# 6   PATIENTS AND METHODS

## 6.1    Patients

**Study I**

The study was based on 553 unselected and consecutive patients treated for thyroid cancers at Tampere University Hospital (TAUH) between 1981 and 2002. Most of the patients (n=498, 90%) had DTC (Table 7). The Finnish Cancer Registry provided information on thyroid cancer cases in TAUH region. Clinical follow-up data, including information on surgery and oncological treatments, cancer recurrences and mortality, was acquired retrospectively from the TAUH medical records. Patients' follow-up was continued until the end of May 2007.

**Study II**

The patients with surgically managed recurrence of DTC in Study **I** were selected for Study **II**. Altogether 27 patients with recurrent DTC had histological samples available for analysis, from primary tumours and metastatic lesions, and the number of samples totalled 60. Twenty-four controls matched for age, gender, tumour type and tumour size with non-recurrent DTC were selected from among the patients of Study **I**. In addition, 24 thyroid adenomas analysed in the pathology laboratory were included as benign controls. A total of 108 samples were analysed for angiogenesis and lymphangiogenesis using CD31 and podoplanin immunohistochemistry.

**Study III**

The DTC patients of Study I were combined with patients in a study conducted at Oulu University Hospital (OUH) (Jukkola, Bloigu et al. 2004) to form the patient material in Study **III**.   A total of 910 consecutive DTC patients (920 patients

before data gathering exclusions) were treated in TAUH (n=486) or OUH (n=424) 1981–2002. Follow-up data was collected from the hospitals' medical records. Five matched controls per patient were acquired from the Population Register Centre of Finland bringing the total number of controls to 4,542.

## Study IV

This study was based on samples of 52 consecutive patients operated on in TAUH with FNA indicating a need for surgical treatment. FNA samples were categorised preoperatively as follicular neoplasms (46%, n=24), suspected malignancies (48%, n=25) or malignancies (6%, n=3). All patients were operated on in TAUH between May 2010 and December 2011.

Table 7.  Summary of study patients, controls and endpoints. DTC = well-differentiated thyroid cancer, CNB = core needle biopsy, FNA = fine-needle aspiration biopsy, TAUH = Tampere University Hospital, OUH = Oulu University Hospital

| Study no. | Patients | Controls, comparison | Objectives, Endpoints |
|---|---|---|---|
| Study I | 498 DTC patients | Patients treated 1981-1991 vs. patients treated 1992-2002 | Recurrence, mortality and treatment-related factors |
| Study II | 27 recurrent DTCs | 24 non-recurrent DTCs and 24 thyroid adenomas | Tumour blood and lymphatic vascularity |
| Study III | 910 DTC patients TAUH 486 OUH 424 | 4542 age, gender and place of residence-matched controls from general population | Incidence of second primary malignancies |
| Study IV | 52 malignancy-suspicious thyroid tumours | Comparison of 52 CNB and FNA samples | Malignancy-specific sensitivity and specificity |

## 6.2 Methods

### 6.2.1 Epidemiology of thyroid cancer (Study I)

A total of 553 consecutive thyroid cancer patients treated in TAUH during the period 1981–2002 was included in Study **I**. Information on patient and treatment related factors was acquired from the medical records. Information collected included symptoms, FNA results, age, gender, surgeries, tumour histology, possible metastases, RAI ablation doses, other oncological treatments, recurrences and mortality. Most patients were operated on in TAUH and all patients were followed up in the TAUH Departments of Oncology or Endocrinology. Histological samples were processed and analysed in the TAUH pathology unit (Fimlab Laboratories).

Patients with DTC (90%, n=498) were grouped into two equivalent time periods, patients diagnosed 1981–1991 (n=207) and 1992–2002 (n=291), to study changes in tumour charasteristics and treatment–related factors in past decades. The WHO criteria for FTC have changed in recent decades, and the FTC samples were re-evaluated by pathologists (Barnes, Eveson et al. 2005).

The material included few rare histopathological cases, 17 thyroid lymphomas and three squamous cell carcinomas, which were excluded from the final analysis. The information included cancer registrations from autopsy findings and these thyroid cancer cases were excluded (n=17).

### 6.2.2 Assessing angiogenesis and lymph angiogenesis (Study II)

Samples of 27 recurrent DTCs, 24 non-recurrent DTCs and 24 thyroid adenomas were analysed in Study I. Althogether 108 paraffin-embedded tissue blocks of tumours and their metastases were cut into 4 μm thick sections and processed with Ventana Life Sciences Benchmark XT Staining module (Ventana Medical Systems, Tucson, Arizona). Sections were incubated with the panendothelial marker CD31 (DAKO, Glostrup, Denmark, clone JC70A, 1:50) for 30 minutes at room temperature. The lymphatic endothelium marker podoplanin (DAKO, Glostrup, Denmark, clone D2-40, 1:50) was detected after 30 minutes of incubation at room temperature. Pre-treatment with 1 mM EDTA (pH 9.0) in a microwave oven followed (Kholova, Dragneva et al. 2011).

Hotspot-areas in CD31 and podoplanin stained sections were analysed for vessel density and vessel area and were photographed at 200x magnification using Cellsens 1.7 software (Olympus Corp., Tokyo, Japan). Intratumoural and peritumoural lymphatic vessel densities (LVDs) were analysed separately in podoplanin hotspots at 200x magnification with Cellsens 1.7 software.

## 6.2.3 Evaluating the incidence of second primary malignancies (Study III)

Patients (n=910) and controls matched for age, gender and place-of-residence (n=4,542) from general population were included in the study. For each patient, clinical follow-up data was ascertained from the TAUH and OUH medical records. Data included date of diagnosis, surgery type and date, date and dose of RAI treatments and last follow-up date. An index date was assigned for each patient, which was the date of RAI treatment or the date of diagnosis in patients without RAI treatment. Patients and corresponding controls started the follow-up at the same time. The Finnish Cancer Registry provided information on malignancies for the patients and controls from 1960 onwards (Engholm, Ferlay et al. 2010) All invasive malignancies were included in the overall cancer incidence except for indolent skin basal cell carcinomas which were excluded. Independent analysis was performed for benign CNS tumours. Ten patients were excluded due to missing information in the registry database, errors in identification numbers or data release limitations. Control subjects having a thyroid cancer diagnosis before the index date were excluded. A total of 910 patients and 4,542 controls were available for analysis.

The time interval for the development of SPM was calculated from the index date to the date of SPM. If the time to SPM was less than a year, SPM was deemed synchronous and excluded to eliminate surveillance bias. For patients with two SPMs, only the first malignancy was taken into account when calculating the overall incidence of SPMs. The end of follow-up was reached at the date of the first SPM, the emigration date from Finland, the date of death or on 31 December 2011, whatever came first.

The Finnish Cancer Registry uses the International Classification of Diseases for Oncology (ICD-O-3), which reports the anatomical region of the cancer (topography) and the cancer histology (morphology) (Fritz, Percy et al. 2000). The topographical classification of ICD-O-3 and the clinical classification (ICD-10) follow each other closely with a few exceptions. The majority of observed SPMs in

this study were organised using the topography. However, for a few malignancies occurring at multiple anatomical sites, morphology was used for grouping. These cancers included haematologic and lymphatic malignancies (morphology [M]9590-M9989), mesenchymal malignancies (bone and soft tissue cancers, M8800-M9262) and a subgroup of the latter, sarcomas and soft tissue cancers (separate ICD-O-3 guidelines) (Fritz, Percy et al. 2000). Additional analysis was performed with a combination of benign and malignant CNS tumours (M9380-M9571) and benign meningiomas (M9530-M9539).

### 6.2.4 Evaluating the effectiveness of core needle biopsy (Study IV)

Immediately after surgery the thyroid nodule was taken into palpation control and biopsied with a 23 Gauge FNA needle and a 20 Gauge CNB needle (Tru-Core II, Medical Device Technologies Inc., Gainesville, FL, USA). Fifty per cent acetone/alcohol was used to fix the FNA samples, which were then centrifuged and stained (Papanicolaou stain). Two slides were prepared from each sample. Buffered formalin (10%) was used to fix the CNB samples, which were then routinely processed and embedded in paraffin. Paraffin blocks were cut into 4 $\mu$m sections and stained with haematoxylin-eosin. A minimum of three sections per sample was analysed. Analysis of CNB and FNA samples was performed blindly without knowledge of the pre-operative FNA or the final histopathological diagnosis. Two cytological and one or two histological research slides in addition to the routine FNA and histology slides were evaluated per patient. Residual material from FNA samples was not used to create cellblocks in this study, because FNA rarely provides sufficient material to produce good-quality cellblocks (VanderLaan, Marqusee et al. 2011, Mills, Poller et al. 2005). The extent of surgery in this study ranged from total thyroidectomy (39%, n=20) to lobectomy of the thyroid gland (56%, n=29) and surgical biopsy of the thyroid gland (6%, n=3).

## 6.3 Statistical methods

Nominal variables were presented as numbers and percentages or proportions. Chi-square test or Fishers's exact test were used when testing significances of binomial variables. Continuous variables with Gaussian distribution were represented as means with standard deviation and t-test was used for testing

significance. For continuous variables with non-Gaussian distribution median with minimum and maximum or quartiles ($Q_1$ and $Q_3$) was given. Mann-Whitney U-test or Kruskal-Wallis test were used to test differences between non-Gaussian groups. P-values less than 0.05 were considered significant.

Kaplan-Meier method was used in survival analysis and tested for significance using log rank test (Study **I, III**). Poisson regression analysis was used when the incidence of thyroid cancer was tested for changes and results were presented using incidence rate ratios (Study **I**). Subgroups of patients were compared using Kaplan-Meier analysis and significance was calculated using log rank test (Studies **I, III**).

Statistical analyses were conducted using SPSS software versions 14 (Study **I**) and 20 (Studies **II-IV**) (IBM Corporation, New York, NY, USA). SPM incidences per 10,000 person-years for both patients and controls and incidence rate ratios (Study **III**) were calculated and compared with Mantel-Haenszel method using Stata software version 8.2 (StataCorp, College Station, TX, USA). Confidence Interval Analysis program (CIA, University of Southampton, UK) was used to calculate sensitivity, specificity, confidence intervals of CNB and FNA samples, with the final histopathological diagnosis as a reference level (Study **IV**).

## 6.4 Ethical aspects

The Ethics Committee of TAUH reviewed and approved the study protocols (R11105 **II**, R12075 **III,** R10013 **IV**). The medical directors of TAUH Research Centre and the OUH administration department granted the permits to use TAUH and OUH medical records (94509, R07521, R12075) (**I-IV**). The permit to use the Finnish Cancer Registry information was received from the Ministry of Social Affairs and Health (32/08/94) (**I**). The National Supervisory Authority for Welfare and Health granted permission to use tissue samples for research purposes (Valvira 3447/06.01.02.01) (**II**). Permission to use the Finnish Cancer Registry, the Population Registry Centre and the Statistics Finland database information was acquired from the National Institute for Health and Welfare (1421/5.05.00/2012) (**III**).

# 7 RESULTS

## 7.1 Thyroid carcinoma in Pirkanmaa Hospital District in recent decades (Study I)

Among thyroid carcinoma patients diagnosed in the period 1981–2002 in Pirkanmaa Hospital District, the type of malignancy was papillary in 427 (77%) patients, follicular in 72 (13%) patients, anaplastic in 40 (7%) patients and medullary in 14 (3%) patients. The majority of patients with PTC, FTC and ATC were female (75-83%), but in MTC gender distribution was equal (Study **I**, Table 1, page 303). Median age at diagnosis was 50 years for PTC, 62 years for FTC, 75 years for ATC and 49 years for MTC. Disease-specific mortality was 5% in PTC, 26% in FTC, 95% in ATC and 29% in MTC.

In patients diagnosed 1981–1991 (earlier group) and 1992–2002 (later group), the median age was the same, 50 years (Study **I**, Table 2, page 303). Lump in the neck, tenderness in palpation, ingestion difficulty and hoarseness were the most common symptoms in the earlier group (p<0.05). More nodular goitre was observed in the earlier group (p<0.001). As seen in Table 8, the share of PTC rose from 81% in the earlier group to 89% in the later group (p=0.02) and the proportion of T1 tumours (≤20mm) increased from 48% to 69% (p<0.001). Median tumour size was 25 mm in the earlier group and 15 mm in the later group (p<0.001). No significant differences between the groups were observed in extrathyroidal extension of the tumour, multifocality or in nodal or distant metastasis (Study **I**, Table 3, page 304).

The incidence of DTC was lower in the earlier group (4.5/100,000) than in the later group (6.0/100,000) (RR 1.33, confidence interval 1.11 - 1.60). A slight peak in incidence was observed 1986-87 (Study **I**, Figure 1, page 303). The extent of surgery was subtotal thyroidectomy in 52% of cases in the earlier group and total or near total thyroidectomy in 83% in the later group. RAI treatments were administered more often in the later group (66% vs. 75%, p=0.045). No differences were observed recurrences between the groups (16% vs. 19%, p=0.29) or in time to recurrence (median 3.1 vs. 3.7 years, p=0.34).

**Table 8.** Major changes in tumour parameters and treatments 1981–2002 in DTC patients (n=498). Data updated until 14.5.2007. PTC=papillary thyroid carcinoma, FTC=follicular thyroid carcinoma, RAI=radioiodine

| | | Earlier group (1981-1991) n=207 | Later group (1992-2002) n= 291 | p-value |
|---|---|---|---|---|
| Histology | PTC | 168 (81%) | 258 (89%) | 0.02 |
| | FTC | 39 (19%) | 33 (11%) | |
| Tumour size | T1a | 40 (23%) | 75 (37%) | <0.001 |
| | T1b | 42 (25%) | 64 (32%) | |
| | T2 | 60 (35%) | 48 (24%) | |
| | T3 | 29 (17%) | 14 (7%) | |
| Median tumour size | mm $(Q_1, Q_3)$ | 25 (15, 40) | 15 (10,25) | <0.001 |
| Surgery | Total thyroidectomy | 81 (42%) | 227 (83%) | <0.001 |
| | Subtotal thyroidectomy | 104 (52%) | 17 (6%) | |
| | Other | 17 (6%) | 39 (11%) | |
| RAI treated | Patients | 137 (66%) | 217 (75%) | 0.045 |
| Cumulative RAI dose | Median mCi, $(Q_1, Q_3)$ | 100 (100, 100) | 100 (100, 193) | 0.040 |
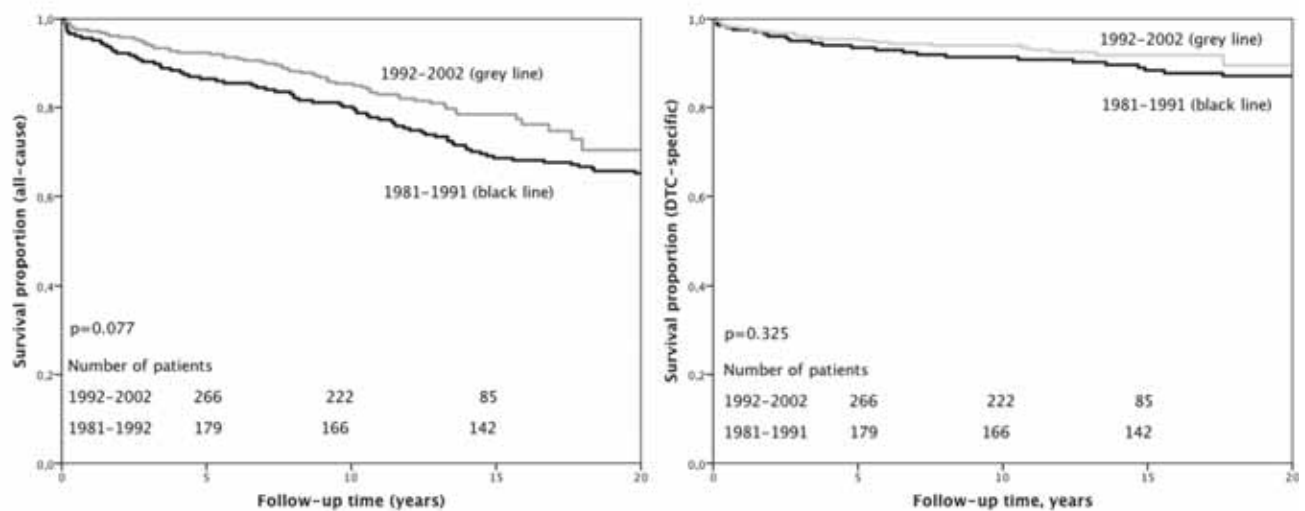


**Figure 3.** Figure on the left shows all-cause survival proportion of the patients treated 1981-1991 (black line) and 1992-2002 (grey line) (p=0.077). Figure on the right shows disease-specific survival between the groups (p=0.325). Survival data is updated until 31.12.2011.

No significant differences were observed in 10-year survival of the DTC patients (Study **I**, Figure 2, page 305). At ten years, all-cause survival was 80% in the earlier group and 86% in the later group (p=0.27) and disease- specific survival

was 92% vs. 94% between the groups (p=0.43). After the release of Study **I**, the survival data was updated until 31 December 2011 and updated survival graphs are presented in Figure 3. All-cause and disease-specific survivals were equal between the groups (p=0.077 and p=0.325 between the groups respectively).

Of 498 DTC patients, 21 (4%) had a persistent disease without clinical remission. Of the rest of the patients, 88 (18%) patients had DTC recurrence. In 71 (14%) patients recurrence was locoregional and in 17 (3%) patients distant metastases were discovered. Recurrences were observed with elevated thyroglobulin in 69 patients (69/88, 78%), with positive scintigraphy in 26 patients (30%) and with US or other radiological examination in 20 patients (23%). Twentytwo (25%) patients had recurrence findings in clinical examination and 10 patients (11%) had symptoms of recurring disease. Althogether 29 recurrences (33%) were treated with surgery.

## 7.2  Angiogenesis and lymphangiogenesis (Study II)

Study **II** consisted of samples from 75 patients in three groups; recurrent DTCs (n=27), non-recurrent DTCs (n=24) and thyroid adenomas (n=24). A total of 51 patients (68%) were female (Study **II**, Table 1, page 827). Age at diagnosis was the same, 52 years in the recurrent and non-recurrent groups and 51 years in the adenoma-group. Most tumours were PTCs, 20 tumours in the recurrent and nonrecurrent groups (74% and 83% respectively). The mean respective tumour sizes were 37.4 mm, 24.2 mm and 31.3 mm in the recurrent, non-recurrent and thyroid adenoma groups. LN metastases were found in 14 (52%) of the recurrent group, while none were observed in the non-recurrent group (p=0.004). Extra-thyroidal extension was found in nine (33%) tumours in the recurrent group and in one (4%) tumour in the non-recurrent group (p=0.004). Multifocality was detected in four (15%) recurrent tumours and in three (13%) non-recurrent tumours. RAI ablation frequency was 100% for the recurrent group and 79% for the non-recurrent group. Time from primary surgery to the surgical treatment for recurrence was 48 months (±37 months). Recurrences were located either in the cervical soft tissue (n=18) or in cervical LNs (n=14).

Primary tumour microvascular density (MVD) varied between the groups, and was 327 vessels (v) per mm2 for recurrent DTC, 362 v/mm2 for non-recurrent DTC and 484 v/mm2 for thyroid adenomas (p=0.017). (Study **II**, Table 2, page 828). Primary tumour median vessel area was 139 μm2/v for recurrent DTC, 173

μm²/v for non-recurrent DTC and 260 μm²/v for thyroid adenomas (p<0.001). Microvascular vessel area was 4.72% for recurrent DTC, 5.45% for non-recurrent DTC and 13.8% for thyroid adenomas (p<0.001). Figure 4 illustrates MVD difference in recurrent PTC and thyroid adenoma.
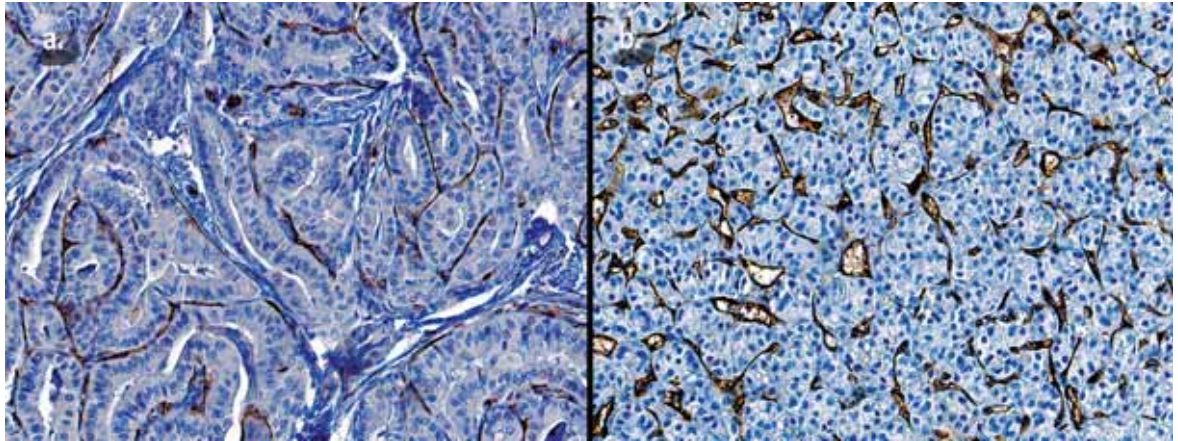


**Figure 4.** **a.** Recurrent PTC with low vascular density on the left picture. **b.** Thyroid adenoma with high vascular density on the right picture. Vascular marker CD31 immunohistochemistry, magnification ad 200x.
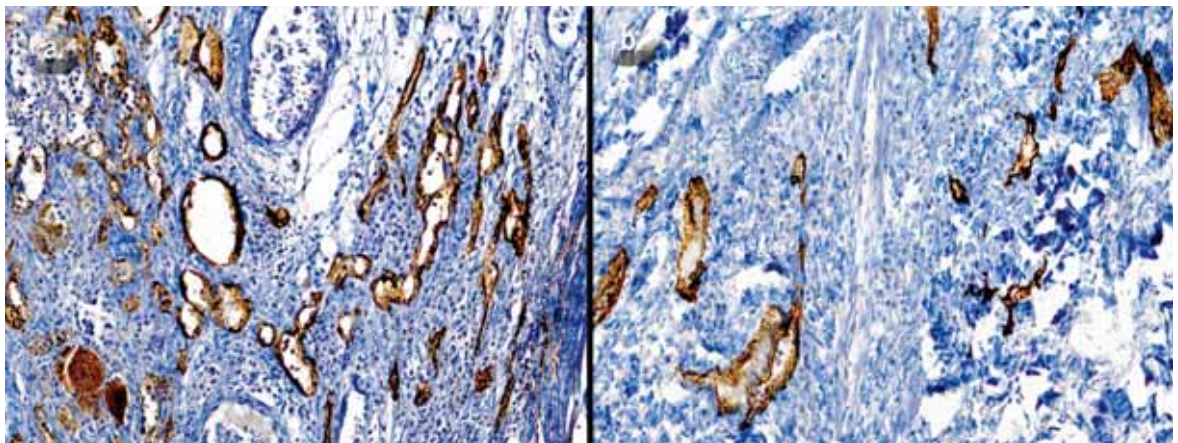


**Figure 5.** **a.** Recurrent PTC with high peritumoural lymphatic vasculature density on the left picture. **b.** Non-recurrent PTC with lower peritumoural lymphatic vasculatur density on the right picture. Lymphatic marker podoplanin (D2-40) immunohistochemistry, magnification ad 200x.

Lymphatic vessel density (LVD) in the peritumoural area of the primary tumour varied between the groups. In recurrent DTC, LVD was 101 v/mm$^2$, in non-recurrent DTC 56.1 v/mm$^2$ and in thyroid adenomas 53.9 v/mm$^2$ (p=0.015). In the histopathological subgroups, LVD was 102 v/mm$^2$ in recurrent PTC, and in non-recurrent PTC significantly lower 56.0 v/mm$^2$ (p=0.044). Peritumoural LVD was also lower in non-recurrent FTC 51.1 v/ mm$^2$, when compared to the LVD of recurrent PTC (102 v/mm$^2$, p=0.045).

Median peritumoural lymphatic vessel area was 340 μm$^2$/v in recurrent DTC, which was significantly smaller than in non-recurrent DTC (647 μm$^2$/v) and in thyroid adenomas (594 μm$^2$/v, p=0.012). In the histopathological subgroups the median vessel area was 328 μm$^2$/v in recurrent PTC, whereas in non-recurrent PTC (705 μm$^2$/v, p=0.001) and in recurrent FTC (827 μm$^2$/v, p=0.031) median vessel areas were significantly larger. Figure 5 illustrates the difference in the peritumoural LVD of recurrent and non-recurrent PTC.

No significant differences were observed in intratumoural LVD between the groups; in recurrent DTC LVD was 30.3 v/mm$^2$, in non-recurrent DTC 21.7 v/mm$^2$ and in thyroid adenomas 16.8 v/mm$^2$ (p=0.777).

Comparison of primary tumours and metastatic lesions revealed no significant differences in the parameters measured (Study **II**, Table 3, page 830). The only significant difference was seen in the median area of the peritumoural lymphatic vessels, which were 328 μm$^2$/v in PTC primary tumours and 1071 μm$^2$/v in PTC metastasis (p=0.008). The peritumoural LVD of FTC primary tumour was 827 v/mm$^2$, significantly higher than the LVD of FTC metastasis (206 v/mm$^2$, p=0.021).

## 7.3    Second primary cancers (Study III)

### 7.3.1    General statistics, follow-up times and antecedent malignancies

Study **III** included 910 DTC patients and 4,542 controls. Eightytwo per cent of the patients and controls were female (Study **III**, Table 1, page 21). Mean age was the same, 49.0 years in patients and 48.9 years in controls. No significant differences were observed in mean follow-up times between patients and controls  (16.2 years for patients and 16.5 years for controls).  Figure 6 illustrates the follow-up times, mortality and number of subjects who emigrated. For most of the patients and

controls the follow-up continued until the study end date (69% and 71%, respectively).

Antecedent malignancies, *i.e.* cancers that occurred before the index date, were observed in 43 patients (4.7%) and 115 controls (2.5%). Therefore, DTC patients had 87% more antecedent malignancies than the controls (p<0.001). Synchronous malignancies (observed during the first 12 months after the diagnosis of DTC) were found in six patients (0.7%) and 17 controls (0.4%).

Total or near-total thyroidectomy was performed on 710 patients (78%), subtotal thyroidectomy on 142 patients (16%) and lobectomy on 38 patients (4%). Twenty patients underwent a biopsy or a small unilateral resection (2%).

Tumour histology was determined to be PTC in 715 cases (79%), follicular variant of PTC in 96 cases (10%) and follicular carcinoma in 99 cases (11%). Histologically confirmed LN metastasis in the cervical region was found in 150 patients (16%). Distant metastatic disease was observed in 61 patients (6.7%).
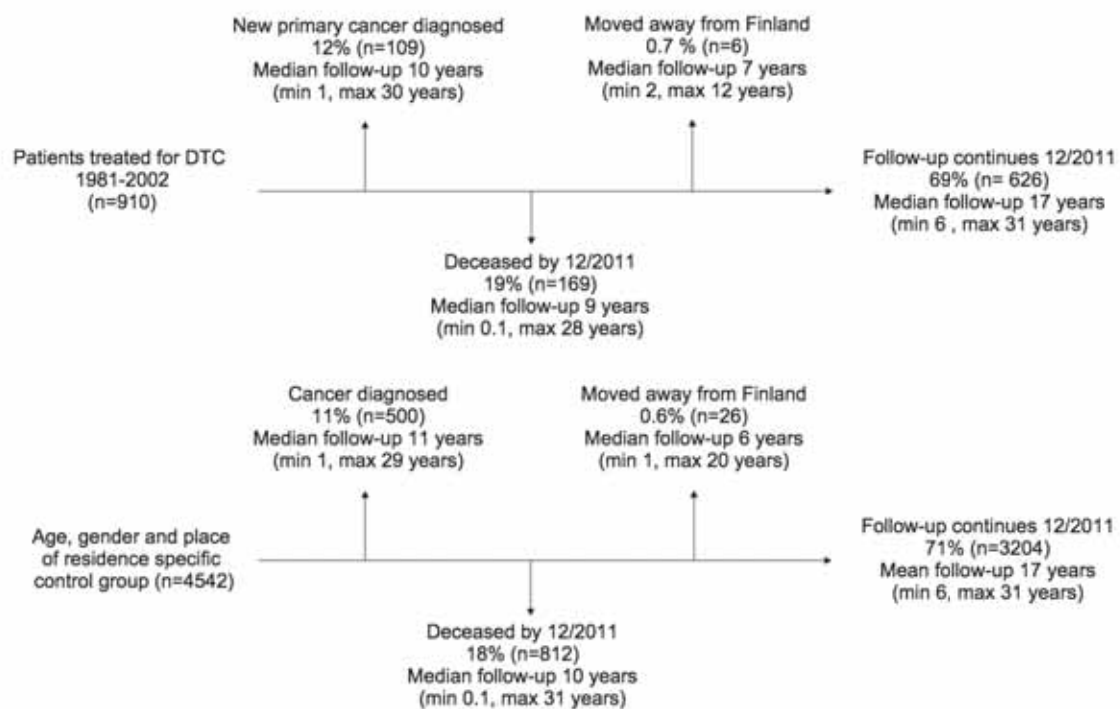


**Figure 6.** Flow chart illustrating the numbers and follow-up times of patients diagnosed with well-differentiated thyroid cancer (DTC) and the control group according to different endpoints.

## 7.3.2 SPMs in subgroups and types of SPM

SPMs were found in 12.0% (n=109) of patients and in 11.0% (n=500) of controls (RR 1.12, p=0.269). Multiple SPMs were detected in three (0.3%) patients and in 35 (0.8%) controls (p=0.144).

Patients younger than 40 years had increased incidence of SPMs when compared to the controls (RR 1.73, p=0.037). Patients older than 40 years did not have a significantly higher risk for SPMs (RR 1.12 for age-group 40-60 years and RR 1.01 for age-group >60 years) (Study **III**, Table 2, page 22).

Year of treatment affected the risk of SPM. If a patient's DTC was treated in the period 1981-1989, the risk of SPM was equal between patients and controls (RR 0.95, p=0.773). Patients treated during the period 1990-1995 were also at equal risk of SPM when compared to controls (RR 1.07, p=0.732). DTC patients treated 1996 onwards had a higher risk of SPM than their conrols (RR 1.51, p=0.029).

Patients had more mesenchyme-derived cancers than the controls (RR 2.74, p=0.025), and the risk of sarcomas and soft tissue tumours was especially high (RR 4.37, p=0.004) (Study **III**, Table 3, page 23). The patients also developed more lymphatic and haematological cancers than the controls (RR 1.87, p=0.035); in particular, the risk for non-Hodgkins lymphoma was higher (RR 2.78, p=0.035). One salivary gland cancer was observed in the patients, while none was observed in controls (RR>1, p=0.024). Although the risk of CNS malignancies was not significantly higher in patients than in controls, the combined incidence of benign and malignant CNS tumours was higher in the patients than in the controls (RR 2.56, p=0.017). Benign meningioma was a more common finding in the patients (RR 3.15, p=0.007).

## 7.3.3 SPMs in RAI treated patients

A total of 740 patients (81%) were treated with RAI postoperatively and 211 patients (23%) were treated with RAI multiple times due to persistent or recurring disease. A minority, 170 patients (19%), did not receive RAI treatment, mainly because of very good prognosis of small, intrathyroidal DTC. External radiotherapy was admsnistered to 28 patients (3%).

The median dose of RAI was 3.7 GBq (100 mCi), and the mean was 5.3 GBq (±3.7GBq). The mean cumulative dose of RAI rose progressively; for patients treated 1981-1989, the dose was 4.4 GBq (±3.1GBq); for those treated 1990-1995, the dose was 5.6 GBq (±4.1GBq); and for those treated after 1996, the dose was

5.8 GBq (±3.5GBq) (p<0.001). No significant difference in the risk of SPMs was observed in the patients who received a cumulative RAI dose of either ≤3.7 GBq (RR 0.94, p=0.650) or >3.7 GBq (RR 1.37, p=0.143) and the corresponding controls. A tendency towards higher risk of SPMs in patients compared to controls was noted with cumulative doses of RAI ≥7.4GBq (≥200mCi, p=0.094).

The overall cancer incidence was equal between the RAI-treated patients and controls (RR 1.04, p=0.721) (Study **III**, Table 4, page 24). The incidence of sarcomas and soft tissue tumours was higher in RAI-treated patients than in controls (RR 6.37, p=0.002). The incidence of hepato-biliary-pancreatic cancers (RR 0.00, p=0.024) and respiratory organ cancers (RR 0.26, p=0.041) was lower in the RAI-treated patients than in the controls. In RAI non-treated patients, the incidence of hepato-biliary-pancreatic cancers was the same (RR 2.54, p=0.114) and the incidence of respiratory organ cancers had a tendency to be slightly higher (RR 5.09, p=0.072) than in corresponding controls.

In patients treated in the TAUH catchment area, the incidence of SPMs was similar in patients and controls (RR 1.24, p=0.133). RAI-treated patients in the TAUH region did not have excess SPMs when compared to controls (RR 1.11, p=0.565). In patients without RAI-treatment in the TAUH catchment area, a slight tendency towards higher incidence of SPMs was observed in patients, when compared to corresponding controls (RR 1.55, p=0.064).

## 7.4 Core needle biopsy (Study IV)

The study material consisted of 52 patients, of whom 40 (77%) were female, and the mean age was 53 years (Study **IV**, Table 1, page 1047). The mean size of tumour in US examination was 25 mm, and most of the tumours (48%) were found in the right lobe. The mean tumour size in the pathology reports was 21 mm (± 16 mm), with a range of 5–75 mm.

The final pathological diagnosis was PTC in 18 (35%) cases; FTC was diagnosed in three (6%) cases, poorly differentiated carcinoma in one (2%) case, ATC in one (2%) case and squamous cell carcinoma in one (2%) case (Study **IV**, Figure 1, page 1048). Benign lesions were classified as follicular adenoma in 16 (32%) cases, adenomatous goitre in nine (17%) cases and thyroiditis in three (6%) cases.

The Bethesda system for cythopathology of the thyroid gland (Cibas, Ali et al. 2009) was used to classify FNA samples. CNB samples were grouped into

categories; non-diagnostic, benign, follicular neoplasm and malignant (Study **IV**, Table 2, page 1049). Of the FNA samples, 10% were non-diagnostic, 15% benign, 17% AUS/FLUS, 35% follicular neoplasms, 13% suspected of malignancy and 10% malignant. Of the CNB samples, 4% were non-diagnostic, 10% were benign, 58% were follicular neoplasms, and 29% were malignant. When compared to the final pathological diagnosis, 14/15 CNB samples (93%) classified as malignant were confirmed to be carcinomas (true positive). One case of thyroiditis with nuclear atypia had originally been classified as malignant (false positive). Of CNBs classified as follicular neoplasms, 8/22 (27%) were malignant. CNBs classified as non-diagnostic or benign had one malignant case each.

Follicular type tumours, *i.e.* FTC, follicular adenomas and adenomatous goitre, were indistinguishable in CNB and FNA, as vascular and capsular invasion could not be reliably observed. To study non-follicular tumours further, we excluded the tumours classified as follicular adenomas, FTCs or adenomatous goitre in the final pathology and determined the results of non-follicular tumours independently (Study **IV**, Table 3, page 1049). For CNB, 14 samples of 21 (67%) were correctly identified as malignant (true positives), whereas for FNA, only five samples out of 21 (24%) were properly specified as malignant. Of FNAs, seven samples were suspected of malignancy, and these samples were all identified as malignant in the final pathology. FNAs classified as follicular neoplasms (two cases) were determined to be either malignant (one case of PTC) or benign (one case of thyroiditis). FNAs classified as AUS/FLUS, all proved to be malignant in the final pathology.

When the sensitivity and specificity of CNBs and FNAs were calculated, only certainly malignant samples were considered positive, and the final pathology was regarded as the variable for comparison and the gold standard (Study **IV**, Table 4, page 1050). Malignancy-specific sensitivity of CNB was 61%, and of FNA 22%. The specificity was 96% for CNB and 100% for FNA. For non-follicular tumours, the malignancy-specific sensitivity was 70% for CNB and 25% for FNA. The specificity was 85% for CNB and 100% for FNA and the accuracy 81% and 65%, respectively.

# 8    DISCUSSION

## 8.1    Increasing incidence and outcome of thyroid cancer

Diagnostic methods in thyroid cancer have changed significantly in recent decades. US became commonly available during the 1990's and made it possible to diagnose non-palpable thyroid nodules (Brander, Viikinkoski et al. 1992). This resulted in a vast increase in the detection of smaller thyroid nodules, which were previously undetectable.

The incidence of DTC in Pirkanmaa Hospital District rose from 4.5 to 6.0 per 100,000 during the time periods 1981-1991 and 1992-2002. A slight peak in incidence was observed in 1986-87 and this may be related to the increased awareness of thyroid cancer risks in general population after the Chernobyl nuclear disaster of April 1986. The fallout after the nuclear plant accident was relatively high in TAUH catchment area (Arvela H., Markkanen M., Lemmelä H. 1990). The time to development of clinically detectable thyroid cancer after radiation exposure may be over 10 years in adults (Williams 2008).

The proportion of PTCs was higher in the period 1992-2002 than 1981-1991. Increased incidence of PTC has been observed worldwide (Colonna, Guizard et al. 2007, Alevizaki, Papageorgiou et al. 2009, Reynolds, Weir et al. 2005, Davies, Welch 2006, Kent, Hall et al. 2007). In an Italian study, Elisei et al. observed an increase in the proportion of PTC from 80.5% to 91.0% (Elisei, Molinaro et al. 2010). The incidence of FTC has remained relatively stable during in recent decades. The reasons for the increase in the incidence of PTC are not completely understood, but improved detection of subclinical small PTCs is the most plausible explanation (Davies, Welch 2006). However, a true increase in the incidence of PTC is a conceivable interpretation, because the incidence of large tumours is also increasing. Environmental carcinogens of industrialised lifestyle, increasing prevalence of chronic autoimmune thyroiditis and obesity may have affected the incidence of DTC (Pellegriti, Frasca et al. 2013).

Median tumour size was significantly smaller in the later time period, as tumour size was 25 mm in 1981-1991 and 15 mm in 1992-2002. A comparable change has been observed in other studies. Although the incidence of all DTCs has increased,

the incidence of small-sized PTCs has risen most markedly. Elisei et al. reported an increase in the proportion of T1 tumours (≤20 mm) from 43.3% to 54.5%. The change was even more marked in thyroid microcarcinomas (T1a, ≤10 mm), as their proportion increased from 7.9% to 28.7%. Our observations were similar; the proportion of microcarcinomas increased significantly, from 23% to 37%, and the percentage of T1 tumours rose from 48% to 69% (p<0.001). Wider availability of US is a probable explanation for the increasing proportion of T1 tumours during the period 1992-2002. In other recent studies, the increase in the incidence of small-sized PTCs is the most prominent feature, but the incidence of larger ≥30 mm thyroid cancers is also rising (Chen, Jemal et al. 2009). On the other hand, in the United States, the incidence of thyroid cancer is rising similarly in all racial and ethnic groups, although the amount and quality of healthcare varies significantly by racial and ethnic group (Aschebrook-Kilfoy, Ward et al. 2011). Furthermore, in the United States between 1980 and 2005, the rate of increase in large ≥50mm thyroid cancers almost equaled that for the smallest cancers in white females (Enewold, Zhu et al. 2009). Medical surveillance and more sensitive diagnostic procedures cannot entirely explain the increase in thyroid cancer incidence. Aetiological investigations focusing on exogenous and endogenous exposures experienced similarly in the whole population, and more strongly in women, are recommended (Aschebrook-Kilfoy, Ward et al. 2011).

RAI-treatments were more numerous and the cumulative median dose rose during the period 1992-2002. Thyroglobulin assays have become more sensitive, which may have an effect on the interpretation of cancer recurrences, and thus the sensitive thyroglobulin assay may lead to an increased number of RAI treatments. Most recurrences (78%) in this study were diagnosed with elevated thyroglobulin levels, and some recurrences were diagnosed with RAI scintigraphy or imaging studies.

The prognosis for DTC patients is favourable in the TAUH catchment area; the results are comparable to those of other recent studies (Lundgren, Hall et al. 2003, Voutilainen, Siironen et al. 2003). All-cause or disease-specific survival did not show significant differences between the time-groups. A minor difference in all-cause survival was observed in short-term follow-up, which may be attributable to other factors, e.g. changes in cardiovascular mortality. No statistically significant change in disease-specific survival was observed. The question arises, as to whether more radical surgery and the increase in RAI treatments observed during the period 1992-2002 are justified. Radical surgery, *i.e.* total thyroidectomy, may not be

beneficial to all DTC patients, especially the subgroup of patients with low-risk disease.

## 8.2     Vascularity of well-differentiated thyroid cancers

In Study **II**, the MVD of recurrent and non-recurrent DTCs was significantly higher than that of thyroid adenomas. The difference between the MVD of recurrent and non-recurrent DTC was not statistically significant, although the lowest MVD was observed in recurrent PTC. The tumours most likely to recur after initial surgery were those with the lowest MVD. This finding suggests that low MVD in a thyroid tumour may be a sign of a more aggressive and recurrence-prone tumour and conversely; high MVD may be a marker of a less aggressive tumour. This finding applies primarily to PTCs, as the MVD of FTCs was closer to that of thyroid adenomas.

Earlier research has reported that high expression of pro-angiogenic mediators (*e.g.* VEGF-A) in thyroid carcinomas (Bunone, Vigneri et al. 1999, de la Torre, Buley et al. 2006, Kilicarslan, Ogus et al. 2003). Despite high VEGF expression of thyroid carcinomas, low MVD has been associated with aggressive histopathological subtypes of PTC, *e.g.* tall cell variant, and intermediate to high-risk AMES risk groups (Stabenow, Tavares et al. 2005).  On the other hand, some recent studies have found no difference in the MVD of PTC and other thyroid tumours (Jebreel, England et al. 2007, Lee, Lee et al. 2012). Gulubova et al. observed that the MVD at the PTC tumour border was higher than at the FTC tumour border (Gulubova, Ivanova et al. 2014).  In our study the intratumoural MVD was lower in PTCs than in thyroid adenomas. Despite high expression of angiogenic factors, DTCs seem to develop a diminished quantity of abnormal intratumoural vasculature, which is associated with small vessels of limited density. This results in poor vascular function and structure. It has been suggested that inhibiting VEGF overexpression may serve to normalize the vascularity of malignant tumours (Carmeliet, Jain 2011). TKI selumetinib has been shown to enhance RAI uptake in advanced thyroid cancer (Ho, Grewal et al. 2013).

TKIs were introduced in the management of DTC, as more was learned about the molecular pathways involved in DTC. In progressive inoperable RAI-refractory DTC, these new drugs may be used after careful consideration of the side effects. Adverse effects of TKIs include hypertension, gastrointestinal symptoms, cardiomyotoxicity, hepatoxicity, renal insufficiency, bone marrow suppression and

dermatological problems. TKIs may cause hypothyroidism and require a higher dose of LT4 in thyroid cancer patients. TKIs improve partial response rates, progression-free survival and stable disease, but the effect on overall survival has not been documented (Haugen, Sherman 2013). Sorafenib targets multiple kinases, and has been studied in RAI-refractory DTC. In the DECISION trial, sorafenib improved progression-free survival to 10.8 months, as opposed to 5.8 months in the placebo-group (Brose, Nutting et al. 2014). Sorafenib is currently approved in the European Union and in the United States to treat DTC, renal cell carcinoma and hepatocellular carcinoma. Lenvatinib is multi-TKI, and in a Phase III trial progression-free survival was 18.3 months in progressing RAI-refractory DTCs and 3.6 months in the placebo-group (Schlumberger, Tahara et al. 2014). Other TKIs in Phase II trials for RAI-refractory DTC include sunitinib, motesanib, axinitib, pazopanib, selumetinib and evorolimus. TKIs are promising targeted therapies in the treatment of RAI-refractory DTC. Current research efforts are focusing on combination treatment strategies and identifying the patients that benefit most from these new treatments with least possible toxities.

## 8.3 Lymphatic vasculaturity of well-differentiated thyroid cancers

The highest peritumoural LVD was observed in recurrent PTC. Lymph vessels were denser in recurrent PTC than in non-recurrent PTC, FTC or thyroid adenomas. The median size of lymph vessels was smallest in recurrent PTC, but the total area of lymph vessels did not differ statistically between the groups. LVD in probably an important factor in the migration of tumour cells to the lymphatic system and LN metastasis of the tumour. In malignant tumours, high expression of lymphatic proliferative factors (VEGF-C, VEGF-D) is probably linked to high LVD and hence to high frequency of LN metastases. In breast cancer, high LVD is associated with high frequency of LN metastasis and poor prognosis (Bono, Wasenius et al. 2004). In head and neck squamous cell carcinoma, lymphatic marker podoplanin is linked to nodal metastasis (Cueni, Hegyi et al. 2010). In thyroid cancer, high expression of VEGF-C and VEGF-D has been associated with increased lymphangiogenesis and metastatic spread via lymphatics (Lee, Lee et al. 2012, Yasuoka, Nakamura et al. 2005), although also contrary evidence has also been reported (Chung, Kim et al. 2012). High intratumoural LVD has been linked to nodal metastasis in thyroid cancer and lymphatic marker D2-40 may be

associated with poor prognosis in thyroid cancer (Wang, Li et al. 2007, Eloy, Santos et al. 2011).

Intratumoural lymph vessels were larger and the area of intratumoural lymph vessels was greater in recurrent PTCs than in recurrent FTCs. Metastasis via the lymphatic system is more common in PTC than in FTC and this finding is consistent with clinical findings. Distant metastasis via the vascular system is more common in FTC than in PTC.

## 8.4　Risk of second primary malignancies in DTC patients

Patient's age at the time of diagnosis affects the risk of SPM. In study **III**, the RR for SPM was 1.73 in patients <40 years; by comparison, the RR in older patients was 1.01-1.12. Comparable findings have been reported previously; Ronckers et al. found that in DTC patients <40 years, SPM risk was 39% higher than in general population. However, in older patients, only a 6% higher risk was observed (Ronckers, McCarron et al. 2005). The reasons for the higher incidence of SPMs in young patients are not fully understood. One explanation may be that genetic, environmental or treatment-related carcinogenic susceptibility is most sensitively observed in younger age groups when the incidence of sporadic malignancies is low. In older age groups a high frequency of sporadic malignancies may impair the detection of genetic, environmental or treatment-related malignancies, expecially if the additional risk of malignancy is relatively low and the sample size is not adequate. The risk of RAI-related SPMs may be highest in young DTC patients, who more frequently have cervical LN and distant metastases, requiring multiple RAI treatments (Jarzab, Handkiewicz-Junak et al. 2005). Furthermore, young patients are more sensitive than older patients to radiation-induced carcinogenesis (Cardis, Kesminiene et al. 2005). On the other hand, in this study the cumulative RAI dose was not higher in the young age group than in the older age groups.

The year of treatment or diagnosis affected the incidence of SPMs. The risk of SPMs was elevated in patients diagnosed from 1996 onwards than in their controls (RR 1.51). However, patients treated prior to 1996 did not have significantly increased risk of SPMs. Kim et al. also show a similar observation in their study (Kim, Bi et al. 2013); the risk of SPMs in DTC patients was lowest in patients diagnosed prior to 1993 (RR 1.03), whereas patients diagnosed later had increased risk of SPMs (RR 1.21-1.45). Possible explanations for increased risk of SPMs in DTC patients in recent decades may be associated with changes in DTC incidence

and tumour characteristics, lifestyle-related factors or changes in the treatment of DTC. The recent increase in the incidence of DTC has been accompanied by an increase in small-size tumours and an increase in the share of PTC. Therefore, the DTCs diagnosed at later time periods have been were on average less aggressive. However, disease-specific mortality has been stable, as observed in Study **I**, and therefore the increase in SPM incidence in recent decades may not be explained by bias caused by increased surveillance time. Changes in lifestyle and nutritional factors in recent decades may also affect the observed increase in incidence of SPMs. Treatment-related factors may also impact the incidence of SPMs. In this study, the mean cumulative dose of RAI was 38% higher in the 1990's than in the 1980's, and the increasing exposure to RAI may be implicated in the incidence of SPMs. Beyond the scope of this study are other treatment-related factors, *e.g.* possible changes in the level and duration of TSH suppression therapy and the resulting therapeutic subclinical hyperthyreosis. Hyperthyreosis has been associated in earlier studies with a 25-34% increased risk of malignancies (Metso, Auvinen et al. 2007, Hellevik, Asvold et al. 2009).

In our study, the overall risk of SPM after diagnosis and treatment of DTC did not differ significantly when compared to that of controls matched for age, gender and place of residence-matched. The excess risk for SPMs in DTC patients had been shown to be fairly low (9-33%), and therefore, a large number of patients were needed to achieve statistical significance. Type 1 error is possible in our study despite the long follow-up time and relatively large number of patients. In that case, the 12% increase in the incidence of SPMs would be in agreement with earlier reports.

In some specific tumour sites and types an elevated risk of SPMs was discovered. In a European study, RAI exposure was associated with leukaemia, colorectal cancer, bone and soft tissue cancer and salivary gland cancer (Rubino, De Vathaire et al. 2003). In the same study, the incidence of breast or kidney cancer was not associated with RAI exposure. Although the salivary glands concentrate and excrete RAI, exposure did not affect the incidence of salivary gland cancer in a large SEER database study (Brown, Chen et al. 2008). In this study, the DTC patients had more sarcomas and soft tissue cancers than did the controls (RR 4.37). Other studies have made similar obervations; Sandeep et al. (Sandeep, Strachan et al. 2006) reported high risk of soft tissue sarcomas (RR 3.63) and bone malignancies (RR 3.62), whereas Rubino et al. (Rubino, De Vathaire et al. 2003) reported an RR of 4.0 for bone and soft tissue cancers. In this study the mean cumulative dose of RAI was 178±105 mCi for patients with sarcomas as an

SPM, which was not statistically higher than the general mean RAI dose of all RAI treated (144±99 mCi, p=0.165).

An increased incidence of lymphatic and haematological malignancies was observed; most notably the risk of non-Hodgkins lymphoma was elevated in patients when compared to controls (RR 2.78). Sandeep, Brown and Lu (Sandeep, Strachan et al. 2006, Brown, Chen et al. 2008, Lu, Lee et al. 2013) each also reported an increased risk of non-Hodgkins lymphoma (SIR 1.68, 1.75 and 2.66, respectively).

RAI-treated patients had a significantly increased risk of sarcomas and soft tissue cancers. This finding is concurs with earlierstudies (Rubino, De Vathaire et al. 2003, Sandeep, Strachan et al. 2006). Sarcoma is a known radiation-induced malignancy. The risk of secondary sarcoma is elevated in childhood cancer survivors treated with radiation (Henderson, Rajaraman et al. 2012).

The risk for hepato-biliary-pancreatic cancers and lung and respiratory organ cancers was lower in RAI-treated patients. These cancers are associated with the consumption of alcohol and tobacco, and the decrease in incidence may be due to lifestyle changes among the patients (Blanchard, Denniston et al. 2003). On the other hand, the small number of patients and observed SPMs may result in incorrect observation. Decreased risk of smoking-related cancers, such as bladder, lung and nonthyroidal head and neck cancer, has been observed in a study on DTC survivors (Brown, Chen et al. 2008).

In recent studies, lower RAI ablation doses (1.11 GBq, 30 mCi) have proven to be as effective as traditional higher doses (3.7 GBq, 100 mCi) (Valachis, Nearchou 2013, Mäenpää, Heikkonen et al. 2008). Although in this study the overall risk of SPMs in RAI-treated patients was not significantly higher than in the controls, in other studies RAI treatment has been associated with 12-25% higher risk of SPMs (de Gonzalez, Curtis et al. 2011, Kim, Bi et al. 2013, Brown, Chen et al. 2008, Sawka, Thabane et al. 2009, Iyer, Morris et al. 2011). Furthermore, patients treated with 3.7 GBq experience more acute and subacute side effects than patients treated with 1.1 GBq dose. In this light, the aim to use lower RAI doses in ablation of the thyroid remnant is well justified, as comparable RAI ablation results can be achieved with a lower dose. RAI clearance is faster with rhTSH than traditional LT4 withdrawal method, which may further mitigate the adverse effects of RAI treatment (Ma, Xie et al. 2010).

## 8.5    Core needle biopsy as an adjuvant method in diagnostics

The study **IV** consisted of 52 CNB and FNA samples of malignant (n=3, 6%) or malignancy-suspicious (n=49, 94%) thyroid nodules. Patients were consecutive and unselected. The final pathology revealed that the majority of tumours (n=28, 54%) were benign. PTC was the most common malignancy (n=18, 35%), followed by FTC (n=3, 6%), ATC (n=1, 2%), poorly differentiated carcinoma (n=1, 2%) and squamous cell carcinoma (n=1, 2%).

Follicular lesions include FTCs, follicular adenomas and adenomaus goitre, and constitute a large proportion of thyroid tumours. In cytological (FNA) samples, benign follicular lesions are indistinguishable from malignant follicular lesions.  In this study, CNB samples did not improve the diagnosis of follicular-type lesions, as FTCs could not be identified among other follicular-type lesions. CNB samples rarely included a capsular area for the evaluation of capsular invasion or enough material for the evaluation of vascular invasion. Similar conclusions have been reached in earlier studies. (Screaton, Berman et al. 2003, Renshaw, Pinnar 2007, Bandyopadhyay, Pansare et al. 2007, Zhang, Ivanovic et al. 2008, Karstrup, Balslev et al. 2001, Na, Kim et al. 2012, Carpi, Nicolini et al. 2000). In a recent meta-analysis, no significant difference was observed in the sensitivity and specificity of FNA and CNB (Li, Chen et al. 2014).

In the diagnosis of non-follicular tumours, the malignancy-specific sensitivity of CNB was better than that of FNA. In high-anaesthesia-risk patients, a benign CNB could be considered a proponent for conservative treatment in spite of a suspicious FNA result. However, it should be noted that a benign CNB did not exclude malignancy in this study, considering that the negative predictive value of CNB was only 76%. CNB did provide more sensitive results than FNA, expecially in non-follicular tumours, and CNB should be considered as a adjuvent method in the diagnosis of thyroid nodules. CNB may provide valuable additional information, especially in ATC, thyroid lymphomas and inflammatory or fibrotic thyroid lesions. Furthermore, recurring insufficient FNAs may benefit from CNB. Immunohistochemistry or genetic tumour profiling may provide additional information on the CNB or FNA biopsy sample. (Ferraz, Eszlinger et al. 2011, Nikiforov, Ohori et al. 2011, Li, Robinson et al. 2011, Kholova, Ludvikova et al. 2003). CNB provides a histological sample of the tumour, which in turn renders tumour immunohistochemistry easy and cost-effective. Immunoshistochemistry may provide additional information on tumour characteristics and may improve preoperative diagnosis.

## 8.6  Strengths and limitations

In Study **I** the patients were consecutive and unselected and the reliability of the material was good. The study time-period and follow-up times of the study were long. Cancer recurrences and information on mortality were available in both clinical and cancer registry databases. The limitations were similar to those of other retrospective clinical studies; information was gathered from medical records and thus uniform and specific information on certain variables, *e.g.* disease symptoms and clinical examination findings might be incomplete for a number of patients. Age-adjusted incidence rate ratios were not calculated in this study.

In Study **II**, all the samples of DTC recurrences of Study **I** were included. The patients with recurrent DTC and the patients with non-recurrent DTC were matched precisely for age, gender and tumour type. The limited number of patients available affected the matching of tumour sizes between the groups. Calculation of vascular and lymphatic vessels was done manually with computer-aided methods, and was rather time-consuming. Fully computerized analysis was not possible due to heterogeneity of samples and unavailability of the precise software as not only immunopositioning, but also morphology is essential to recognize the vessels.

In Study **III** the quality of the register data and the matching of the patients and controls were good. The strengths include the long follow-up time and good coverage of clinical and registry data throughout the study period. Less than one per cent of patients were lost from follow-up due to emigration. The follow-up period was long, with a median of over 16 years, and the clinical follow-up was carried out at the same two university hospitals. Limitations included the relatively small number of patients and observed SPMs included in this study and, therefore, the lack of statistical power to demonstrate smaller differences between the groups. The use of morphology coding in some cancer types may differ from those of some earlier studies relying solely on topographical classification.

The material of Study **IV** consisted of consecutive patients with malignancy-suspicous thyroid nodules. FNA and CNB samples were acquired under palpation control from the thyroid gland after surgery and the study did not cause any complication risks to the patients. In vivo, the most common complication in FNA and CNB is haematoma formation, the risk of which is slightly higher in CNB. No degradation or autolysis of the biopsy materials occurred when samples were taken without delay after specimen removal

# 9 SUMMARY AND CONCLUSIONS

The major findings of this study were:

- The incidence of well-differentiated thyroid cancer has increased in Pirkanmaa Hospital District, with a notable increase in the share of small-sized papillary thyroid cancers. Cancer-related mortality has been stable in recent decades. Although the share of low-risk cancers has increased and the median tumour size has dimished, the cumulative dose of radioiodine treatments has increased. Indications for radioiodine treatment should be considered in light of these observations.

- Recurrent papillary thyroid cancers expressed more peritumoural lymphatic vasculature than non-recurrent papillary cancers. Less vascularity was observed in recurrent thyroid cancers. High lymphatic vasculature may be considered a prognostic marker in the treatment plan of the PTC patient.

- The overall incidence of second primary malignancies was not significantly higher in patients with well-differentiated thyroid cancer compared to that of controls matched for age, gender and place of residence. The incidence of second primary malignancies was higher in young patients less than 40 years old and patients diagnosed or treated after 1995. The incidence of sarcomas and soft tissue cancers and lymphatic and haematological malignancies was higher in patients with well-differentiated thyroid cancer than in their controls. Special attention should be paid to the treatment and follow-up of younger thyroid cancer patients.

- Core needle biopsy provides more accurate malignancy-specific results than fine-needle aspiration biopsy in non-follicular thyroid tumours and this method should be validated in a prospective diagnostic setting.

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Tommi Hakala

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# 12  ORIGINAL COMMUNICATIONS

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Rising incidence of small size papillary thyroid cancers with no change in disease-specific survival in Finnish thyroid cancer patients

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Abstract

Background: The aim of this study was to investigate trends in the incidence, diagnostics, treatment and survival of thyroid cancer in Tampere University Hospital (TAUH) region in recent decades.

Material and Methods: New thyroid cancer cases from 1981 to 2002 were ascertained from the Finnish Cancer Registry. Follow-up data was collected from medical records of TAUH. Differentiated thyroid cancer (DTC; consisting of papillary thyroid cancer (PTC) and follicular thyroid cancer (FTC)) patients' data was analyzed and divided into two equal time periods (1981-1991 and 1992-2002).

Results and Conclusions: The total amount of thyroid cancer cases was 553, of which 427 (77%) were papillary and 72 (13%) follicular.  Thyroid cancer was four times more common in females than in males and the median age at the time of diagnosis was 52 years.

The incidence of DTC was 4.5/100000 in the earlier group and 6.0/100000 in the later group (IRR 1.33, CI 1.11-1.60). The proportion of papillary thyroid cancer rose from 81% to 89% (p=0.02) in two study periods. Median tumour size became smaller, from 25 mm to 15 mm (p<0.001). Surgery became more radical as total thyroidectomies were performed almost exclusively on the later group (p<0.001). Median cumulative dose of radio-iodine ($I^{131}$) therapy was higher in the later group (p=0.04). There was no difference in number of cancer recurrences (p=0.54). The prognosis of DTC was good; 10-year disease-specific survival was 92% in the earlier group and 94% in the later group (p=0.43).

Conclusions: The incidence of thyroid cancer has risen and proportion of papillary cancer has increased, however, median size of tumour has decreased. No difference was seen in either all-cause or disease-specific survival.

Keywords: Adenocarcinoma, Follicullar; Carcinoma, Papillary; Carcinoma, Medullary; Head and Neck Neoplasms; Humans; Neoplasms, Glandular and Epithelial; Prognosis; Survival Analysis; Radionuclide Imaging; Thyreoglobulin; Thyreoidectomy; Thyroid Diseases; Thyroid Gland; Thyroid Neoplasms; Thyroid Nodule; Treatment Outcome

## Introduction

Thyroid cancer is the most frequent endocrine gland malignancy accounting for 0.5% to 1.5% of all malignancies. Incidence of thyroid cancer has increased worldwide in recent years (1,2). The Finnish Cancer Registry reported 359 new cases of thyroid carcinoma in 2008. Thyroid carcinoma is almost four times more frequent in females than in males in Finland, 7.9/100 000 vs. only 2.1/100 000 in men. In Finland the 5-year survival rate in thyroid cancer is 93% for women and 90% for men. (3)

Malignant tumours of the thyroid gland are of either follicular, parafollicular or stromal origin. The majority of thyroid carcinomas are differentiated papillary (PTC, 70-80%) and follicular (FTC, 10-20%) thyroid carcinomas. Highly aggressive and fatal anaplastic carcinoma (ATC) accounts for 5-10% of thyroid malignancies (4-6). Medullary carcinoma (MTC, 5-10%) derives from the parafollicular cells (7).

Recent studies have reported a steady increase in thyroid cancer incidence over time (8,9). This increase has been accompanied by a change in the distribution of histological types with a notable increase in PTCs (10-12). One of the factors considered to be responsible for the increase of PTC appears to be an increase in the supply of iodine in the general population (13). Additionally, genetic factors have also been proposed as a contributory factor (14-16), between 5 and 10% of PTCs are known to be familial (17). Furthermore, increased awareness for thyroid cancer risks after the Chernobyl nuclear accident may be an additional factor. Finally, the wider availability of diagnostic tools may have also been important. (18)

Despite the generally good prognosis for most patients, approximately 8–10% will eventually die of thyroid cancer (19). The primary treatment modalities used for thyroid carcinoma (i.e., surgery, radioiodine, and thyroid hormone) have been used for over 50 years, yet controversy persists regarding the most appropriate choice of therapies for different groups of patients. (20)

Objective of this study was to evaluate the incidence, diagnostics, treatment and survival of thyroid cancer patients in TAUH region.

Material and methods

The material consists of 553 unselected consecutive patients with thyroid cancer in Tampere University Hospital (TAUH) diagnosed 1981-2002 and followed up until 2007. The total population of the area was approximately 0.48 million in 2008 and has been stable over past decades. (Statistics Finland, www.stat.fi).   Thyroid cancer cases from TAUH region were obtained from the Finnish Cancer Registry.  All patient data was obtained from the medical records in TAUH. Surgery was mainly performed in TAUH and a few patients with occult carcinomas were operated on in regional hospitals. The pathological diagnosis was performed in TAUH pathology unit. Patients were followed up in the TAUH Department of Oncology with the exception of some occult cancers that were followed up at the TAUH Department of Endocrinology. Permission to use cancer registry databases was obtained from the Ministry of Social Affairs and Health.

The following epidemiological and tumour data were collected: pre-operative symptoms, fine-needle aspiration cytology, age at diagnosis, gender, type and number of surgical interventions, histological type and size of tumour, lymph node metastasis and distant metastasis, number and dose of $I^{131}$ ablation therapy, chemotherapy or external radiotherapy, follow-up time and number of hospital visits, cancer recurrences, all-cause and cancer-specific deaths.

For further analysis, patients with PTC or FTC (n=498) were classified into two time period groups according to year of diagnosis: earlier group 1981-1991 (n=207); later group, 1992-2002 (n=291). WHO criteria for histopathology have changed during past

decades mainly regarding criteria for FTC. FTC cases were re-evaluated in order to assimilate the groups for the histopathological diagnosis. (21)

Primary thyroid lymphomas (n=17) and squamous cell carcinomas of the thyroid (n=3) were excluded from the final analysis. Patients whose diagnosis was established post mortem (n=17), were also excluded from further analysis. In most of these cases, thyroid cancer was an occult finding in autopsy, but in five of these cases thyroid cancer was a cause of death (papillary carcinoma, follicular carcinoma, medullary carcinoma, one each of them and two anaplastic carcinomas).

Continuous variables are represented as medians with quartiles ($Q_1$, $Q_3$). T-test or Mann-Whitney U-test was used when appropriate. Nominal variables are presented as numbers of patients (%) and were compared using Fisher's exact test. Survival results were analysed using the Kaplan-Meier method. Survival curves were compared using the log-rank test. The incidence of thyroid cancer was analysed by Poisson regression analysis. Results are given as incidence rate ratios (IRR) with 95% confidence intervals. The differences were considered to be statistically significant if p-values were less than 0.05. The statistical analyses were conducted with the SPSS 14.0 software (SPSS Inc., Chicago, IL, USA) and Stata 8.2 (StataCorp, College Station, TX, USA).

Results

Basic characteristics of the whole study group are summarized in Table 1. The only histological type without female predominance was medullary carcinoma.

We scoped in the analysis on patients with DTC (n=498) as the other cancer histological cases are less common. Patients with DTC were divided into two groups according to the year of diagnosis. The earlier group consisted of cases diagnosed between 1.1.1981 and 31.12.1991 and the later group of cases diagnosed between 1.1.1992 and 31.12.2002. Incidence of DTC was 4.5/100000 in earlier group and 6.0/100000 in the later group. The incidence rate ratio was 1.33 (1.11 - 1.60) between groups. Incidence was on a steady increase as seen in Figure 1. Two slight peaks in incidence were observed around the periods 1986-87 and 1997-98.

Basic characteristics of patients in both groups are summarized in Table 2. Median age at diagnosis was the same in both groups. The most common symptom of DTC was a lump in the neck. This finding was more common in the earlier group than in the later group (77% vs. 68%, p=0.03). Tenderness in palpation, hoarseness and difficulty in swallowing were more common in the earlier group (p<0.01). Nodular goiter was common in both groups, more in the earlier group (52% and 29%, p<0.001). Pre-operative FNB results were obtained in 159 (77%) cases in the earlier group and in 239 (82%) in the later group (p=0.17).

Information on tumour characteristics is summarized in Table 3. Type of cancer was papillary in 168 (81%) in the earlier group and in 258 (89%) in the later group (p=0.02). Histology was re-evaluated in all FTC cases (n=72). Tumour size was T1 (≤20mm) in 82

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(48%) patients in the earlier group and 139 (69%) in the later group (p<0.001). The median tumour size was significantly larger in earlier group (25 mm and 15 mm, p<0.001). No significant differences were found in nodal metastasis, distant metastasis or rate of occult cancers. Location of tumour was right lobe in 90 (46%) and 135 (49%) cases, left lobe in 55 (28%) and 84 (30%) cases, isthmus in 12 cases in both groups (6% and 4%), bilateral in 23 (12%) and 29 (10%) and multifocal in 13 (7%) and 15 (5%) cases. Three ectopic cancers were found, one in the earlier group and two in the later group.

Patient's treatment modalities are summarized in Table 4. Preferred surgical procedure was subtotal thyreoidectomy in the earlier group (52% vs. 6%) and total or near total thyreoidectomy in the later group (42% vs. 83%). In the earlier group 137 (66%) patients and in the later group 217 (75%) patients received $I^{131}$—treatment (p=0.045). Most of the patients had only one $I^{131}$-ablation treatment, 126 (61%) in the earlier group and 153 (53%) in the later group. Two or more ablations were given in 12 (6%) and 67 (23%) of cases. The most common dose was 100 mCi (3.7 GBq) in both groups. Median cumulative $I^{131}$ dose was 100 mCi in both groups, with $Q_3$ higher in the later group (100 and 193 respectively) (p=0.040). Local radiotherapy was administered to 10 patients in both groups (4.9% and 3.4%). Only one patient (0.5%) in the earlier group and four patients (1.4%) in the later group received chemotherapy for differentiated thyroid cancer.

There were 32 (16%) cancer recurrences in the earlier group and 56 (19%) cancer recurrences in the later group (p=0.29). Most recurrences were detected by increasing

thyreoglobulin values. Median time for disease recurrence was 3.1 (0.3, 11) years in the earlier group and 3.7 (0.4, 12) years in the later group. The survival of the DTC patients is illustrated with Kaplan-Meier graphs in Figures 2a and 2b. Ten-year all-cause survival was 80% for the earlier group and 86% for the later group (p=0.27). Ten-year disease-specific survival was 92% for the earlier group and 94% for the later group (p=0.43). Median follow-up time was 15.8 (0.1, 26) years in the earlier group and 9.0 (0, 16) years in the later group.

Discussion

Significant changes have occurred in past decades in the diagnostics and treatment of thyroid cancer. Neck ultrasound started to be commonly available at the beginning of the 1990's, thus making it possible to detect and diagnose smaller subclinical thyroid nodules.

In our series, median age at diagnosis was 50 years in both groups. This finding is consistent with other recent studies (22,23).

The incidence of DTC was rising from 4.5/100000 in the earlier group to 6.0/100000 in the later group. Slight peaks in incidence were observed around the periods 1986-87 and 1997-98. The earlier peak may be due to the increased awareness of thyroid cancer in general population due to the Chernobyl nuclear plant accident in April 1986. The later peak may also be related to the Chernobyl nuclear accident, as the fallout after the accident in TAUH region was the highest in Finland (24). The nationwide register also shows a slight peak in incidence in mid-1990s. Thus no significant difference exists with our series and nationwide register. The latent period of thyroid cancer after nuclear plant accident radiation exposure in adults is typically over 10 years, consistent with the findings (25).

There were differences in symptoms between groups. In the earlier group more patients felt a lump in the neck, had tenderness in palpation, hoarseness, and difficulty swallowing. The more frequent symptoms can most likely be explained by the fact that in the earlier group more patients had nodular goiter and the tumours were significantly bigger.

More PTCs were observed in later group. In DTCs, the proportion of PTC is increasing worldwide (10-12,26,27). In Elisei's study the proportion of papillary thyroid cancer increased from 80.5% to 91.0%. (1) Reasons for this change are not fully understood.

Tumour size was significantly smaller in the later group. In Elisei's study the proportion of T1 tumours (<20 mm) had increased from 43.3% to 54.5% and the increase in microcarcinomas (T1a, <10 mm) was even more marked, from 7.9% to 28.7%. In our study, the findings were similar. The proportion of T1a microcarcinomas rose from 23% to 37% and the proportion of T1 tumours rose from 48% to 69% ($p<0.001$). The availability and use of ultrasound may explain why smaller tumours were detected in the later group. Partially those tumours were also incidental findings; however incidentalomas' incidence stayed stable.

I[131]-treatments were more numerous in the later group and there was a statistically significant difference in cumulative median dose of I[131]. This may be due to more sensitive thyreoglobulin monitoring and interpretation of cancer recurrences. There was slightly more thyroid cancer recurrences in the later group, although the difference was not statistically significant. Most of the recurrences were detected by increased thyreoglobulin levels with a minority of recurrences detected by imaging studies or verified by cytology or histology. The reason for increasing trend in cancer recurrences, despite more aggressive surgical therapy and smaller primary tumours, warrants further studies.

Overall, the prognosis of thyroid cancer patients is good in TAUH region, the data are comparable to other recent studies (28,29). No significant difference was seen in

either all-cause or disease-specific survival. There was a slight difference in all-cause survival in short-term follow-up, but this could be explained with other factors, e.g. lower cardiovascular mortality during these time periods. The question arises whether more radical surgery, as seen in the later group, is justified or necessary when there is no statistical difference in disease-specific survival. Radical surgery may not benefit all patients, especially patients with low-risk DTC.

The reliability of the material in this study is good and the patients were non-selected. The study is retrospective and was conducted over long time-period.

The main findings of our study are as follows: The incidence of papillary thyroid cancer has increased, however the median tumour size has dimished. Surgery for thyroid cancer has become more radical, nevertheless no significant change in survival of thyroid cancer patients was detected.

Acknowledgements

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Tables

Table 1. Basic characteristics of the whole study group according to histological type.

| Histology | No. of patients | Female patients | Median age at diagnosis, years $(Q_1, Q_3)$ | Disease-specific mortality[*] | All-cause mortality[*] |
|---|---|---|---|---|---|
| papillary | 427 (77%) | 353 (83%) | 50 (37, 63) | 23 (5%) | 82 (19%) |
| follicular | 72 (13%) | 58 (81%) | 62 (48, 71) | 19 (26%) | 39 (54%) |
| medullary | 14 (3%) | 7 (50%) | 49 (34, 71) | 4 (29%) | 5 (36%) |
| anaplastic | 40 (7%) | 30 (75%) | 75 (63, 78) | 38 (95%) | 40 (100%) |
| Total | 553 (100%) | 448 (81%) | 52 (39, 67) | 84 (15%) | 166 (30%) |

* Follow-up data until 15.5.2007

Table 2. All patients with DTC, background characteristics, symptoms and diagnostic procedures.

| | Earlier Group (1981-1991) n=207 | Later Group (1992–2002) n=291 | p-value |
|---|---|---|---|
| **Female patients** | 164 (79%) | 246 (85%) | 0.15 |
| **Median age at diagnosis** $(Q_1, Q_3)$ | 49.9 (37.5, 65.9) | 49.8 (37.3, 63.1) | 0.83 |
| **Symptoms and findings** | | | |
| Lump in neck | 160 (77%) | 197 (68%) | 0.03 |
| Pain in neck | 13 (6%) | 17 (6%) | 0.85 |
| Tenderness in palpation | 37 (18%) | 9 (3%) | <0.001 |
| Hoarseness | 26 (13%) | 14 (5%) | 0.002 |
| Difficulty swallowing | 26 (13%) | 13 (5%) | 0.001 |
| Shortness of breath | 12 (6%) | 13 (5%) | 0.54 |
| Occult finding | 62 (30%) | 84 (29%) | 0.84 |
| **Anamnestic information** | | | |
| Earlier irradiation of neck region | 3 (1%) | 6 (2%) | 0.74 |
| Graves´ disease | 7 (3%) | 1 (0.3%) | 0.01 |
| Nodular goiter | 108 (52%) | 85 (29%) | <0.001 |
| Thyreostatic medication | 8 (4%) | 8 (3%) | 0.61 |

Table 3. Patients with DTC, tumour characteristics.

| | Earlier Group (1981-1991) n=207 | Later Group (1992–2002) n=291 | p-value |
|---|---|---|---|
| **Histology** | | | 0.02 |
| Papillary | 168 (81 %) | 258 (89 %) | |
| Follicular | 39 (19 %) | 33 (11 %) | |
| **Tumour size** | | | <0.001 |
| ≤10 mm(T1a) | 40 (23%) | 75 (37%) | |
| >10mm and ≤20 mm (T1b) | 42 (25%) | 64 (32%) | |
| >20 and ≤40 mm (T2) | 60 (35%) | 48 (24%) | |
| >40 mm (T3) | 29 (17%) | 14 (7%) | |
| **Median tumour size mm** $(Q_1, Q_3)$ | 25 (15,40) | 15 (10,25) | <0.001 |
| **Tumour staging** | | | 0.26 |
| Tumour confined within thyroid gland | 116 (56%) | 172 (59 %) | |
| Tumour invasion to thyroid capsule or beyond | 12 (6%) | 28 (10 %) | |
| Nodal metastasis | 31 (15%) | 52 (18%) | |
| Distant Metastasis | 11 (5.3 %) | 11 (3.8 %) | |
| Occult cancers | 25 (12%) | 22 (8%) | |

Table 4. DTC patients: different treatment modalities and follow-up.

| | Earlier Group (1981-1991) n=207 | Later Group (1992–2002) n=291 | p-value |
|---|---|---|---|
| **Surgery** | | | <0.001 |
| lobectomy | 10 (5 %) | 22 (8 %) | |
| subtototal thyreoidectomy | 104 (52 %) | 17 (6 %) | |
| total thyreoidectomy | 81 (42 %) | 227 (83 %) | |
| palliative | 2 (1 %) | 9 (3 %) | |
| no operation | 4 (2%) | 7 (2%) | |
| no information | 1 (0.5%) | 1 (0.3%) | |
| **I$^{131}$ treated patients** | 137 (66 %) | 217 (75 %) | 0.045 |
| Median cumulative dose, mCi ($Q_1$, $Q_3$) | 100 (100, 100) | 100 (100, 193) | 0.040 |
| **Local radiation therapy** | 10 (5%) | 10 (3%) | 0.49 |
| **Chemotherapy** | 1 (0.5%) | 4 (1.4%) | 0.41 |
| **Cancer recurrences** | 32 (16 %) | 56 (19 %) | 0.29 |
| **10-year all-cause survival** | 0.802 | 0.859 | 0.27 |
| **10-year disease-specific survival** | 0.918 | 0.938 | 0.43 |

Figure Legends



Figure 1. Increased incidence of DTC in TAUH region, 1981-2002 (black line). Incidence

rate ratio=1.33, CI (1.11 - 1.60) for time periods 1981-1991 and 1992-2002. For

comparison incidence of DTC in Finland (grey line, data acquired from the Finnish
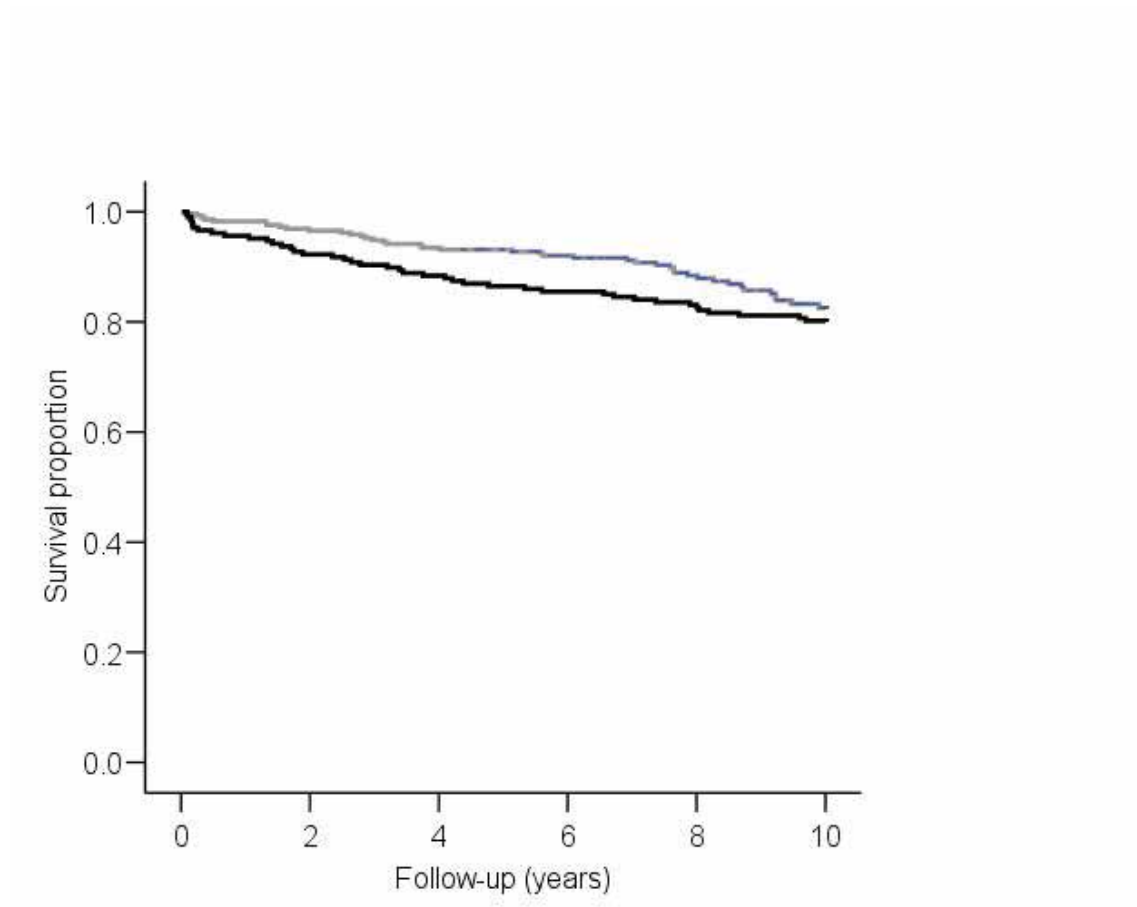
Cancer Registry).

Figure 2a. Overall survival was good throughout the study period with no difference between earlier and later group (p=0.27). Earlier group (black line) and later group (grey line).
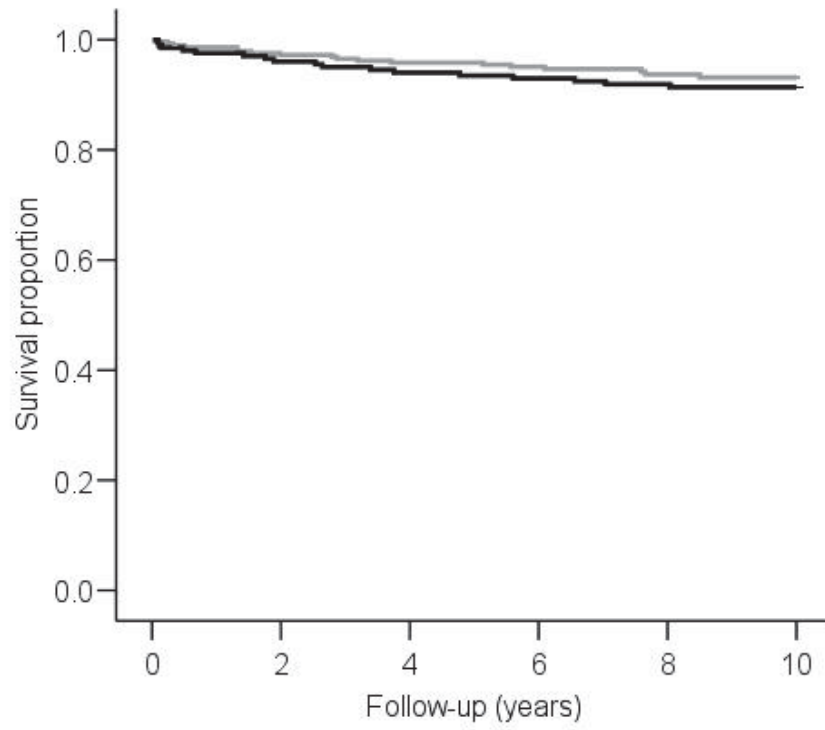
Figure 2b. Disease-specific survival was almost identical in both groups. Earlier group (black line) and later group (grey line). p=0.43

## ORIGINAL ARTICLE

# Recurrent thyroid cancers have more peritumoural lymphatic vasculature than nonrecurrent thyroid cancers

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**ABSTRACT**

**Background** The goal of the study was to evaluate angiogenesis and lymphangiogenesis in differentiated thyroid cancer and recurrences.

**Methods** Twenty-seven patients with recurrent differentiated thyroid cancer (20 papillary and seven follicular thyroid carcinomas) and 24 nonrecurrent thyroid cancers were included in this study. Additionally, 24 thyroid adenomas were included as benign controls. All thyroid cancer recurrences were operatively managed, and local recurrences in cervical lymph nodes or cervical soft tissue were histologically confirmed. Altogether, a total of 108 samples were evaluated using CD31 and D2-40 immunohistochemical staining and microscopy.

**Results** As measured in primary tumours, the median density of CD31-positive vascular structures was 327 vessels (v)/mm$^2$ for recurrent cancers, 362 v/mm$^2$ for nonrecurrent cancers and 484 v/mm$^2$ for thyroid adenomas ($P = 0.017$). Among the subgroups, the lowest median vascular density of 316 v/mm$^2$ was found in recurrent papillary cancers and the highest vascular density of 604 v/mm$^2$ was observed in nonrecurrent follicular cancers ($P = 0.018$). The median density of D2-40-positive peritumoural lymphatic vessels was 101/mm$^2$ in recurrent cancers, 56.1/mm$^2$ in nonrecurrent cancers and 53.9/mm$^2$ for adenomas ($P = 0.015$). In the subgroups, peritumoural lymphatic vascular density was 102 v/mm$^2$ in recurrent papillary cancers and 56.0 v/mm$^2$ in nonrecurrent papillary cancers ($P = 0.044$).

**Conclusions** Recurrent thyroid cancers expressed less intratumoural microvessels than thyroid adenomas. A high density of peritumoural lymphatic vessels was found in recurrent papillary cancers. High blood vessel density may be a marker for less aggressive tumours, while high peritumoural lymphatic vasculature is a marker for more aggressive and recurrence-prone tumours.

**Keywords** Angiogenesis, head and neck cancer, histopathology, lymphangiogenesis, thyroid, thyroid cancer.

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## Introduction

Thyroid cancer accounts for 1% of all cancer cases and is the most common endocrine malignancy. The incidence of thyroid cancer is rising, especially among younger patients, and it is the 6th most common cancer in patients less than 40 years old [1–3]. Most thyroid cancers are follicular cell-derived papillary (PTC) or follicular (FTC) carcinomas, whereas medullary thyroid cancers arise from parafollicular C cells. Anaplastic thyroid carcinomas are poorly differentiated

aggressive tumours. PTC and FTC are referred to as differentiated thyroid cancer (DTC), and the prognosis of DTC is generally favourable [4,5].

Although the prognosis of DTC is generally good, a small proportion of patients suffer from recurrent and persistent cancer, ultimately resulting in death. Treatment of DTC consists of thyroid surgery, which is followed by radioactive iodine (RAI) ablation. The extent of surgery has been frequently debated, and no clear consensus exists for example regarding the role of prophylactic lymph node dissection [6,7]. Some

tumours are less sensitive to RAI, and recurrent disease may develop in those patients insensitive to RAI treatment [8,9]. Once recurrent or persistent disease becomes RAI refractory, there are no known available curative treatment alternatives. Local recurrences may be treated with surgery or ultrasound-guided local treatments, for example ethanol injections [10,11].

Invasion of tumour cells to blood vessels and lymphatic vessels is a marker of aggressive tumours. Angioinvasion is a risk factor for metastatic spread of disease, and lymphatic vessel invasion is a risk factor for local lymph node metastases [12,13]. Lymphangiogenesis of primary tumours is a predictive indicator for lymph node metastasis and relatively poor prognosis [14,15]. Tumour cells secrete vascular endothelial growth factors (VEGFs), which promote angiogenesis (VEGF-A) and lymphangiogenesis (VEGF-C, VEGF-D) [16–18]. Novel treatments, including monoclonal antibodies against VEGF, such as bevacizumab, are used in the treatment of solid tumours to inhibit angiogenesis [19].

The aim of the present study was to evaluate whether angiogenesis and lymphangiogenesis, measured in both tumour tissue and surrounding tissue, correlate with local recurrences and prognosis.

## Materials and methods

### Patients and samples

Samples were retrieved from Tampere University Hospital pathology archives. All patients had been treated for thyroid cancer in our hospital between 1981 and 2002. The clinical database contained 495 DTC patients, 29 of whom were re-operated on due to recurrence of cancer in the neck region [2]. Samples were available for 27 re-operated patients, with a total of 60 histological samples of primary and recurrent tumours. Age, gender, tumour type and tumour size-matched samples of nonrecurrent thyroid cancers ($n = 24$) were acquired from the same clinical database. For benign controls, 24 thyroid adenoma samples were included in the study. Altogether, 108 samples were analysed for angiogenesis using CD31 and D2-40 immunohistochemical staining.

### Immunohistochemistry

Four-micrometre-thick sections were cut from paraffin-embedded tissue blocks of all of the tumours. Immunohistochemistry was performed using Ventana Life Sciences Benchmark XT Staining module (Ventana Medical Systems, Tucson, AZ, USA). Sections were incubated with the panendothelial marker CD31 (clone JC70A, 1 : 50; DAKO, Glostrup, Denmark) for 30 min at room temperature. The lymphatic endothelium marker D2-40 (clone D2-40, 1 : 50; DAKO) was detected after 30 min of incubation at room temperature following a 1 mM EDTA (pH 9·0) pretreatment in a microwave oven [20].

### Morphometry

Hotspot areas on CD31 stained sections were analysed for the vessel density and area and were photographed at 200× magnification using Cellsens 1.7 software (Olympus Corp., Tokyo, Japan). Lymphatic vasculature was evaluated in intratumoural and peritumoural areas, in D2-40 stained sections. Lymphatic vessel density and area were analysed at 200× magnification with Cellsens 1.7 software. A pathologist and a PhD student, blinded to the tumour type, conducted analyses.

### Ethics

The ethics committee of Tampere University Hospital approved this study (R11013). Permission to use tissue samples for research purposes was acquired from the National Supervisory Authority for Welfare and Health (Valvira 3447/06.01.03.01).

### Statistical analysis

Continuous variables are represented as the medians with quartiles ($Q_1$, $Q_3$) or the means with standard deviation. Independent samples were tested for significance with a Mann–Whitney U-test and a Kruskal–Wallis test when three or more samples were compared. Categorical variables were tested for significance with Fisher's exact test. SPSS software version 20 (IBM Corporation, New York, NY, USA) was used for statistical analysis. *P*-values <0·05 were considered significant.

## Results

The majority of the patients in this study were female; 17 (63%) in the recurrent DTC group, 15 (63%) in the nonrecurrent group and 19 (79%) in the thyroid adenoma group, as shown in Table 1. The mean age at diagnosis was similar between the groups, 51·9, 52·1 and 51·4 years in the recurrent, nonrecurrent and adenoma group, respectively. Most of the tumours were PTCs; 20 (74%) in the recurrent group and 20 (83%) in the nonrecurrent group. The mean tumour size was 37·4 mm in recurrent cancers, 24·2 mm in nonrecurrent cancers and 31·3 mm in thyroid adenomas. At the time of primary operation, nodal metastasis was observed in 14 (52%) of the recurrent cases and in 0% of the nonrecurrent cases ($P = 0·004$). The tumours had extra-thyroidal invasion in 9 (33%) of the recurrent group cases and in 1 (4%) from the nonrecurrent group ($P = 0·004$). The tumours were multifocal in 4 (15%) recurrent cases and 3 (13%) nonrecurrent cases. All of the patients in the recurrent DTC group had postoperative RAI ablation, and 19 (79%) patients in the nonrecurrent group were RAI ablated. The mean time from primary operation to recurrence operation was 48 months (±37 months). Recurrent tumours were located either in cervical lymph nodes ($n = 14$) or in cervical soft tissue ($n = 18$).

**Table 1** Basic characteristics of the study groups reveal equal groups with slightly smaller primary tumours in the nonrecurrent cancer group

| | Recurrent DTC *n* = 27 | Nonrecurrent DTC *n* = 24 | Thyroid adenomas *n* = 24 |
|---|---|---|---|
| Number of women | 17 (63%) | 15 (63%) | 19 (79%) |
| Mean age at diagnosis (SD) | 51·9 (18·3) | 52·1 (17·3) | 51·4 (17·0) |
| Tumour type | | | |
| Papillary | 20 | 20 | N.A. |
| Follicular | 7 | 4 | |
| Mean tumour size at diagnosis, mm (SD) | 37·4 (17·4) | 24·2 (12·8) | 31·3 (15·2) |

DTC, differentiated thyroid cancer; N.A., not applicable.

Subtypes of recurrent PTCs were as follows: 10 classical PTCs, four follicular variant PTCs, three oncocytic PTCs, two Warthin-like PTCs and one diffuse sclerosing PTC. Subtypes of nonrecurrent PTCs were similar: nine classical PTCs, eight follicular variant PTCs, one oncocytic PTC, one diffuse sclerosing PTC and one cribriform-morular PTC. Thyroid adenoma subtypes were as follows: 10 foetal, six oncocytic, four simple, two embryonal, one colloidal and one atypical.

## Primary tumour vascularity

Primary tumour vasculature in recurrent tumours, nonrecurrent tumours and thyroid adenomas is summarised in Table 2. Primary tumour microvascular density was lowest in recurrent DTC (327 vessels (v)/mm$^2$), followed by nonrecurrent DTC (362 v/mm$^2$) and thyroid adenomas (484 v/mm$^2$, $P = 0·017$). Recurrent DTC had significantly smaller vessels (median 139 μm$^2$/v) than nonrecurrent DTC (median 173 μm$^2$/v) or thyroid adenomas (median 260 μm$^2$/v, $P < 0·001$). The median proportional vessel area of recurrent DTC was 4·72%, which was lower than that observed in nonrecurrent DTC (5·45%) or adenomas (13·8%, $P < 0·001$). Among the subgroups, the median proportional vessel area was largest in nonrecurrent FTC (14·6%), significantly larger than in recurrent PTC (4·56%, $P = 0·018$) or nonrecurrent FTC (5·05%, $P = 0·013$). Figs 1a–d and 2a illustrate these differences.

## Primary tumour lymphatic vasculature

Peritumoural lymphatic vascular density in recurrent DTC was 101 v/mm$^2$, significantly higher than that of nonrecurrent DTC (56·1 v/mm$^2$) or thyroid adenomas (53·9 v/mm$^2$,

$P = 0·015$). Among the subgroups, the peritumoural lymphatic vascular density was higher in recurrent PTC (102 v/mm$^2$) than in nonrecurrent PTC (56·0 v/mm$^2$, $P = 0·044$) or nonrecurrent FTC (51·1 v/mm$^2$, $P = 0·045$), as illustrated in Figs 1e–h and 2b. Peritumoural lymphatic vessel size was smaller in recurrent DTC than in nonrecurrent DTC or thyroid adenomas (medians 340, 647 and 594 μm$^2$/v, $P = 0·012$). Among subgroups, the median vessel size in recurrent PTC was 328 μm$^2$/v, which was significantly smaller than in nonrecurrent PTC 705 μm$^2$/v ($P = 0·001$) or in recurrent FTC 827 μm$^2$/v ($P = 0·031$). There were no significant differences in the proportional areas of lymphatic vessels. Intratumoural lymphatic vasculature density was low in all of the groups: 30·3 v/mm$^2$ in recurrent DTC, 21·7 v/mm$^2$ in nonrecurrent DTC and 16·8 v/mm$^2$ in adenomas ($P = 0·777$). Intratumoural lymphatic vessels were similar in size, 240 μm$^2$/v in recurrent DTC, 354 μm$^2$/v in nonrecurrent DTC and 280 μm$^2$/v in adenomas. As for the subgroups, the median vessel size was higher in recurrent PTC (272 μm$^2$/v) than in recurrent FTC (123 μm$^2$/v, $P = 0·013$), and the median proportional area of intratumoural lymphatic vessels was higher in recurrent PTC (0·90%) than in recurrent FTC (0·46%, $P = 0·036$). See also Fig. 2c.

## Metastatic lesions

The microvasculature of primary tumours and metastatic lesions are presented in Table 3. Although the differences in blood vessel density, vessel size and proportional area of vessels were not statistically significant, differences were observed in the lymphatic vasculature. The median size of peritumoural lymphatic vessel was 328 μm$^2$/v in PTC primary tumours, lower than in PTC metastasis (1071 μm$^2$/v, $P = 0·008$) or in FTC primary tumours (827 v/mm$^2$, $P = 0·031$). FTC metastasis had a peritumoural lymphatic vessel density of 206 v/mm$^2$, lower than in FTC primary tumour (827 v/mm$^2$, $P = 0·021$). The peritumoural lymphatic vessel area was higher in PTC metastasis (10·3%) than in FTC metastasis (0·91%, $P = 0·025$), as shown in Fig. 1k–l. The intratumoural lymphatic vessel size was 272 μm$^2$/v in PTC primary tumours, significantly higher than in FTC primary tumours (123 μm$^2$/v, $P = 0·013$). Additionally, the proportional area of intratumoural lymphatic vessels was higher in PTC than in FTC primary tumours (0·90% and 0·46%, respectively, $P = 0·036$).

## Survival

The disease-specific mortality of patients with recurrent DTC was 37%, according to data collected from the clinical database. No disease-specific mortality was observed in patients with nonrecurrent DTC. All-cause mortality was 41% in the recurrent DTC group and 33% in the nonrecurrent group.

**Table 2** A summary of the microvascular structures of differentiated thyroid cancers (DTC, $n = 51$) and thyroid adenomas, as measured from primary tumours. Breakout data on papillary thyroid cancer (PTC, $n = 40$) and follicular thyroid cancer (FTC, $n = 11$) are also presented, along with the corresponding *P*-values

| | | Recurrent DTC ($n = 27$) | Nonrecurrent DTC ($n = 24$) | Adenomas ($n = 24$) | *P* |
|---|---|---|---|---|---|
| CD31, v/mm$^2$ | All | 327 (256, 468) | 362 (303, 457) | 484 (340, 592) | 0·017 |
| | PTC | 315 (255, 392) | 353 (303, 447) | | 0·194 |
| | FTC | 431 (300, 545) | 604 (315, 873) | | 0·648 |
| CD31, μm$^2$/v | All | 139 (88·8, 204) | 173 (113, 204) | 260 (211, 363) | <0·001 |
| | PTC | 153 (89·6, 219) | 173 (110, 201) | | 0·978 |
| | FTC | 139 (88·8, 195) | 177 (124, 288) | | 0·527 |
| CD31, area % | All | 4·72 (3·77, 6·91) | 5·45 (4·43, 8·56) | 13·8 (10·7, 18·2) | <0·001 |
| | PTC | 4·58 (3·78, 6·82) | 5·05 (4·28, 7·69) | | 0·387 |
| | FTC | 5·84 (3·41, 8·16) | 13·56 (7·49, 14·64) | | 0·230 |
| D2-40 PT, v/mm$^2$ | All | 101 (57·2, 186) | 56·1 (43·8, 96·0) | 53·9 (42·9, 82·6) | 0·015 |
| | PTC | 102 (69·3, 245) | 56·0 (44·6, 108) | | 0·044 |
| | FTC | 57·2 (47·1, 141) | 51·1 (38·7, 72·7) | | 0·527 |
| D2-40 PT, μm$^2$/v | All | 340 (229, 764) | 647 (342, 1310) | 594 (462, 1480) | 0·012 |
| | PTC | 328 (215, 445) | 705 (508, 1310) | | 0·001 |
| | FTC | 827 (401, 1196) | 337 (324, 3889) | | 0·412 |
| D2-40 PT, area % | All | 4·37 (2·48, 11·3) | 4·94 (2·03, 7·88) | 3·95 (2·62, 7·14) | 0·923 |
| | PTC | 3·86 (2·27, 10·7) | 5·04 (3·55, 7·88) | | 0·626 |
| | FTC | 5·35 (4·01, 12·7) | 1·67 (1·31, 29·9) | | 0·527 |
| D2-40 IT, v/mm$^2$ | All | 30·3 (8·42, 65·6) | 21·7 (10·8, 41·2) | 16·8 (10·9, 37·0) | 0·777 |
| | PTC | 30·3 (7·58, 66·5) | 23·4 (12·7, 41·2) | | 0·533 |
| | FTC | 16·8 (0, 37·0) | 13·5 (4·21, 381) | | 0·788 |
| D2-40 IT, μm$^2$/v | All | 240 (354, 280) | 354 (141, 516) | 280 (186, 529) | 0·363 |
| | PTC | 272 (159, 614) | 355 (159, 426) | | 0·685 |
| | FTC | 123 (0, 258) | 406 (112, 2700) | | 0·164 |
| D2-40 IT, area % | All | 0·67 (0·24, 1·38) | 0·74 (0·41, 1·74) | 0·63 (0·31, 1·23) | 0·913 |
| | PTC | 0·90 (0·35, 2·06) | 0·74 (0·42, 1·74) | | 0·626 |
| | FTC | 0·46 (0, 0·67) | 0·80 (0·27, 0·54) | | 0·315 |

CD31, panendothelial marker (vascular structures); D2-40, lymphatic endothelium marker; v/mm$^2$, vessels per square millimetre; μm$^2$/v, area per one vessel in micro square metres; area %, proportion of vessel area in per cents; PT, peritumoural; IT, intratumoural.
Figures are medians with quartiles ($Q_1$, $Q_3$). *P*-values are calculated with a Mann–Whitney U-test, except for the 'All'-row, where a Kruskal–Wallis test was used.

## Discussion

### Vasculature

In the present study, thyroid carcinomas had relatively small microvessels with lower density, whereas thyroid adenomas had larger and denser microvessels. When measuring the proportional area of tumour microvessels, PTCs and recurrent FTCs had significantly less vasculature than nonrecurrent FTCs or thyroid adenomas. The tumours that had the lowest microvascular density were the ones most likely to have recurrence after initial surgery. This finding indicates that low vasculature in the thyroid gland may be a marker for more aggressive
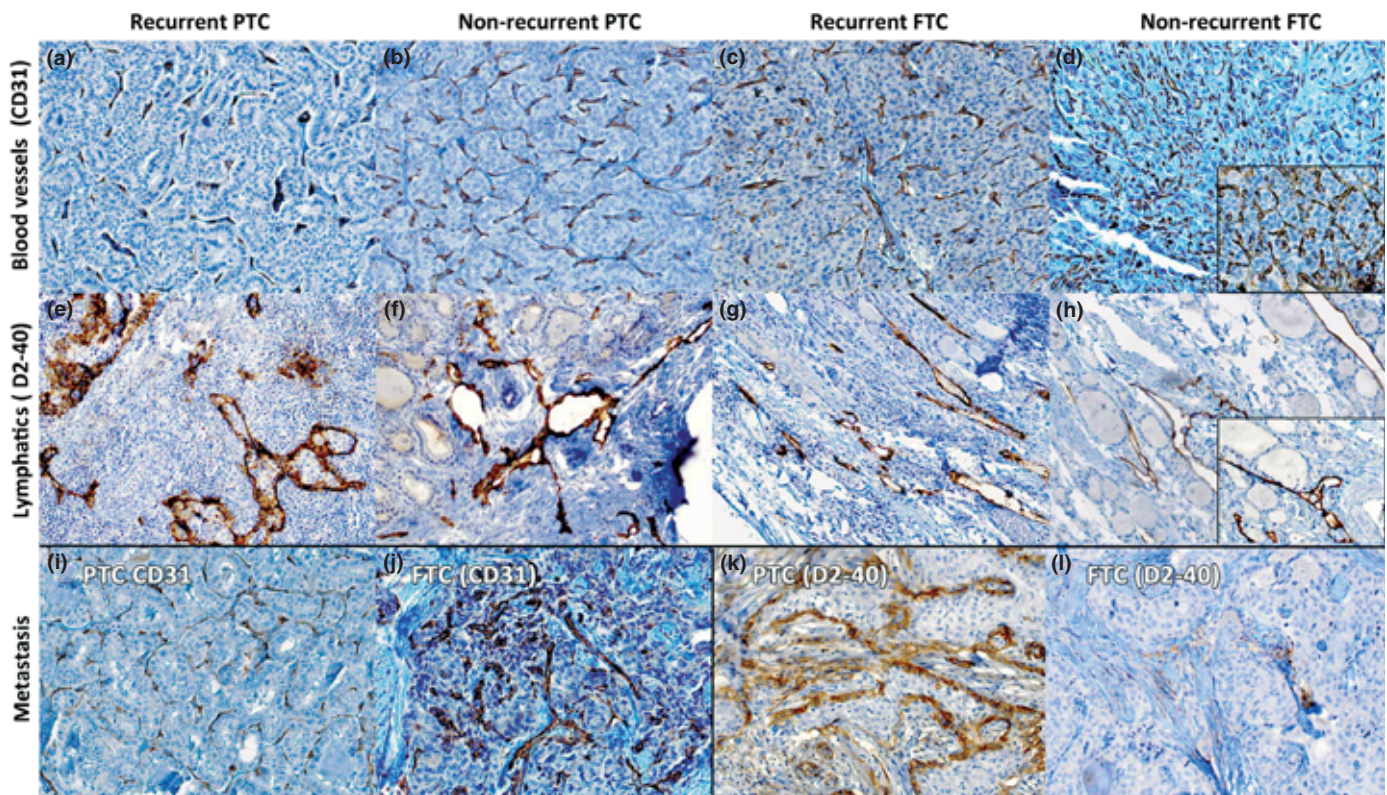
**Figure 1** (a) CD31 immunohistochemical (IHC) staining shows relatively low intratumoural microvessel density (MVD) in a primary tumour of recurrent papillary thyroid cancer (PTC). (b) Slightly more vessels are visible in nonrecurrent PTC. (c) Recurrent follicular thyroid cancer (FTC) has moderate MVD. (d) Nonrecurrent FTC has the high MVD and also thyroid adenoma shown in the insert has a high density of large microvessels. (e) D2-40 IHC staining shows high peritumoural lymphatic vessels density (LVD) in recurrent PTC. Tumour cell infiltration in lymphatic vessels is evident, and lymphocytic infiltrates are present. (f) Nonrecurrent PTC has lower peritumoural LVD. (g, h) Peritumoural LVD of recurrent and nonrecurrent FTC is relatively low. Thyroid adenoma shown in the insert has low peritumoural LVD. (i) PTC metastasis in cervical lymph node shows relatively low MVD in CD31 IHC. (j) FTC metastasis in soft tissue (ST) of cervical area shows higher MVD in CD31 IHC. (k) PTC lymph node metastasis has high LVD in D2-40 IHC. (l) FTC metastasis lacks lymphatic vasculature almost entirely in D2-40 IHC. Magnification 200×.



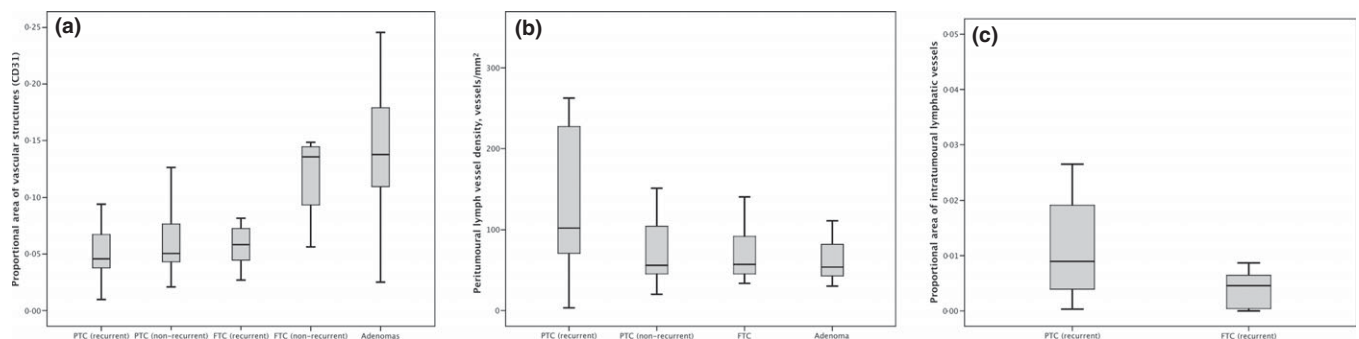**Figure 2** (a) Recurrent papillary thyroid carcinomas (PTC) had less vasculature than nonrecurrent follicular thyroid carcinoma (FTC) or thyroid adenomas. ($P < 0.001$) (b) Highest peritumoural lymph vessel density was observed in recurrent PTC. ($P = 0.020$) (c) Although intratumoural lymphatic vasculature was scarce in all tumour types, more lymphatic vessels were found in recurrent PTC than in recurrent FTC. ($P = 0.036$).

**Table 3** Data of papillary thyroid cancer (PTC, 20 primary tumours and 20 metastases) and follicular thyroid cancer (FTC, 7 primary tumours and 8 metastasis) revealed that peritumoural lymphatic vessels were larger in metastases of PTC; the opposite was true for FTC

|  |  | Primary tumours ($n = 27$) | Metastasis ($n = 28$) | *P* |
|---|---|---|---|---|
| CD31, v/mm$^2$ | PTC | 315 (255, 392) | 334 (201, 292) | 0·968 |
|  | FTC | 431 (300, 545) | 325 (209, 430) | 0·232 |
| CD31, μm$^2$/v | PTC | 153 (89·6, 219) | 163 (111, 196) | 0·829 |
|  | FTC | 139 (88·8, 195) | 202 (126, 308) | 0·463 |
| CD31, area % | PTC | 4·58 (3·78, 6·82) | 4·88 (3·12, 7·54) | 0·914 |
|  | FTC | 5·84 (3·41, 8·16) | 5·42 (4·14, 9·41) | 0·694 |
| D2-40 PT, v/mm$^2$ | PTC | 102 (69·3, 245) | 74·1 (34·5, 154) | 0·194 |
|  | FTC | 57·2 (47·1, 141) | 45·1 (42·0, 192) | 0·536 |
| D2-40 PT, μm$^2$/v | PTC | 328 (215, 445) | 1071 (306, 1980) | 0·008 |
|  | FTC | 827 (401, 1196) | 206 (163, 313) | 0·021 |
| D2-40 PT, area % | PTC | 3·86 (2·27, 10·7) | 10·3 (3·90, 15·2) | 0·062 |
|  | FTC | 5·35 (4·01, 12·7) | 0·91 (0·86, 6·26) | 0·094 |
| D2-40 IT, v/mm$^2$ | PTC | 30·3 (7·58, 66·5) | 20·2 (13·5, 35·3) | 0·674 |
|  | FTC | 16·8 (0, 37·0) | 26·5 (15·1, 36·2) | 0·536 |
| D2-40 IT, μm$^2$/v | PTC | 272 (159, 614) | 320 (221, 785) | 0·344 |
|  | FTC | 123 (0, 258) | 272 (182, 334) | 0·054 |
| D2-40 IT, area % | PTC | 0·90 (0·35, 2·06) | 0·81 (0·51, 1·31) | 0·978 |
|  | FTC | 0·46 (0, 0·67) | 0·95 (0·29, 1·18) | 0·094 |

CD31, panendothelial marker (vascular structures); D2-40, lymphatic endothelium marker; v/mm$^2$, vessels per square millimetre; μm$^2$/v, area per one vessel in micro square metres; area %, proportion of vessel area in per cents; PT, peritumoural; IT, intratumoural.
Figures represent the medians with quartiles (Q$_1$, Q$_3$).

It has been demonstrated that vasculature of malignant tumours is abnormal, resulting in a hostile tumour environment, which is associated with high interstitial fluid pressure, low pH and hypoxia [22]. This induces changes in cells, and tumours begin to secrete excess vascular proliferative meditators (e.g., VEGF-A) to attract more vasculature [18]. To inhibit these pro-angiogenic factors, novel therapies against angiogenesis have been recently introduced. VEGF humanised monoclonal antibodies and tyrosine kinase inhibitors work by inactivating VEGF or by inhibiting the effects of VEGF on the tyrosine kinase portion of VEGF receptors. The tyrosine kinase inhibitor sorafenib has passed phase III trials and has been proved to improve progression-free survival by 5 months in locally advanced or metastatic RAI refractory DTC [23,24]. It remains unclear whether sorafenib improves overall survival. Sorafenib has affinity to multiple tyrosine kinases and thus inhibits a number of kinases in thyroid cancer cells and adjacent vascular endothelial cells in addition to VEGF tyrosine kinase receptors [25,26]. When vascular growth is inhibited, the tumour blood flow diminishes and the tumour suffers from depletion of oxygen and nutrients. It has been speculated that by blocking the effects of VEGF, tyrosine kinase inhibitors may promote hypoxic conditions that trigger tumour cell mesenchymal transformation and metastatic process [27].

According to our results, differences in the vasculature of primary tumours and metastatic lesions were minimal in both PTCs and FTCs. Thus, DTC metastases appear to 'inherit' vasculature from primary tumours, and RAI treatments consequently did not alter vasculature. The majority of malignant thyroid primary tumours had lower vasculature than thyroid adenomas. It may be possible that RAI penetrates cancerous tissue in inadequate quantities if tumour vasculature and tissue conditions are insufficient. This may lead to inadequate effects of RAI ablation and a 'stunning' effect on cancerous tissue, which can eventually lead to RAI refractory disease. It has been speculated that VEGF inhibition may normalise tumour vasculature by reducing VEGF overexpression [22]. This is an interesting theory that gained credibility with the discovery of improved uptake of RAI in advanced thyroid cancers after treatment with the tyrosine kinase inhibitor selumetinib [28].

### Lymphatic vasculature

In the present study, the tumour lymphatic vasculature was highest in malignant tumours. Peritumoural lymph vessels in recurrent PTC were denser and smaller than in nonrecurrent PTC or thyroid adenomas. In recurrent FTC, lymph vessels were larger but less dense than in recurrent PTC. The density of lymphatic vessels is likely a key element in predicting tumour aggressiveness because most lymphatic vessels in benign lesions were large and hollow, as determined by microscopy. The lymphatic vessel density is most likely connected to

tumours and that high vasculature may be a marker for less aggressive tumours. In previous studies, DTCs have been associated with high expression of vascular proliferative factors, for example VEGF-A [21]. In the present study, PTCs and recurrent FTCs did not express more vasculature; in fact, the density of vasculature was well below that of nonrecurrent FTCs or benign thyroid adenomas. High expression of proliferative factors seems to be associated with formation of abnormal tumour vasculature with less dense and smaller vessels, hence poor function and structure [22].

Development of tumour vasculature, that is tumour angiogenesis, has been studied intensively in recent decades [12,13].

vascular growth factor stimulation, as a high local concentration of lymphatic vessels after local gene transfer of vascular growth factors has been previously described [29,30]. In the present study, the expression of high local concentrations of lymphatic vessels was primarily observed in recurrent PTCs. In recurrent PTC cases, the size and proportional area of intratumoural lymphatic vessels were larger than that observed in recurrent cases of FTC. This finding is consistent with the fact that the main route of metastatic spread of PTC is via the lymphatic system to local lymph nodes in the neck region. Distant blood-borne metastasis is less common in PTC. This effect was also shown by the fact that the recurrent tumours had more often lymph node metastases at the time of primary operation in our study population.

Other recent studies have demonstrated that a high density of lymphatic vasculature or lymphatic vasculature promoting factors is associated with a higher frequency of nodal metastasis. In breast cancer, high lymphatic vessel density correlates with lymph node metastasis [14]. The lymphatic vessel marker podoplanin predicts nodal metastasis in head and neck squamous cell carcinoma [31]. In thyroid cancer, high intratumoural lymphatic microvascular density has been linked to nodal metastasis, and the lymphatic marker D2-40 may predict a poor prognosis [32,33]. Additionally, VEGF-C and VEGF-D have been associated with increased lymphangiogenesis and lymph node metastasis in PTC [34,35]. However, in another study, VEGF-C or VEGF–D expression did not correlate with lymph node metastasis but a high peritumoural lymph vessel density did [36].

Metastatic lesions in PTC had more lymphatic vasculature than primary tumours. A large portion of PTC metastases was found in local lymph nodes, which may explain this finding. It is probable that RAI treatments did not lessen the stimulus for VEGF production. PTC appears to have a constant stimulus for lymphatic vasculature promoting factors, the effects of which are more pronounced in peritumoural areas.

## Conclusion

Angiogenesis of recurrent DTC was characterised by a diminished quantity of small intratumoural microvessels. A high quantity of large intratumoural microvessels was found in benign thyroid adenomas and in nonrecurrent FTC. A high density of peritumoural lymphatic vessels was found in recurrent PTCs. High blood vessel density may be a marker for less aggressive tumours, but high lymphatic vasculature appears to be a marker for more aggressive and recurrence-prone tumours.

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Increased risk of certain second primary malignancies in patients treated for well-differentiated thyroid cancer

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Abstract

Background: The objective was to evaluate the incidence of second primary malignancies (SPMs) in thyroid cancer patients compared to age- and gender-matched controls from the same region and to investigate which subgroups had the highest risk of SPMs.

Methods: Patients with well-differentiated thyroid cancer (n=910) were treated in Tampere or Oulu University Hospitals 1981–2002. Patients and controls from the general population (n=4542) were followed for an average of 16 years through the Finnish Cancer Registry. The incidence of invasive malignancies per 10 000 person-years was calculated and compared between patients and controls. The follow-up period ended December 31st, 2011.

Results: Increased incidence of SPMs was observed in young patients <40 years old (Rate Ratio [RR] 1.73, p=0.037) and patients diagnosed since 1996 (RR 1.51, p=0.029). Patients had increased risk of sarcomas and soft tissue tumours (RR 4.37, p=0.004) and haematologic and lymphatic malignancies (RR 1.87, p=0.035), in particular non-Hodgkin lymphomas (RR 2.78, p=0.035). The overall incidence of SPMs was not statistically higher in patients (109 SPMs/910 patients vs. 500 SPMs/4542 controls, RR 1.12, p=0.269). Most patients were radioiodine-treated (81%), and when compared to the controls, the risk of SPMs with low cumulative doses was 0.94 (≤3.7 GBq, p=0.650) and with high doses 1.37 (>3.7 GBq, p=0.143). Cumulative radioiodine dose increased during the study period.

Conclusions: The incidence of SPMs in thyroid carcinoma patients was higher in patients <40 years old and patients diagnosed since 1996. The incidence of sarcomas and lymphomas was higher in patients than in controls.

Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for 1-2% of all cancer cases. Incidence of thyroid cancer has increased in western countries during the past few decades, especially among young people (1-3). In Finnish patients less than 40 years old, thyroid cancer is the 4th most common malignancy in females and 7th most common malignancy in males (4). Well-differentiated malignancies arising from follicular thyroid cells and are referred to as differentiated thyroid cancers (DTCs), which are further classified as papillary thyroid cancers (80-90%) and follicular thyroid cancers (10-20%). The prognosis of DTC is generally favourable (5,6).

The treatment of DTC consists of total thyroidectomy, followed by radioactive iodine (RAI) ablation, which destroys residual thyroid tissue after surgery (7). In addition, RAI eradicates and destroys possible DTC metastasis in local lymph nodes and in distant sites. Most frequent sites of distant metastasis are the lungs and bone (8). Serum thyroglobulin may be used as a tumour marker after RAI ablation. RAI ablation may cause irritation of the salivary glands and mucous membranes of the gastrointestinal and respiratory tract acutely or subacutely (9). There is also concern about radiation induced second primary malignancies (SPMs) because DTC affects relatively young patients (3,10). In previous studies DTC patients have been associated with 9-33% greater risk of SPM than general population (10-16). The aim of this study was to evaluate the risk of

SPMs in a cohort of Finnish DTC patients compared to age- and gender-matched controls of the same region, and to investigate which subgroups of patients had the highest risk of SPMs.

Materials and Methods

Study population and the follow-up

The study included 920 consecutive patients treated for DTC at two Finnish university hospitals between 1981 and 2002. A total of 493 patients were treated at Tampere University Hospital and 427 at Oulu University Hospital (2,17). Follow-up data were collected from the hospitals' medical records and included the date of birth, date of cancer diagnosis, date and extent of surgery, cumulative dose of RAI treatments and the latest date of follow-up. Each patient was assigned an index date, which was the date of RAI ablation for patients treated with RAI and the date of diagnosis of DTC for those who did not receive RAI treatment. Ten patients were excluded because of missing information in the registry database, errors in identification numbers or data release limitation. A total of 910 patients were available for analysis.

For each patient, 5 control subjects from general population were selected from the databases of Population Register Center of Finland and matched for age, gender and place of residence. The follow-up period of the control subjects started at the index date of the corresponding patients. Cancer statistics for the patients and controls were acquired from the Finnish Cancer Registry (4) since 1960. Only invasive cancers were included into the overall cancer incidence. In situ-cancers, suspicions of cancer and benign tumours were excluded from the

Finnish Cancer Registry data. Basal cell carcinomas, considered as indolent skin cancers, were also excluded. Benign CNS tumours were analysed independently.

Second cancers and categories

The time interval for the development of SPM was calculated from the index date to the date of SPM. Antecedent malignancies, cancers occurring before the index date, were analysed separately. If the time between the index date and the date of SPM was ≤12 months, SPM was considered synchronous and excluded to eliminate surveillance bias. The follow-up period ended either at the date of the first SPM, the emigration date from Finland, the date of death or on December 31, 2011, whichever came first.

Classification system used by the Finnish Cancer Registry is the International Classification of Diseases for Oncology (ICD-O-3), which identifies the anatomical region of the cancer (topography) and the cancer histology (morphology) (18). Topographical classification of ICD-O-3 follows closely the clinical classification (ICD-10) with a few exceptions. Majority of observed SPMs were categorised using the topography. However, morphology was used in some cancers occurring in multiple anatomical sites. These cancers included hematologic and lymphatic malignancies (morphology [M]9590-M9989), mesenchymal malignancies (bone and soft tissue cancers, M8800-M9262) and subgroup of latter, sarcomas and soft tissue cancers (separate ICD-O-3

guidelines) (18). Accessory analysis was conducted with a combination of malignant and benign CNS tumours (M9380-M9571) and benign meningiomas (M9530-M9539).

Ethics

The Ethics Committee of the Pirkanmaa Hospital District approved the study protocol. In addition, the National Research and Development Center for Welfare and Health gave permission to use data from the Population Register Center and the Finnish Cancer Registry. The study was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

SPM incidences per 10,000 person-years were calculated for both patients and controls using Stata software (StataCorp, College Station, TX, USA). Incidences between groups were compared using rate ratios according to the Mantel-Haenszel method.

Other statistical analyses were conducted using SPSS software version 20 (IBM Corporation, New York, NY, USA). Continuous variables are represented as the means with standard deviation. Kaplan-Meier analysis and the log rank test were used when comparing various subgroups of patients. The Chi-square test

was used for categorical variables. The Mann-Whitney U-test was used to

compare RAI doses, with skewed distributions, between the time periods.

Significances are two-tailed and p-values < 0.05 were considered statistically

significant.

Results

Second primary malignancies were studied in 910 patients with DTC and 4542 controls as seen on Table 1. The mean age of patients and controls at the beginning of follow-up was 49 years and 82% were female. Mean follow-up time, mean time to SPM and mortality were equal between the groups. Only few patients and controls emigrated (0.7% vs. 0.6%, p=0.736) and follow-up continued for the majority of patients and controls (68.8% vs. 70.5%) at the study end-date of the 31st of December 2011.

The surgical treatment of DTC did not differ significantly between the two hospitals participating in the study. Total or near-total thyroidectomy was performed in 78% of the patients (n= 710) and subtotal thyroidectomy was used in 16% (n=142). Thyroid cancer type was papillary in 79% (n=715), follicular variant of papillary carcinoma in 10% (n=96) and follicular carcinoma in 11% (n=99). Majority, 53% (n=480) of tumours were ≤20mm (T1) and 17% (n=158) were 21-40mm in size (T2). Lymph node metastasis in the cervical region was found in 16% (n=150) of patients and distant metastatic disease was found in 6.7% (n=61) of patients.

Forty-three antecedent cancers were observed in 910 DTC patients (4.7%), compared to 115 antecedent cancers in 4542 controls (2.5%). Before the diagnosis of DTC, 87% more malignancies were detected in patients than

controls (p<0.001). Number of synchronous malignancies was 6 (0.7%) in patients and 17 (0.4%) in controls (p=0.226).

Second primary malignancies

SPMs were observed in 12.0% (n=109) of patients and in 11.0% (n=500) of controls (Rate ratio [RR] 1.12, p=0.269). Multiple second cancers were observed in 3 (0.3%) patients and in 35 (0.8%) controls (p=0.144). Table 2 illustrates the risk for SPMs in various subgroups of patients. Increased risk of SPMs was observed in patients <40 years (RR 1.73, p=0.037), whereas patients 40–60 years or >60 years did not have a significantly higher risk for SPMs than the corresponding controls (RR 1.12 and 1.01, respectively). If the patient was treated 1996 onwards, the risk for SPMs was significantly higher than in the controls (RR 1.51, p=0.029). The risk of SPMs in the patients diagnosed during 1981–1989 and 1990–1995 was not significantly higher than in the controls (RR 0.95, p=0.773 and RR 1.07, p=0.773 respectively). There were no differences in the incidence of SPMs in patients with a papillary or a follicular thyroid cancer compared to the controls. Figure 1 illustrates the observed differences between various groups of patients and controls.

Specific tumour sites

Incidences of SPMs in specific tumour sites in patients and corresponding controls are shown in Table 3. The risk of sarcomas and soft tissue tumours was higher in patients than in controls (RR 4.37, p=0.004). More lymphatic and hematologic malignancies were observed in patients when compared to controls (RR 1.87, p=0.035). The risk for non-Hodgkin lymphoma was higher in patients (RR 2.78, p=0.035). The combined incidence of benign and malignant CNS tumours was higher in patients than in controls (RR 2.56, p=0.017), and benign meningioma was a more common finding in patients (RR 3.15, p=0.007). The incidence of thyroid cancer as a SPM was higher in the patients than in controls (RR 2.99, p=0.016). One salivary gland cancer was observed in the patients.

RAI treated patients

RAI ablation was administered to 81% of the patients (n=740). Patients with persistent or recurrent disease (n=211, 23%) had multiple RAI treatments. The median dose of RAI was 3.7 GBq (100 mCi), and the mean was 5.3 GBq (±3.7GBq). The cumulative dose of RAI was ≤3.7 GBq (≤100 mCi) in 71% of the patients (n=526) and >3.7 GBq in 29% of the patients (n=214). The mean cumulative dose of RAI rose during the study period; during 1981-1989 the dose was 4.4 GBq (±3.1GBq); during 1990-1995 5.6 GBq (±4.1GBq) and 1996

onwards 5.8 GBq (±3.5GBq) (p<0.001). Patients with a good prognosis, *i.e.,* patients with no lymph node metastasis and those with an intra-thyroid and small-sized cancer were not treated with RAI (n=170, 19%). External radiotherapy was used for 3% of the patients (n=28).

The incidences of SPMs in RAI-treated patients and controls are shown in Table 4. The overall cancer incidence was equal between patients and controls (RR 1.04, p=0.721). The incidence of sarcomas and soft tissue tumours was higher in RAI-treated patients than in controls (RR 6.37, p=0.002). One salivary gland cancer was observed in the RAI treated patients. The incidence of hepato-biliary-pancreatic cancers was lower in the RAI-treated patients than in controls (RR 0.00, p=0.024), but not in RAI-non-treated patients (RR 2.54, p=0.114). The incidence of lung and respiratory tract cancers was lower in the RAI-treated patients than in controls (RR 0.26, p=0.041), but a tendency to opposite direction was observed in patients without RAI treatment (RR 5.09, p=0.072). When compared to the controls, the RR of SPMs in patients with ≤3.7 GBq RAI dose was 0.94 (p=0.650) and with >3.7 GBq RAI dose 1.37 (p=0.143).

Discussion

In the present study, the risk of SPMs in DTC patients did not differ significantly when compared to age, gender and place of residence-matched controls of general population. Patients less than 40 years old and those treated since 1996 had an increased incidence of SPMs when compared to controls.

The strengths of this study include the excellent quality of the register data, the good matching of the patients and controls. The follow-up time was long, with median follow-up of 16 years, and maximum of over 30 years. Clinical follow-up was carried out at the same two university hospitals. The coverage of the study was good, and less than 1% of patients were lost from the registry follow-up due to immigration.

Patient's age affected the risk of SPM. Patients <40 years old had 73% higher risk for SPM than controls, whereas in older patients the risk was not significantly higher. Ronckers et al. (19) reported that in DTC patients <40 years, the risk of SPM was 39% higher than in the general population, whereas in older patients, the risk was only 6% higher. In a study by Lu et al. (15), the risk of SPMs was highest in patients less than 50 years old. It may be speculated that the young DTC patients have genetic, environmental or lifestyle-related factors, which make them more susceptible to carcinogenesis than their peers on general population. Higher incidence of antecedent malignancies in DTC

patients suggests that the general carcinogenic susceptibility of DTC patients is higher than in general population. Bidirectional association between the incidence of DTC and non-thyroidal cancers has been discovered in other studies (19,20). Hence, patients with DTC have more SPMs than general population, and patients with other malignancies have more thyroid cancers than general population. This difference may be most marked in young patients, when the overall cancer incidence is lower than in older patients.

Patients treated since 1996 had increased risk of SPMs in this study (RR 1.51), whereas patients treated before 1996 had equal risk of SPM when compared to controls.  Kim et al (21) also made a similar observation; risk for SPMs in DTC patients was higher in patients diagnosed after 2003 when compared to controls (RR 1.45) and the risk was lower for patients diagnosed earlier (RR 1.03- 1.21). Higher incidence of SPMs in DTC patients in recent decades is an interesting, yet poorly understood, observation. Proportion of small-size papillary carcinomas with favourable prognosis has rapidly increased during recent decades  (22,23). Good prognosis and longer survival of DTC patients may contribute to the increased risk of SPMs. On the other hand, disease-specific mortality has not changed significantly during the past decades  (2,24). In our study, RAI dose has increased during past decades, which may have affected the incidence of SPMs.

In some cancer sites and types, an increased incidence of SPMs in patients was observed. Sarcomas and soft tissue tumours were more common in DTC patients than in controls (RR 4.37). This finding is consistent with previous studies; Sandeep et al. (10) reported SIRs of 3.63 for soft tissue sarcoma and 3.62 for bone malignancies, whereas Rubino et al. (12) observed an RR of 4.0 for bone and soft tissue cancers. The risk of lymphatic and haematologic malignancies was elevated, especially the risk of non-Hodgkin lymphoma (RR 2.78). Sandeep et al., Brown et al. and Lu et al. (10,11,15) each also reported an increased risk of non-Hodgkin lymphoma (SIR 1.68, 1.75 and 2.66, respectively). We observed increased incidence of meningiomas in patients when compared to controls. In other studies, high incidence of CNS cancers (RR 2.2-4.0) has been reported in DTC patients. (12,15)

RAI-treated patients did not have higher overall incidence of SPMs when compared to controls or patients without the RAI treatment. In subgroups, RAI-treated patients had an increased incidence of sarcomas and soft tissue cancers. Lower incidence of hepato-biliary-pancreatic cancers and lung and respiratory organ cancers was observed in patients than in controls. These malignancies are associated with the use of alcohol and tobacco, and the decrease in incidence may be due to lifestyle changes among the patients (25). In other studies, RAI treatment has been associated with 12-19% higher incidence of SPMs when compared to DTC patients without radiation therapy (26,27). Decreased

incidence of lung cancers in DTC survivors has been reported previously. (11,19)

Due to limited number of patients and observed SPMs in this study, the results of subgroup analyses must be interpreted with caution. Risk of false positive results exists due to the limited number of events. Increased incidence of thyroid carcinomas as SPMs is explained by the date inconsistencies between the clinical and registry databases. The use of morphological classification in some cancer types may differ from some previous studies, which rely solely on topographical classification.

In conclusion, our results show that DTC patients <40 years old, as well as patients diagnosed since 1996, had a higher risk for second primary malignancy, when compared to age, gender and region-matched controls of general population. DTC patients had increased incidence of sarcomas and soft tissue cancers and hematologic and lymphatic malignancies. RAI treated patients had markedly increased incidence of sarcomas and soft tissue cancers. On contrary, the risk of hepato-biliary-pancreatic cancers and respiratory organ cancers was decreased in radioiodine treated patients. However, the results must be interpreted cautiously due to limited patient and event count. The overall incidence of second primary malignancies was not significantly higher in DTC patients.

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Tables and Figures

Table 1. General statistics and follow-up times for the patients with differentiated thyroid cancer and controls from general population. The controls were matched for age, gender and place of residence at the corresponding patient's date of diagnosis or date of radioiodine ablation.

|  |  | Patients (n=910) | Controls (n=4542) | p |
|---|---|---|---|---|
| Age | Years, mean (SD) | 49.0 (15.9) | 48.9 (15.8) | -* |
| Gender | Females | 742 (82%) | 3702 (82%) | -* |
| Follow-up time | Years, mean (SD) | 16.2 (6.9) | 16.5 (6.6) | 0.187 |
| Time to SPM | Years, mean (SD) | 10.7 (7.4) | 11.6 (6.4) | 0.240 |
| Observed SPMs |  | 109 (12%) | 500 (11%) | 0.397 |
| Mortality, all-cause |  | 214 (23.5%) | 1073 (23.6%) | 0.944 |
| Surgery | Total or near total thyroidectomy | 710 (78%) | - |  |
|  | Subtotal thyroidectomy | 142 (16%) | - |  |
|  | Lobectomy | 38 (4%) | - |  |
|  | Biopsy / Inoperable | 20 (2%) |  |  |
| Pathology | PTC | 715 (79%) | - |  |
|  | PTC follicular variant | 96 (10%) | - |  |
|  | FTC | 99 (11%) | - |  |
| TNM | T1 | 480 (53%) | - |  |
|  | T2 | 158 (17%) | - |  |
|  | T3 | 75 (8%) | - |  |
|  | T4 | 22 (2%) | - |  |
|  | Nodal metastasis (N+) | 150 (16%) | - |  |
|  | Distant metastasis (M+) | 61 (6.7%) | - |  |
| Antecedent cancers | (Before index date) | 43 (4.7%) | 115 (2.5%) | **<0.001** |
| Synchronous cancers | (Index date +12 months) | 6 (0.7%) | 17 (0.4%) | 0.226 |

*matched groups, PTC=papillary thyroid carcinoma, FTC=follicular thyroid carcinoma

Table 2. Different subgroups of patients (n=910) and the corresponding controls (n=4542), second primary malignancies and cancer incidence per 10000 person-years. Person-years at risk 14707 for the patients and 74902 for the controls.

| Subgroup of patients | Patients | | Controls | | Patients vs. controls | |
|---|---|---|---|---|---|---|
| | Cases | Incidence | Cases | Incidence | Rate ratio | p |
| All | 109 | 77.28 | 500 | 68.76 | 1.12 | 0.269 |
| Gender | | | | | | |
| Females, n=742 | 88 | 75.28 | 368 | 60.78 | 1.24 | 0.071 |
| Males, n=168 | 21 | 86.99 | 132 | 108.43 | 0.80 | 0.348 |
| Age | | | | | | |
| <40 years, n=279 | 19 | 37.47 | 59 | 21.69 | 1.73 | **0.037** |
| 40-59 years, n=391 | 51 | 77.96 | 231 | 69.59 | 1.12 | 0.463 |
| ≥60 years, n=240 | 39 | 156.55 | 213 | 155.51 | 1.01 | 0.969 |
| Year of diagnosis | | | | | | |
| <1990, n=293 | 41 | 72.34 | 226 | 75.99 | 0.95 | 0.773 |
| 1990-1995, n=294 | 32 | 67.64 | 151 | 63.28 | 1.07 | 0.732 |
| ≥1996, n=323 | 36 | 97.11 | 123 | 64.34 | 1.51 | **0.029** |
| Primary thyroid cancer type | | | | | | |
| PTC, n=715 | 91 | 79.77 | 392 | 67.49 | 1.18 | 0.150 |
| PTC, follicular variant, n=96 | 3 | 20.81 | 39 | 55.38 | 0.38 | 0.089 |
| FTC, n=99 | 15 | 119.59 | 69 | 90.89 | 1.32 | 0.334 |
| Radioiodine treatment (RAI) | | | | | | |
| No RAI, n=170 | 26 | 89.08 | 90 | 59.88 | 1.49 | 0.072 |
| ≤3.7 GBq (≤100 mCi)*, n=526 | 56 | 67.41 | 306 | 72.01 | 0.94 | 0.650 |
| >3.7 GBq (>100 mCi)*, n=214 | 27 | 93.79 | 104 | 68.47 | 1.37 | 0.143 |

PTC=papillary thyroid carcinoma, FTC=follicular thyroid carcinoma, *Cumulative dose

Table 3. Patients with differentiated thyroid cancer (n=910) and in age, gender and place of residence-matched controls (n=4542), incidence of second primary malignancies per 10,000 person years.

| Cancer type | Patients | | Controls | | Patients vs. controls | |
|---|---|---|---|---|---|---|
| | Cases | Incidence | Cases | Incidence | Rate Ratio | p |
| Head and neck | 2 | 1.36 | 13 | 1.74 | 0.784 | 0.748 |
| Oesophagus and stomach | 2 | 1.36 | 25 | 3.34 | 0.41 | 0.206 |
| Small intestine and Colorectal | 9 | 6.14 | 46 | 6.16 | 1.00 | 0.99 |
| Hepato-biliary-pancreatic | 4 | 2.72 | 34 | 4.54 | 0.60 | 0.327 |
| Respiratory organ | 4 | 2.72 | 42 | 5.61 | 0.49 | 0.157 |
| Skin | 8 | 5.45 | 38 | 5.09 | 1.07 | 0.860 |
| Breast (female) | 25 | 20.75 | 122 | 19.86 | 1.05 | 0.841 |
| Gynaecological | 11 | 9.05 | 61 | 9.86 | 0.92 | 0.795 |
| Prostate | 6 | 24.3 | 48 | 38.45 | 0.63 | 0.286 |
| Kidney and renal pelvis | 6 | 4.09 | 16 | 2.14 | 1.91 | 0.167 |
| Urinary tract | 1 | 0.68 | 3 | 0.40 | 1.70 | 0.643 |
| Central nervous system | 2 | 1.36 | 4 | 0.53 | 2.55 | 0.26 |
| Thyroid | 7 | 4.79 | 12 | 1.60 | 2.99 | **0.016** |
| Miscellaneous | 3 | 2.04 | 17 | 2.27 | 0.90 | 0.865 |
| Morphological classification | | | | | | |
| Mesenchymal tumours | 7 | 4.76 | 13 | 1.74 | 2.74 | **0.025** |
|    Sarcomas and soft tissue | 6 | 4.08 | 7 | 0.94 | 4.37 | **0.004** |
| Lymphatic or haematopoietic | 15 | 10.25 | 41 | 5.49 | 1.87 | **0.035** |
|    Non-Hodgkin lymphoma | 6 | 4.09 | 11 | 1.47 | 2.78 | **0.035** |

Table 4. Radioiodine-treated patients with differentiated thyroid cancer (n=740) and in age, gender and place of residence matched controls (n=3692), incidence of second primary malignancies per 10,000 person-years.

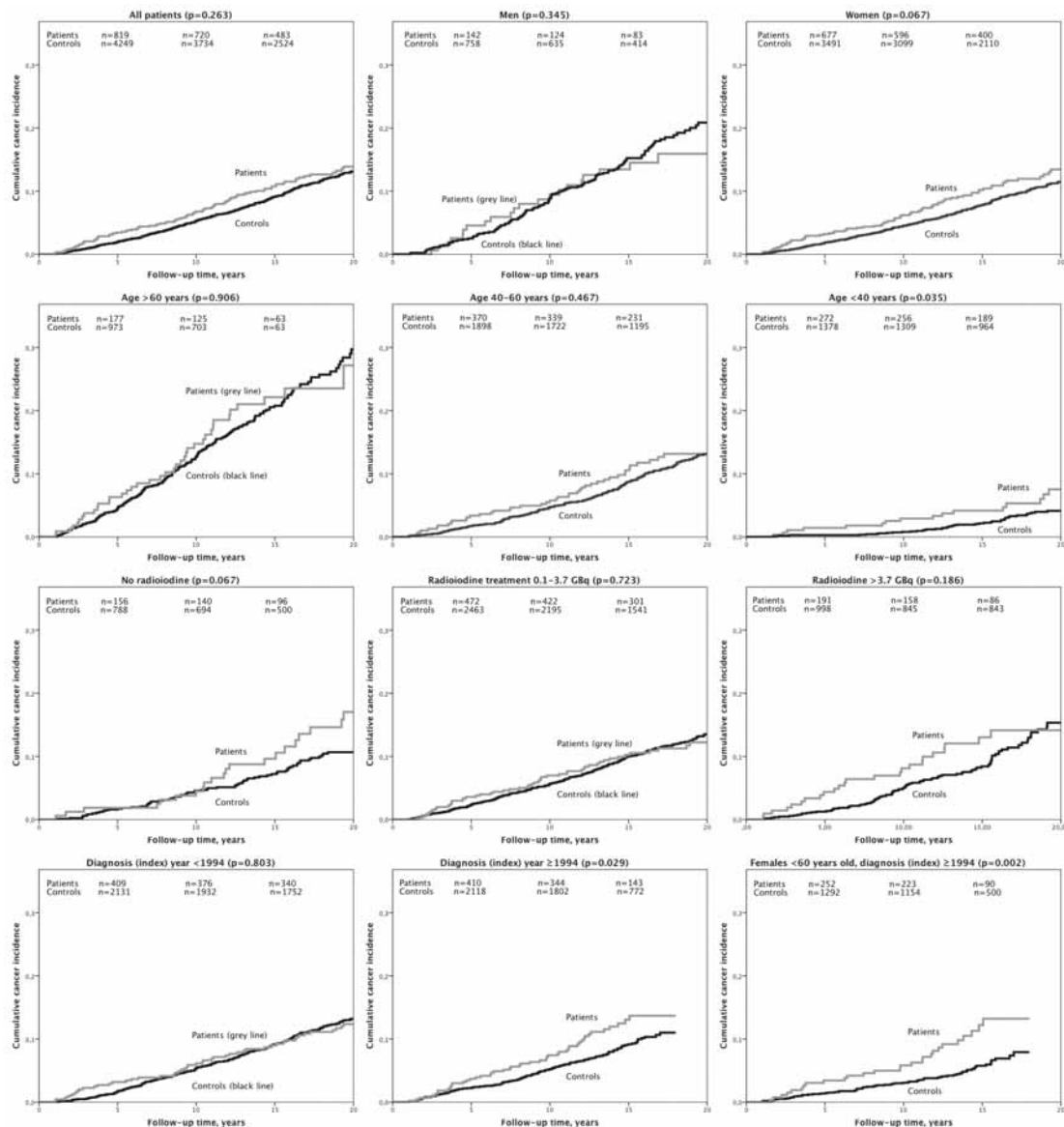| | Patients | | Controls | | Patients vs. Controls | |
|---|---|---|---|---|---|---|
| Cancer type | Cases | Incidence | Cases | Incidence | Rate ratio | p |
| All | 83 | 74.20 | 410 | 71.08 | 1.04 | 0.721 |
| Head and neck | 2 | 1.72 | 12 | 2.02 | 0.85 | 0.831 |
| Oesophagus and stomach | 2 | 1.71 | 21 | 3.53 | 0.49 | 0.318 |
| Small intestines and colorectal | 8 | 6.88 | 38 | 6.41 | 1.08 | 0.853 |
| Hepato-biliary-pancreatic | 0 | 0 | 26 | 4.37 | 0.00 | **0.024** |
| Respiratory organ | 2 | 1.71 | 40 | 6.73 | 0.26 | **0.041** |
| Skin | 6 | 5.15 | 29 | 4.89 | 1.05 | 0.907 |
| Breast (female) | 18 | 19.04 | 100 | 20.71 | 0.92 | 0.742 |
| Gynaecological | 9 | 9.47 | 49 | 10.08 | 0.94 | 0.863 |
| Prostate | 6 | 28.87 | 39 | 37.50 | 0.77 | 0.550 |
| Kidney and renal pelvis | 4 | 3.44 | 14 | 2.35 | 1.46 | 0.502 |
| Urinary tract | 1 | 0.86 | 3 | 0.50 | 1.70 | 0.641 |
| Central nervous system | 2 | 1.71 | 4 | 0.67 | 2.55 | 0.263 |
| Thyroid | 6 | 5.18 | 12 | 2.02 | 2.56 | 0.051 |
| Miscellaneous | 3 | 2.57 | 15 | 2.52 | 1.02 | 0.975 |
| Morphological classification | | | | | | |
| Mesenchymal tumours | 6 | 5.15 | 9 | 1.51 | 3.40 | **0.014** |
| Sarcomas and soft tissue | 5 | 4.29 | 4 | 0.67 | 6.37 | **0.002** |
| Lymphatic or haematopoietic | 11 | 9.48 | 31 | 5.22 | 1.82 | 0.085 |
| Non-Hodgkin lymphoma | 3 | 2.58 | 8 | 1.35 | 1.92 | 0.328 |

Figure 2. The top left figure illustrates all patients and corresponding controls, followed by comparison of subgroups of women and men with controls. In the second row, patients in the age groups >60 years, 40-60 years and <40 years are compared with the corresponding controls. In the third row, patients with no radioiodine treatment, with a cumulative radioiodine dose of ≤3.7 GBq and with a cumulative radioiodine dose of >3.7 GBq are compared with controls. In the fourth row, patients diagnosed before and after January 1994 are compared with controls. The last image on the fourth row illustrates a subgroup of women <60 years old diagnosed after January 1994 with the corresponding controls. The log-rank test was used for testing statistical significance.